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Highlights

- Anti-Helicobacter Pylori
- Respiratory Syncytial Virus
- Haematological Profile
- Penicillin and Cephalosporin

The Blood Plasma

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A Study of Rational Prescriptions of Penicillin and Cephalosporin Antibiotics in a Secodary Health Care Facility in South West Nigeria

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Abstract - The purpose of this study is to assess the prescriptions pattern of penicillin and cephalosporin antibiotics among physicians at Sacred Heart Hospital Lantoro Abeokuta and determine their conformity with standard guidelines and principles of antibiotic use. It was a retrospective study involving data obtained from outpatient case notes that were prescribed with cephalosporin and penicillin antibiotics during the 6 months period of January to June 2010. A total of six hundred and fourteen (614) case notes were randomly selected and used for the study. One hundred and sixty seven (167) (**27%**) patients were of the age group 0 - 9 years, 31(**5%**) patients were aged 10 – 19 years and 226 (**37%**) patients were of age group 20 – 29 years . Four hundred and forty (440) (**71.7%**) patients were males while 174 (**28.3%**) patients were females. Among diagnosis studied were upper respiratory tract infection **(URTI)**238 (38.8%) and lower respiratory tract infection **(LRTI)**(**21.0%**) ($p = 0.00393$) ($p<0.05$). There was no definite diagnosis **(NDD)** made in 37 (**6.02%**) cases.

Keywords : Antibiotics, Penicillins, Cephalosporins, Rational, Prescriptions.

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Omole, Moses Kayode Pharm. D α & Adeola Adebisi Michael M. Pharm σ

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Four hundred and forty (440) (71.7%) patients were males while 174 (28.3%) patients were females. Among diagnosis studied were upper respiratory tract infection (URTI) 238 (38.8%) and lower respiratory tract infection (LRTI) (21.0%) ($p = 0.00393$) ($p<0.05$). There was no definite diagnosis (NDD) made in 37 (6.02%) cases.

Penicillins were prescribed for 474 (77.2%) patients and cephalosporins were prescribed for 140 (22.8%) patients. Both classes of antibiotics were prescribed most frequently for URTI. The cost of filling a prescription followed a normal curve distribution with the peak at the age group 35 – 49 years for both penicillins and cephalosporins antibiotics. Generic prescriptions were found to be 95(15.5%) for penicillins and 103(16.7%) cephalosporins. Prescriptions by proprietary names were 379(61.8%) for penicillins and 37(6.0%) for cephalosporins. Mean duration of prescription for penicillins was 6.65 ± 1.95 days while it was 5.5 ± 1.5 days for the cephalosporins. There was neither a case of microbial culture sensitivity test (MCS) nor a case of adverse effect documented.

The study showed that prescriptions pattern of penicillin and cephalosporin antibiotics were not completely in line with standard guidelines of antibiotic therapy. Measures should be taken to detect and document adverse drug reactions and consideration should be given to microbial culture sensitivity test.

Keywords : Antibiotics, Penicillins, Cephalosporins, Rational, Prescriptions.

Running Title : Rational prescriptions of Penicillins and Cephalosporins.

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I. Introduction

Antibiotics are the most frequently prescribed and misused drugs and there are reported concerns about the continuous indiscriminate and excessive use of antibiotics leading to emergence of antibiotic-resistant organisms (N J Mclellan2001)(Krivoy et al 2007).

Despite the wide range of antibiotics available for treatment for infections, therapeutic failure has been increasingly experienced. This can largely be attributed to their irrational usage leading to development of resistant strains of bacteria. (N J Mclellan2001).

Rational use of antibiotics means that right antibiotics should be prescribed for the right patient in adequate dose for the sufficient duration as appropriate to the clinical needs of the patient at the lowest cost (WHO 1988). Many people are dying from infectious diseases that are curable but which no longer have the correct treatment.(Gaash B.2008). (Abdelsalam mohamed hamed Elfaki.2010)This is because certain bacteria are transforming themselves and developing increasing resistance to antibiotics. More than 80% of the most common bacteria, Staphylococcus aureus are now resistant to penicillin such as ampicillin (Henry F chembers.2004). The problem of irrational use of antibiotics is both complex and multi- faceted, but whatever its complexity, it should not be underestimated because it has a harmful influence on certain prevalent conditions such as caused by Staphyloccus aureus infections that can successfully be treated. Inappropriate drug prescriptions has been identified in many health facilities in Nigeria (Erah et al 2003) (Akande and Medubi 2009).,(Omole and Bello 2011).

The discovery of penicillin by Sir Alexander Fleming in 1928 ushered in the antibiotic era and transformed the practice of medicine (EH Decloedt et al 2008). It was however in 1940 that penicillin was produced in sufficient pure form to treat human infections. Many useful antibiotics have since been developed that belong to the penicillins. According to figures gathered during surveys by WHO in 2000, about 60% of antibiotics in Nigeria were prescribed unnecessarily. (Gaash B 2008).

The penicillins are classified as B-lactam drugs because of their four- membered lactam ring. They shared features of chemistry, mechanism of action, pharmacologic, clinical effect and immunologic characteristics with cephalosporins which are also B-lactam compound.

Studies had confirmed that these two classes of antibiotics (penicillins and cephalosporins) are widely prescribed. In a study (Palikhe N 2004) at pediatric hospital of Kathmada valley.,cephalosporins was the top most frequently prescribed antibiotics followed by penicillins. Penicillins were found to be more frequently prescribed than the cephalosporins in a study conducted at University of Ilorin teaching hospital (Akande et al 2009). Penicillins and cephalosporins were prescribed in more than half of the estimated emergency department visits for antibiotic-associated adverse events (E H Decloedt 2008). There is the need to prevent the age long antibiotics (Penicillins and Cephalosporins) from loss of efficacy by ensuring that they are rationally prescribed.

Not many studies have been conducted on rational prescriptions of these two classes of antibiotics by the Physicians in Nigeria. This study therefore examines the rational prescriptions of penicillin and cephalosporin antibiotics at Sacred Heart Hospital Abeokuta in Ogun State of Nigeria and determine their conformity with standard guidelines and principles of antibiotic use with the goal of providing and promoting pharmaceutical care.

II. Patients and Methods

Sacred Heart Hospital (SHH) is a secondary mission healthcare institution situated in Abeokuta, Ogun State in Nigeria. It was established in the year 1805 and currently attracts patients throughout the state and the neighboring states.

The study was a retrospective study involving data obtained from outpatients case notes. The data were collected from case notes of patients prescribed with either penicillin antibiotics or cephalosporin antibiotics or both antibiotics during the six months period of January to June 2010.

A total of 626 outpatient's case notes were randomly selected from the outpatients medical record. Twelve (12) patients' case notes were excluded from the study due to incomplete data. A total of 614 outpatient case notes were therefore used for the study.

Information obtained from the case notes were demographic data including age, sex, presenting complaint, laboratory investigations, penicillin and cephalosporin antibiotics prescribed in generic and proprietary names, dosage regimen, change in therapy, educational status of patients, marital status, microbial culture sensitivity (MCS) test, diagnosis made and adverse drug reactions.

The information obtained from each outpatient case note was entered using Epi info. Analysis was done using the Statistical Package for Social Sciences (SPSS) Version XII (12). Results were presented in frequencies, percentages, means and standard deviations. Two categorical variables were compared using the Chi-Square test and two unrelated variables were compared using Pearson correlation. Statistical significance was decided at the 5% level ($p<0.05$).

The management of Sacred Heart Hospital Lantoro Abeokuta granted the ethics to carry out this study.

III. Results

One hundred and sixty seven (167) (27%) patients were aged 0- 9years, 31 (5%) patients aged 10 – 19 years, 226 (37.0%) patients aged 20 – 29 years, 111 (18%) aged 30 – 39 years, 52 (8.5%) aged 40-49 years, 13 (2.2%) aged 50-59 years, 11 (1.8%) aged 60 - 69 years and 3 (0.5%) patients aged 70 years and above. Four hundred and forty (440) (71.7%) were males while 174 (28.3%) were females. (Table 1)

One hundred and eighty seven (187) (30.4%) patients aged 19 years and above had URTI. Patients aged 0 – 9 years had no UTI and PID. Two hundred and seventy nine (279) (45.3%) patients were diagnosed of URTI while pelvic inflammatory disease (PID) was diagnosed in 12 (2%) patients.(Table 2)

The penicillins and the cephalosporins were prescribed mostly for upper respiratory tract infection (URTI). Amoxycillin (penicillin) was prescribed for 61(9.9%) patients, Ampiclox (Ampicillin + Cloxacillin) for 99 (16.1%) patients and Amoxiclav for 30(4.8%) patients. The penicillins were not prescribed for pelvic inflammatory disease (PID) but Amoxiclav (Amoxicillin + Clavuric acid) was prescribed for 2 (0.3%) patients with PID. Cefuroxime the only cephalosporin was prescribed for all the conditions diagnosed. (Table 3)

The penicillins were prescribed for mean duration of 4.7 days for patients aged 0 - 5 years and 8.6 days for patients aged 18 – 34 years. The mean duration for cephalosporins prescribed ranged between 4.0 days for patients aged 0-5years and 7 days for patients aged 35-49 years.Both penicillins and cephalosporins were prescribed for the longest mean duration for patients in the age group 19 – 34 years and were prescribed for least mean duration for patients in the the age group 0 - 5 years. The average cost of filling each antibiotic prescription with the cephalosporins was found to be N1400.00 for patient in the age group 0 - 5 years. With the penicillins, the highest mean cost was found to be N655.00 for patients in the age group 35-49 years (Table 4). Table 4 further showed that the cost of filling each prescription followed a normal distribution with a peak at the age group 35 – 49 for both penicillins and cephalosporins.(Table 4)

Total prescriptions by proprietary names 416 (67.8%) were higher than generic prescriptions 198(32.2). Prescriptions by proprietary 379 (61.7%) were higher than the generic prescriptions 95 (15.4) for the penicillins whereas it was found to be lower 37(6%) than generic prescriptions 103 (16.7%) for the cephalosporins.(Table 5)

IV. Discussion

The number of male patients that were prescribed with penicillin and cephalosporin antibiotics was higher 440(71.7%) than female patients 174(28.3%). There was a significance association between drugs prescribed and sex (P= 0.00393) ($p<0.05$) (Table 1). This result was similar to the study conducted by Palikhe in 2004 in Kathmandu medical college, and study conducted at the university of Ilorin teaching hospital by Akande et al in 2009 which showed higher antibiotics prescriptions for males to be 54.0%. Patients in the age group 0 - 9 years were prescribed antibiotics 167 (27.0%) more frequently than older children aged 10 - 19 years 31(5.0%) There was a significance association between drugs prescribed and age. (P = 0.00393) ($p<0.05$). This was similar to the result obtained by Marlies et al in 1999 which showed that patients aged below ten years were treated with antibiotics more frequently 25% than those above ten years (11%) (P = 0.0256) ($p<0.05$) and the study conducted by Palikhe in 2004 which showed that patients below one year received antibiotic treatment more frequently than older patients.(Table 1).

Cephalosporins and penicillins were prescribed mostly for upper respiratory tract infections (URTI) 279(45.3%) and least prescribed for pelvic inflammatory disease (PID) 121(2.0 %). There was no definite diagnosis made in 45 patients (7.4%) (Table 2). This study showed that patients in the age group 0 – 9 years presented more frequently with upper respiratory tract infection 77 (12.5%) than for other infections. This may be due to the fact that infants have less developed respiratory organs and may be more prone to respiratory infections. There were no cases of pelvic inflammatory disease (PID) and urinary tract infection in this group of patients. PID and UTI were only presented by patients in the age group 18 years and above (Table 2)

Ampiclox® (Ampicillin + cloxacillin) 99(16.1%) was the most frequently prescribed penicillins. (Table 3) for URTI while Amoxiclav (Amoxycillin + clavulanic acid) 30(4.8%) was the least prescribed penicillins for the same condition. Cefuroxime in the form of suspension and tablet was the only cephalosporin prescribed in this study. Cefuroxime was prescribed mostly for URTI and least prescribed for PID. This was similar to the pattern seen with the penicillins. All the conditions were treated with single cephalosporin antibiotics (Table 3). This was similar to the study conducted by Palikhe in 2004 who reported that 93% of the patients studied were prescribed only with one antibiotics. He also reported that 75% cases of enteric fever was treated with single antibiotics. Other studies showed lower percentage treatment with single antibiotics 60.6% (Josefina and Caminnal et al 2005) and 36% (Marlies et al 199l). In the study conducted, penicillins were more frequently prescribed 474 (77.2%) than the cephalosporins 140 (22.8%). (Table 3). A study conducted at University of Ilorin Teaching Hospital (Akande et al 2009) reported higher prescription for penicillins (72%) than cephalosporins (28%.) Another study reported by Palikhe in 2004 indicated higher prescription for cephalosporins. The prescriptions of antibiotics in this study which was based mainly on clinical judgment (empirical treatment) without microbial culture sensitivity (MCS) test was similar to study conducted by Palikhe 2004. There was also a similar study conducted by Suping Hu et al 2002 which showed collection of specimen for culture to be only 8.4% among the patients prescribed with antibiotics. In all the cases considered in this study specimen for culture were not obtained. It is very necessary to ensure that specimen are obtained and cultured before initiating antibiotic therapy in some of the cases. Measures should be taken to avoid the inappropriate use of antibiotics to prevent antibiotics resistance, high health care costs and possible side effect including gastrointestinal side effect (Sneha et al 2006) (Saping 2009). Among the penicillins, the mean duration of prescription for penicillins was 6.3 days and 5.1 days for the cephalosporins (Table 4). This differed from mean duration of prescription 10.59 days reported by study conducted at University of Ilorin Teaching Hospital (Akande et al 2009).

Except for few conditions, the optimum duration of antibiotic treatment is unknown. Many antibiotics are often prescribed for duration of 5-7 days (Krivoy et al 2007) (Lim V Ket al 2009). Nevertheless it is reasonable to discontinue therapy even after a shorter period if patients' symptoms have resolved. There are however certain infections where prolonged treatment is necessary. In some conditions such as uncomplicated cystitis in women and gonococcal urethritis in males, single dose regimen have been shown to be effective for shorter duration.

Duration of therapy depends on the site and severity of infection such as tonsillitis – 10 days, bronchitis 5-7 days, urinary tract infection single shot to 21 days, lung abscess 2-8 weeks and tuberculosis 6-24 months. The frequency of administration was found to range from six- hourly of four times in twenty four hours to twelve-hourly of two times in twenty four hours with the penicillins and from twelve – hourly to once daily of one dose in twenty four hours with the cephalosporin. These are concurrent with the recommended standard

doses in literature (BNF 2009) (EMDEX 2006). Frequency of administration could be increased in cases of severe, deep seated and sequestrated infections and reduced in cases of renal failure. Only 198(32.2%) of the prescribed penicillins 95(15.5%) and cephalosporins 103(16.7%) were in generic names (Table 5). This is similar to 37.2% generic prescription reported by Abdel Salam in 2010. A similar study conducted at the teaching hospital Ilorin reported generic antibiotic prescription to be 45.6 %.(Akande et al 2009). There was a similar report in some African Countries where generic prescription of drugs is not a popular practice (Akande et al 2009).

Proprietary prescrition was relatively high (67.8%) (Table5). Generic prescription however, is the internationally accepted method of prescription. It follows therefore that the choice of either the cephalosporins or the penicillins was based on the discretion of the physicians. Oral route was used in all the cases considered for both penicillins and cephalosporins. This is expected as the patients were out patients and were able to tolerate oral medications.Exactly 6.1% of the antibiotics prescriptions were for common cold and catarrh. This is unnecessary as antibiotics are not appropriate for viral infections. In a Kentucky study, 60% of patients were prescribed antibiotics for common cold (Marlies A. Van 1999) (Sharm Rashimi et al 2005) (Sujit J Chandy 2008). There was no case of adverse drug reaction documented. This may be that there was no adverse drug reaction or that the case was not reported or documented. In a similar study by Palikhe in 2004,only 9% cases of adverse drug reaction were reported.

Antibiotic monotherapy was found to be high. Cases where combination therapy was used include Ampiclox (Ampicillin + cloxacillin). This combination displays synergy against some B-lactamase producing organisms since cloxacillin potentuates ampicillin antibacterial activity (Emdex 2006). Amoxiclav (Amoxycillin + clavulanic acid) was another combination therapy prescribed. Although clavulanic acid is not an antibiotic, it protects amoxicillin from enxymatic destruction by binding to them resulting in potentiation or synergistic effect.

There was no case of change in therapy probably because most of the cases might not have warranted antibiotics prescription in the first instance and due to the fact that culture sensitivity test was not done.

V. Conclusion

The use of antibiotics in the outpatient department (OPD) of Sacred Heart Hospital was not completely in line with the standard guidelines of antibiotic therapy as regards the generic prescriptions, cost and frequency of use. There is need in some cases to carry out a culture sensitivity test before prescribing antibiotics especially in children as their organs are not fully developed and they can easily suffer from toxic and adverse effects of drugs.

VI. Acknowlegdment

We hereby acknowledge the technical support and cooperation of the members of staff of Sacred Heart Hospital, Lantoro Abeokuta Ogun State, Nigeria.

Table 1 : Ages and sex distributon of patients prescribed with penicillins and cephalosporins. (N = 614).

AGE (YRS)	FREQUENCY			PERCENTAGE OF PEN AND CEP PRESCRIBED	
	PEN	CEP	TOTAL		
0 - 9	129	38	167	27.0	
10 - 19	24	7	31	5.0	
20 -29	174	52	226	37.0	
30 - 39	86	25	111	18.0	P = 0.00393
40 - 49	40	12	52	8.5	
50 - 59	10	3	13	2.2	
60 - 69	8	3	11	1.8	
70 & abv.	3	0	3	0.5	
Total			614	100.0	
SEX					
Male	249	91	440	71.7	
Female	130	44	174	28.3	
Total			**614**	**100.00**	

PEN = penicillin, CEP = cephalosporin.

Table 2 : Diagnoses based on different age group.

Diagnosis	Age (yrs)	Frequency		Percentage	
URTI	0 – 9	77		12.5	
	10 - 18	15		2.4	
	19 & above.	187	(279)	30.4	(45.3%)
LRTI	0 – 9	2		0.3	
	10 - 18	1		0.2	
	19 & abv.	20	(23)	3.3	(3.8%)
ENTERITIS	0 - 9	59		9.6	
	10 - 18	12		2.0	
	19 & abv.	62	(133)	10.0	(21.6%)
UTI	0 - 9	-		0.0	
	10 - 18	-		0.0	
	19 & abv.	73	(73)	11.9	(11.9%)
PID	0 - 9	-		0.00	
	10 - 18	-		0.00	
	19 & abv	12	(12)	2.0	(2.0%)
ENT. FEVER	0 - 9	7		1.1	
		1		0.2	
	19 & abv	41	(48.2)	6.7	(7.7%)
NDD	0 - 9	20		3.3	
	10- 18	4		0.7	
	19 & abv.	21	(45)	3.4	(7.4%)
TOTAL		**614**		**100.0**	**(100%)**

URTI : *Upper respiratory tract infection*
LRTI : *Lower respiratory tract infection*
ENTERITIS : *Enteritis*
UTI : *Urinary tract infection*
PID : *Pelvic inflammatory diseases*
ENT. FEVER : *Enteric fever*
NDD : *No definite diagnosis*

Table 3 : Penicillins and cephalosporins prescribed for different diagnosis N = 614.

Diagnosis	Amoxycillin (Penicillin)		Ampicillin+ cloxacillin		Amoxycillin+ clavulanic acid		Cefuroxime	
	Freq.	%	Freq.	%	Freq.	%	Freq,	%
URTI	61	9.9	99	16.1	30	4.8	48	7.8
LRTI	26	4.3	49	8.1	14	2.3	22	3.7
Enteritis	31	5.1	52	8.5	21	3.4	25	4.0
UTI	15	2.4	7	1.1	17	2.8	15	2.4
PID	0	0.0	0	0.0	2	0.3	5	0.8
Ent. Fev.	21	3.4	0	0.0	0	0.0	17	2.7
Others	10	1.6	16	2.6	3	0.5	8	1.4
Total	164	26.7	223	36.4	87	14.1	140	22.8

URTI : *Upper respiratory tract infection*
LRTI : *Lower respiratory tract infection*
ENTERITIS : *Enteritis*
UTI : *Urinary tract infection*
PID : *Pelvic inflammatory diseases*
ENT. FEVER : *Enteric fever*

Table 4 : Mean duration and cost of prescribed antibiotics.

AGE GRP.(yrs)	MEAN DURATION OF ANTIBIOTCS PRESCRIPED (DAYS)		MEAN COST OF ANTIBIOTICS PRESCRIBED Naira (Dollar)		MEAN COST OF ALL DRUGS PRESCRIBED
	PENICILLINS	CEPHALOSPORINS	PENICILLINS	CEPHALOSPORINS	
0 - 5	4.7	4.0	410(2.6)	1400(8.8)	1700(10.6)
6 - 10	5.1	6.0	430(2.7)	1125(7.0)	1345(8.4)
11 - 18	8.2	5.1	565(3.5)	950(5.9)	1100(6.9)
19 - 34	8.6	5.1	600(3.8)	950(5.9)	1105(6.9)
35 - 49	6.8	7	655(4.1)	1100(6.9)	1250(7.8)
50 - 69	5.2	4.3	450(2.8)	485(3.0)	523(3.3)
70 – abv	5.0	4.1	420(2.6)	475(3.0)	495(3.1)
MEAN %			20.40(0.1)	36.87(0.2)	42.73(0.3)

Convert to Naira to Dollar.

Table 5 : Prescriptions according to generic and proprietary.

PENICILLINS					CEPHALOSPORINS				
	GENERIC		PROPRIETARY			GENERIC		PROPRIETARY	
	freq	%	freq	%		freq	%	freq	%
Amoxycillin susp.	44	7.2	26	4.3	Cefuroxime susp.	41	6.7	13	2.1
Amoxycillin caps	51	8.3	43	7.0	Cefuroxime tab	62	10.0	24	3.9
Ampiclox susp			201	32.7					
Ampiclox caps	-	-	22	3.6					
Amoxiclav susp.		-	35	5.7					
Amoxiclav tablet	-	-	52	8.5					
Total %	95	15.5	379	61.8		103	16.7	37	6.0

Ampiclox = (ampicillin + cloxacillin), Amoxiclav = (amoxicillin + clavulanic acid).

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Haematological Profile in Children with Protein Energy Malnutrition in North Central Nigeria

By Saka A.O , Saka M J, Ojuawo A , Abdulkarim Aa , Bilamin Sa , Latubosun L & Adeboye Man

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Abstract - Background : Protein Energy Malnutrition **(PEM)** is associated with various changes in the body systems including changes in the haematologic system. These changes affect all the blood cells.. Observations about haematological changes in this group of children have been inconsistent due to frequent and constant changes in haemopoesis resulting from this condition. This has limited the usefulness of these parameters in the anticipatory care of these patients thus the need to describe and validate the changes and possible haematological disturbance among children with **PEM** informed this study in Ilorin, North-central Nigeria.

Keywords : *Children, Protein Energy Malnutrition, Haematologic profiles.*

G JMR - B Classification: *NLMC Code: WM 175, WS 115, WT 115*

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Haematological Profile in Children with Protein Energy Malnutrition in North Central Nigeria

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Abstract - Background : Protein Energy Malnutrition **(PEM)** is associated with various changes in the body systems including changes in the haematologic system. These changes affect all the blood cells.. Observations about haematological changes in this group of children have been inconsistent due to frequent and constant changes in haemopoesis resulting from this condition. This has limited the usefulness of these parameters in the anticipatory care of these patients thus the need to describe and validate the changes and possible haematological disturbance among children with **PEM** informed this study in Ilorin, North-central Nigeria.

Methodology : All children admitted into the Emergency Paediatric Unit **(EPU)** with a diagnosis of **PEM** were enrolled over a period of one year (January – December 2009). Controls were well children attending the routine clinic without haematologic or infectious condition. Haematological profiles were determined using auto-analyzer SMX 60. Data entry and analysis were carried out with a micro-computer using the Epi info version 3.5 (2008) software packages and p value of < 0.05 was regarded as significant.

Results : Ninety children with **PEM** and 90 age and sex matched controls were studied. Children with **PEM** had lower mean values for haemoglobin, haematocrit and mean corpuscular haemoglobin ($p<0.05$) when compared with controls. The mean value of **WBC** in the children with PEM was 12.8±11.6 ×10 3 cell/mm 3 while it was 5.9±8.7 ×10 3 cell/mm 3 among the controls($p= 0.001$). The mean value of platelet counts were 291.8 ±131.7 × 10 9 / L and 326.4 ±133.9× 10 9 / L for the subjects and controls respectively ($p=0.0001$). A statistical significant difference was observed in the lymphocyte count of the various classes of PEM with the edematous forms having higher counts ($p= 0.0001$). *Conclusion / Recommendation :* In conclusion, Children with Protein Energy Malnutrition had lower red cell indices and platelet count, and a higher white cell count than the controls. Also the edematous forms of **PEM** had higher granulocyte and lymphocyte counts when compared to the non edematous forms.

The study hereby recommends that more frequent studies be carried out to describe in more details the trend of such changes in these conditions. This would enhance the anticipatory care and outcome of the children affected.

Keywords : Children, Protein Energy Malnutrition, Haematologic profiles.

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I. Introduction

Protein Energy Malnutrition **(PEM)**, is defined as a spectrum of diseases arising as a result of an absolute, or relative deficiency of calories and or protein in the diet[1,2] . It is globally the most important risk factor for illness and death, with hundreds of millions of young children affected[3].

According to **UNICEF** in 2005, malnutrition was associated with approximately 50% of child deaths worldwide[4].It has been estimated that **PEM** affect every fourth child in the developing world[4], with the regional prevalence for the severe forms ranging from 1-7%[5]. It is associated with 49% of the 10 million deaths occurring in children in the developing world and 52% of all under five deaths in Nigeria[6], with 24% and 16% of the total under-5 Nigerian population estimated to have suffered from mild-moderate and severe malnutrition respectively from 1973 to 1983[7,8] .The hospital based incidence of severe **PEM** in Nigeria varies from 3.18% in Ilorin[9]. 4.39% in Ibadan8 and 4.5% in Ife[10].

Protein Energy Malnutrition results in various changes in the body including changes in haematologic profile of the body. Low red cell count resulting in anaemia has always been a constant feature of protein energy malnutrition and may be normochromic normocytic, microcytic hypochromic, or, macrocytic [11,12.] The anaemia of malnutrition may be attributable to various factors such as iron deficiency, and /or reduced red cell production in adaptation to a smaller lean body mass[2,12]. Erythropoietin deficiency, deficiencies of vitamins (folic acid, B12,) or trace elements (copper, zinc), infections and chronic diseases have also been implicated[2,12,13].

White cell changes seen in protein energy malnutrition varies and such changes have been attributed to various factors. These include the synergist relationship which **PEM** has with infections and thymic atrophy seen in children with **PEM**[16].

This paper set to validate existing literature on these changes especially in a condition where possible

adaptational changes development can occur frequently. It will also evaluate further , possible haematologic changes in the sub classes of protein energy malnutrition.

II. Methodology

The study was a case control study carried out at the University of Ilorin Teaching Hospital among children (6 -59 months old) with PEM and controls were children with normal nutritional status without haematological or infectious conditions. All consecutive admissions into the Emergency Paediatric Unit with a diagnosis of PEM based on the Wellcome classification that fulfilled the inclusion criteria were enrolled. Controls were well children attending the routine clinic without haematologic or infectious condition. Children with history suggesting ongoing haemolysis and haemoglobinopaties were excluded.

The minimum calculated sample size with 10% attrition rate was 90, thus 90 subjects each were selected for the study and control groups with a total of 180 participants for the study.

A semi–structured questionnaire (proforma) was used to obtain information from the subjects using interview method. Relevant information on the child's socio-demographic characteristics, nutritional indices and laboratory findings were documented.

Study participants were grouped into upper, middle and lower socioeconomic classes based on the Oyedeji socio –economic classification scheme[13].

Under aseptic conditions, after cleaning the venepuncture site with 70% alcohol, 5ml of venous blood was collected by venepucture using a fixed hypodermic needle. The blood specimen was decanted into a sample bottle containing ethylene diamine tetra acetate (EDTA) and gently mixed to prevent clotting. The sample was analysed using an automated blood analyzer model/Symax KX 21® .

Data entry and analysis were carried out with a micro-computer using the Epi info version 3.5 (2008) software packages .Chi-square test and student t-test were used to test for statistical significance of the difference for discreet and continuous variable respectively. A p value of < 0.05 was regarded as significant. Analysis of variance (ANOVA) was used for some comparisons.

The study was approved by the Ethics and Research Committee of the University of Ilorin Teaching Hospital. Informed consent was obtained from the parents/caregivers of participants.

III. Results

A total of 180 children - 90 with Protein Energy Malnutrition and 90 controls- were studied. Among the PEM group, 59 (65%) were males and 31 (34.4%) were females with a male to female ratio of 1.9:1. The mean age of the children with PEM was 22.7 + 14.4 months compared to 29.3 + 16.9 months for the controls and the difference was not significant (p=0.08)(Table 1).

Table 1 : The socio-demographic characteristics of the subject and controls.

Variable	PEM (n=90)	Controls (n=90)	χ^2	*P*
Age (month)				
Range	9.0-59.0	6.0-59.0		
Mean± S.D	22.7±14.4	29.3±16.9	t=7.95	0.08
Gender				
Male	59(65.6%)	53(58.9%)		
Female	31(34.4%)	37(41.1%)	0.85	0.356
Social Economic Class				
I	2(2.2)	4(4.4)		
II	6(6.8)	27(30.0)		
III	26(28.8)	35(38.9)	**28.17**	**0.000012**
IV	38(42.2)	16(17.8)		
V	18(20.0)	8(8.9)		
Maternal Educational Status				
None	25(27.8)	9(10)		
Primary	29(32.2)	20(22.2)	**19.025**	**0.0002**
Secondary	21(23.3)	23(25.6)		
Post secondary	15(16.7)	38(42.2)		

Thirty eight (42%) of the children with PEM were in socio-economic class (SEC) IV, 26 (28.8%) in SEC III, 18(20%) in SEC V and only 2(2.2%) in SEC I. The subjects were of a lower socioeconomic class compared to the controls (p=0.00001)(Table 1).

Of the 90 mothers interviewed, 29 (32.2%) had primary education, 25 (27.8%) had no form of education, while 21(23.3%) and 15(16.7%) had secondary and tertiary education respectively. The educational status of mothers of children with PEM were lower compared to that of controls (p=0.0002) (Table I).

The mean haematocrit values for the subjects and controls were 30.4 ± 6.3% and 32.0 ± 6.1% respectively while their mean haemoglobin values were 10.1±2.1g/dl and 10.9±15.0g/dl respectively and the difference was statistically significant (p=0.019 and 0.003 respectively) with the subject having a lower value (Table 3) The mean values of the mean corpuscular volume were 72.4 ± 10.9fl and 72.6 ± 13.6fl in the subjects and controls respectively and the values were similar (p = 0.913 ,while he mean values for mean corpuscular haemoglobin concentration and mean corpuscular haemoglobin were 30.4±2.8g/dl RBC and 24.3±10.5fl for subjects and0.3±1.8 g/dl RBC and25.6±1.6g/dl RBC, for controls and both were comparable (p>0.05) (Table 2),.

The mean value of platelets count were 291.8 ±131.7× 10^9 /L and 326.4 ±133.9 × 10^9 /L for the subjects and controls respectively and the difference was statistically significant (p=0.0001) with PEM children having a lower platelet count compared to the controls(Table 2).

Table 2 : Haematologic profile of the PEM versus the Control.

Haematological Parameters	PEM mean±S.D	Controls mean±S.D	*t*	*P*
RBC (x10^0 cell/mm^3)	4.0±0.9	4.2±0.9	2.39	0.123
Haemoglobin (gldl)	**10.1±2.1**	**10.9±15.0**	**18.58**	**0.019**
Haematocrit (%)	**30.4±6.3**	**32.0±6.1**	**2.97**	**0.003**
MCV(fl)	72.4±10.9	72.6±13.6	0.01	0.091
MCH (pg/cell)	24.3±10.5	25.6±10.6	0.68	0.41
MCHC(gHbldl RBC)	30.4±2.8	30.3±1.8	0.68	0.411
Platelet count(x10^3 cell/mm^3)	**291.8±131.7**	**326.4±133.9**	**180.18**	**0.0001**
White cell count(x10^3 cell/mm^3)				
Neutrophils% Lymphocyte%				
	12.8±11.6	**5.9±8.7**	**20.38**	**0.001**
	49.5±12.3	**43.8±5.9**	**15.64**	**0.001**
	52.7±12.3	**59.4±7.5**	**23.80**	**0.00002**

The mean value of WBC in the children with PEM was 12.8±11.6×10 ^{3} cell/mm ^{3} and 5.9±8.7×10 ^{3} cell/mm ^{3} among the controls (p= 0.001) (Table 2). The subjects had higher mean values of total white cell count, neutrophil and lower lymphocytes counts compared with controls (p<0.05)(Table 2).

Children with Kwashiokor had the highest mean for haemoglobin, (31.6±1.6g/dl) and haematocrit (10.7±0.4%), while subjects with marasmus had the lowest mean for haematocrit (27.6±5.8%), haemoglobin (9.1±2.1g/dl) and mean corpuscular haemoglobin (22.9±2.3pg/cell).(Table 2). The subjects with kwashiokor and marasmic-kwashiokor had the highest lymphocyte counts while underweight had the lowest lymphocyte count with a statistical significant difference (p=0.0001) (Table 3). Underweight children had the highest mean of white cell count (13.8±14.5×10^3 cell/mm^3) while Marasmic –Kwashiokor had the lowest mean count, however, the difference is not statistically significant (p=0.750). The neutrophils counts were similar in all the types of Protein Energy Malnutrition (p=0.438) with subjects with kwashiorkor having the highest value. (Table 3)

Table 3 : Haematolgic Profile of Children According to the Types of PEM.

Haematologic Parameters	Marasmus n=21 Mean±S.D	Kwashiokor n=8 Mean±S.D	Marasmic-kwashikor n=11Mean±S.D	Underweight n=50 Mean±S.D	*T*	*p*
RBC(×10 ^{6} cell/mm^3)						
Haemoglobin (g/dl)	3.82±0.91	4.28±0.05	4.28±0.05	4.08±9.5	0.85	0.47
Haematocrit (%)	9.1±2.1	10.7±0.4	10.4±1.7	10.3±2.4	1.89	0.1575
MCH(pg/cell)						
MCHC(hb/dlRBC)	27.6±5.8	31.6±1.6	31.1±5.1	31.08±21	1.77	0.157
MCV(fl)	22.9±2.3	22.4±0.5	23.5±1.3	25.2±13.9	0.36	0.7815
Total WBC (x10^3 cell mm^3)	**31.2±2.9**	**31.5±2.1**	**33.1±1.5**	**29.4±2.9**	**4.43**	**0.006**
Neutrophils%	68.1±11.9	73.0±3.6	74.4±6.2	73.3±2.1	1.231	0.3015
Lymphocyte%						
Platelet (x10^3 cell mm^3)	12.9±8.8	11.2±0.7	9.9±1.3	13.8±14.5	0.405	0.750
	47.2±17.9	52.3±13.4	44.7±7.4	51.2±9.8	0.915	0.438
	51.8±13.9	**67±4.1**	**59.9±4.2**	**49.1±7.1**	**11.62**	**0.0001**
	273.9±156.2	270.0±5852	226.5±48.7	316.8±137.6	1.7022	0.171

IV. Discussion

This study confirms that anaemia as well as high white cell count are near constant features of protein energy malnutrition as reported by previous studies.[10,14] Lower mean values were also observed in the haematocrit and haemoglobin values of children with PEM as compared to controls a finding similar to previous studies.[10,14] Other red cell changes observed from this study includes a significantly lower mean values for MCH, MCV and RBC count in children with PEM when compared to the controls. These red cell changes can be attributed to adaptation to lower metabolic oxygen requirements and decrease in lean body mass seen in PEM.[15] These changes have also been attributed to changes in the plasma volume as well as the intracellular body water in the body.[{16,20}] An increase in plasma volume is seen and is said to be responsible for changes in haematocirt and haemoglobin levels while a concomitant decrease in intracellular water is said to be responsible for changes seen in MCHC.[23] Micronutrient deficiencies such as iron, zinc, have also been implicated.[2,12,13]

This study also found a significant leucocytosis and neutrophilia among children with PEM as compared to controls, this is similar to a previous study where there was a significant rise in leukocyte count in the patients with PEM compared to the controls.[16] Leucocytosis in these children can be a result of infection which is seen commonly in PEM: both PEM and infection, either clinical or subclinical have been reported to act synergistically.[16] This has been an important factor in determining morbidity and mortality attributed to PEM.[14] However, several other studies revealed leucopenia as well as neuropenia as a common finding in malnutrition.[16,21,22]

Furthermore, a lower lymphocyte count was observed in the malnourished children compared to controls. The lower lymphocyte count can be attributed to changes in the thymus which is greatly reduced in children during severe PEM. The degree of thymic atrophy correlates closely with depletion of lymphocytes and a decrease in the thymic dependent lymphocyte is also associated with impaired immunity.[17]

However, among the various classes of PEM, the study found that children with the edematous forms of PEM had the highest mean values for neutrophils as well as lymphocytes count and a significant difference was observed in the lymphocyte count among the various classes of PEM. These findings are not in consonance with that of a previous study which found no difference in the lymphocyte count of children with malnutrition and concluded that a suppression in both granulocyte and lymphocyte functions occurred in malnutrition;[18] another study also reported lower white cell counts in Protein energy malnutrition.[19] The findings in this study can be explained by some possible adaptive mechanism which attempts to maintain some degree of immunocopetence in the edematous forms of malnutrition. This assumption can be corroborated by that of another study where CD4 counts were higher in malnourished children with edema compared to the non edematous types.[18] No significant changes were observed in the platelet of the various classes of PEM but there was a significant difference in the controls compared to PEM. Children with PEM had a significantly lower platelet count. This decrease in platelets seen in PEM can be attributed to a purported decrease in bone marrow activities which indirectly affect megakaryocyte functions. A similar finding has been reported by a previous study.[20]

In conclusion, Children with PEM had lower red cell indices and platelet count, and a higher white cell count than the controls. Also the edematous forms of PEM had higher granulocyte and lymphocyte counts when compared to the non edematous forms of malnutrition.

Also, PEM is a condition that constantly modifies the body's defense mechanism and thus altering the haemtopoesis at all levels, thus this studies recommends that more frequent studies be carried out to describe in more detailed the trend of such changes in this part of the world. This would enhance anticipatory care and outcome of the children affected.

V. Authors Contribution

Saka AO, Ojuawo A, Abdulkarim and Adeboye MAN, were involved in conceptualizing the research work as well as carrying out the research work. Bilamin and Latubosun were invoveled in the laboratory analysis while Saka MJ was the biostatistician involved in study design, data collation as well as analysis.

The authors declare that we have no competing interest.

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A Study of Mode of Origin of Inferior Phrenic Artery in 30 Adult Human Cadavers - Clinical Implications

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Abstract - Keeping in view the paucity of information related to inferior phrenic arteries, the present study has been carried out to provide a detailed account of variation in the mode of origin of inferior phrenic artery. The study was carried out on 30 adult human cadavers of known sex. On the right side, the inferior phrenic artery arose independently in 20 cases (66.6%) and by a common trunk in 10 cases (33.3%). On the left side the artery arose independently in 20 cases (66.6%) and by a common trunk in 10 cases (33.3%). The renal artery was seen as the source of the inferior phrenic artery on 3 sides. The inferior phrenic artery usually originates from the aorta or celiac trunk and less frequently from the renal, hepatic or left gastric arteries. This artery is a major source of collateral or parasitized arterial supply to hepatocellular carcinoma, second only to the hepatic artery. Recognition of variations enables clinicians to distinguish features which merit further investigations or treatment from those which do not .Clinical implications of variations in this artery have been stressed upon.

Keywords : *Inferior phrenic artery, Hepatocellular carcinoma, Aorta, Coeliac trunk.*

G JMR - B Classification: *NLMC Code: WG 25, WG 106*

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A Study of Mode of Origin of Inferior Phrenic Artery in 30 Adult Human Cadavers - Clinical Implications

Ambica Wadhwa [α] & Sandeep Soni [σ]

Abstract - Keeping in view the paucity of information related to inferior phrenic arteries, the present study has been carried out to provide a detailed account of variation in the mode of origin of inferior phrenic artery. The study was carried out on 30 adult human cadavers of known sex. On the right side, the inferior phrenic artery arose independently in 20 cases (66.6%) and by a common trunk in 10 cases (33.3%). On the left side the artery arose independently in 20 cases (66.6%) and by a common trunk in 10 cases (33.3%). The renal artery was seen as the source of the inferior phrenic artery on 3 sides. The inferior phrenic artery usually originates from the aorta or celiac trunk and less frequently from the renal, hepatic or left gastric arteries. This artery is a major source of collateral or parasitized arterial supply to hepatocellular carcinoma, second only to the hepatic artery. Recognition of variations enables clinicians to distinguish features which merit further investigations or treatment from those which do not .Clinical implications of variations in this artery have been stressed upon.

Keywords : Inferior phrenic artery, Hepatocellular carcinoma, Aorta, Coeliac trunk.

I. Introduction

In anatomy, normality embraces a range of morphologies. It includes those that are most common and others called variations which are less frequent but not considered abnormal. Variations ranging from subtle to remarkable affect every part of the human body. They may have important influences on predisposition to illness, symptomatology, clinical examination, investigation and patient management including operative surgery. Recognition of variations enables clinicians to distinguish features which merit further investigations or treatment from those which do not (Willan and Humpherson, 1999).

Anomalous blood vessels are always interesting from a purely scientific point of view, especially since they so often shed light on obscure problems of phylogeny and ontogeny. They may also be of considerable significance from a clinical or a surgical standpoint (Dawson and Reis, 1922)

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The knowledge of the arterial anatomic variations is very important for the clinical, radiological and surgical diagnosis. Regarding inferior phrenic arteries, which irrigate the diaphragm, it is known that they vary in relation to their origin. The purpose of the present study is to verify these variations. Vascular variations are constantly observed in dissection of adult cadavers (Lipshutz, 1917). Recent advances stress upon the fact that right inferior phrenic artery is the most common extrahepatic feeding artery supplying the hepatocellular carcinoma. The great importance of such knowledge lies in the fact that an unresectable hepatocellular carcinoma can be treated by transcatheter embolization of not only its typical blood supply, the right or left hepatic arteries, but also by embolization of a right inferior phrenic artery, if involved (Tanabe et al, 1998).

These arteries also contribute to arterial supply of adrenal glands are of thus important in angiographic examination for adrenal lesions (Kahn,1967).

According to Pick and Anson (1941), these arteries may arise from the coeliac artery (34.8%), aorta (26.3%) or from a common trunk that stems from the aorta (18.5%) or coeliac trunk (13.0%). Rarely, it may arise from the renal artery (5.8%). The purpose of the present study is to analyse the variations in mode of origin of inferior phrenic artery and its clinical implications thereof.

II. Material and Methods

The material for the study comprised of 30 adult well-embalmed human cadavers from Department of Anatomy , Governmentt Medical College, Amritsar, Punjab. They were serialized from 1-30 with suffix 'M' for male and 'F' for female. The abdominal cavity was opened by a cruciform incision passing through the whole thickness of the anterior abdominal wall. Flaps were reflected. The abdominal viscera i.e. stomach, intestines liver, pancreas and spleen were systematically removed according to Cunningham's Manual of Practical Anatomy (Romanes, 2000). The crus of the diaphragm with the inferior phrenic artery was traced and cleaned. All the ganglions and the nervous tissue around the arteries were removed. After resection of subjacent tissues to the diaphragmatic crura and

adhesion of tissues all along the median arcuate ligament, the arteries were exposed and their mode of origin was studied.

III. Results

In the current study, the origin of the artery though variable, there was a marked tendency for the origin of inferior phrenic arteries of the right and left sides to be symmetrical; the most common source of origin being the abdominal aorta independently.

Table 1 : Incidence of source of origin of inferior phrenic artery.

Parent artery	Independent origin		Common trunk
	Right	Left	
Abdominal aorta	11 (55%)	13 (65%)	6
Coeliac trunk	7 (35%)	6 (30%)	4
Renal artery	2 (10%)	1 (5%)	-

On the right side, the inferior phrenic artery arose independently in 20 cases (66.6%) and by a common trunk in 10 cases (33.3%). On the left side the artery arose independently in 20 cases (66.6%) and by a common trunk from the abdominal aorta in 10 cases (33.3%). The renal artery was seen as the source of the inferior phrenic artery on 4 sides – 3 on the right side and 1 on the left side. It is clear from Table 1 that independent origin of inferior phrenic artery from abdominal aorta is more common than coeliac trunk on both the sides.

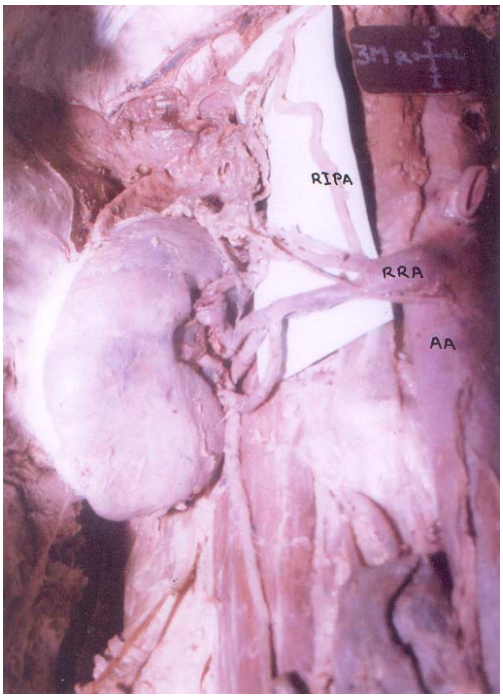

Figure 1 : Right inferior phrenic artery (RIPA) arising from right renal artery (RRA).

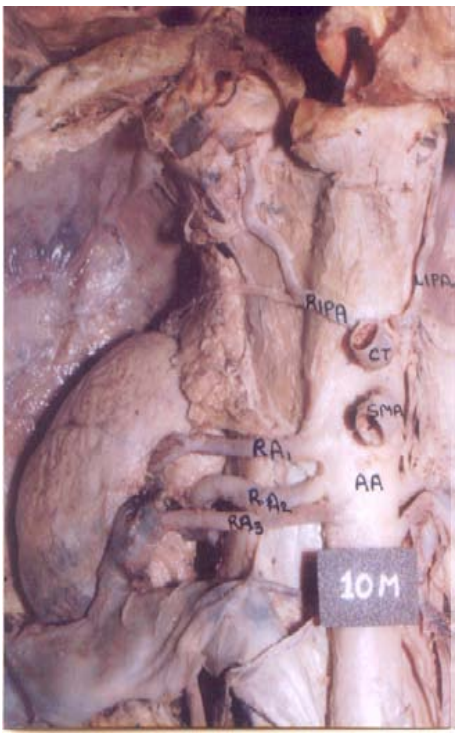

Figure 2 : Right inferior phrenic artery (RIPA) arising from coeliac trunk (CT) and left inferior phrenic artery (LIPA) arising directly from abdominal aorta (AA)

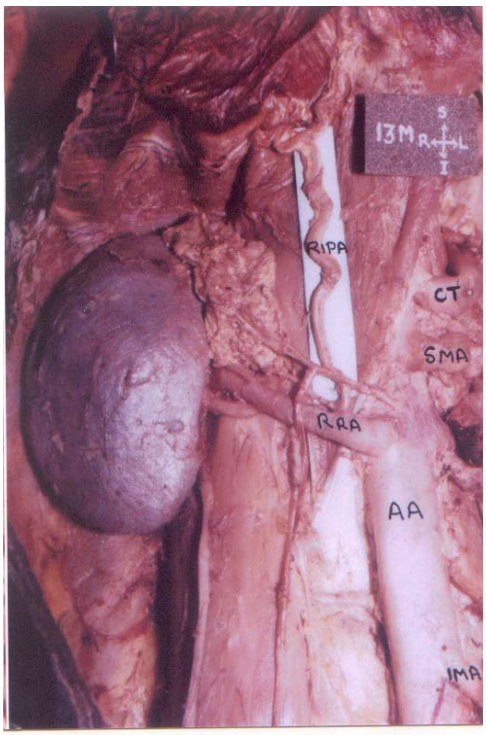

Figure 3 : Right inferior phrenic artery (RIPA) arising from right renal artery (RRA).

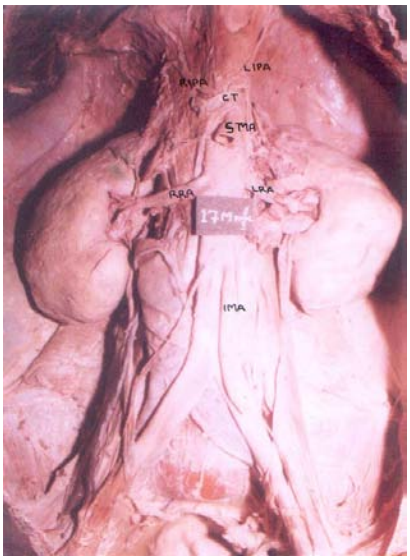

Figure 4 : Origin of Inferior phrenic artery from abdominal aorta.
RIPA - Right Inferior phenic artery
LIPA - Left inferior phrenic artery

IV. Discussion

Considering the paucity of information presently available concerning these arteries, a more definitive study seemed appropriate and necessary, both for its potential clinical applications and to provide additional data to contemporary anatomical literature. The **Gray's Anatomy** gives the most complete textbook account, claiming origins from both the celiac trunk and aorta, as well as describing common trunk origins and mentioning alternative origins, including the renal or accessory renal arteries, the left gastric, hepatic, and gonadal arteries. The computed tomography (CT) study by **Gokan et al (2001)** described these arteries with slightly greater detail and included actual percentages.

Table 2 : Comparison of the incidence of source of inferior phrenic artery.

Author (years)	No. of dissections	Source of the artery				
		Aorta		Coeliac trunk		Renal
		Indepen-dently	Common trunk	Indepen-dently	Common trunk	
Adachi (1928)	34	-	6	16	12	-
Cauldwell and Anson (1936)	106	31	23	25	27	-
Michels (1955)	60	6	12	18	24	-
Merklin & Michels (1958)	44	8	8	8	14	8
Present study (2004)	60	23	6	13	4	4

The results of the present study corroborate with the findings of **Merklin and Michels (1958).**

Inferior phrenic artery may arise more frequently from the coeliac axis than directly from the aorta **(Rossi & Cova, 1904; Adachi, 1928 and Pick & Anson, 1941);** However **Quain, 1844; Descomps, 1910; and Lipshutz, 1917** commented that the inferior phrenic artery arises more commonly from the aorta than from coeliac trunk.

Those instances in which the inferior phrenic artery arises from the renal artery, suprarenal arteries are rarely derived from other than renal sources. This fact may be of surgical importance, in clamping renal pedicle, in nephrectomy, when the entire blood supply of the suprarenal gland on that side could be ligated by tying the renal artery proximal to its inferior phrenic branch. Fortunately with the phrenic artery arising from the renal artery more commonly on the right side, the proximal segment of right renal artery, where the phrenic usually takes root, is covered by inferior vena cava anteriorly and thus preventing them from trauma in manipulation of renal pedicle. Contrary, the hazard is greater on the left side **(Pick and Anson, 1941).**

Modern surgical techniques depend in part on knowledge of both the normal and the anomalous arterial blood supply. The inferior phrenic artery is a major source of collateral or parasitized blood supply to hepatacellular carcinoma , second only to hepatic artery. This is useful to evaluate the efficacy and safety of transcatheter oily chemoembolization therapy (TOCE) via the inferior phrenic artery (IPA) in hepatocellular carcinoma (HCC).

The knowledge of this type of variation shows that surgeons must be cautious to avoid unintentional sectioning of small caliper arteries, as it may occur during the celiac artery decompression in the compression syndrome of the celiac trunk by the median arcuate ligament.

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Rp-Hplc Method for the Determination of Pramipexole Dihydrochloride in Tablet Dosage Form

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Abstract - A simple, sensitive, rapid, selective, precise and accurate high performance liquid chromatographic method was developed and validated for the determination of Pramipexole dihydrochloride in bulk and tablet dosage forms. HPLC separation was carried out by reversed phase chromatography on a Thermo Scientific C18 column (250 mm × 4.6 mm, 5 µm), held at ambient temperature. The mobile phase consisted of methanol: acetonitrile (40:60 v/v), run at a flow rate of 1.0 ml/min and with UV detection at 263 nm. The method was found to be linear over an analytical range of 1-100 µg/ml with LOD = 0.075 µg/ml and LOQ = 0.227 µg/ml, respectively. The proposed method was validated successfully and applied to the quantification of the drug in tablet dosage forms.

Keywords : *Pramipexole dihydrochloride, RP-HPLC, development, Validation*

GJMR-C Classification : *NLMC Code: QV 785 QV 786, QV 787*

Strictly as per the compliance and regulations of:

RP-HPLC Method for the Determination of Pramipexole Dihydrochloride in Tablet Dosage Form

P.Lavudu [α], A.Prameela Rani [σ], C.Balashekaran [ρ] & V.Venumadhav [ω]

Abstract - A simple, sensitive, rapid, selective, precise and accurate high performance liquid chromatographic method was developed and validated for the determination of Pramipexole dihydrochloride in bulk and tablet dosage forms. HPLC separation was carried out by reversed phase chromatography on a Thermo Scientific C18 column (250 mm × 4.6 mm, 5 µm), held at ambient temperature. The mobile phase consisted of methanol: acetonitrile (40:60 v/v), run at a flow rate of 1.0 ml/min and with UV detection at 263 nm. The method was found to be linear over an analytical range of 1-100 µg/ml with LOD = 0.075 µg/ml and LOQ = 0.227 µg/ml, respectively. The proposed method was validated successfully and applied to the quantification of the drug in tablet dosage forms.

Keywords : Pramipexole dihydrochloride, RP-HPLC, development, Validation.

I. Introduction

Pramipexole dihydrochloride (PPD) [1-6], a nonergot dopamine agonist approved in the US (1997), is used as an antidyskinetic for treatment of Parkinson's disease. Its chemical name is (S)-N^6-propyl-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine dihydrochloride (Fig. 1). The ability of PPD to alleviate the signs and symptoms of Parkinson's disease is supposed to be linked to its ability to stimulate dopamine receptors in the striatum.

Various analytical methods have been reported in the literature for the assay of PPD in pure and in its pharmaceuticals preparations. Procedures using UV-spectrophotometry [7], visible spectrophotometry [8,9], HPTLC [10] have been reported by several workers. High-performance liquid chromatography with mass spectrometer (HPLC-MS) [11-13], capillary electrophoresis with laser-induced fluorescence detection [14], gas chromatography with mass spectrometer (GC-MS) [15] and Ultra-performance liquid chromatography with mass spectrometer (UPLC-MS) [16] have been used for the analysis of PPD in biological samples.

Only few HPLC methods with UV detection have been described in the literature for determination of PPD. Pathare et al [17] developed a chiral liquid chromatographic method for the enantiomeric resolution of Pramipexole dihydrochloride monohydrate on a Chiralpak AD (250 mm × 4.6 mm, 10 µm) column using a mobile phase system containing n-hexane:ethanol: diethylamine (70:30:0.1 *v/v/v*). A method developed for determination of PPD and its impurities by Jačić et al [18] was carried out using a C18 column with mobile phases containing different ratios of acetonitrile and water phase (aqueous triethylamine/orthophosporic acid). Yau et al [19] reported a HPLC method for the determination of pramipexole in human plasma and urine. Separation is achieved by ion-pair chromatography on a Zorbax Rx C8 column (250 mm × 4.6 mm, 5 µm) and a Brown lee RP-8 pre-column (15 mm x 3.2 mm, 7 µm) with electrochemical detection at 0.6 V for plasma and ultraviolet detection at 286 nm for urine. A RP-HPLC [20] method for PPD in pure and in its pharmaceutical dosage forms has been reported by RAO et al and was carried out on an hypersil ODS-C18 (250 mm × 4.6 mm, 5µm) column with acetonitrile and acetate buffer (90:10 *v/v*) as the mobile phase and a detection wavelength of 260 nm. Srinubabu et al [21] have reported an RP-HPLC method for the assay of PPD in tablet formulations on an ODS-C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase of acetonitrile and phosphate buffer (60:40 *v/v*) and detection at 260 nm. The reported HPLC methods for the determination of PPD in pharmaceutical dosage forms suffer from one or more disadvantages like preparation of buffer, rigid pH control, narrow linear concentration range and less sensitivity.

In this paper, an attempt is made to develop and validate a simple, efficient and reliable method, without incorporating the use of an internal standard, for the determination of PPD in tablet dosage forms by HPLC using UV detection.

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II. Materials and Methods

a) Apparatus

All HPLC experiments were carried out on a isocratic High Pressure Liquid Chromatography system (Shimadzu HPLC class VP series, Shimadzu Corporation, Kyoto, Japan) with two LC-10 AT, VP pumps, variable wavelength programmable UV/Visible detector SPD-10A, VP, CTO-10AS VP column oven, SCL-10A, VP system controller. The HPLC system was equipped with the software "class VP series version 5.03" (Shimadzu). The analytical column used for the separation was 250 mm × 4.6 mm I.D., 5 µm particle size, Thermo Scientific C18 (Phenomenex, Torrance, CA, USA).

b) Chemicals and reagents

All chemicals and reagents were of HPLC grade quality. Milli-Q-water was used throughout the process and it was obtained from Merck Specialties Private Ltd, Hyderabad, and Andhra Pradesh, India. Methanol and acetonitrile of HPLC grade were from Rankem laboratories, Mumbai, India.

c) Preparation of Mobile phase

Mobile phase 'A' consisted of methanol. Mobile phase 'B' was acetonitrile. The mobile phase used for analysis was prepared by mixing mobile phase 'A' and mobile phase 'B' in the ratio, 40:60 v/v. The same mobile phase was also used as a diluent for the sample preparations.

d) Standard solutions and tablet dosage forms

Pharmaceutical grade PPD was kindly gifted by Matrix laboratories, Hyderabad, India, and was used as received. The following available pharmaceutical dosage forms containing 0.5 mg and 1 mg of active ingredient were purchased from the local pharmacy and used in the present investigation:

- Parpex (1 mg, Zydus cadila, Ahmedabad, India)
- Pramipex (0.5 mg and 1 mg, Sun pharma, Mumbai, India)

Stock solution of PPD (1 mg/ml) was prepared by dissolving 100 mg of PPD in 50 ml of diluent in a 100 ml volumetric flask and then made up to the mark with diluent.

e) Chromatographic conditions

The mobile phase was a mixture of methanol and acetonitrile (40:60 v/v). The contents of the mobile phase were filtered before use through 0.45 µm membrane filter, degassed with a helium sparge for 15 min and pumped from the respective solvent reservoirs to the column at a flow rate of 1 ml/min. The column temperature was maintained at 25±10C. The injection volume of samples was 20 µl. The analyte was monitored at a wavelength of 263 nm.

f) Recommended procedure

Working standard solutions equivalent to 1 to 100 µg/ml PPD were prepared by appropriate dilution of the stock standard solution (1 mg/ml) with the diluent. Prior to injection of the drug, the mobile phase was pumped for about 30 minutes to saturate the column thereby to get the base line corrected. 20 µl of each solution was injected automatically onto the column in triplicate and the peaks were determined at 263 nm. The peak areas of PPD were plotted against the corresponding nominal concentration to obtain calibration graph. The concentration of the drug was obtained from the calibration graph or the regression equation.

g) Procedure for tablet dosage forms

Fifty tablets containing PPD were exactly weighed and ground into a fine powder. From this powder, an amount of the tablet powder equivalent to 25 mg PPD was transferred to a 25 ml standard flask containing 10 ml of diluent and shaken for 10 minutes. The volume was made up to the mark with diluent and mixed well. The solution was filtered through a 0.45 µm membrane filter. The filtered solution was appropriately diluted with diluent to obtain a concentration of 100 µg/ml. From this solution, 20 µL was injected into the HPLC system. The area under the peak was noted and the drug content in the tablets was quantified using the calibration graph or regression equation.

III. Results and Discussion

a) Method development

In order to develop an efficient and simple RP-HPLC method for the analysis of the drug in bulk and in its tablet dosage forms, preliminary tests were conducted to select satisfactory and optimum conditions. HPLC parameters, such as detection wavelength, ideal mobile phase & their proportions and flow rate were carefully studied.

Preliminary experiments indicated that the Thermo Scientific C18 (250 mm × 4.6 mm, 5 µm) column provides efficient and reproducible separation of PPD at ambient temperature. Hence Thermo Scientific C18 column was selected for method development and validation. PPD was determined by injecting the drug solution on to Thermo Scientific C18 column with UV detector set at 263 nm. After trying different ratios of mixtures of methanol and acetonitrile, the best results were achieved by using a mixture of methanol-acetonitrile (40:60 *v/v*) as mobile phase. At a flow rate of 1.0 ml/min, the retention time for PPD was 4.458 min. The analyte peak area was well defined and free from tailing under the described experimental conditions.

b) System suitability

System suitability test was carried out on freshly prepared solution of PPD (50 µg/ml) to ensure the

validity of the analytical procedure. Data from five injections were used to confirm system suitability parameters like retention time, peak area, peak asymmetry, theoretical plates, plates per meter and height equivalent to theoretical plate. The results are presented in Table 1. The values obtained demonstrated the suitability of the system for the analysis of the PPD.

c) Selectivity

Selectivity is the ability of an analytical method to distinguish between the analyte of interest and other components present in the sample. To identify the interference by the excipients in the tablet dosage form, the tablet extract was prepared according to procedure described under "Procedure for tablet dosage forms" and injected. The resulting chromatogram (Fig. 2) did not show any peak other than that of PPD, which confirmed the selectivity of the method. The selectivity of the method was also demonstrated by interference check by injecting the diluent blank to determine whether any peaks in the diluent are co-eluting with PPD peak. No interference of peaks eluted in the diluent blank with PPD peak was observed (Fig.3).

d) Linearity

The linearity was determined by constructing calibration curve. A calibration curve was constructed using least squares method by plotting the peak area vs concentration of PPD. The calibration curves (Fig.5) for PPD show good linearity with excellent regression coefficient (0.9993) in the concentration range of 1-100 µg/ml. The linear regression equation and regression coefficient of the calibration curve is presented in Table 2.

e) LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of y-intercepts of regression lines or standard deviation of blank readings and the slope of the calibration curve by using three calibration curves. Results of LOD and LOQ for PPD are shown in Table 2.

f) Accuracy and precision

The precision and accuracy of the method was determined by performing five repeated analysis of three different standard solutions containing 5, 50, 90 µg/ml PPD, on the same day, under the optimized experimental conditions. The precision and accuracy are expressed as RSD and relative error, respectively. The results of this study are presented in Table 3. The values of the relative standard deviation and relative error were found satisfactory. Hence the proposed method is precise and accurate.

g) Recovery studies

The accuracy of the proposed method was also further assessed by performing recovery experiments using the standard addition method. Known amount of the pure PPD was added to pre-analyzed formulation and the total concentration was once again determined by the proposed method. The obtained mean recoveries and relative standard deviations were in the range 99.66–100.33 and 0.378–0.614 %, respectively (Table 4). The results revealed that any small change in the drug concentration in the solutions could be accurately determined by the proposed method. The closeness of the recoveries suggests lack of interference from tablet excipients and thereby establishes some degree of selectivity.

h) Application to tablet dosage forms

To find out the suitability of the proposed method for the assay of tablet dosage forms containing PPD was analyzed by the proposed method. The results obtained from the proposed method were compared statistically with reference method7 by applying Student's t-test for accuracy and F-test for precision. From the results (Table 5) it was found that the proposed method does not differ significantly in precision and accuracy from the reference method.

IV. Conclusion

A simple, sensitive, selective, accurate and precise RP-HPLC method was developed for the determination of PPD in bulk and in tablet dosage forms. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. The short chromatographic time makes this method appropriate for the processing of numerous samples in a limited time. The method has wider linear range with good accuracy and precision. The method shows no interference from tablet excipients. Hence, the proposed method could be useful and fit for the quantification of PPD in bulk and tablet dosage forms.

V. Acknowledgements

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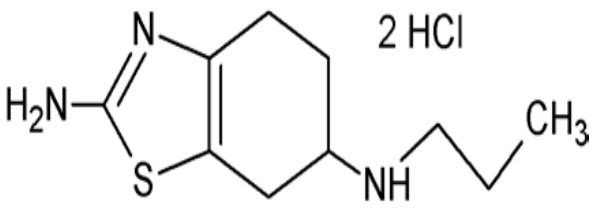

Figure 1 : Structure of pramipexole dihydrochloride.

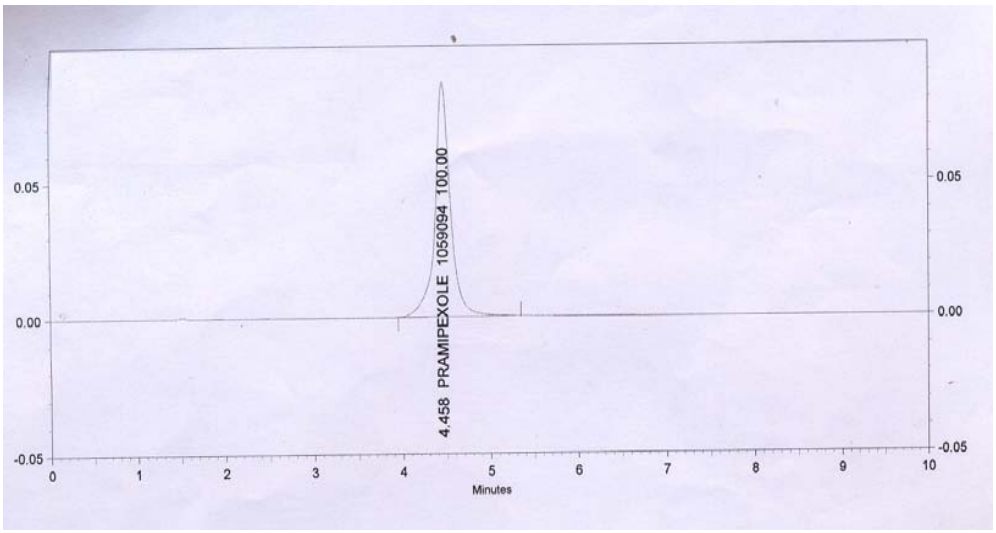

Figure 2 : Chromatogram of standard PPD (100 µg/ml).

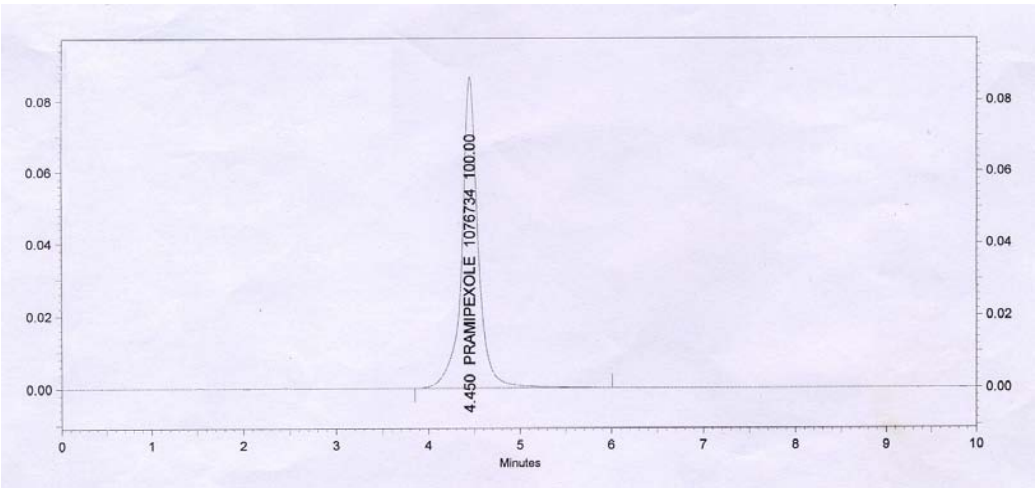

Figure 3 : Chromatogram of PPD tablet dosage form (100 µg/ml).

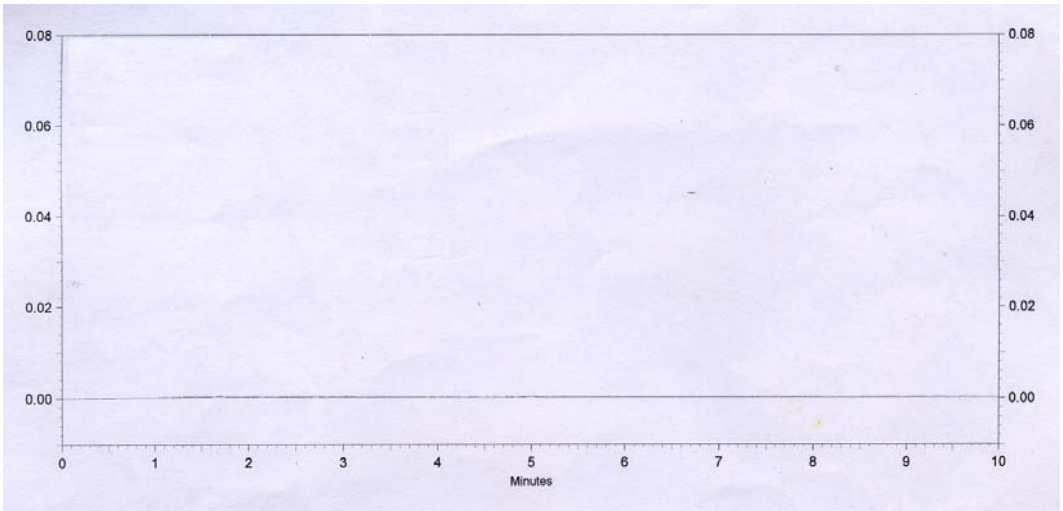

Figure 4 : Chromatogram of diluent blank (methanol and acetonitrile in the ratio of 40:60 v/v).

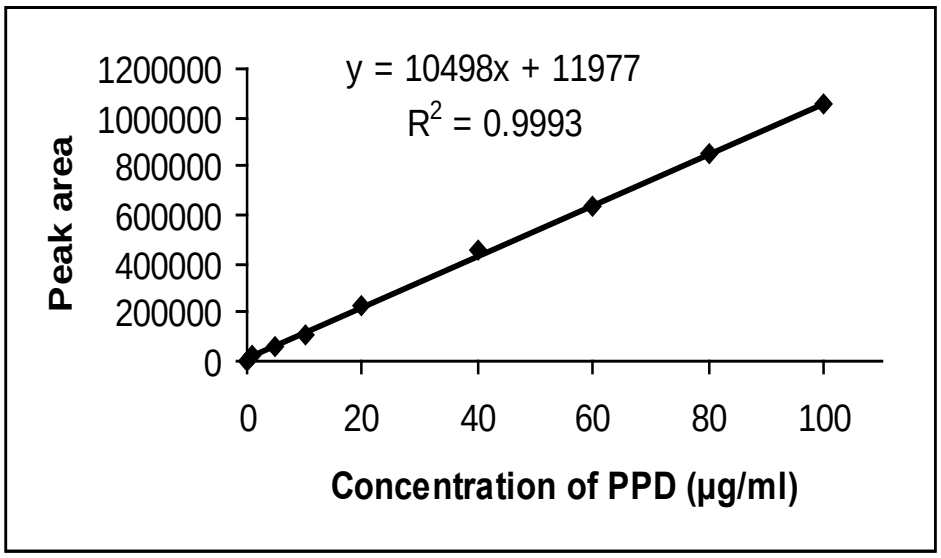

Figure 5 : Calibration curve for assay of PPD.

Table 1 : System suitability studies

Parameter	Value	RSD (%)
Retention Time (t) (Min)	4.458	1.052
Peak area	541238	0.957
Theoretical Plates (n)	8464	0.834
Plates per Meter (N)	17500	1.127
Height equivalent to theoretical plate (HETP) (mm)	2.958×10^{-5}	1.096
Peak asymmetry	1.95	0.946

Table 2 : Linearity and regression characteristics.

Parameter	Value
Linearity range (µg/ml)	1-100
Regression equation (Y = a + bc):	
Slope (b)	10498
Intercept (a)	11977
Regression Coefficient (r^2)	0.9993
Limit of Detection (µg/ml)	0.075
Limit of Quantification (µg/ml)	0.227

Table 3 : Precision and accuracy studies.

Concentration of PPD (µg/ml)		RSD (%)	Recovery (%)	Error (%)
Taken	Found ± SD(n=5)			
5	4.92 ± 0.067	1.361	98.40	1.60
50	50.09 ± 0.459	0.916	100.18	0.18
90	91.02 ± 0.729	0.800	101.13	1.13

Table 4 : Recovery studies.

Formulation	Labelled claim (mg)	Concentration of PPD (mg)		RSD (%)	Recovery (%)
		Added	Found ± SD (n=5)		
Parpex	1.0	0.50	1.495 ± 0.0083	0.555	99.66
Pramipex	1.0	0.50	1.505 ± 0.0057	0.378	100.33
Pramipex	0.5	0.25	0.748 ± 0.0046	0.614	99.73

Table 5 : Assay of PPD in tablet dosage forms.

Formulation	Labelled claim (mg)	Found (mg) ± SD (n=5)		t Value*	F value*
		Proposed method	Reference method		
Parpex	1.0	0.992 ± 0.0056 %R=99.20 %RSD=0.564	0.995 ± 0.0036 %R=99.50 %RSD=0.361	1.56	3.61
Pramipex	1.0	1.051 ± 0.0037 %R=105.10 %RSD=0.352	0.997 ± 0.0028 %R=99.70 %RSD=0.280	1.20	3.08
Pramipex	0.5	0.509 ± 0.0067 %R=101.80 %RSD=1.316	0.502 ± 0.0038 %R=100.40 %RSD=0.756	0.96	2.51

R – Recovery

**Tabulated t value at 95 % confidence level = 2.77 and Tabulated F value at 95% confidence level = 6.39.*

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Respiratory Syncytial Virus Infection: IgA & LT-E4 Responses and Effects of Host Factors for Infants with Acute Bronchiolitis in Two Iraqi Pediatric Hospitals

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Abstract - Background : Respiratory syncytial virus **(RSV)** is a leading cause of lower respiratory tract disease in infants and young children. Both the magnitude , intensity of infection and the host response to **RSV** infection determine the severity and intensity of disease.

Objective : Our goal was to evaluate the effect of immune response (**RSV** IgA) and inflammatory mediators (LT-E4), in addition to the influence of host factors on the severity of the disease.

Keywords : Pediatric; RSV, Respiratory Syncytial Virus IgA (RSV-IgA); Leukotriene E-4(LT-E4), Bronchiolitis.; Cysteinyl Leukotrienes; Secondhand Cigarette Smoke; Infants; Parental Asthma.

GJMR-B Classification : FOR Code: WF 140, WC 515, WC 505

Strictly as per the compliance and regulations of:

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Afrah,G.Salih [α] , Kassim,J.Al-Shamma [σ] , Mahir,M.Hassan [ρ] , Raed ,Y.Salman [ω] , & Israa ,M.Salih [¥]

Abstract - Background : Respiratory syncytial virus (RSV) is a leading cause of lower respiratory tract disease in infants and young children. Both the magnitude , intensity of infection and the host response to RSV infection determine the severity and intensity of disease.

Objective : Our goal was to evaluate the effect of immune response (RSV IgA) and inflammatory mediators (LT-E4), in addition to the influence of host factors on the severity of the disease.

Methods : This was a randomized, prospective study in two Iraqi pediatric hospitals. One hundred and twenty –three infants (mean age: 6.99±0.62 ,71 boys & 52 girls) ,with a first episode of acute bronchiolitis were randomly divided into eight treatment groups: oxygen plus intravenous fluid, montelukast pediatric chewable tablet,salbutamol syrup,salbutamol nebulizer ,combination of both oral plus nebulized salbutamol,dexamethasone IV injection, hydrocortisone IV injection, and azithromycin suspension. Control infants with non respiratory diseases were also studied for comparisons. The measured parameters was RSV IgA titer, LT-E4 titer, and a variety of environmental and host factors that may contribute to the severity of RSV bronchiolitis.Severity of bronchiolitis was based on the quantization of lowest O_2 saturation and the length of hospital stay.

Results : There were significant increase in RSV IgA values in patients (1.58 ± 0.24 U/mL) compare to the control (0.36 ± 0,03 U/mL);also there were a significant increase in the leukotriene E 4 values in patients (2.66 ± 0.52 ng/ml) compared to the control infants(0.15 ± 0.007 ng/ml). Age was found to be a significant factor in the severity of infection. The younger an infant was, the more severe the infection tended to be as measured by the lowest oxygen (O_2) saturation. We also found that infants exposed to postnatal cigarette smoke from the mother had a lower O_2 saturation than those not exposed. Although a history of maternal atopy seemed to be protective.

Conclusion : Secretory IgA antibodies level was found to be a good indicator to respiratory syncytial virus infection as seen by significantly higher levels in patients compared to the control infants. The severity of RSV bronchiolitis early in life seems modified by postnatal maternal cigarette smoke exposure, atopy and age of the infants.

Keywords : Pediatric; RSV, Respiratory Syncytial Virus IgA (RSV-IgA); Leukotriene E-4(LT-E4), Bronchiolitis.; Cysteinyl Leukotrienes; Secondhand Cigarette Smoke; Infants; Parental Asthma.

I. Introduction

Respiratory syncytial virus (RSV) is the leading cause of serious respiratory tract infections in infants and young children throughout the world (1). RSV replicates for 1–3 days before producing lower respiratory tract symptoms affecting almost 60% of infants and up to 25% of toddlers and preschoolers. Current treatment approaches for severe RSV induced disease are ineffective. Therefore, prevention of disease is a high priority .Immunoglobulin A(IgA) is the most abundant immunoglobulin in mammals. Unlike other antibody isotypes, IgA is targeted to mucosal tissues, and virus-specific IgA in mucosal secretions has been shown to protect from reinfection. IgA, unlike IgG, is able to bind and neutralize viral proteins intracellularly at the site of initial replication in epithelial cells .Therefore; mucosal IgA may be of particular importance in immunity against RSV, which is a mucosally restricted pathogen (2,3). Inflammatory mechanisms in bronchiolitis have been documented recently, including increased airway secretion, mucosal edema, and infiltration of inflammatory cells. Cysteinyl leukotrienes (CysLTs) are released during respiratory syncytial virus (RSV) airway infection in infants, and their levels are significantly elevated. CysLTs are known to cause bronchial obstruction, mucosal edema, and infiltration of eosinophilic granulocytes and to increase bronchial responsiveness (4). CysLTE4 (LTE4), one of the terminal cysLT metabolites, is significantly increased in the infants hospitalized with RSV bronchiolitis (5). The risk of severe RSV disease is increased by factors that compromise the ability to control and withstand a respiratory tract infection. Therefore; environmental factors also play a role, including ones that affect lung

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function (e.g., household tobacco use) or that increase exposure to infection (e.g., day care, hospitalization, multiple siblings, crowding) (6,7). The objective of the present randomized, prospective study was to evaluate the effects of immune response, inflammatory mediators, host and environmental factors on the severity of the acute viral bronchiolitis.

II. Materials & Methods

This prospective study was conducted in two Iraqi pediatric hospitals. Baghdad Health Office/Karkh, Child's Central Teaching Hospital & Karbala Health Office, Karbala Pediatric Teaching Hospital. Inclusion criteria were infants' patients aged >8 weeks and <2 years with a respiratory symptom duration of<4 days. Additional inclusion criteria included first episode of wheezing or shortness of breath, randomization within 12 hours of admission and informed consent. Exclusion criteria were any previous hospital admissions with respiratory illnesses, had ever been treated with anti-asthma medications before the current illness, corticosteroids treatment in any form during current illness, and underlying cardiopulmonary disease. Gender, age, weight, height , body temperature ,family history in (first-degree relatives), of asthma ,atopy, tobacco smoking, usage of kerosene heater ,type of feeding ,duration of exclusive breast feeding, concurrent diseases, and concomitant medications, were recorded for each infants .A total number of 123 patients mean age: 6.99±0.62 with mild to moderate bronchiolitis were divided randomly into eight treatment groups:

Group A: Ten infants' patients had received oxygen + intravenous fluid.Group B: Fourteen infants' patients had received study treatment, montelukast pediatric chewable tablet 4mg once daily, if vomiting occurred one additional dose was given (4).Group C: Ten infants patient had received azithromycin suspension (200mg/5ml) ,with a dose of 10 mg /kg once daily. Group D: Ten infants' patients had received hydrocortisone vial (100 mg/2ml), with a dose of, 5-10 mg/kg/dose q 6hour IV.Group E: Twenty infants' patients had received salbutamol syrup (2mg/5ml), with a dose of , 0.1-0.3 mg/kg/dose q 8 hour; the method of syrup administration was taught to the caregiver. Any patient who vomited the drug within 15 minutes of ingestion was advised to have a repeat dose (8). Group F: Twenty infants' patients had received salbutamol given in combination as syrup & by nebulization, (oral salbutamol 0.1-0.3mg/kg/dose q8 hour+salbutamol nebulizer 0.01-0.02 mg/kg /dose q6hour). Group G: Twenty infants' patients had received dexamethasone ampule (4mg/1ml), with a dose of, 0.25 -0.5 mg/kg/dose q 12 hours intravenously.Group H: Nineteen infants' patients had received salubutamol solution for nebulizer (5mg/ml), with a dose of, 0.01-0.02 mg/kg /dose q 6 hour.

From all enrolled infants, blood samples were taken and try to measure both (RSV IgA) & LT-E4, antibody to RSV & inflammatory mediators that release during RSV acute bronchiolitis, respectively. These parameters were measured by the enzyme linked immunoassays (ELISA), to investigate the etiology of acute respiratory infections in hospitalized infants .The test was explained to the parents and they signed the informed consent form. The obtained optical density (OD) of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method [9,10].

Other type of samples that taken from the patients that put on the study treatment, montelukast pediatric chewable 4mg tablets, was the nasal swab. In the present study, we prospectively tried to examine the association between the presence of nasal eosinophils and severity of acute bronchiolitis and the effect of montelukast on nasal eosiophil. In this study we tried to quantify the number of neutrophils and eosinophils in nasal secretions by utilizing the semiquantitative nasal cytology grading score by Meltzer[11,12].

III. Results

Table 1 : Demographic data and baseline characters of patients and control infants. The data were expressed as number (n), and percentage (%).

	Patients (n_{total} =123)	Control Infants (n_{total} =10)
Characteristics	(n,%)	(n,%)
Age <6months.	73 (59)	4(40)
Age >6months.	50 (41)	6 (60)
Male.	71 (58)	7 (70)
Female.	52 (42)	3 (30)
Family history of Asthma.	64 (52)	4 (40)
Family history of Atopy.	78 (63)	6 (60)
History Of Passive Tobacco Smoking.	91(74)	8 (80)
Family history of Kerosene Heating.	95 (77)	7 (70)
Presence of pets at house.	55 (45)	5 (50)
Breast Feeding.	67 (54)	1 (10)
Bottle feeding.	37 (30)	7 (70)
Mixed Feeding.	16 (13)	2 (20)
< 5 Member.	16(13)	2(20)
>5 Member.	107(87)	8(80)
Mean Weight, kg.	7.2 ± 0.79	9.3 ± 1.14
Duration Of Exclusive Breast Feeding, months.	4.53± 0.303	8 ± 2.68
The values of weight,& duration of exclusive were expressed as mean ± standard error of mean (SEM).		

Table (1), demonstrated that, there were no significant differences between the groups in terms of demographic variables.

Table 2 : Relationships between host factors and RSV IgA titer for infants' patients with acute viral bronchiolitis and for the control infants. Data were expressed as mean ± standard error of mean (SEM), number (n) and percent (%).

		RSV IgA		RSV IgA
	Patients	U/ml	Control	U/ml
	n ,(%)	Patient	n ,(%)	Control
	total = 45		total = 10	
RSV IgA High Titer (mean ± SEM)		1.58* ± 0.24		0.36 ± 0.03
RSV IgA Low Titer (mean ± SEM)		0.39 ± 0.02		
Age <1year.	34 (75.6)	1.24* ± 0.28	7,(70)	0.31 ± 0.03
Age >1 year.	11 (24.4)	2.63 * ± 0.39 †	3,(30)	0.36 ± 0.06
Male.	34 (75.6)	1.37 * ± 0.19	7,(70)	0.35 ± 0.03
Female.	11 (24.4)	2.23* ± 0.78	3,(30)	0.29 ± 0.03
Positive Family history of Asthma.	21,(46.6)	1.21* ± 0.21	3,(30)	0.36 ± 0.06
Negative Family history of Asthma.	24(53.3)	1.91 * ± 0.41	7,(70)	0.32 ± 0.03
Positive Family history of Atopy.	33 (73.3)	1.18* ± 0.15	6,(60)	0.33 ± 0.03
Negative Family history of Atopy.	12 (26.6)	2.69 *± 0.74 †	4,(40)	0.33 ± 0.05
Positive History Of Passive Tobacco Smoking.	35 (77.7)	1.79* ± 0.30	8,(80)	0.32 ± 0.03
Negative History Of Passive Tobacco Smoking.	10(22.2)	0.86* ± 0.13 †	2,(20)	0.36 ± 0.09
Positive Family history of Kerosene Heating.	38 (84.4)	1.49 *± 0.19	7(70)	0.32 ±0.03
Negative Family history of Kerosene Heating.	7 (15.6)	2.21* ± 1.19	3 (30)	0.36 ± 0.001
Positive Presence of Animal in the house.	20(44.4)	1.26* ± 0.22	3 (30)	0.30 ± 0.029
Negative Presence of Animal in the house.	25 (55.5)	1.84* ± 0.39	7(70)	0.35 ± 0.04
Positive Breast Feeding.	22(48.8)	1.73* ± 0.43	3,(30)	0.26 ± 0.006
Negative Breast Feeding.	23 (51.1)	1.44 * ± 0.25	7,(70)	0.36 ± 0.03 †
Positive Bottle feeding.	21,(46.6)	1.41* ± 0.25	7,(70)	0.35 ± 0.03
Negative Bottle feeding.	24,(53.3)	1.73* ± 0.4	3,(30)	0.26 ± 0.006
Number Of Family Member > 5.	36 ,(80)	1.5 * ± 0.28	8 ,(80)	0.35 ± 0.03
Number Of Family Member < 5.	9 ,(20)	1.91* ± 0.53	2 ,(20)	0.26 ± 0.009 †

*P<0.05: Significant difference between patients and control group.
†P<0.05: significant difference from preceding value.
RSV IgA = Respiratory Syncytial Virus Immunoglobuline A.
Control :infants with non respiratory illness

The table (2) showed the RSV IgA values for infants' patients with acute viral bronchiolitis, together with RSV IgA values for the control infants. There was a significant increase in RSV IgA values in patients compared to the control infants.

There was a significant relationsihips between titer of the antibody against RSV(RSV IgA) and family history of atopy,tobacco smoking ,and the ages of infants patients.

Table 3 : Relationships between host factors and leukotriene -E4 for infants' patients with acute viral bronchiolitis and for the control infants. Data were expressed as mean ± standard error of mean (SEM), number (n) and percent (%).

	Patients	Patients	Control	Control
	n ,(%)	LTE4	n ,(%)	LTE4
	total = 48	ng / ml	total = 10	ng / ml
LT-E4 High Titer.		2.66 * ± 0.52		0.15 ± 0.007
LT-E4 Low Titer.		0.142±0.004		
Age <1year.	36 (75)	2.46* ± 0.6	7,(70)	0.14 ± 0.006
Age >1 year.	12 (25)	3.28 * ± 1.1	3,(30)	0.15 ± 0.02
Male.	34,(70.8)	2.10* ± 0.56	7,(70)	0.14 ± 0.01
Female.	14,(29.1)	4.25 * ± 1.07 †	3,(30)	0.15 ± 0.01
Positive Family history of Asthma.	23,(47.9)	1.93* ± 0.60	3,(30)	0.15 ± 0.02
Negative Family history of Asthma.	25 (52.1)	3.31 * ± 0.84	7,(70)	0.14 ± 0.006
Positive Family history of Atopy.	34 (70.8)	2.47 *± 0.65	6,(60)	0.15 ± 0.01
Negative Family history of Atopy.	14 (29.1)	3.11* ± 0.91	4,(40)	0.14 ± 0.008
Positive History Of Passive Tobacco Smoking.	38 (79.2)	2.87 *± 0.63	8,(80)	0.15 ± 0.008
Negative History Of Passive Tobacco Smoking.	10(20.8)	1.84* ± 0.77 †	2,(20)	0.15 ± 0.02
Positive Family history of Kerosene Heating.	40 (83.3)	2.68 *± 0.60	7(70)	0.15 ± 0.01
Negative Family history of Kerosene Heating.	8 (16.6)	2.64* ± 1.04	3 (30)	0.13 ± 0.005
Positive Presence of Animal in the house.	20(41.6)	2.33 *± 0.63	5 (50)	0.15 ± 0.01
Negative Presence of Animal in the house.	28 (58.3)	2.89 * ± 0.78	5 (50)	0.14 ± 0.006
Positive Breast Feeding.	29 (60.4)	3.26* ± 0.74	3 (30)	0.15 ± 0.01
Negative Breast Feeding.	19 (39.6)	1.74* ± 0.66	7(70)	0.15 ± 0.01
Positive Bottle feeding.	20 (41.6)	2.09 *± 0.71	7,(70)	0.15 ± 0.01
Negative Bottle feeding.	28 (58.3)	3.06* ± 0.74	3,(30)	0.15 ± 0.01
Number Of Family Member > 5.	36 ,(75)	2.45* ± 0.59	8 ,(80)	0.15 ± 0.009
Number Of Family Member < 5.	12 ,(25)	3.43* ± 1.31	2 ,(20)	0.14 ± 0.002
*P<0.05: Significant difference between patients and control group.				
†P<0.05 : significant difference from the preceding value				
LT-E4= Leukotriene E 4				
Control :infants with non respiratory illness				

Table (3) showed the leukotriene E4 values in infants patients with acute bronchilitis, together with leukotriene values of the control infants. There was a significant increase in the leukotriene E4 values in patients compared to the control infants As the table shown, only the gender and family history of tobacco smoke showed significant differences.

Table 4 : Effects of host factors on length of stay (LOS) and oxygen saturation (S_pO2) for the infants patients with acute viral bronchiolitis .Data were expressed as mean ± standard error of mean (SEM) ,number (n) and percent (%).

	Patients	Patients	Patient
	n ,(%)	LOS (day)	S_pO2
	total = 60		
Male.	41 (68.3)	2.9 ± 0.18	94.14 * ± 0.34
Female.	19 (31.7)	3.3 ± 0.32	93.84 * ± 0.39
Age > 1 year.	14 (23.3)	2.86 ± 0.38	94.8 * ± 0.64
Age < 1 year.	46 (76.7)	3.11 ± 0.17	93.7 * ± 0.28
Positive Family history of Asthma.	27 (45)	3.18 ± 0.24	94.41 * ± 0.35
Negative Family history of Asthma.	33 (55)	3.2 ± 0.20	93.75 * ± 0.38
Positive Family history of Atopy.	42 (70)	2.5 ± 0.23	94.16 * ± 0.31
Negative Family history of Atopy.	18 (30)	3.16 ± 0.21 †	93.77 *± 0.51
Positive History Of Passive Tobacco Smoking.	46 (76.7)	3.26 ± 0.19	94.04 * ± 0.29
Negative History Of Passive Tobacco Smoking.	14 (23.3)	3.25 ± 0.29	94.07 * ± 0.61
Positive Family history of Kerosene Heating.	51 (85)	3.22 ± 0.19	93.90 * ± 0.28
Negative Family history of Kerosene Heating.	9 (15)	2.83 ± 0.18	94.88 * ± 0.69
Positive Presence of Animal in the house.	27 (45)	3.22 ± 0.26	94.29 * ± 0.34
Negative Presence of Animal in the house.	33 (55)	3.19 ± 0.21	94.36 * ± 0.32
Positive Breast Feeding.	31 (51.6)	2.86 ± 0.23	94.58 * ± 0.38
Negative Breast Feeding.	29 (48.3)	3.09 ± 0.23 †	93.54 * ± 0.34 †
Positive Bottle feeding.	30 (50)	3.17 ± 0.28	93.83 *± 0.35
Negative Bottle feeding.	30 (50)	2.8 ± 0.18	94.26 * ±0.39
Number Of Family Member > 5.	48 (80)	3.18 ± 0.19	94.12 * ± 0.28
Number Of Family Member < 5.	12 (20)	3.13 ± 0.27	93.7 * ± 0.7
RSV IgA high titer (mean ± SEM).	1.58 * ± 0.24	2.9 ± 0.14	94.2 ± 0.29
RSV IgA low (mean ± SEM).	0.39 ± 0.02	3.9 ± 0.47 †	93.06 *± 0.58 †
LTE4 high titer (mean ± SEM).	2.66* ± 0.52	3.26 ± 0.20	94.16 ± 0.27
LTE4 low titer (mean ± SEM).	0.142 ± 0.004	2.79 ± 0.18 †	93.58 * ± 0.72

*P<0.05: Significant difference between patients and control group.
†P<0.05: significant difference from the preceding value.
RSV IgA : Respiratory Syncytial Virus Immunoglobuline A.
LT-E4: Leukotriene E4.
LOS: of hospital stay; S_paO2= blood oxygen saturation.

Table (4) showed the effects of host factors on the length of hospital stay (LOS) and oxygen saturation (S_pO2) in infants patients with acute viral bronchiolitis. As the table shown, only the host factors of family history of atopy and breast feeding of infants showed significant effects on duration of hospital stay and oxygen saturation of blood.

Concerning nasal swab from infants' patients with acute viral bronchiolitis before and after treatment with montelukast chewable 4 mg tablets once daily; according to Meltzer grading there was a significant differences in the count of eosinophils –neutrophils before and after treatment with montelukst ; 1.6 ± 0.32 versus 0.33 ± 0.16 respectively. This could indicated eosinophil-recruiting chemokines were strongly produced and released from bronchial epithelial cells after in vivo stimulation with RSV.

IV. Discussion

RSV is a highly infectious and prevalent virus. More than other respiratory viruses, RSV infection can occur very early in life despite maternal antibodies, and reinfection can readily occur throughout life without significant antigenic change .The relative contribution of viral versus various host factors to RSV pathogenesis remains controversial (6). The immune response to primary RSV infection is generally inefficient and consequently subsequent reinfections are common throughout life.In RSV infection, innate and adaptive immunity are out of balance (13).

Comparing the risk factors with RSV IgA values of infants' patients , only the age, history of atopy and passive tobacco smoking showed significant differences (14). In the age category older infants' patients (over 1 year) had significantly higher RSV IgA value compared to younger patients (below 1 year) . Patients with negative family history of atopy had significantly higher RSV IgA value compared to patients with positive history of atopy. On the other hand patients with positive history of passive tobacco smoking had significantly higher RSV IgA value compared with those of negative history of passive tobacco smoking. This could indicate that, parental smoking did not inhibit the production of anti-microbial IgA, suggesting that other factors are responsible for the increased susceptibility to infection in these infants. Infants who lived in tobacco smoking environments had increased severity of disease, as results of Th2 predominance, with decreased expression of Th1 cytokines [15] , and IgA titer was less effective for protecting against RSV infection [2] . Lanari et al. (2002) [14], demonstrated that exposure to cigarette smoke, in general, seems to worsen the severity of the viral bronchiolitis.

Comparing the risk factors with LTE4 values, only the gender and family history of tobacco smoke showed significant difference. Concerning the gender, the value in female babies was significantly higher than male babies. This could indicate that the females infants had more sever RSV infections compared to male infants; this has been attributed to the tendency of parents to bring sick male babies to the hospital earlier than female babies [3].CysLT increased in infants who exposed to the tobacco smoke. This could indicated that, the exposure to the tobacco smoke increases the severity of RSV bronchiolitis, which was described here by the increased level of LTE4 in the infants who lived in tobacco smoking environments [16,17,18].

Comparing the effects of host factors (age, sex, family history of asthma, atopy, tobacco smoking, kerosene heating, presence of pets at home, breast or bottle feeding and number of family members) on the length of hospital stay and oxygen saturation in infants with acute viral bronchiolits; only the host factors of family history of atopy and breast feeding of infants showed a significant effects on duration of hospital stay and oxygen saturation of blood [19] . Infants with a positive family of atopy showed a shorter duration of hospital stay and a higher value of blood oxygen saturation compared to infants with acute viral bronchiolitis and have no family history of atopy. Breast feeding of infants with acute viral bronchiolitis showed a significant effects on the blood oxygen saturation and length of hospital stay. Breast feeding is protective, through either transfer of maternal antibody or enhancement of virus-specific lymphocyte transformation activity. Infants with breast feeding have a shorter length of stay and higher value of blood oxygen saturation relative to infants without having breast feeding and have bottle fed. This finding is substantiated further by the fact that infants with a higher O_2 saturation spent less time in the hospital than infants with a lower O_2 saturation[14].

Regarding to the effects of RSV IgA level on the length of hospital stay and patients oxygen saturation, there were a significant effects on both length of hospital stay and patient oxygen saturation. infants with low titer of RSV IgA showed longer period of hospital stay & lower values of oxygen saturation compared to the patients with a high titer of RSV IgA , which could indicated effects of immune response of the patients on the resolution of symptoms and the time at which patients were fit to the discharge [7,20]. Regarding to the effects of inflammatory mediators cysteinyl leukotriene and its metabolite LTE4 on the period of hospital stay and oxygen saturation of infants patients with acute viral bronchiolitis, there were a significant effects. High titers of LTE4 associated with prolong hospital stay and lower value of blood oxygen saturation .Female,younger infants ,negative family history of atopy, and absence of breast feeding ,showed longer period of hospital admission & lower value of blood oxygen saturation.

According to Meltzer grading there were a significant differences in the counts of eosinophils – neutrophils before and after treatment with montelukst tablet for the infants patients with acute viral bronchiolitis. This could indicated that eosinophil-recruiting chemokines are strongly produced and released from bronchial epithelial cells after stimulation with RSV[12] ;and montelukast treatment has been shown to reduce eosinophils in nasal mucosa of infants [21].

V. Conclusion

The relationships between risk factors and RSV IgA titer in infants with viral bronchiolitis, only age, family history of atopy and tobacco smoking showed significant effects.Patients with low titer of RSV IgA showed longer period of hospital stay & lower values of oxygen saturation comparing to the patients with a high

titer of RSV IgA. Concerning the relationships between risk factors of infants with bronchilitis and leukotriene E4 level, only the gender and family history of tobacco smoke showed significant difference. There were a significant effects of high level of LTE4 on the period of hospital stay compared to the low level of LTE4.

Host factors of family history of atopy and breast feeding of infants showed significant effects on duration of hospital stay and oxygen saturation of blood.Infants exposed to postnatal cigarette smoke from the mother had a lower O_2 saturation than those not exposed. Infants with a family history of atopy especially a maternal history of asthma had a higher O_2 saturation.Infants with highest blood oxygen saturation, have shorter length of hospital stay.

There were significant differences in the count of eosinophils –neutrophils before and after treatment with montelukst,which could indicated that, there was a correlation between nasal eosinophil and severity of viral bronchiolitis .

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Exploring Medicinal Plants for Anti-Helicobacter Pylori Activity

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Abstract - Helicobacter pylori is a class 1 carcinogen that requires targeted therapeutic strategy. A number of drugs including proton pump inhibitors, antibiotics and antiprotozoals are available for the treatment of Helicobacter pylori infections like chronic gastric irritation, gastro duodenal ulcers and low grade gastric mucosa associated lymphoid tissue lymphoma. Clinical evaluation of these drugs has shown the incidence of relapses, side effects and drug interactions. Multi drug resistance to Helicobacter pylori has been the main reason for treatment failure. This has been the rationale for the development of new anti- Helicobacter pylori drugs and search for novel molecules has been extended to medicinal herbs that offer better protection, decreased relapse and undevelopment of resistance towards bacteria. The present article reviews the medicinal herbs from global perspective for their anti- Helicobacter pylori activity and active compounds from the plants responsible for this activity. We have highlighted some of the important plants and their active constituents reported for their anti- Helicobacter pylori activity. Ancient system of medicine (Ayurvedic and Unani) supported by modern science is necessary to isolate, characterize and standardize the active constituents from herbal sources for anti-Helicobacter pylori activity.

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Exploring Medicinal Plants for Anti-Helicobacter Pylori Activity

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Abstract - *Helicobacter pylori* is a class 1 carcinogen that requires targeted therapeutic strategy. A number of drugs including proton pump inhibitors, antibiotics and antiprotozoals are available for the treatment of *Helicobacter pylori* infections like chronic gastric irritation, gastro duodenal ulcers and low grade gastric mucosa associated lymphoid tissue lymphoma. Clinical evaluation of these drugs has shown the incidence of relapses, side effects and drug interactions. Multi drug resistance to *Helicobacter pylori* has been the main reason for treatment failure. This has been the rationale for the development of new anti- *Helicobacter pylori* drugs and search for novel molecules has been extended to medicinal herbs that offer better protection, decreased relapse and undevelopment of resistance towards bacteria. The present article reviews the medicinal herbs from global perspective for their anti- *Helicobacter pylori* activity and active compounds from the plants responsible for this activity. We have highlighted some of the important plants and their active constituents reported for their anti- *Helicobacter pylori* activity. Ancient system of medicine (Ayurvedic and Unani) supported by modern science is necessary to isolate, characterize and standardize the active constituents from herbal sources for anti-*Helicobacter pylori* activity.

I. Introduction

Helicobacter pylori (H. pylori), a Gram - negative spiral bacterium which was first detected in 1984 by Marshall et al, is one of the most common chronic bacterial pathogens in humans.[1] Approximately 50% of people in the world are infected with it, and its prevalence is significantly higher in developing countries than in developed countries.[2] Once a person is infected, the organism can live in the stomach indefinitely and may not cause clinical illness. It is still not clear how *H.pylori* are transmitted or why some people infected with bacteria become sick and others do not.[3] *H. pylori* infection is an important etiologic impetus usually leading to chronic gastritis, gastroduodenal ulcer and low grade gastric mucosa associated lymphoid tissue lymphoma. Epidemiological data shows that a high *H. pylori* infection rate is related to the high incidence of gastric cancer and gastric adenocarcinoma.[4] World Health Organization has categorized *H. pylori* as a class 1 carcinogen.[5] Eradication of the organism has been shown to result in ulcer healing, prevention of peptic ulcer reoccurrence and may also reduce the prevalence of gastric cancer in high-risk populations.[6] Many clinical trails involving patients with gastric and duodenal ulcers show that curing the infection is associated with a significant reduction in ulcer reoccurrence rates.[7-8]

II. Current Treatment Regimens

Since 1984 physicians prescribing triple therapy to treat *H. pylori* infections which includes three options. First option includes the combination of proton pump inhibitor (PPI), clarithromycin and ampicillin. Second option includes PPI, clarithromycin and metronidazole. Third option includes bismuth subsalicylate, metronidazole and tetracycline, but the cure rate from standard triple therapy has been low as 50%.[9-10] However, eradication by the triple therapy is not always successful and acquisition by *H. pylori* resistance to antibiotics could present a serious problem that may reduce treatment efficiency.[11] Quadruple therapy, where three antibiotics are taken alongside the PPI, has also been used in cases where triple therapy has not been successful. But the success rate was only 67%.[12]

III. Multidrug Resistance to H.Pylori

Many strains of *H. pylori* are now developing resistance to commonly used antibiotics. *H. pylory* acquires resistance by mutations to all the antibiotics used in the treatment regimens. The mechanism of resistance involves point mutations which are transmitted vertically, however transformation may be possible if two strains are present simultaneously in the stomach. Drug efflux proteins also can contribute to natural insensitivity to antibiotics and to emerging antibiotic resistance. Efflux pump gene hef A of *H. pylori* play an important role in multidrug resistance. Global resistance of *H. pylori* to metronidazole, clarithromycin, amoxicillin and tetracycline was also reported. One person may have more than one strain of *H. pylori*. Here the antibiotics may kill one strain, but not the other.[13-14] Furthermore, undesirable side effects of the drugs and the significant cost of combination therapy require the

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exclusive need to search alternative approaches of eradicating or preventing infection.

As phytomedicine has proved to be an untapped treasure for the discovery of lead compounds to cure gastrointestinal disorders. Hence several studies have been aimed to evaluate the anti-helicobacter pylori activity of medicinal herbs.[15] To the best of our knowledge, there is no extensive global view on exploring medicinal plants for anti-helicobacter pylori activity. List of medicinal herbs with anti-Helicobacter pylori activity including their source and active extracts are given in table 1.

IV. Solvents Employed in the Study of Plant Extracts Against H.Pylori

The insolubility of non-polar extracts makes it very difficult for the investigators to be used in an aqueous medium during the study of anti-*Helicobacter pylori* activity.[26] Water or alcohol (methanol/ethanol) are used mainly for a large number of crude extract preparations.[27] The type of solvent used may have an effect on the nature of the compounds extracted and the resulting bioactivity of the extract.[28] To estimate the value of each extract therefore, several factors, including the rate of extraction, the quantity extracted (yield), the diversity of compounds extracted, the diversity of inhibitory compounds extracted, the ease of subsequent handling of the extracts, toxicity of the solvent in the bioassays and the potential health hazards of the extractants have to be evaluated. In many research works, methanol/ethanol is used for alkaloid extraction; acetone for flavonoids and steroids; hexane, diethyl ether and chloroform for fat soluble oils, wax, lipids and esters. Dichloromethane for terpenoids, ethylacetate for esters, ethanol may be used for sterols, poly phenols, tannins and water for water soluble components like glycosides, polysaccharides, polypeptides and lectins.[29] Hundreds of plants with antimicrobial compounds have been reported. However, very few of these studies have reported the *in vivo* anti-*Helicobacter pylori* activity of these compounds. It is very important to know whether these compounds will still maintain their maximum activity in the gastric mucus niche of *H. pylori.* Anti-*Helicobacter pylori* compounds from plants and their mechanism of action are given in table 2.

Curcumin, biologically active poly phenolic from *Curcuma longa* has recently been shown to arrest *H. pylori* growth. The anti-*Helicobacter pylori* activity of curcumin against 65 clinical isolates of *H. pylori in vitro* was examined. Minimum inhibitory concentration ranging from 5-50 μg/ml, showing its effectiveness against *H. pylori* growth *in vitro* irrespective of genetic makeup of strains. Curcumin showed immense therapeutic potential against *H.pylori* infection as it was highly effective in eradication of *H.pylori* from infected mice as well as restoration of *H.pylori* induced gastric damage.

Curcuma longa extract was the most efficient in killing the seven strains of H.pylori within 15 minutes followed by chilli and ginger.[46-48] *Mallotus phillipinesis* is (Lam) Muell. Exhibited the most potent bactericidal activity against *H.pylori* which completely killed the bacteria at the concentration of 15.6-31.2 μg/ml.[16] There is no evidence of *in vivo* effectiveness of this plant. Antibacterial activity of *Allium sativum* L(garlic) against *H.pylori* is well documented (40 μg/ml) and resistance has not been reported.The synergistic action of garlic and omeprazole against *H.pylori* was also reported. Thiosulfinates play an important role in the antibiotic activity of garlic. Further clinical evaluation seems warranted.[49-51] A mixture of tannic acid and n-propyl gallate can limit the gastric mucosa deterioration induced by *H.pylori* infection and vac A administration, suggest that vac A inhibition plays a role in this protective activity. So, polyphenols from plant sources may contribute to limit the pathological outcomes of *H.pylori* infection.[52] Successive extracts of *Sapindus mukorossi* and *Rheum emodi* inhibited the growth of 30 resistant clinical isolates of *H.pylori in vitro* and *in vivo* studies and there was no acquired resistance against these herbal extracts even after ten consecutive passages.[53]

V. Conclusion

The evidence summarized above tentatively suggests possible benefits from some herbal sources with anti- *Helicobacter pylori* activity. Herbal science, Ayurvedic knowledge supported by modern science is required to standardize the plant extracts and to isolate, characterize and standardize the active constituents from plant sources for anti- *Helicobacter pylori* activity. Extensive investigations and large scale well designed clinical trails are required to provide more conclusive proof to explore medicinal herbs for anti-*Helicobacter pylori* activity.

Table1 : Medicinal herbs having anti-*Helicobacter pylori* activity (global perspective).

Botanical name	Source	Part used	Extract	Reference
South Asian Herbs				
Mallotus phillipinesis	Pakistan	covering fruit	Aqueous ethanol (70%)	16,17,18
Curcuma amada Roxb.	Pakistan	rhizome		
Myristica fragrans Houtt.	Pakistan	seed		
Psoralea corylifolia	Pakistan	seed		
Glycyrrhiza glabra L	India,Srilanka	root		
Terminalia chebula	Pakistan	fruit		
Curcuma longa L	India	rhizome		
Cuminum cyminum	Srilanka	seed		
Coccinia grandis	India	leaves	Ethanol	
Terminalia arjuna	India	bark	Methanol	
East Asian Herbs				
Rhizoma coptidis	China	rhizome	Aqueous	19,20
Radix scutellariae	China	root		
Radix isatidis	China	root		
Asasarum sieboldi	Korea	root	Methanol	
Lindera strychifolia	Korea	root		
Angelica tenuissima	Korea	root		
Alpinia oxyphylla	Korea	fruit		
American Herbs				
Zingiber officinale	USA	rhizome	Methanol	21
Rosmarinus officinalis	USA	rosemary leaf		
Foeniculum vulgare	USA	seed		
Nigella sativa	USA	seed		
African Herbs				
Terminalia spinosa	East Africa	young branches	Aqueous	22
Harrisonia abyssinica	East Africa	root		
Ximenia caffra	East Africa	root		
Azadirachta indica	East Africa	leaves, stem bark		
Combretum molle	South Africa	stem bark	Acetone	23
Sclerocarya birrea	South Africa	stem bark		
Carica papaya	Nigeria	leaf	Aqueous& ethanol	24
Morinda lucida	Nigeria	leaf		
Octimum gratissimum	Nigeria	leaf		
Phyllanthus amarus	Nigeria	leaf		
Brazelian Herbs				
Bixa orellana L	Brazil	seed	Aqueous ethanol (96%)	25
Chamonilla recutita L	Brazil	inflorescence		
Ilex paraguariensis A	Brazil	green leaves		
Malva sylvestris L	Brazil	inflorescence & leaves		

Table 2 : Anti-*Helicobacter pylori* compounds from plants.

Compound name	Examples	Mechanism of action	reference
Quinones	Quinones,idebenone,duroquinone, menadione,juglone,coenzyme Q_1	inhibition of respiration and cellular ATP level	30-32
Flavones, flavonoids and flavonols	Quercetin, catechins, myristin, rutin	Ability to complex with extracellular and cellular proteins	33-34
Phenolics and polyphenols	Catechol, pyrogallol, curcumin	Enzyme inhibition by the oxidized compounds possibly through reaction sulfhydryl or nn-specific interaction with proteins	35-36
Tannins	Polymeric phenols, hydrolysable tannins	Ability to inactivate microbial adhesins, enzymes, cellenvelop transport proteins	37-39
Coumarins	7-hydroxy-4-methyl coumarin, 6,7-hydroxy-4-methyl coumarin, 6-hydroxy-7-methoxy-4-methyl coumarin and 5,7-dihydroxy cyclopentano coumarin	Not known	40-42
Terpenoids and essential oils	Di, tri, tetra and hemi terpenes Quinoline alkaloids, alkylmethyl quinoline	Decrease the risk of associated pathologies	43
Alkaloids	Not explored	Selective against *H.pylori* Without affecting the intestinal flora	44
Lectins and poly peptides		May be formation of ion channels in the microbial membrane or competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors	45

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The Beneficial Effects of Herbs in Cardiovascular Diseases

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*Introduction -*The history of medicine dates back perhaps to the origin of human race and since time immemorial, man has made use of plants for the treatment of disease. Application of various herbal preparations which are highly effective for curing many diseases seem to have been in practice as early as 400 BC. The earliest reference to the use of medicinal herbs as a cure for a disease was found in Ebers Papyrus (2600 BC). In India, references to the curative properties of herbs in Rig Veda (period estimate between 3500-1800 BC) seem to the earliest records of use of plants in medicine. However, these references are very brief. More detailed account is available in the Ayur veda (about 2500 BC), the Indian System of Medicine. After the Vedas, appeared the two most important works on Indian System of Medicine, the Charak-Samhita (1000 BC) and Susruta-Samhita (800 BC). The Unani system of medicine further enriched the Herbal Materia Medica. Sheikh Abu Ali Seena (980-1033 AD), the author of AL QANOON described various plant medicines in his book Adviya Qalbia (Mamtani and Mamtani, 2005).

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The Beneficial Effects of Herbs in Cardiovascular Diseases

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I. Introduction

The history of medicine dates back perhaps to the origin of human race and since time immemorial, man has made use of plants for the treatment of disease. Application of various herbal preparations which are highly effective for curing many diseases seem to have been in practice as early as 400 BC. The earliest reference to the use of medicinal herbs as a cure for a disease was found in Ebers Papyrus (2600 BC). In India, references to the curative properties of herbs in Rig Veda (period estimate between 3500-1800 BC) seem to the earliest records of use of plants in medicine. However, these references are very brief. More detailed account is available in the Ayur veda (about 2500 BC), the Indian System of Medicine. After the Vedas, appeared the two most important works on Indian System of Medicine, the Charak-Samhita (1000 BC) and Susruta-Samhita (800 BC). The Unani system of medicine further enriched the Herbal Materia Medica. Sheikh Abu Ali Seena (980-1033 AD), the author of AL QANOON described various plant medicines in his book Adviya Qalbia (Mamtani and Mamtani, 2005).

Herbs have been used in medical treatment and some derivates (aspirin, digitalis) have become the mainstay of pharmacology. Medicinal plants have been observed to possess numerous activities with regard to cardiovascular system viz. antiplatelet, hypolipidemic, anti-inflammatory, hypoglycemic and hypotensive actions. For cardiovascular diseases, herbal treatments have been used in patients with congestive heart failure, systolic hypertension, angina pectoris, atherosclerosis, cerebral insufficiency, venous insufficiency, and arrhythmia.

This review compiles herbal medicines that affect the cardiovascular system both in terms of efficacy and safety as gleaned from the scientific literature that is available. The purpose of this review article is to critically evaluate the available evidence for the role of medicinal herbs in prevention and treatment of cardiovascular diseases. In order to simplify, these herbs are categorized under the primary diseases they treat. Nonetheless, most herbal medicines have multiple cardiovascular effects that may frequently overlap.

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II. Angina Pectoris

a) *Carthamus tinctorius extract*

Carthamus tinctorius L. (safflower), a Chinese herbal medicine is widely used to prevent and treat cardiac disease in clinical practice. The anti-ischemic effects of a purified extract of C. tinctorius (ECT) both in vivo and in vitro was investigated. For in-vivo studies, an animal model of myocardial ischemic injury induced by left anterior descending coronary artery occlusion was studied. Pretreatment with ECT (100, 200, 400, 600 mg/kg body wt.) protected the myocardium from ischemia injury by limiting infarct size and improving cardiac function. For the in vitro experiment, neonatal rat ventricular myocytes were incubated in H2O2 and the direct cytoprotective effect of ECT against H2O2 exposure was studied. Pretreatment with 100-400 microg/ml ECT prior to H_2O_2 exposure significantly increased cell viability as revealed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. ECT significantly reduced H2O2 induced cardiomyocyte apoptosis, as detected by Annexin V and flow cytometry. Phosphatidylinositol 3 kinase (PI3K) play a role in the signaling cascade involved in ECT mediated anti-apoptotic effects as the P13K inhibitor (LY294002) blocked the cytoprotective effect conferred by ECT. It was also observed that the rise in the intracellular level of reactive oxygen species (ROS) as assessed by 2',7'-dichlorofluorescin diacetate (DCFH-DA), was significantly inhibited by ECT treatment. The study provides evidence that the cardioprotective effect of ECT in myocardial ischemia is mediated via reducing oxidative stress induced damage and apoptosis (Han et al., 2009).

b) *Sini Decoction*

The cardioprotective activity and mechanism of Sini Decoction (SND) against anti-mitochondrial oxidation injury caused by myocardial ischemia and reperfusion (I/R) was investigated. Kun ming mice were randomly allocated to three groups: Control group, I/R

group and SND-treated group. At the end of experiment, hearts of mice were taken out for estimation of myocardial and mitochondria superoxide dismutase (SOD) activity, myocardium and mitochondrial malondialdehyde (MDA) content, mitochondrial swelling, lactic acid content of myocardium and Mn SOD mRNA expression. SND treatment increased the activity of myocardium and mitochondrial SOD ($P<0.01$), decreased the content of myocardium and mitochondrial MDA ($P<0.01$), decreased the lactic Acid content of myocardium, lighted the swelling of mitochondria ($P<0.01$) and altered the expression of Mn SOD mRNA ($P<0.01$). Sini decoction treatment prevented the mitochondrial oxidation injury caused by myocardial I/R. Cardioprotective effects may be attributed to increase in the expression of MnSODmRNA (Zhao et al., 2008).

c) Sanwei Tanxiang

The effect of Sanwei Tanxiang powder on myocardial pathologic change, myocardium lipid peroxidation and oxidative stress in an anesthetized rat model of I/R was studied. A rat model of regional myocardial I/R was established by 30 min occlusion of the left anterior descending coronary artery followed by 40 min reperfusion. The experimental animals were randomly divided into the sham operation, IR control group, positive control group and Sanwei Tanxiang treatment groups. The changes in myocardial creatine phosphokinase (CK), antioxidant enzymes and lipid peroxidation along with the ultrastructural changes were studied. In Sanwei Tanxiang group's significant myocardial protection, reduction in oxidative stress and improvement in ultrastructral pathological changes was observed as compared with the I/R model group. The authors conclude that the protective effects of Sanwei Tanxiang powder on anesthetized rat's hearts against myocardial I/R injury may be related to the antioxidant activity of Sanwei Tanxiang powder (Kou et al., 2008).

d) Curcumin

The protective effect of curcumin against myocardial injury was studied. A rat model of myocardial I/R injury was established by occluding the left anterior descending branch of coronary artery for 60 min and subsequently reperfusing for 60 min. Different dose of curcumin (20, 40 mg/kg) were administered by intravenous injection 5 min before the onset of ischemia. The changes in myocardial infarct sizes, the serum CK and lactate dehydrogenase (LDH), the myocardial lipid peroxidation and free fatty acid (FFA) content, the myocardial SOD and glutathione peroxidation (GSHPx) activity were estimated. Curcumin (20, 40 mg/kg) reduced the myocardial infarct sizes, the serum CK and LDH activity. The myocardial lipid peroxidation and FFA content declined significantly. Upregulation in antioxidant enzyme activity was observed. Curcumin exerted protective effects on myocardial I/R injury, which may be attributed to inhibition of lipid peroxidation, augmentation of endogenous antioxidants and improving myocardial metabolism (Cheng et al., 2005).

e) Chuanxiong-phthalide

The cardioprotective effect of Chuanxiong-pathalide A on endothelial cell injury induced by I/R was studied. Myocardial injury was induced of a 30-min normothermic global ischemia followed by 60 min reperfusion. The isolated rat hearts were perfused under constant pressure with Chuanxiong-pathalide A at the concentrations of 0.012 5 mg/ mL, 0.025 mg/mL and 0.05 mg/mL within 10 min followed by a 10-min washout period before the induction of I/R. Pretreatment with Chuanxiong-pathalide A produced a reduction in the incidence of reperfusion-induced ventricular fibrillation (VF) and ventricular tachycardia (VT). Pretreatment of the hearts with high dose of Chuanxiong-pathalide A (0.05 mg/mL) prior to the I/R, reduced the incidence of reperfusion-induced ventricular fibrillation (VF) and ventricular tachycardia (VT) to 37.5% as compared with non-pretreated control group ($P < 0.05$). The duration of occurrence of VF and VT in the group pretreated with Chuanxiong-pathalide A at dosages was significantly shorter than the non-pretreated control group. In the Chuanxiong-pathalide A treated group increase in coronary flow and significant reduction in the oxidative stress in the group pretreated with Chuanxiong-pathalide A as compared to control group was observed. In addition, enzyme immunoassays showed decrease in IL-1beta and TXB2/6-Keto-PGF1alpha ratio. Results demonstrated that chuanxiong-pathalide A pretreatment protected the endothelial function from the injury caused by I/R (Gao et al., 2005).

f) Acanthopanax senticosus saponins

The protective effect of Acanthopanax senticosus saponins (ASS) on myocardial I/R injury was investigated. The myocardial ischemia-reperfusion model was induced by ligating the left anterior descending coronary for 30 min and thereafter reperfusing for 120 min. The changes in myocardial infarct size, the serum CK and lactate LDH activity, serum lipid peroxidation content, SOD and GSH-Px activity and plasma endothelin (ET), angiotensin II (Ang II), prostacycline (PGI2) and thromboxane A2 (TXA2) levels and myocardial FFA content of infarct and non-infarct area were determined. In rats treated by ASS (in a dosage of 25, 50 and 100 mg/kg i.v. at 30 min after coronary occlusion), the myocardial infarct size was significantly reduced, the serum CK and LDH activity, the plasma ET, Ang II and TXA2 level and myocardial FFA content declined, while plasma PGI2 level and PGI2/TXA2 was significantly increased. In addition, serum MDA content declined SOD and GSH-Px activity increased markedly. ASS has protective effect on myocardial I/R injury, which may be due to its function of improving free radicals and myocardial metabolism,

decreasing plasma ET, Ang II and TXA2 levels and increasing plasma PGI2 level and PGI2/TXA2 ratio (Sui et al., 2004).

g) Shuangshen tongguan

The study was conducted to observe the effects of Shuangshen tongguan (SSTG) on infarction size and tumor necrosis factor-alpha (TNF-alpha), intercellular adhesion molecular-1 (ICAM-1) levels in serum during reperfusion injury of acute myocardial ischemia. To induce myocardial I/R injury, anterior descending branch of coronary artery was ligated and released. The size and weight of infarction area and the contents of TNF-alpha, ICAM-1 in serum were assayed by Nitroblue tetrazolium (N-BT) staining and ELISA respectively. The size and weight of infarct area and the contents of TNF-alpha, ICAM-1 in serum were significantly increased in the control group compared with the normal group. However, following treated with SSIG a decrease in TNF-alpha and ICAM-1 was observed. I/R injury resulted in release of TNF-alpha, ICAM-1. SSTG protected myocardium from I/R injury by suppressing over-secretion of TNF-alpha and ICAM-1 and reduced the size and weight of infarct area (Han et al., 2004).

h) Sasanquasaponin

The effects of sasanquasaponin (SQS), a traditional Chinese herb's in ameliorating I/R injury was assessed. Further, the possible role of intracellular Cl- homeostasis on SQS's protective effects during I/R was also elucidated. An in vivo experimental ischemia model was induced in mice (weight 27-45 g) using ligation of left anterior descending coronary artery. In vitro model of isolated perfused heart and isolated cultured ventricular myocytes were used. The in vivo results showed that SQS inhibited cardiac arrhythmias during I/R. Incidence of arrhythmias during I/R, including ventricular premature beats and ventricular fibrillation, was significantly decreased in the SQS-pretreated group ($P<0.05$). Results in perfused hearts showed that SQS suppressed the arrhythmias, prevented I/R induced decrease in contract force and promoted the force recovery from reperfusion. Furthermore, in-vitro intracellular Cl- concentrations ([Cl-]i) were measured using a fluorescence method in isolated ventricular myocytes. SQS slightly decreased [Cl-]i in non-hypoxic myocytes and delayed the hypoxia/reoxygenation-induced increase in [Cl-]i during ischemia and reperfusion ($P<0.05$). Our results showed that SQS protected mice against I/R-induced cardiac injury. Modulation of intracellular Cl- homeostasis by SQS plays a role in its anti-arrhythmia effects during I/R (Lai et al., 2004).

i) Psidium guajava L, Limonium wrightii and Okinawan medicinal plants

Effects of the aqueous extracts of Psidium guajava L. and Limonium wrightii, (medicinal herbs growing in Okinawa) at concentrations known to possess antioxidant activity were evaluated in an in vivo model of global I/R. Results were further compared with those of quercetin and gallic acid, major antioxidative components of P. guajava L. and L. wrightii, respectively. Both extracts significantly attenuated ischemic contracture during ischemia and improved myocardial dysfunction after reperfusion. Both plant extracts restored high-energy phosphates and reduced lipid peroxidation in the reperfused hearts. Quercetin and gallic acid also exerted similar beneficial effects. These results indicate that P. guajava L. and L. wrightii both have cardioprotective effects against myocardial I/R injury in isolated rat hearts, primarily through their antioxidant actions (Sakanashi, 2003).

j) Astragalus membranaceus

The effect of components isolated from Astragalus membranaceus on myocardial I/R injury was investigated. Myocardial I/R injury was induced by ligating the left anterior descending coronary artery. The effect of total saponins, total flavonids and astragaloside i.v. isolated from A. membranaceus on hemodynamics during acute myocardial ischemia, Na(+)-K(+)-ATPase activity, cAMP and MDA contents in the ischemic myocardium were assessed. The total saponins, total flavonids and astragaloside i.v. prevented the decline in cardiac function in rat heart injured by I/R in vivo, and decreased Na(+)-K(+)-ATPase activity in the ischemic myocardium. Results demonstrate that the total saponins increased the cAMP content and the total flavonids decreased the level of MDA production in the ischemic myocardium. The cardioprotective effects of different components isolated from A. membranaceus on the cardiac function in the process of I/R may be attributed to improving energy metabolism and antioxidant activity in the ischemic myocardium (Zhou et al., 2000).

k) Ginkgo biloba extract

Ginkgo biloba leaf extract (GBLE) contains many different flavone glycosides and terpenoides. GBLE showed significant antioxidant activity, exerted an anti-inflammatory effect on inflammatory cells (by suppressing the production of active oxygen and nitrogen species), a relaxing effect on vascular walls, an antagonistic action on platelet-activating factor, an improving effect on blood flow or microcirculation, and a stimulating effect on neurotransmitters. GBLE inhibited the oxidative decomposition of low-density lipoprotein (LDL), reduced the cell death in various types of neuropathy, and prevented the oxidative damage to mitochondria. The study using a model of I-R injury has also demonstrated the protective effect of GBLE on cardiac muscle and its antioxidative action in vivo. Favorable results have been obtained in double-blind, placebo-controlled, comparative trials of patients with memory disorders, obstructive arteriosclerosis, and

dementia. GBLE shows a very strong scavenging action on free radicals, and is thus considered to be useful for the treatment of diseases related to the production of free radicals, such as ischemic heart disease, cerebral infarction, chronic inflammation, and aging (Yoshikawa etal., 1999).

The cardioprotective efficacy and the total plasma antioxidant activity of a standardized Ginkgo biloba L. extract (GB) (300 mg/kg/day) or complexed with phosphatidylcholine (GB-PC; 1:2 w/w), after a 5 days oral administration was studied. On the 6th day, the total plasma antioxidant defence was determined by the TRAP and FRAPS assay. The hearts from all groups of animals were subjected to moderate ischemia (flow reduction to 1 ml/min for 20 min) and reperfusion (15 ml/min for 30 min). The recovery of left ventricular developed pressure (LVDP) at the end of reperfusion was 35-40% of the preischemic values in both control and vehicle rats, 50.2% in the GB group and 72.5% in the GB-PC pre-treated animals. CK outflow in the perfusate from the hearts of GB and GB-PC treated animals were restrained to a different extent vs. controls (by 71% GB-PC; by 22% GB); the rate of prostacyclin (6-keto-PGF1 alpha) release was far greater in GB-PC than in GB hearts. In parallel, the GB extract significantly increased the total antioxidant plasma capacity only when complexed with phospholipids. This indicated that there was an increase in bioavailability of phenolic antioxidants when suitably embedded within a lipophilic carrier. The results of this study demonstrated that complexation of Ginkgo biloba with phospholipids provided superior cardioprotection perhaps due to an increased plasma antioxidant activity (Carini et al., 2003).

The cardioprotective effects of EGb 761 on the release of nitric oxide (NO), the concentration of serum thiobarbituric acid reaction substance (TBARS), the activity of CK and the incidence of ventricular arrhythmias were investigated in an in vivo model of myocardial I/R injury. The hearts of the Wistar rats were subjected to 30 min of ischemia and 10 min of reperfusion in vivo. Different doses of EGb 761 (25, 50, 100, 200 mg/kg i.p.), SOD, l-arginine (50 mg/kg i.p.) and nitric oxide synthase inhibitor NG-nitro-l-arginine (NNA, 50 mg/kg i.p.) were administered to the I/R rats. EGb 761 (100 mg/kg) increased the signal intensity of NOFe2+(DETC)2 complex, while EGb 761 at 200 mg/kg showed an effect of decreasing the signal intensity of NOFe2+(DETC)2 complex. EGb 761 inhibited the formation of TBARS, the release of CK, and mitigated the incidence of ventricular arrhythmias in a dose dependent way. Both l-arginine and SOD increased the signal intensity of NOFe2+(DETC)2 complex and inhibited the formation of TBARS, the leakage of CK and the incidence of ventricular arrhythmia. In conclusion, EGb 761 demonstrated significant cardioprotective effects by means of adjusting the level of NO and inhibiting oxygen free radicals induced lipid peroxidation in myocardial I/R injury in vivo (Shen et al., 1999).

Ginkgo biloba extract, a containing kaempferol and quercetin esters, which are potent radical scavengers, was studied on various models of cardiac ischaemia, both in vitro and in vivo. Ginkgo biloba extract showed no significant effect on the cardiac function in vitro models of I/R injury. However, a significant decrease in the intensity of ventricular fibrillation during the reperfusion stage was observed. On normal or hypertrophied heart in vivo, Ginkgo biloba extract provided effective protection against the electrocardiographic disorders induced by ischaemia. On the different models of global or localized ischaemia (followed or not by reperfusion), a decrease of arrhythmia without change in cardiovascular parameters was regularly noted (Guillon et al., 1986).

To assess the cardioprotective and antioxidant effects of therapeutically relevant concentrations of Ginkgo biloba extract (EGb 761; 5, 50 or 200 microg/ml), its terpenoid constituents (ginkgolide A; 0.05 microg/ml and ginkgolide B; 0.05, 0.25 or 0.50 microg/ml), and a terpene-free fraction of EGb 761 (CP 205; 5 or 50 microg/ml), hemodynamic and electron spin resonance (ESR) analyses were performed on isolated ischemic and reperfused rat hearts. Hearts underwent 10 min of low-flow ischemia, 30 min of no-flow global ischemia, and 60 min of reperfusion. Test substances were added to the perfusion fluid during the last 10 min of control perfusion, low-flow ischemia and the first 10 min of reperfusion. The study results showed that in vitro exposure of hearts to EGb 761 (5 or 50 microg/ml) or to ginkgolides A and B (both at 0.05 microg/ml), or in vivo pretreatment of the rats with CP 205 delayed the onset of contracture during ischemia. A significant decline in left ventricular end-diastolic pressure was observed in the EGb 761, by ginkgolide A, and to a lesser extent by ginkgolide B, or by prior oral treatment with CP 205 treated hearts. Post-ischemic functional recovery was significantly improved by in vivo administration of CP 205, by perfusion with 5 microg/ml of EGb 761 or with both terpenoids as compared to untreated group but in vitro CP 205 was not effective. ESR analyses revealed that free radical concentrations in coronary effluents were markedly decreased by all treatments, except for the lowest concentration of ginkgolide B. The findings provide the first evidence that part of the cardioprotection afforded by EGb 761 involves a mechanism independent of direct free radical-scavenging property. These effects may partly be due to a specific action of its terpenoid constituents and the flavonoid metabolites that are formed after in vivo administration of the extract. These may act in a complementary manner to protect against myocardial I/R injury (Liebgott et al., 2000).

l) Polygonum multiflorum extract

'Dang-Gui Decoction for Enriching the Blood' (BE), is a traditional Chinese formulation consisting of Angelica sinensis and Astragalus membranaceus. It is used for stimulating red blood cell production as well as enhancing cardiovascular function. In the present study, the myocardial protection afforded by BE pretreatment against I/R injury in isolated-perfused rat hearts was studied. Polygonum multiflorum extract supplemented BE preparation (BEA) demonstrated a more complete and potent myocardial protection against IR injury. The results suggest that superior cardioprotective effects demonstrated by BEA may be linked to its ability to sustain the myocardial glutathione antioxidant status under conditions of I/R-induced oxidative stress. These beneficial effects may be because of synergistic interaction between the BE and Polygonum extract (Yim et al., 2000).

m) Panax ginseng

The protective effect of oral administration (one week) of Panax ginsengs (PG) extract (10 mg/ml in drinking water; 1.6 g/kg/day) on myocardial post-ischemic damage induced by hyperbaric oxygen (HBO) and on the loss in functionality of the endothelium in aorta ring preparations was investigated. The hearts from control rats (no-HBO and no-HBO-PG), and from rats exposed to HBO and to HBO after PG treatment were isolated and subjected to mild ischemia and then reperfused. Exposure to HBO greatly worsened the post-ischemic damage in controls. A significant rise of left ventricular end diastolic pressure (LVEDP) and coronary perfusion pressure (CPP) was observed in the control group. PG significantly attenuated the increase in LVEDP and CPP with respect to HBO-untreated rats. In HBO control rats the reduction of the vasorelaxant effect of acetylcholine on norepinephrine precontracted aortic rings, was markedly recovered by PG. A similar trend was observed in aortic rings challenged with the nitric oxide synthase inhibitor NG-monomethyl-L-arginine (56% recovery). These results strongly indicate that through an antioxidant intervention, PG prevented the myocardial I/R damage and the impairment of endothelial functionality induced by reactive oxygen species following exposure to HBO. The antioxidant activity of PG seems to be too weak (0.05-0.5 mg/ml). This suggests the indirect antioxidant action of the drug (endothelial nitric oxide synthase stimulation) also plays an important role (Maffei Facino et al., 1999).

n) Scutellaria baicalensis Georgi

Scutellaria baicalensis Georgi is a Chinese herbal medicine used to treat allergic and inflammatory diseases. The constituent flavones reported to have antioxidant properties may be responsible for the medicinal effects of S. baicalensis root. It was investigated whether S. baicalensis could confer protection in a cardiomyocyte model of ischemia and reperfusion. The intracellular fluorescent probes 2',7'-dichlorofluorescin diacetate (sensitive to H2O2 and hydroxyl radicals) and dihydroethidium (sensitive to superoxide) were used to assess intracellular reactive oxygen species, and propidium iodide was used to assess cell viability in cultured embryonic cardiomyocytes. S. baicalensis extract (SbE) significantly attenuated generation of free radicals during transient hypoxia and during exposure to the mitochondrial site III inhibitor antimycin A, as measured by fluorescent probes. Reduced oxidative stress was associated with improved survival and function. Cell death after ischemia/reperfusion decreased significantly in S. baicalensis treated cells ($p<0.001$). After antimycin A exposure, S. baicalensis decreased cell death from 49+/-6 % in untreated to 23+/-4 % in treated cells. Return of contraction occurred in S. baicalensis-treated cells but was not observed in control cells. Studies have revealed that baicalein, a major flavone component of SbE possess antioxidant property and can directly scavenge superoxide, hydrogen peroxide, and hydroxyl radicals. Collectively, these findings indicate that SbE and its constituent flavones such as baicalein can attenuate oxidant stress and protect cells from lethal oxidant damage in an I/R model (Shao et al., 1999).

o) Crataegus oxyacantha

The effect of water-soluble fraction of Crataegus (Crataegus extract) was studied on the cardiac mechanical and metabolic function in the isolated, perfused working rat heart. Ischemia for 15 min was induced by removing afterload pressure, and reperfusion of 20 min was produced by returning it to the original pressure. In the control (no drug) heart, ischemia decreased mechanical function to the lowest level, which did not recover even after the end of reperfusion. Crataegus extract (0.01 or 0.05%) was applied to the heart from 5 min before ischemia through the first 10 min after reperfusion. With the high concentration of Crataegus extract (0.05%) the mechanical function recovered during reperfusion incompletely without increasing coronary flow, but the low concentration of Crataegus extract (0.01%) did not. In the heart treated with the high concentration of Crataegus extract, the reperfusion-induced recovery of the energy metabolism was accelerated. The level of lactate during ischemia was lower than that in the control heart, though the myocardial levels of free fatty acids during I/R were not greatly affected. These results demonstrate that Crataegus extract (0.05%) has a cardioprotective effect on the ischemic-reperfused heart. However, the cardioprotective effect is not accompanied by an increase in coronary flow (Nasan et al., 1993).

The effect of the pretreatment with the powder of crataegus oxyacantha on the release of LDH during I/R was studied in an isolated rat heart model. Male Wistar rats were divided into control and crataegus

treated group. For the control group, the standard diet was mixed with a 2% crataegus powder standardized to 2.2% flavonoids. The investigations started 3 months after commencing the treatment. The hearts were isolated and a retrograde perfusion was performed at constant pressure according to the technique of Langendorff. The experimental protocol comprised of 10 min equilibration, according to the technique of Langendorff. The experimental protocol comprised of 10 min equilibration, 110 min occlusion of the left anterior descending coronary artery, and 30 min reperfusion. The coronary effluent was sampled for the LDH determination at various time points (5, 30, 90, 120 and 150 min). The LDH activity increased slightly during the ischemia, and markedly as soon as the heart was reperfused. Crataegus pretreatment resulted in significant decrease in LDL activity. The attenuation of the LDH release by crataegus pretreatment suggests preservation of the cell membrane and significant myocardial protection (Al Makdessi et al., 1996).

The cardioprotective effects of a standardized extract from leaves with flowers of Crataegus (WS-1442; content of oligomeric procyandins [OPC]: 18.75%) have been documented in various studies. To elucidate its cardioprotective mechanism, the active constituents involved in these effects of WS-1442 were identified. Exhausting partitioning between ethyl acetate/water and successive ultrafiltration of the aqueous layer led to the quantitative recovery of three fractions, which were tested for their in vitro radical scavenging (RS) and human neutrophil elastase (HNE) inhibitory activity. The OPCs of Crataegus extracts possess superior antioxidant activities than flavone derivatives or other constituents. In addition, the oligomeric components are more potent inhibitors of HNE. Oral administration of 20 mg/kg/d of the OPC-rich fraction to rats afforded comparative protection against I/R induced pathologies as treatment with 100 mg/kg WS-1442. These observations indicate that radical scavenging and elastase inhibitory activities could indeed be involved in the observed cardioprotective effects of WS-1442. The study emphasizes that OPCs are major orally active constituents of WS-1442. Thus, Crataegus extracts used therapeutically for cardiovascular diseases should be analyzed and standardized for their OPC-content (Chatterjee et al., 1997).

p) Panax pseudoginseng

Trilinolein, a natural plant triacylglycerol, known to have myocardial protective effects was evaluated in vivo. This study investigated if inhibition of calcium influx and alteration of SOD activity are involved the myocardial protection mechanism of trilinolein. In isolated cardiomyocytes, pretreatment with 10(-9) M trilinolein significantly reduced Ca2+ influx stimulated by hypoxia/normoxia. Pretreatment with 10(-7) M trilinolein (for 15 min) in isolated perfused rat heart subjected to 60 min global hypoxemia without reperfusion significantly reduced infarct size. SOD-mRNA assay was analysed by Northern blot. Pretreatment with 10(-7) M trilinolein to in vivo rat heart subjected to 30 min ischaemia and 10 min reperfusion, significantly reduced oxidative stress. It prevented the rise in SOD-mRNA. These results reconfirm the myocardial protection of trilinolein. Cardioprotection may be attributed to antioxidant activity and inhibition of Ca2+ influx (Chan et al., 1999-2006).

q) Withania somnifera

The cardioprotective effects and mechanisms of Withania somnifera (Ws), in the setting of I/R injury were assessed. Wistar rats were divided into three groups and received orally saline (sham, control I/R) and Ws-50 mg/kg (Ws-I/R), respectively, for 1 month. On the 31st day, in the control IR and Ws-IR group rats, left anterior descending coronary artery occlusion was undertaken for 45 min followed by 1 h reperfusion. Subsequently, all the animals were sacrificed for biochemical, immunohistochemical {Bax and Bcl-2 protein}, terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) positivity and histopathological studies. Post-ischemic reperfusion injury resulted in significant cardiac necrosis, apoptosis, and decline in antioxidant status and elevation in lipid peroxidation in the I/R control group as compared to sham. Ws prior-treatment favorably restored the myocardial oxidant-antioxidant balance, exerted marked anti-apoptotic effects {upregulated Bcl-2 ($p<0.001$) protein, decreased Bax ($p<0.01$) protein, and attenuated TUNEL positivity ($p<0.01$)}, and reduced myocardial damage as evidenced by histopathologic evaluation. It is speculated that the antioxidant and anti-apoptotic properties of Ws may contribute to the observed cardioprotective effects (Mohanty IR et al., 2008).

r) Bacopa monniera

Bacopa monniera(Bm), a medicinal herb commonly known as Brahmi is widely used in the Indian system of medicine. The cardioprotective effects of Bm was studied in the Langendorff model of myocardial I/R injury. Effect of Bm on cardiomyocyte apoptosis and antioxidant status following I/R injury was investigated. Forty-eight rats were randomly divided into four groups (12 in each group): sham group (no I/R injury), Bm control group (orally fed Bm at a dose of 75 mg/kg, for three weeks); I/R control group (subjected to I/R-induced myocardial injury) and Bm-treated group (same protocol as I/R control group except that rats also fed Bm). Post-ischaemic reperfusion injury resulted in significant cardiac necrosis, apoptosis, depression of heart rate, decline in antioxidant status and elevation in lipid peroxidation. Oral administration of Bm per se for three weeks to healthy rats caused augmentation of myocardial antioxidants, SOD, catalase and glutathione, along with induction of heat shock protein 72 (HSP72).

I/R induced biochemical and histopathological perturbations were significantly prevented by Bm(75 mg/kg) pre-treatment. Interestingly, Bm also restored the antioxidant network of the myocardium and reduced myocardial apoptosis, caspase 3 and Bax protein expression. Histopathological studies and myocardial creatine phosphokinase content further confirmed the cardioprotective effects of Bm (75 mg/kg) in the experimental model of I/R injury. The study provides scientific basis for the putative therapeutic effect of Bm in ischaemic heart disease (Mohanty IR et al., 2010).

s) Curcuma longa

The cardioprotective potential of Curcuma longa (Cl) in the I/R model of myocardial infarction was investigated. Wistar rats were divided into three groups and received saline orally (sham, control I/R group) and Cl 100 mg/kg (CL-100 treated group) respectively for one month. On the 31st day, rats of the control I/R and Cl treated groups were subjected to 45 min of occlusion of the LAD coronary artery and were thereafter reperfused for 1 h. I/R resulted in significant cardiac necrosis, depression in left ventricular function, decline in antioxidant status and elevation in lipid perodixation in the control I/R group as compared to sham control. Myocardial injury due to I/R was significantly prevented by Cl treated group. Cl treatment resulted in restoration of the myocardial antioxidant status and favorable modulation of hemodynamic parameters as compared to control I/R. Furthermore, I/R-induced lipid peroxidation was significantly inhibited by Cl treatment. The beneficial cardioprotective effects also translated into the functional recovery of the heart. Cardioprotective effect of Cl may be attributed to the suppression of oxidative stress and improvement in ventricular function. Histopathological examination further confirmed the protective effects of Cl on the heart (Mohanty et al., 2004). Further, the effect of Cl on myocardial apoptosis was studied in the I-R model of myocardial injury. Cl pre-treatment reduced the Bax/Bcl-2 ratio and demonstrated significant ant-apoptotic activity. The antioxidant and anti-apoptotic properties of Cl may contribute to the cardioprotective effects (Mohanty et al., 2006).

III. Congestive Cardiac Failure (Chf)

a) Corydalis yanhusuo

Corydalis yanhusuo, a Chinese medicinal plant is reported to possess significant cardioprotective effects. The main active principle, l-tetrahydropalmatine, is responsible for its pharmacological effects. The protective effects of Corydalis yanhusuo was evaluated in a rat heart failure model. Rats were orally fed with 50, 100, or 200 mg/ kg of ethanolic extract of Corydalis yanhusuo daily, from the 7th day after surgery and thereafter subjected to coronary artery ligation. The cardiac function, plasma atrial natriuretic peptide (ANP), relative heart and lung weights, infarct size and ventricular dilatation after treatment for 8 weeks were measured. Corydalis yanhusuo treatment led to a significant reduction in infarct size and improvement in cardiac function as demonstrated by lower LVEDP and elevated +/-dp/dt(max). Corydalis yanhusuo significantly reduced left ventricular (LV)/body weight ratio, lung/body weight ratio and inhibited neurohormonal activation. The study concluded that Corydalis yanhusuo exerted salutary effects on heart failure induced by myocardial infarction in rats (Wu L et al, 2007).

b) Shenqi Fuzheng

The effect of Shenqi Fuzheng injection (SFI) on the humoral immunity (IgG IgM IgA), cellular immunity (T-lymphocyte subsets), SOD activity and plasma viscosity in CHF patients were studied. Sixty patients with CHF, with heart function of NYHA grade II-IV were randomly divided into two groups. The treaded group was treated with SFI 100 ml, and the control group was treated by 10 mg nitroglycerine injection. To detect the IgG, IgM, IgA, T-lymphocyte subsets, SOD, lipid peroxidation and plasma viscosity, venous blood from cubital vein was collected before and after treatment. Results demonstrate that the heart function improved markedly in the treated group as compared to the control group ($P < 0.05$). The left ventricular ejecting fraction (LVEF) and end systolic volume (ESV) were improved in both group ($p < 0.05$, $p < 0.01$), and the improvement in the treated group was superior to the control group ($p < 0.05$). In the treated group, the CD4, SOD level and CD4/DC8 ratio increased ($p < 0.05$), whereas lipid peroxidation, IgG and IgM reduced ($p < 0.05$) significantly compared to the control group. Significant improvement in the plasma viscosity was seen in the treatment group. SFI improved the immune function of CHF patients. Shenqi Fuzheng injection (SFI) has potential as an adjuvant therapy in the treatment for CHF (Liu H et al., 2005).

c) Manshuailing

The clinical effect of manshuailing in patients with CHF was evaluated. A total of 90 heart failure patients were randomly divided into 2 groups: 45 cases in the routine treatment group (RT) received general therapy including diuretics and digitalis, and 45 cases in the Chinese herbal medicine group (CH) were treated for six weeks with the above medicine, with additional manshuailing oral liquid for six weeks. The clinical effect was summarized six weeks after treatment. Total effect rate was 82.2% and 62.2% in CHF and RT group respectively. Compared with pretreatment, heart function including stroke volume (SV), stroke volume index (SVI), cardiac index (CI), distance of inter-ventricular septal to mitral valve (EPSS) were all improved significantly in both groups ($p < 0.05$ or $p < 0.01$). The cardiac function was superior in the CH group as compared to the RT group ($p < 0.05$ or $p<$

0.01). Manshuailing oral liquid alleviated clinical symptom, decreased EPSS, and improved heart function (Yang et al., 2003).

d) Zhimu and huangqi combination

The efficacy of Zhimu in treating cardiac hypertrophy associated with CHF was evaluated. Mice cardiac hypertrophy model was established by s.c. Isoproterenol (ISO), 2 times per day for 14 days and heart-weight-index was measured. Zhimu and Huangqi were given orally alone or jointly for 14 days. Abdominal aorta banding operation was done in mice and 3 weeks after operation, they were administrated for 2 weeks, and then run-time (exercise capacity), quiet heart rate, heart rate after ISO and heart-weight-index were measured. Cardiac hypertrophy model mice were administrated for 12 days, and the mortality and dying time of mice in cold (-20 degrees C) and heat (45 degrees C) stimulative condition were observed. Zhimu could cut down the increasing of heart rate induced by ISO, decreased significantly heart-weight-index in cardiac hypertrophy mice, reduced the quiet heart rate and prolonged the run time in abdominal banding model. Zhimu combined with Huangqi improved the ISO response in abdominal banding model mice, reduce the mortality and delayed dying time of mice in stimulative condition. Zhimu combined with Huangqi slowed down heart rate, enhanced the reserve force of the heart, and improved the response capacity of cardiac hypertrophy mice in stimulative condition (Hu etal., 2003) .

e) Crataegus oxyacantha (aubepine)

Crataegus oxyacantha (Aubepine, Hawthorn), was used by European herbalist in the first century A. D. Until the 19th century, its true potential for treatment of heart disease was not fully explored. The leaves, flowers, and berries of hawthorn contain a variety of bioflavonoid-like complexes that appear to be primarily responsible for the cardiac actions of the plant. Bioflavonoids found in C. oxyacantha include oligomeric procyanidins (OPCc), vitexin, quercetin, and hyperoside. These ingredients are responsible for its beneficial cardiovascular effects (Ju LY et al., 2005). A placebo controlled, randomized, parallel group, multicentre trial was conducted to assess the efficacy and safety of a standardized extract of fresh berries of Crataegus oxyacantha L. and monogyna Jacq. (Crataegisan) in patients with grade NYHA class II cardiac failure. A total of 143 patients (72 men, 71 women, mean age of 64.8 (8.0 years) were recruited and treated with 3 times 30 drops of the extract (n = 69) or placebo (n = 74) for 8 weeks. The primary endpoint included the evaluation of change in exercise tolerance determined with bicycle exercise testing; secondary variables included the blood pressure-heart rate product (BHP). Subjective cardiac symptoms at rest and at higher levels of exertion were assessed by the patient on a categorical rating scale. The difference between the treatment groups was 8.3 watts in favor of the standardized extract of fresh Crataegus berries ($p = 0.045$). Although, the results were not statistically significant, changes in BHP at 50 watts and at comparable maximum load were in favour of Crataegus extract. The subjective assessment of cardiac symptoms at rest and at higher levels of exertion did not change significantly and the patient and investigator overall assessment of efficacy were similar for the two groups. The medication was well tolerated and had a high level of patient acceptability. These results are clinically significant as the symptoms of dyspnoea and fatigue do not correct until a significantly higher wattage has been reached in the bicycle exercise test. The study concluded that NYHA II patients showed improvement in their heart failure condition under long term therapy with the standardized extract of fresh Crataegus berries (Degenring et al., 2003).

f) Berberine

Berberine, is an alkaloid from Hydrastis canadensis L., a Chinese herb Huanglian. It is widely used in traditional Chinese medicine as an antimicrobial for the treatment of dysentery and infectious diarrhea. Berberine and its derivatives, tetrahydroberberine and 8-oxoberberine have significant beneficial cardiovascular effects. Berberine has positive inotropic, negative chronotropic, antiarrhythmic, and vasodilator properties. Both derivatives of berberine have antiarrhythmic activity. Cardiovascular effects of berberine and its derivatives are attributed to the blockade of K+ channels (delayed rectifier and K(ATP)) and stimulation of Na+ -Ca(2+) exchanger and prolongation of the duration of ventricular action potential. Its vasodilator activity has been attributed to multiple cellular mechanisms. The cardiovascular effects of berberine suggest its possible clinical usefulness in the treatment of heart failure (Lau et al, 2001).

g) Digitalis purpurea

Digoxin has been commonly used to treat patients with CHF, over the past 200 years. William Withering was able to identify Digitalis purpurea as the essential ingredient in a prescription dispensed by a herbalist, and systematically proceeded to show its value in patients with cardiac failure (Krikler, 1985). He identified the cardinal symptoms of digitalis intoxication and worked out effective rules for the prescription of an infusion of digitalis.

Use of digitalis for the treatment of patients with CHF and sinus rhythm remains controversial. To ascertain the proper therapeutic role of digitalis, the published clinical evidence of digitalis was critically appraised. A search of the English literature from 1960 to 1982 identified 736 articles, of which 16 specifically addressed the clinical evaluation of digitalis therapy for patients with CHF and sinus rhythm. Only two double-blind, placebo-controlled trials provided clinically useful information. One study showed that digoxin therapy

could be withdrawn successfully in elderly patients with stable CHF. The other showed that patients with chronic heart failure and an S3 gallop benefited from digoxin therapy (Wray et al., 1985; Mulrow et al., 1984).

Clinical trials have demonstrated the benefits of the use of digoxin on exercise tolerance, ejection fraction, and neurohormone production. The Digoxin Investigators Group trial has recently provided strong evidence for the long-term benefits of digoxin on morbidity for patients with heart failure (Demers et al., 1999).

h) Red Ginseng

The beneficial effect of red ginseng in CCF was evaluated and compared with Ginseng. Forty-five patients with class IV cardiac function were divided into three groups of fifteen patients each: group I (digoxin group), group II (Red Ginseng group) and group III (Red Ginseng plus digoxin group). After treatment, the improvement in the hemodynamic and biochemical indexes in Red Ginseng group and Red Ginseng plus digoxin group were greater than those of digoxin group, and group Red Ginseng plus digoxin group was the most significant amongst all. The results suggested that Red Ginseng and digoxin act synergistically in the treatment of CCF. Red Ginseng is an effective and safe adjuvant for effective management of CHF (Ding et al., 1995).

i) Terminalia Arjuna

The beneficial effect of Terminalia Arjuna, an Indian medicinal plant, in CCF was studied in a double blind cross over study. Terminalia Arjuna was administered to twelve patients with refractory chronic CHF (Class IV NYHA), related to idiopathic dilated cardiomyopathy (10 patients); previous myocardial infarction (one patient) and peripartum cardiomyopathy (one patient). Terminalia Arjuna bark extract (500 mg 8-hourly) or placebo was administered for 2 weeks each, separated by 2 weeks washout period. The clinical, laboratory and echocardiographic evaluation was carried out at baseline and at the end of Terminalia Arjuna and placebo therapy. Thereafter, the results were compared. Terminalia Arjuna, compared to placebo, was associated with improvement in symptoms and signs of heart failure, improvement in NYHA Class (Class III vs. Class IV), decrease in echo-left ventricular end diastolic and end systolic volume ($P < 0.005$) indices, increase in left ventricular stroke volume index $P < 0.05$) and increase in left ventricular ejection fractions ($P < 0.005$). Further, on long term evaluation in an open design Phase II study, participants continued Terminalia Arjuna in fixed dosage (500 mg 8-hourly) in addition to flexible diuretic, vasodilator and digitalis dosage for 20-28 months (mean 24 months) on outpatient basis. Patients showed continued improvement in symptoms, signs, effort tolerance and NYHA Class, with improvement in quality of life (Bharani et al., 1995).

j) Sini decoction

The study was conducted to investigate the protective effects of Sini decoction (SND) on Adriamycin-induced heart failure and also elucidate its cardioprotective mechanism. SD rats were randomly divided into three groups: control group, heart failure model group and SND group. Adriamycin was injected in the rats of Adriamycin model group and SND group by caudal vein. After injection, the rats in SND group were given SND (3.75 g/kg) per day, per orally. Three weeks later, protein expressions of Bid and Bcl-xl were detected by immunohistochemistry; mRNA expression ratio of Bcl-xl/Bcl-xs was detected by RT-PCR and apoptosis rate was determined by flow cytometry. The protein expression of Bcl-xl and mRNA ratio of Bcl-xl/Bcl-xs decreased, while the protein expression of Bid and apoptosis rate significantly increased in the SND treatment group as compared with the control group. SND could decrease cell apoptosis, increase the protein expression of Bcl-xl, increase bcl-xl/bcl-xs mRNA ratio and decrease Bid protein expression. Bcl-xl plays an important role in ADR-induced heart failure in rats. The mechanism of SND cardioprotection may be related to modulation of key regulatory proteins of apoptosis, Bcl-xl and Bid (Zhao et al., 2009).

k) Wenxin Keli

The effect of Wenxin Keli treatment on ISO induced heart failure was studied in rats. Sixty six-week old male Wistar rats were randomized to six groups. The rats of control group received distilled water every day. Wenxin Keli (9 mg/kg) was administered for 2 weeks every day. The rats in Wenxin Keli and control group received two subcutaneous injections (85 mg/kg of ISO, separated by a 24 hour interval). The rats in valsartan and ISO group received two subcutaneous injections (85 mg/kg) of ISO, and received valsartan 30 mg/kg for 2 weeks every day. Echocardiogram measurement in rats was carried out after 4 weeks and 10 weeks feeding. In the In the ISO group, echocardiogram indicated that left ventricular internal diameter at diastolic phase (LVIDd), left ventricular internal diameter at systolic phase (LVIDs), LV percent fractional shortening (FS) and LV ejection fraction (EF) were reduced. Treatment with valsartan for 4 weeks significantly increased FS and EF as compared with the ISO group. However, treatment with Wenxin Keli for 10 weeks did not significantly change the LVIDs, FS, EF compared to the ISO group. 10 weeks of treatment with valsartan and Wenxin Keli resulted in significant improvement in the hemodynamic parameters: LVEDP, left ventricular systolic pressure (LVSP), and dp/dt(max). It was concluded that Wenxin Keli significantly improves the ISO induced cardiac dysfunction (Zhou et al., 2007).

IV. Hypertension

a) Asttragalus complanatus

The effects of total flavonoid fraction of Asttragalus complanatus on blood pressure in conscious spontaneously hypertensive rats (SHR) and hemodynamics in anesthetized SHR was investigated. It was observed that the total flavonoid fraction of Asttragalus complanatus (100, 200 mg/kg) decreased the blood pressure of conscious SHR significantly (decreasing 7.1%, $P < 0.05$ and 9.3%, $p < 0.01$ respectively) and total peripheral resistance (decreasing 20%, $P < 0.05$). However, there was no significant change in heart rate and cardiac output. It was concluded that the total flavonoid fraction of Asttragalus complanatus possesses significant antihypertensive effects by virtue of decreasing the total peripheral resistance (Xue et al., 2002).

b) Allium sativum

Allium sativum commonly referred to as garlic, possess a number of beneficial cardioprotective effects. The active ingredient allicin is responsible for its therapeutic effects. Qidwai et al, 2000 conducted a study to find out whether individuals with blood pressure (BP) on the lower side consume more garlic in their diets. A questionnaire was developed and was administered to 101 adult subjects, presenting to the Family Practice Centre of a hospital in the city of Karachi, Pakistan. It was estimated that average garlic use was 134 grams per case per month. Subjects with BP on the lower side were found to consume more garlic in their diets ($p < 0.05$). This study demonstrates that individuals whose blood pressures (BP) are on the lower side are likely to consume more garlic in their diets.

The effect of garlic on pulmonary pressures in rats subjected to alveolar hypoxia and on vasoconstriction in isolated pulmonary arterial rings was also studied (Fallon et al., 1998). Garlic gavage (100 mg/kg wt) for 5 days resulted in complete inhibition of acute hypoxic pulmonary vasoconstriction compared with the control group. These studies document that garlic blocks hypoxic pulmonary hypertension in vivo and demonstrates a combination of endothelium-dependent and -independent mechanisms responsible for the effect in pulmonary arterial rings. Meta-analysis concluded that garlic possess significant hypotensive effects only in patients with increased systolic pressure (Reinhart et al., 2008). Compared to placebo, garlic preparations were found to be superior in reducing BP in individuals (Ried et al, 2008). The beneficial cardioprotective action of garlic in essential hypertension (HTN) was studied. The antihypertensive effect of garlic was observed in 20 patients with HTN receiving garlic pearls preparation for a period of two months (Dhawan and Jain, 2008).

c) Apium graveolens

Apium graveolens, commonly known as celery, according to Chinese theory is known to be effective for HTN associated with liver disease. In Mainland China, celery was useful in reducing HTN in 14 out of 16 patients. The juice was mixed with equal amount of honey and about 8 ounces were taken orally three times each day for up to one week. It significantly reduced systolic and diastolic BP. The difference of BP in human beings before and after treatment was found to be significant ($p<0.05$), indicating that seeds of A. graveolens possess significant hypotensive effect. Fresh celery juice can be mixed with vinegar to relieve dizziness and headache and shoulder pain associated with HTN. It is also effective in HTN associated with pregnancy and climacteric (Gharooni and Sarkarati, 2000).

d) Artemisia vulgaris L.

Artemisia vulgaris L. dried leaves were extracted in distilled water and chloroform. Two partition fractions from the aqueous extracts and four partition fractions from the chloroform extracts were tested on male Sprague-Dawley rats using both the in situ mesenteric circulation and the isolated perfused mesentery. Administration of 10% w/v solutions of water extract fractions FGN 63-1 and FGN 63-2 of A. vulgaris in the isolated perfused rat mesentery model was highly effective in reversing the hypertensive action induced by norepinephrine with no significant effect on heart rate in either the normotensive or hypertensive states (Tigno et al, 2000).

e) Ajmaloon

Ajmaloon, an herbal drug, was studied in anesthetized rabbits and monkeys for its effect on the arterial BP, heart rate and baroreceptor-heart rate reflex. Intravenously administered Ajmaloon produced a dose-dependent hypotensive response in both the species without any significant effect on the heart rate. In Ajmaloon treated animals, loss of tachycardia response to fall in arterial pressure indicated that the drug suppresses normally existing sympathetic excitatory influence in response to hypotension. Even after intravenous administration of 100 mg/kg Ajmaloon (a dose much higher than the prescribed highest oral dose for humans), Baroreflex regulatory heart rate response to hypertension remained intact. Intact baroreflex regulation of arterial BP in response to hypertension in Ajmaloon treated group suggests that Ajmaloon does not interfere with the normal BP regulatory mechanism through arterial baroreceptors during hypertension. Study concluded that Ajmaloon possess significant hypotensive effect (Fahim et al, 2005).

f) Bidens pilosa Linn

The hypotensive effect of Bidens pilosa Linn (Asteraceae) leaves was evaluated in SHR, salt-loading hypertensive rats (SLHR) and normotensive Wistar rats, using the indirect (tail-cuff) method. Acute changes in urine volume and urinary excretion of Na+ and K+ were also studied. It was found that the hypotensive effect of the extract was more remarkable in hypertensive than in normotensive rats. Although not statistically significant, the urinary excretion of Na+ was decreased by 36% in SHR and the excretion of K+ increased by 35% in normotensive rats. These results suggest that the extract has significant hypotensive effect by virtue of its vasodilatory property (Dimo et al., 1999)

g) Cecropia obtusifolia (Moraceae)

The antihypertensive efficacy of the leaf extract of Cecropia obtusifolia was evaluated. Cecropia obtusifolia leaf extract demonstrated significant antihypertensive when administrated intravenously to conscious spontaneous hypertensive rats. Forty-five minutes after injection, the maximum fall in arterial pressure (-23.5% relative to pre-injection values) was seen. At the end of the 180 min observation period, recovery was not complete. The extract was administered to pre-hypertensive SHR and normotensive rats. The fall in BP was more conspicuous in the two SHR groups and was not accompanied by changes in cardiac frequency in any group (Salas et al, 1888).

h) Crataegus pinnatifida

Crataegus pinnatifida, commonly known as hawthorn's decoction has been used in China for treatment of HTN for thousands of years. The active ingredients that contribute to hawthorn's beneficial effects on heart are flavonoids and oligomeric procyanidins. In experiments with anesthetized rabbits, intravenous administration of the extract preparation lowered the BP for up to 3 hours (Bensky and Gamble, 1990). Grataegic acid was identified as the hypotensive principle. Mechanisms of action of Crataegus involve a broad-based influence on the cardiovascular system. The hypotensive activity is mediated via vasorelaxation resulting from nitrous oxide stimulation, significant antioxidant activity, and a tonic action on cardiac myocytes (Schüssler et al, 1995).

i) Carica papaya (L.)

The hypotensive action of crude ethanolic extract from the unripened fruit of Carica papaya was evaluated and compared with hydrallazine. Both hydrallazine (200 microg/100 g i. v) and Carica papaya extract (20 mg/kg. i.v) produced a significant depression of mean arterial pressure (MAP) in all groups ($p < 0.01$ vs controls). The hypotensive effect of the extract was more profound. It produced about 28% more depression of MAP than hydrallazine in the hypertensive groups. The extract (10 microg/mL) produced relaxation of vascular muscle tone in vitro studies using isolated rabbit arterial (aorta, renal and vertebral) strips. These effects were however, attenuated by phentolamine (0.5-1.5 microg/mL). Based on the study results it is concluded that the fruit juice of C. papaya produces significant hypotension attributed to mainly the inhibition of alpha-adrenoceptor activity (Eno et al, 2000).

j) Casimiroa edulis

Casimiroa edulis seed is reported to possess hypotensive activity. The methanolic extract of Casimiroa edulis contains many active ingredients: synephrine acetonide, N-monomethyl histamine, N,N-dimethyl histamine, proline, N-methylproline, gamma-aminobutyric acid and casimiroedine. These components were isolated. Their antihypertensive activity was tested in experimental animals. In anesthetized rats, both histamine derivatives produced transient hypotension mediated via H1-histaminergic receptors and in the case of N,N-dimethyl histamine, via nitric oxide release. Synephrine acetonide produced transient hypertension and tachycardia, mediated via alpha and beta-adrenergic receptors, respectively. N-methyproline, proline and gamma-aminobutyric acid elicited pronounced and prolonged hypotension. Casimiroedine did not significantly affect on the BP of anesthetized rats. However, it was capable of lowering blood pressure persistently in anesthetized guinea pigs. It was concluded that several active components of C. edulisis are responsible for its hypotensive effects. Histamine derivatives acting on H1-receptors are responsible for its immediate effect. More prolonged hypotension is attributed to the mixture of amino acids through an unknown mechanism, as well as by casimiroedine, possibly by activation of H3-receptors (Magos et al, 1999).

k) Cecropia lyratiloba

The effect of methanol extract (ME) of Cecropia lyratiloba and its flavonoid fraction (FF) on the contractility of cardiac, vascular and tracheal smooth muscles was evaluated. Adrenaline-induced contractions of the aorta were inhibited by both ME and FF in a concentration-dependent manner. The flavonoids isolated from FF, namely iso-orientin and a mixture of orientin and isovitexin, were also tested in the aorta. Results show that this flavonoid is not responsible for the vasorelaxant effects of ME and FF. The vascular relaxation of FF was abolished in the presence of N(omega)-nitro-L-arginine methyl ester, an inhibitor of nitric oxide synthase. It was concluded that the endothelium-dependent vasodilation induced by FF is mediated by the release of nitric oxide (NO). The vascular relaxation demonstrated by ME and FF validate its traditional use for treatment of arterial hypertension (Ramos et al 2006).

l) Panax ginseng

Panax ginseng is well known to enhance the release of NO from endothelial cells of the rat aorta and to reduce BP in experimental animals. To further confirm the efficacy of the Panax ginseng extract, clinical studies were conducted. The effects of water extract of Korea red ginseng (KRG) on NO concentration levels in the exhaled breath, BP, and heart rate of human volunteers was studied. It was also investigated whether NO level in exhaled breath was increased by KRG extract, and if any correlation between BP and heart rate. A single administration of KRG water extract (500 mg/50 kg) increased NO levels in exhaled breath, and concomitantly decreased mean blood pressure and heart rate of twelve healthy, non-smoking male volunteers. The correlation value between NO levels and heart rate ($r = 0.94$), and the correlation value between NO levels and heart rate ($r = 0.84$) were found to be significant ($P < 0.01$). Linear regression analysis shows the clear conversed correlation between NO levels and BP as well as heart rate. The results support the claim that KRG may be useful for the treatment of HTN and pulmonary vascular obstruction (Han et al, 2005)

Han et al, 1998, evaluated the changes in diurnal blood pressure pattern (24 hour ambulatory blood pressure monitoring) after 8 weeks of red ginseng medication (4.5 g/day). Study was conducted among 26 subjects with essential hypertension. Their 24 hour mean systolic blood pressure decreased significantly ($p = 0.03$) while diastolic blood pressure showed only a slight decline ($p = 0.17$). The decrease in pressures were observed at daytime (8 A.M.-6 P.M.) and dawn (5 A.M.-7 A.M.). 8 subjects were probably of white coat hypertension, as no significant change in BP was observed. Based on the results, it was concluded that red ginseng might be useful as a relatively safe medication adjuvant to current antihypertensive medications.

m) Ginkgo biloba

The acute effect of ginkgo (Ginkgo biloba L.) ethanolic extracts on arterial BP, and heart rate in anesthetized normotensive rats was examined and compared. The left carotid artery was used for the measurement of arterial BP. The intravenous administration of the extracts produced a statistically significant dose-dependent and reversible hypotensive and bradycardic effects (Brankovic et al, 2011). The effects of Ginkgo biloba extract (GBE) on the development of hypertension, platelet activation and renal dysfunction in deoxycorticosterone acetate (DOCA)-salt hypertensive rats was also studied (Umegaki et al, 2000). Both DOCA-salt hypertensive rats and normotensive rats were fed a 2% GBE diet for 20 days. The tail-cuff and telemetry methods were used for the measurement of BP. Rats fed a 2% GBE diet did not develop significant hypertension. In addition, an increase in heart weight, an indicator of sustained high BP, was inhibited significantly by feeding of the GBE diet. Feeding of the GBE diet also decreased 5-hydroxytryptamine content in platelets, a marker of platelet activation in vivo associated with hypertension. The telemetry study demonstrated that BP and heart rate showed a clear circadian rhythm and the antihypertensive effect of GBE was prominent in the daytime, a resting period for rats. This anti-hypertensive effect of GBE was not detected in normotensive rats (Umegaki et al, 2000).

n) Guazuma ulmifolia

The hypotensive effect of procyanidin fraction (PCF) obtained from acetone extract of Guazuma ulmifolia bark was studied. 10 mg/kg PCF dose was orally administered to sugar-fed hypertensive rats. PCF significantly decreased both the systolic arterial pressure and the heart rate, whereas the same doses administered intravenously induced arterial hypotension. Hypotensive effect was attenuated by NG-nitro-L-arginine methylester (L-NAME) pretreatment. The PCF reduced the contraction induced by norepinephrine (1x10(-7) M) in isolated aortic rings of normotensive and sugar-fed hypertensive rats. Vascular endothelium removal or L-NAME (30 microM) pretreatment inhibited the relaxant activity of PCF. Procyanidin oligomers consisting mainly of tetramers and trimers are the active ingredients of PCF responsible for its hypotensice effects. Guazuma ulmifolia bark possesses long-lasting antihypertensive and vasorelaxing properties. These beneficial effects can be linked to the endothelium related factors; involving nitric oxide (Magos et al, 2008).

o) Hibiscus sabdariffa

The antihypertensive effect of the plant extract of Hibiscus sabdariffa was evaluated. It was observed that in experimentally induced hypertensive rats, an intravenous administration of 20 mg/kg aqueous extract of dry H. sabdariffa calyx produced a significant fall in the BP. The hypotensive effects of the crude extract of H. sabdariffa may be mediated through direct vasorelaxant effects of acetylcholine and histamine. Earlier report showed that the petal crude extract of same plant produced a direct relaxant effect on the aortic smooth muscle of rats (Herrera-Arellano et al., 2004). The chronic administration of aqueous extract of HS has been reported to reverse cardiac hypertrophy in reno-vascular hypertensive rats. A clinical trial of the plant extract has shown reliable evidence of antihypertensive effect. A standardized dose of H. sabdariffa (9.6 mg per day) given to 39 patients and captopril, 50 mg per day, given to the same number of patients did not show significant difference relative to hypotensive effects, antihypertensive effectiveness and tolerability (Odigie et al., 2003).

p) Herniaria glabra

The antihypertensive effects of Herniaria glabra saponins was studied and compared with that of furosemide. Spontaneously hypertensive rats were treated with Herniaria glabra saponins at a dosage of 200mg/Kg of body weight. Treatment with Herniaria glabra saponins led to significant decline in both systolic and diastolic blood pressures after 1 month. However, no significant change in heart rate was observed. It was concluded that H. glabra saponins lowered blood pressure by multifactorial mechanism (Rhiouani et al, 2001).

q) Olea. africana (Oleaceae)

The effects of crude extract of the root and stem of Olea africana on MAP and heart rate in normo and hypertensive rats was studied in experimental rats. An immediate and dose dependent fall in MAP and heart rate in anaesthetised normotensive rats was produced by intravenous administration of aqueous and ethanolic extracts of Olea Africana. The efficacy of the aqueous extract was more superior to the ethanolic extract. Orally administered aqueous extract produced lowering of MAP and HR in DOCA-salt hypertensive rats (Osim et al, 1999).

r) Rauwolfia serpentina

Reserpine, was the purified alkaloid of Rauwolfia serpentina. It was the first potent drug widely used for the long-term treatment of hypertension. In Europe, Georg Eberhard Rumpf first reported about rauwolfia in his Herbarium amboinense, 1755. The first modern paper about therapeutic applications of the whole root of rauwolfia was published in 1931 in the Indian Medical Journal by Sen and Bose, and many papers dealing with botanics, chemistry and pharmacology then appeared in Indian and European periodics. In 1949, Vakil published the first report of the antihypertensive effect of rauwolfia in the British Heart Journal. In the Ciba laboratories in Basel, Switzerland, Mueller, Schlittler and Bein analyzed various rauwolfia alkaloids and published in 1952 the first complete report about their chemistry and pharmacology. In the same year, reserpine was introduced under the name Serpasil for the treatment of hypertension, tachycardia and thyreotoxicosis (Jerie et al, 2007).

In a carefully controlled series of 39 severe cases of hypertension (38 with essential hypertension and 1 with nephritic hypertension) treated for 6 to 20 months with rauwolfia preparations, a fall in BP in 67% of cases was observed. In most cases there was a proportionate fall in both systolic and diastolic BP, but in several the fall in the diastolic appeared to be relatively greater than in the systolic. The fall was slight (10-20 mm. Hg diastolic) in 21 % but appreciable or marked in 46% (greater than 20 mm. Hg diastolic), and in four patients the diastolic BP fell to below 100 mm. Hg (S. Locket, 1955).

s) Terminalia superba

Terminalia superba, is used in traditional Cameroonian medicine as an antihypertensive remedy. Tom et al., 2010 investigated the hypotensive efficacy of the aqueous extract of Terminalia superba. Rats were orally administered 10% D-glucose for 3 weeks to induce hypertension. The antihypertensive effects were studied after oral administration of the extract (50 and 100 mg/kg/day) or nifedipine (10 mg/kg/day) for 3 weeks. BP and heart rate were measured along with the antioxidant parameters in the heart, aorta, liver and kidney at the end of the experiment. Intravenous administration of the aqueous extract of Terminalia superba induced a significant hypotensive response without any significant change in HR. The oral administration of the extract significantly prevented the rise in BP in glucose-hypertensive rats. Treatment with plant extract resulted in withdrawal of sympathetic tone and an improvement in the antioxidant status as it significantly reduced the oxidative stress associated with hypertension. The present study demonstrates that the aqueous extract of the stem bark of Terminalia superba exhibits significant hypotensive effects that are, at least in part, related to a withdrawal of sympathetic tone and to an improvement of the antioxidant status (Tom et al., 2010).

t) Xingnao Qingxuan

Zhou et al., 1999 studied the effect of Xingnao Qingxuan capsules (XQC) in decreasing BP of normal and anesthetized cats. Oral administration of XQC, 2.8 g/kg produced a decrease in BP of normal cats. XQC 1.4, 2.8 and 5.6 g/kg once a day for 14 days, produced a dose-dependent reduction of BP in SHR. Although after 3-4 days of administration the BP returned to the baseline values but the change was not statistically significant. With oral administration of 2.8 and 5.6 g/kg XQC, the incubation period of eyeball tremor induced by chloroform by dropping into the ear was prolonged by 14.4% and 13.0%, and the keeping time shortened by about 33.3% and 23.3% respectively. Brain basic arterial spasm induced by KCl or 5-HT in dog was relaxed significantly by XQC in vitro experiment. Results demonstrate that XQC reduces blood pressure resisting dizziness (Zhou et al., 1999).

u) Stephania tetrandra S Moore

The hypotensive effect of the extract of Radix Stephaniae Tetrandrae (RST), the root of a Chinese hero Stephania tetrandra S Moore was evaluated experimentally. Results were compared to those of tetrandrine (Tet), active component of RST (Wong et al., 2000). The RST extract inhibited Ca2+ influx into the myocyte and reduced protein release during reperfusion. RST extract suppressed elevation of arterial blood pressure in DOCA-salt hypertensive rats. The results suggest that the efficacy of the RST extract cannot be accounted for by Tet alone. Some of the

effects may be due to an interaction between the components of the extract. The RST extract produced similar hypotensive effects as verapamil, a prototype Ca2+ channel antagonist widely used in the treatment of hypertension.

v) Solanum sisymbriifolium

S. sisymbriifolium Lam., root, a perennial herb, has been used as a traditional medicine in Paraguay. It possesses diuretic and antihypertensive properties. The hypotensive effect of the crude hydroalcoholic extract from root was investigated both in normotensive and hypertensive rats. The intravenous administration of the extract (50 and 100 mg/kg) produced a significant decrease in BP in anesthetized DOCA hypertensive rats. Oral administration of the extract (10, 50, 100, and 250 mg/kg) produced a dose-dependent hypotensive effect in conscious hypertensive animals. In anesthetized normotensive rats, the extract (50 and 100 mg/kg, i.v) induced hypotension in a dose-dependent manner. When administered orally (10, 50, 100, 250, 500, and 1000 mg/kg) to conscious normotensive rats, no significant effect on BP was produced by the extract. In another study, the active ingredient nuatigenosido was isolated from the extract. Nuatigenosido at 100 g/kg and 1 mg/kg i.v lowered BP in rats and at 10−6 and 10−5 M augmented the contractile force in the right atrium of a bullfrog. Nuatigenosido at 10-7 M increased the overshoot amplitude in frog atrial myocytes, the action potential duration was shortened, the calcium current was increased, and the delayed outward potassium current was increased. The study concluded that nuatigenosido may play an important role in the therapeutic effects of this herb (Ibarrola et al., 2003).

w) Uncaria rhynchophylla

U. rhynchophylla has been used in traditional oriental medicine to lower BP and relieve various neurological symptoms. The indole alkaloid called hirsutine acts on calcium chaneels and is responsible for its hypotensive activity. The effects of hirsutine on cytosolic Ca2+ level ([Ca2+]cyt) were studied by using fura-2- Ca2+ fluorescence in smooth muscle of the isolated rat aorta. Noradrenaline and high K+ solution produced a sustained increase in [Ca2+]cyt. Application of hirsutine after the increases in [Ca2+]cyt induced by noradrenaline and high K+ notably decreased [Ca2+]cyt. Results suggest that hirsutine inhibits Ca2+ influx through voltage-dependent Ca2+ channel. Furthermore, hirsutine had profound effect on intracellular Ca2+ stores. It significantly reduced the caffeine-induced contraction under the Ca2+ -free nutrient condition in the rat aorta. During Ca2+ loading when hirsutine was added it augmented the contractile response to caffeine. It was concluded that hirsutine reduces intracellular Ca2+ level through its effect on the Ca2+ store as well as through its effect on the voltage-dependent Ca2+ channel. In another study, the methanolic extract of the hooks of an Uncaria species was found to have a potent and long-lasting hypotensive effect in rats. Further studies of the extract resulted in the isolation of 3-indole alkaloid, glycoside, cadambine, dihydrocadambine, and isodihydrocadambine. The active ingredients dihydrocadambine, and isodihydrocadambine were found to possess significant hypotensive property, whereas cadambine was inactive (Endo et al., 1983).

x) Zingiber officinale

Zingiber officinale (Zo.Cr), commonly known as Ginger is used in Asian cooking. It is known to improve the blood circulation. In anesthetized rats, the crude extract of Zo.Cr induced a dose-dependent (0.3-3 mg/kg) fall in the arterial BP. In guinea pig paired atria, Zo.Cr exhibited a cardiodepressant activity on the rate and force of spontaneous contractions. In rabbit thoracic aorta preparation, Zo.Cr inhibited the phenyl ephrine-induced vascular contraction at a dose ten times higher than that required against K+ (80 mM)-induced contraction. Similar to the effect of verapamil, Zo.Cr shifted the Ca2+ dose-response curves to the right, confirming the Ca2+ channel-blocking activity. The results suggest that the hypotensive effect of Zo.Cr is mediated via blockade of voltage-dependent calcium channels. Chronic administration of Pet ether extract (PE) (50 mg/kg/day; po), toluene fraction (10 mg/kg/day; po) of ginger rhizome, and Korean ginseng extract (KGE) (30 mg/kg/day; po) significantly reduced the BP in DOCA salt-induced hypertensive rats. The PE (50 mg/kg/day; po) and KGE (30 mg/kg/day; po) produced significant hypotensive effects in fructose-induced hypertensive rats. It was also speculated that the hypotensive mechanism of action may partly be attributed to serotonin antagonism. Few clinical trials using low dose Zo.Cr have been undertaken with inconclusive results (Nicoll and Henein, 2009).

y) Zygophyllum coccineum

Gibbons and Oriowo, 2001, studied the effects of an aqueous extract of Zygophyllum coccineum L. on rat BP and on the mesenteric vascular bed. The extract dose-dependently reduced BP and heart rate in normotensive and spontaneously SHRs. It also reduced BP in pithed SHRs. In vitro, the extract had no effect on basal perfusion pressure of the mesenteric vascular bed. However, when the perfusion pressure was raised with noradrenaline or potassium chloride, the extract produced a dose-dependent reduction in perfusion pressure. It was concluded that extracts of Z. coccineum possess significant hypotensive activity which may be attributed to membrane hyperpolarization (Gibbons and Oriowo, 2001).

z) Withania somnifera and Terminalia Arjuna combination

In Ayurveda, medicinal plants, Withania somnifera (Ws) and Terminalia Arjuna (Arjuna) have been described to be beneficial for cardiac ailments. Ashwagandha is categorised as Rasayana, known to promote health and longevity and Arjuna primarily for treatment of heart ailments (coronary artery disease, heart failure, hypercholesterolemia, anginal pain and can be considered as a useful drug for coronary artery disease, hypertension and ischemic cardiomyopathy). The present investigation assessed the effects of Ws and Arjuna individually and as a combination on maximum velocity, average absolute and relative power, balance, maximum oxygen consumption (VO2 max) and blood pressure in humans. Ws and Arjuna were administered in the form of capsules (dosage 500mg/day). Thirty participants were assigned to experimental group of which 10 received standardized root extracts of Ws, 10 received standardized bark extract of Arjuna and the rest of the 10 received standardized root extract of both Ws and Arjuna. Ten participants received placebo (capsules filled with flour). All the subjects continued the regimen for 8 weeks. All variables were assessed before and after the course of drug administration. The results showed that Ws increased velocity, power and VO2 max whereas Arjuna increased VO2 max and lowered resting systolic blood pressure. When given in combination, the improvement was seen in all parameters except diastolic blood pressure. Ws were found to be useful for treating generalized weakness, improving speed and lower limb muscular strength and neuro-muscular co-ordination. Arjuna was found to be beneficial in improving cardio-vascular endurance and lowering systolic blood pressure (Sandhu et al., 2010).

IV. Hypolipidemics

a) Bougainvillea spectabilis

The active ingredient, D-pinitol (3-O-methyl-chiroinositol), of the traditional antidiabetic plant, Bougainvillea spectabilis, has significant antidiabetic effects. This study was undertaken to evaluate the effect of D-pinitol on lipids and lipoproteins in streptozotocin (STZ)-induced diabetic Wistar rats. Type II diabetic was induced by a single intraperitoneal injection of 40 mg/kg STZ. In diabetic rats, a significant increase in blood glucose, total cholesterol, triglycerides, free fatty acids, phospholipids in the liver, kidney, heart, and brain was observed. In diabetic rats, a significant increase in the levels of low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) cholesterol and a decrease in the high-density lipoprotein (HDL) were seen in diabetic rats. Oral administration of D-pinitol to STZ-induced diabetic rats showed significant ($p < 0.05$) decrease in the levels of blood glucose and total cholesterol, triglycerides, free fatty acids, and phospholipids in serum, liver, kidney, heart, and brain. Treatment with D-pinitol significantly ($p < 0.05$) lowered LDL and VLDL cholesterol levels and significantly ($p < 0.05$) increased HDL cholesterol levels in the serum of diabetic rats. Thus, the present study clearly demonstrates the antihyperlipidemic effect of D-pinitol in STZ-induced type II diabetic rats (Geethan et al., 2008).

b) Daming capsule

The hypolipidemic effect of darning as well as the mechanism of its hypolipidemic effect was elucidated. The expression of connexin43 in the myocardium before and after using the capsule was studied. Forty Wistar rats were randomly divided into 5 groups: control group, hyperlipemia model group and Daming capsule treatment (200, 100, 50 mg/kg) groups. The indexes of total cholesterol (TC), TG, LDL, HDL and non-esterified free fatty acid (NEFA) in the serum were measured via vena caudalis. The myocardial total RNA was extracted by Trizol method. RT-PCR, immunostaining and microconfoul was used to study the expression of connexin 43. The concentrations of TC, TG, LDL and NEFA in hyperlipemic serum were significantly increased ($P < 0.05$), while that of HDL decreased ($P < 0.05$). Daming capsule treatment decreased the concentration of the preceding four indexes. The concentration of HDL was increased up to baseline levels. No significant difference was found in the ECG of the three groups. The mRNA expressions of connexin43 in hyperlipemia group was weakened ($P < 0.05$), while that of the drug group was enhanced ($P < 0.05$) as compared with the normal group. The study demonstrates that changes in Cx43is responsible for the hypolipidemic activity of Darning capsule (Xing et al., 2007).

c) 'Trikatu'

'Trikatu' is an indigenous preparation containing Piper longum (fruit), Piper nigrum (fruit) and Zingiber officinale (rhizome) dry powder. To ascertain its efficacy as a hypolipidaemic agent, Trikatu' was fed to normal and cholesterol fed male Rattus norvegicus. Its effects on body weight, blood and tissue (aortic, cardiac and hepatic) lipids--total, free and esterified cholesterol, LDL and HDL cholesterol, TG and phospholipids—and the atherogenic index were measured. 'Trikatu' reduced triglycerides and LDL cholesterol and increased HDL cholesterol. Hence Trikatu' can reduce the risk of hyperlipidaemia and atherosclerosis. It was concluded that 'Trikatu' possess significant hypolipidaemic activity and it reduces the atherosclerosis associated with a high fat diet (Sivakumar and Sivakumar, 2004).

d) Garlic

Bordia et al., 1981 were the first to evaluate the hypolipidemic activity of garlic. A clinical study using garlic was conducted on two groups of individuals.

Group A consisted of 20 healthy volunteers who were fed garlic for 6 months and then followed for another 2 months without garlic. Administration of garlic significantly lowered the serum cholesterol and TG while raising the HDL. Group B consisted of 62 patients with coronary heart disease with elevated serum cholesterol. They were randomly divided into two subgroups: B1 was fed garlic for 10 months while B2 served as a control. Results demonstrated that garlic intake decreased the serum cholesterol ($p < 0.05$), TG ($p < 0.05$) LDL ($p < 0.05$) while increasing the HDL fraction ($p < 0.001$). These changes in lipid profile were statistically significant at the end of 8 months and persisted for the next 2 months of follow-up. This study demonstrates that the essential oil of garlic has distinct hypolipidemic action in both healthy individuals and patients of coronary heart disease (Bordia et al., 1981).

Hyperlipidemia and oxidative stress may be involved in coronary heart disease and the progression of renal damage in Nephrotic syndrome (NS) patients. Studies have documented that hypolipidemic and antioxidant properties of Garlic may be responsible for its beneficial effects. In the present study the effect of a 2% garlic diet on acute and chronic experimental NS induced by puromycin aminonucleoside (PAN) was studied. Acute NS was induced by a single injection of PAN to rats and sacrificed after 10 days. Chronic NS was induced by repeated injections of PAN to rats and sacrificed 84 days after the first injection. Results indicate that garlic treatment was unable to modify proteinuria in either acute or chronic NS, and hypercholesterolemia and hypertriglyceridemia in acute NS. However, garlic intake diminished significantly total-cholesterol, LDL-cholesterol and TG, but not HDL-cholesterol in chronic NS. Garlic significantly prevented the oxidative stress (in vivo renal H_2O_2 production and the diminished renal Cu, Zn-SOD and catalase activities in acute NS). Results demonstrate that garlic treatment ameliorates hyperlipidemia and renal damage in chronic NS (Pedraza-Chaverrí et al., 2000).

e) Red ginseng

Red ginseng is the steamed and dried root of Panax ginseng. Active ingredient (ginseng saponin) isolated from red ginseng was studied in a cyclophosphamide (CPM)-induced hyperlipidemia model in fasted rabbits. In this model, chylomicrons and VLDL accumulation occurs as a result of release of lipoprotein lipase from the heart. Oral administration of ginseng saponins at a dose of 0.01 g/kg for 4 weeks reversed the increase in serum TG and concomitant increase in cholesterol produced by CPM treatment. In addition, ginseng saponins treatment led to a recovery in post heparin plasma lipoprotein lipase activity and heparin-releasable heart lipoprotein lipase activity, which were markedly reduced by CPM treatment. In rats given 15% glycerol/15% fructose solution, postheparin plasma lipoprotein lipase activity declined to two third of normal rats, whereas ginseng saponins reversed it to normal levels. This study demonstrates that ginseng saponins sustained lipoprotein lipase activity at a normal level. It maintained the lipoprotein lipase activity and produced significant hypolipidemic activity (Inoue et al., 1999).

f) Tinospora cordifolia

Tinospora cordifolia is an indigenous plant widely used in Ayurvedic medicine in India. The present study was undertaken to evaluate the hypolipidaemic effect of an aqueous extract of Tinospora cordifolia roots. A significant reduction in serum and tissue cholesterol, phospholipids and free fatty acids was seen in alloxan diabetic rats after administration of the extract of T. cordifolia roots (2.5 and 5.0 g/kg body weight) for 6 weeks. The root extract at a dose of 5.0 g/kg body weight showed significant hypolipidaemic effect (Stanely Mainzen et al., 1999).

g) T. arjuna

The effect of orally administered indigenous drugs Terminalia arjuna, T. belerica and T. chebula were investigated on experimental atherosclerosis. Rabbits were fed a cholesterol-rich diet to induce atherosclerosis. The three drugs (Terminalia arjuna, T. belerica and T. chebula) were orally fed along with cholesterol to these rabbits. At the end of the experimental period, the plasma lipid profile and lipid peroxidation were assessed. Atherosclerotic lesions of the aorta were examined histologically. T. arjuna significantly inhibited rabbit atheroma formation. The results indicate that T. arjuna has significant hypolipidemic activity (Shaila et al., 1998).

h) Ocimum sanctum

Ocimum sanctum is commonly known as Tulsi. In the present study, 1% Tulsi leaf powder was fed to normal and diabetic rats for a period of one month to explore the effect on fasting blood sugar, uric acid, total amino acids, and the lipid profile in serum and tissue lipids. The results indicated a significant reduction in fasting blood sugar, uric acid, total amino acids, TC, TG, phospholipids and total lipids. In liver, total cholesterol, triglyceride and total lipids were significantly lowered. Total lipids were significantly reduced in kidney. In heart, a significant fall in total cholesterol and phospholipids was observed. Study observations confirm the hypoglycemic and hypolipidemic effect of Tulsi in diabetic rats (Rai et al., 1997).

i) Curcuma longa and Nardostachys jatamansi

The hypolipidemic activity of Curcuma longa and Nardostachys jatamansi was studied in triton-induced hyperlipidaemic rats. Oral feeding of fifty per cent ethanolic extract of Curcuma longa and Nardostachys jatamans resulted in elevation of HDL-cholesterol/total cholesterol ratio. The extracts also caused a significant reduction in the ratio of total

cholesterol/phospholipids. The cholesterol and triglyceride lowering activity of Curcuma longa was superior as compared to N. jatamansi in triton-induced hyperlipidaemic rats. It was concluded that Curcuma longa possesses significant hypolipidemic activity and has protective action against heart disease and atherogenecity (Dixit et al., 1988).

V. Conclusion

The renewed interest in the search for new drugs from natural sources, especially from plant sources for the treatment of cardiovascular conditions, has gained global attention during the last two decades. Development of such indigenous herbal products with potential cardioprotective effects may be a boon in developing countries like India and South East Asian Nations as the synthetic drugs are comparatively costly and therefore patients belonging to weaker sections of the society may be non-complaint in therapy on long term basis. India is blessed with natural resources, primarily due to the rich biodiversity they harbor, which may be sources of new drugs with potential novel structures. However, of this rich biodiversity, only a small portion has been studied for its medicinal potential. Thus, a major opportunity exists in our natural resources for identifying and selecting efficacious, inexpensive and safer approaches for cardioprotection.

There is a paucity of scientific data on herbal medicines as few systemically designed studies on herbal medicines are currently available and their risk-versus-benefit ratios are not clearly elucidated. These medicinal plants need to be investigated scientifically and rigorously to define their role in prevention and treatment of cardiovascular conditions and to stimulate future pharmaceutical development of therapeutically beneficial herbal drugs. At the same time, legal surveillance of herbal medicine use with low safety margins, adverse cardiovascular reactions and drug interactions should be instituted.

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Protective Effects of Diallyl Disulfide Against Experimentally Induced Hepatoma in Mice

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Abstract - Many herbal extracts have been reported to modify significantly, the transformation of normal cells into neoplastic cells. Garlic and its extracts are known for their hypolipidemic, hypoglycemic, antiplatelet aggregating effect as well as for its anticancer effects. Many of these health beneficial effects of garlic are attributed to its principle organosulfur compound diallyl disulfide(DADS). It was thought that DADS may be involved in anticarcinogenic & antitumorogenic effect of garlic, hence the present work was undertaken to assess the protective effects of DADS in ehrlich ascites carcinoma (EAC) cells induced hepatoma in mice. The study has three groupsnormal group (group1), the EAC cells implanted mice (group 2) & DADS-treated EAC cells implanted mice (group 3). The results indicate a significant decrease in ascitic fluid volume, ascitic fluid cell count, liver tissue amino acid nitrogen levels, liver tissue glutaminase activity & liver tissue lactate levels as well as a increase in life span observed in group 3 mice as compared to group 2 mice, suggesting that DADS gives a significant protection in group3 mice probably by decreasing the anaerobic glucose utilization as well as by interfering with protein & deoxy ribonucleotide synthesis.

Keywords : *Herbal extracts, garlic, diallyl disulfide, anti-*tumorogenic effects ., EAC cells, liver, *hepatoma*

GJMR-D Classification : *NLMC Code: QY 60.R6, QY 140*

Strictly as per the compliance and regulations of:

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Divya.D [α], Vickram [σ] & Kashinath.R.T [ρ]

Abstract - Many herbal extracts have been reported to modify significantly, the transformation of normal cells into neoplastic cells. Garlic and its extracts are known for their hypolipidemic, hypoglycemic, antiplatelet aggregating effect as well as for its anticancer effects. Many of these health beneficial effects of garlic are attributed to its principle organosulfur compound diallyl disulfide(DADS). It was thought that DADS may be involved in anticarcinogenic & antitumorogenic effect of garlic, hence the present work was undertaken to assess the protective effects of DADS in ehrlich ascites carcinoma (EAC) cells induced hepatoma in mice. The study has three groups-normal group (group1), the EAC cells implanted mice (group 2) & DADS-treated EAC cells implanted mice (group 3). The results indicate a significant decrease in ascitic fluid volume, ascitic fluid cell count, liver tissue amino acid nitrogen levels, liver tissue glutaminase activity & liver tissue lactate levels as well as a increase in life span observed in group 3 mice as compared to group 2 mice, suggesting that DADS gives a significant protection in group3 mice probably by decreasing the anaerobic glucose utilization as well as by interfering with protein & deoxy ribonucleotide synthesis.

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I. Introduction

The transformation of normal cells into neoplastic cells involves at least three distinctive phases, namely- initiation, promotion and progression. Many dietary components have been reported to significantly modify each of these phases (30). Garlic (Allium sativum) a common dietary component, is known to modify the cancer process. Epidemiologic and clinical studies have shown that consumption of garlic reduced the risks of cancer incidence (5, 17, 29). A number of studies have demonstrated the chemoprotective activity of garlic by using different garlic preparations including fresh garlic extract, aged garlic, garlic oil and a couple of organosulfur compounds derived from garlic (1,18). The chemoprotective activity has been attributed to the presence of organosulphur compounds in garlic (6, 25, 31). The principle organosulphur compound present in garlic is diallyl disulphide [DADS] (3, 22). Hence it was thought DADS may be responsible for garlic's cancer protective activity. The present work was undertaken to assess the chemoprotective effects of DADS in Ehrlich ascites cells induced hepatoma in mice.

II. Materials and Methods

a) Tumor cell line & their Maintenance :

The inoculum of EAC cells was kindly provided by Amala Cancer Research Institute, Thrissur Kerala (India). EAC cells were thereafter propagated by weekly intraperitoneal injection of freshly drawn ascitic fluid (0.5 ml) from a donor mice bearing ascites tumor of 8-10 days old into healthy swiss albino male mice. Transplantation was carried out using sterile disposable syringes under aseptic conditions

b) Chemicals :

All the chemicals employed in the present study were of Analar grade (A.R). Dially disulphide (DADS) was procured from Sigma-Aldrich chemicals Pvt. Ltd. USA.

c) Animals :

In the present study, 18 Swiss male albino mice weighing 25-30g were randomly selected from animal house of Basaveshwara Medical College & Hospital, Chitradurga. The experiments were conducted according to the norms of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), New Delhi and Ethical clearance was obtained from IAEC (Institutional Animal Ethical Committee) of Basaveshwara Medical College.

d) Experimental design :

The mice were divided into 3 groups (6 animals per group)–normal group (group 1), control group [EAC cells implanted mice] (group 2) and protective group [DADS-treated EAC cells implanted mice] (group 3).

i. *Normal group*

This group consists of 6 swiss albino male mice that received 5.0 ml of normal saline /kg body weight orally by gastric intubation daily for a period of 10 days.

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ii. *Control group*

This group consists of 6 swiss albino male mice with experimentally induced hepatoma. About 3x 10^6 EAC cells were injected intraperitoneally into healthy mice. These mice also received 5.0 ml of normal saline / kg body weight orally by gastric intubation daily for a period of 10 days. A well grown tumor was observed within 7-10 days.

iii. *Protective group*

This group consists of 6 swiss albino male mice, received 5.0 ml of warm aqueous solution of DADS (100mg/kg body weight) orally by gastric intubation daily for a period of 4 days. On the 4th day 3x 10^6 EAC cells were injected intraperitonially. Later 5.0 ml warm aqueous solution of DADS (100 mg)/kg body weight was given orally further for a period of 6 days.

The mice of all the three groups were maintained on standard lab feed (Amruth Rat Feed, supplied by Pranav Agro Industries, Pune, India) and tap water adlibitum throughout the study. On the 11th day, body weights of mice of all the groups were noted & abdominal circumferences were recorded. Then the mice were anaesthetized & sacrificed. The ascitic fluid was immediately collected in a clean dry graduated tube by puncturing the abdomen. The fluid volume was noted. The ascitic fluid was assayed for total proteins (11) & total cell count was assessed microscopically using Neubauer chamber. The mice were dissected & livers were procured. Blood stains of liver tissues were removed by smooth blotting & were immediately transferred into a clean pre weighed beaker. The weights of liver of individual groups were noted. Later, the liver tissues were refrigerated at 0-2°C in cold phosphate buffer pH 7.4 till further use. Each individual liver tissue procured was processed to analyze various biochemical parameters as follows:

a) To 0.2 g of liver tissue slice, 4.8 ml 10% TCA was added & allowed to stand at room temperature for 10 minutes & homogenized thoroughly in a Potter Elvehjam tissue homogenizer for 5 minutes & centrifuged at 3000 rpm for 5 minutes. The supernatant was employed for the estimation of lactate (15).

b) 0.5 g of liver tissue slice was employed for the estimation of glycogen content as explained in David Plummer – Practical manual (8).

c) To 0.3 g liver tissue, 4.7 ml of cold phosphate buffer, pH 7.4, was added & thoroughly homogenized for 5 minutes & centrifuged at 3000 rpm for 5 minutes. The supernatant was employed for the estimation of tissue total proteins (11), transaminase activity- alanine transaminase (ALT) & aspartate transaminase (AST) (13), total thiol (-SH) groups (7) & glutaminase activity (21).

d) To 0.2 g of liver tissue slice, 1.0 ml of 2/3 N H2SO4 + 1.0 ml of 10% sodium tungstate + 7.8 ml of distilled water were added, mixed well & allowed to stand for 10 minutes at room temperature. Later the contents were thoroughly homogenized & centrifuged at 3000 rpm for 5 minutes. The clear supernatant was employed for the estimation of amino acid nitrogen (AAN) (12).

e) *Data management & Statistical Evaluation :*

The data entry was carried out using MS Office Exel worksheet and statistically evaluated. The P value was calculated using 'student t' test.

III. Results

The results of the present study are given in table 1 & 2.

Table 1 narrates the bodyweight, abdominal circumference of group 1, group 2 & group 3. Also, the table gives the ascitic fluid volume, ascitic fluid total proteins, ascitic fluid cell count in group 2 & group 3. It is evident from the table that both bodyweight & abdominal circumference are significantly raised ($p< 0.001$) in group 2 as compared to group 1, whereas the same parameters are significantly lowered ($p<0.001$) in group 3 as compared to group 2. It is also evident from the table that a significant decrease ($p<0.001$) in ascitic fluid volume, ascitic fluid protein content & cell count in group 3 as compared to group 2.

Table 2 gives the liver tissue levels of glycogen, lactate, total proteins, aminoacid nitrogen, total thiol groups & activity of glutaminase, AST & ALT in group1, group 2 & group 3. It is evident from table that the levels of lactate, total proteins, amino acid nitrogen & glutaminase activity in liver tissue are significantly raised ($p<0.001$) in group 2 as compared to group 1, whereas the same parameters are significantly lowered ($p<0.001$) in group 3 as compared to group 2. It is also seen from the table that a significant decrease ($p<0.001$) in liver glycogen content & total thiol groups observed in group 2 as compared to group 1 & the same parameters are significantly raised ($p<0.001$) in group 3 as compared to group 2. However there is no significant change seen in transaminases (ALT & AST) activity in all the three groups.

IV. Discussion

The eukaryotic cell cycle normally consists of series of events involving – growth stimulus, replication & division (3, 23, 24). It is known that many allyl sulphur compounds of herbal origin reduce the growth rate of neoplastic cells in culture as well as invivo (26), probably by blocking certain events of cell cycle. The results of the present study with 100 mg DADS/ kg bodyweight given to EAC implanted mice (refer table-1) suggests that at this dosage DADS significantly retards the development of ascites. DADS might have interfered with the cell cycle at G2/M phase as it is known that DADS arrest the cell growth at G2/M phase of cell cycle

in human colon cancer (18, 4, 19), by decreasing the kinase activity of CDK1/cyclin B complex. Further DADS is a disulphide and like any other disulphides can undergo sulphydryl exchange rections with cellular proteins & enzymes (33) as follows-

$$\text{Enz-SH} + \text{RSSR} \longleftrightarrow \text{Enz-SSR} + \text{RSH}$$

$$\text{Enz (SH)}_2 + \text{RSSR} \longleftrightarrow \text{Enz S}_2 + 2\text{RSH}$$

$$2\text{RSH} + \text{DADS} \longleftrightarrow \text{RS-SR} + \text{Allyl thiol}$$

A similar sulphydryl exchange reaction with kinases & other growth factors involved in cell cycle may suppress cell multiplication causing a reduction in tumor growth, indicating DADS has a significant chemoprotective action against EAC induced hepatoma in mice, which is evident from the results obtained in the present studies (refer table 1 & 2). Cell proliferation as well as cell multiplication requires increased DNA produ--ction (14) which means increased synthesis of deoxy-ribonucleotides. This process requires the participation of nucleotide reductase enzyme, which requires thioredoxin, a sulphydryl compound for its activity. A possible sulphydryl exchange reaction of DADS with thioredoxin as proposed above may reduce its availability hence decreases the production of deoxy ribonucleotides thus reducing the available DNA levels in cancer cell development which is evident from results depicted in table 1.

Tumor cells do act as nitrogen trappers (22) which is a required phenomenon for increased protein synthesis essential for rapid cell proliferation as well as cell multiplication. Liver tissue protein levels in group 2 shows a significant raise ($P<0.001$) as compared to group 1 (refer table 2), indicating a rise in protein synthesis, a normal requirement of increased cell multiplication. The amino acids which are essential for increased protein synthesis might have derived from an increased proteolysis of host tissue. The results given in table 2 shows a significant raise ($p<0.001$) in liver tissue amino acid nitrogen levels. This increase may partly be due to increase in glutamic acid formation through an increased activity of enzyme glutaminase (28). A significant decrease in liver tissue amino acid nitrogen levels, seen in the present studies, in group 3 mice as compared to group 2 mice suggests that DADS might have interfered with host tissue proteolysis hence causing a decrease in liver tissue amino acid nitrogen levels. This decrease in liver tissue amino acid nitrogen levels in group 3 mice, in part, may be due to decreased glutaminase activity (refer table 2) resulting in a lowered glutamic acid levels.

It is known that tumor cells prefer anaerobic glycolytic breakdown of glucose as compared to glucose oxidative pathways. The observed increase in liver tissue Lactate content in group 2 mice is clearly suggestive of the above statement whereas a significant decrease ($P<0.001$) in liver tissue lactate levels in group 3 mice as compared to group 2 mice (refer table 2) indicates probably DADS might have interfered with cellular glycolytic pathways. Many enzymes of glycolytic pathway including hexokinase, phospho fructo kinase (PFK) & pyruvate kinase (PK) are thiol enzymes (09). DADS, a disulfide might have undergone sulfhydryl exchange reaction similar to any other disulfide (27) as proposed above with glycolytic thiol enzymes hence reducing their activities which results in decreased anaerobic glycolysis thus a decrease in lactate output. This decrease in lactate level in group 3 mice as compared to group 2 mice may also be due to lowered cellular NADPH or NADH levels in group 3 mice as DADS is known to undergo reductive cleavage to its thiols using cellular NADPH or NADH, thus reducing the available NADPH or NADH causing a decrease in lactate formation.

A reliable criteria for assessing the potential use of any anticancer agent is the prolongation of life span of animals (16). Andreani et al (2) has suggested that an increase in lifespan of ascites bearing animals by 25% is considered as indicative of significant drug activity. An increase in life span by 50% i.e. 25.6 ± 0.81 days in group 3 against 16 ±0.89 days in group 2 (refer table 1) in present study suggesting that DADS has a significant protective effect on EAC induced hepatoma bearing mice. Further, a decrease in tumor volume & viable tumor cell count observed in present study (refer table 1) can also be considered as an important marker of reduced tumor burden & enhanced life span of EAC bearing mice. Increased life span % [ILS] & inhibitory growth rate % [IRT%] was calculated as given by Gupta et al (20) to evaluate the effect of DADS on life span of hepatoma induced mice. The percent increase in life span was found to be 50% and inhibitory growth rate percent was found to be 45.43% suggesting that DADS has a significant inhibitory effect on tumor development.

In conclusion, DADS by interfering with protein synthesis as well as with the glucose breakdown in cancer cells, results in reduced cancer cell proliferation & multiplication. Thus shows significant protection against EAC induced hepatoma bearing mice.

Table 1 : The table showing the body weight, abdominal circumference, ascitic fluid volume, cell count, ascitic fluid total proteins & mean survival days of - Group 1, Group 2 & Group 3 mice.

Group	*Body weight(g)*	*Abdominal circumference (cm)*	*Ascitic fluid volume (ml)*	*Ascitic fluid Cell count (Cells/mm³)*	*Ascitic fluid total proteins(mg/ml)*	*Mean survival time(days)*
Group 1 n=6	23.11 ± 2.02	7.75 ± 0.41	--	--	--	--
Group 2 n=6	32.48*** ± 1.99	12.83*** ± 0.5	10.08 ± 0.97	220,815.00 ± 44,645.32	187.5 ± 22.36	16.00 ± 0.89
Group 3 n=6	26.50*** ± 2.09	9.08*** ± 0.39	5.50*** ± 1.18	70,158.83*** ± 14,990.15	108.33*** ± 17.07	25.66*** ± 0.81

Note :

- Number in parenthesis indicate the number of liver specimen
- The values are expressed as their mean ± SD
- Statistical evaluation : probability level - * $p<0.05$, ** $p< 0.01$, *** $p<0.001$

Table 2 : The table showing the liver tissue levels of glycogen, lactate, total proteins, aminoacid nitrogen, total –SH groups, glutaminase activity & transaminases activity (ALT, AST) in - Group 1, Group 2 & Group 3 mice.

Group	*Glycogen Content (mg/g)*	*Lactate Content (mg/g)*	*Total Proteins (mg/g)*	*Aminoacid nitrogen (µgAAN/g)*	*Total SH groups (mg SH/g)*	*Glutaminase units*	*ALT (IU)*	*AST (IU)*
Group 1 n=6	12.29 ± 2.01	1.81 ± 0.27	144.09 ± 12.17	550.0 ± 32.86	2.24 ± 0.20	18.37 ± 1.17	21.1 ± 0.4	28.8 ± 1.17
Group 2 n=6	1.17*** ± 0.24	2.91*** ± 0.10	201.28*** ± 8.50	680.0*** ± 17.88	1.28*** ± 0.15	31.12*** ± 1.52	19.14 ± 0.45	30.05 ± 1.04
Group 3 n=6	3.14*** ± 0.43	2.20*** ± 0.10	164.92*** ± 7.84	586.6*** ± 27.32	1.78*** ± 0.17	22.11*** ± 0.95	20.02 ± 0.74	28.04 ± 0.65

Note :

- Number in parenthesis indicate the number of liver specimen
- The values are expressed as their mean ± SD
- Statistical evaluation : probability level - * $p<0.05$, ** $p< 0.01$, *** $p<0.001$
- Glutaminase : 1 unit = µg NH4 liberated /g liver/hr.

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9. Use and get big pictures: Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

10. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right! It is a good habit, which helps to not to lose your continuity. You should always use bookmarks while searching on Internet also, which will make your search easier.

11. Revise what you wrote: When you write anything, always read it, summarize it and then finalize it.

12. Make all efforts: Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

13. Have backups: When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

14. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating "hotchpotch." So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

15. Use of direct quotes: When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

16. Use proper verb tense: Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

17. Never use online paper: If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

18. **Pick a good study spot:** To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

19. Know what you know: Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

20. Use good quality grammar: Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

21. Arrangement of information: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

23. Multitasking in research is not good: Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. Go for seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. **Think technically:** Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be

sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page

- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to

shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.
- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw th

principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.

- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

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- The **major constraint** is that you must independently make all content, tables, graphs, and facts that are offered in the paper. You must write each part of the paper wholly on your own. The Peer-reviewers need to identify your own perceptive of the concepts in your own terms. NEVER extract straight from any foundation, and never rephrase someone else's analysis.

- Do not give permission to anyone else to "PROOFREAD" your manuscript.

- Methods to avoid Plagiarism is applied by us on every paper, if found guilty, you will be blacklisted by all of our collaborated research groups, your institution will be informed for this and strict legal actions will be taken immediately.)
- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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