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DISCOVERING THOUGHTS AND INVENTING FUTURE

Highlights

- Artemisinine Combination Therapy (Act)
- Peyer's Patches of Sheep (Ovis aries)
- Markers of Diabetic Retinopathy
- Root Bark of Azadirachta Indica

The Blood Plasma

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A Study of Cost Effectiveness of Artemisinine Combination Therapy (Act) Among Paediatric Patients at a Tertiary Medical Centre in South West Nigeria

By OMOLE Moses Kayode & LAWAL Tajudeen A.
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Abstract - This retrospective study was to compare the cost effectiveness of oral ACT as a first line of treatment of malaria with non-oral ACTs in paediatric patients aged 5 years and below. The study was carried out at Children Outpatients Clinic of Federal Medical Centre, Abeokuta Ogun State in Nigeria between April and September 2005. Clinical case notes of paediatric patients aged 5 years and below were used. Three hundred and fifty (350) patients were randomly selected with only 328 patients meeting the inclusion criteria. The number consisted of 153 (46.6%) males and 175 (53.4%) females. The mean age of patients was 2.3 years (SD 1.4). Oral Chloroquine/Sulphadoxine and Pyrimethamine (CQ/SP) was administered to 127 (38.7%) patients, 113 (34.5%) patients had Quinine/Sulphadoxine and Pyrimethamine (QSP) while 88 (26.8%) patients had Arthemisine/ Sulphadoxine, Pyrimethamine (ASP). The cure rate for CQ/SP, Quinine/SP and Arthemisine/SP were (11.0%), (22.1%) and (97.7%) respectively. It was only Artemisinine/SP that met the 7.5% efficacy rate recommended by WHO. Data on the therapeutic efficacy of the treatment given to the patients were collected and analyzed using descriptive statistics of frequency distribution and percentages with statistical package Microsoft SPSS 10.0 window version.

Keywords : *ACTs, Non ACTs, Chloroquine, Quinine, Sulphadoxine, Pyrimethamine.*

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A Study of Cost Effectiveness of Artemisinine Combination Therapy (Act) Among Paediatric Patients at a Tertiary Medical Centre in South West Nigeria

OMOLE Moses Kayode [α] & LAWAL Tajudeen A. [σ]

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Oral Chloroquine/Sulphadoxine and Pyrimethamine (CQ/SP) was administered to 127 (38.7%) patients, 113 (34.5%) patients had Quinine/Sulphadoxine and Pyrimethamine (QSP) while 88 (26.8%) patients had Arthemisine/ Sulphadoxine, Pyrimethamine (ASP). The cure rate for CQ/SP, Quinine/SP and Arthemisine/SP were (11.0%), (22.1%) and (97.7%) respectively. It was only Artemisinine/SP that met the 7.5% efficacy rate recommended by WHO. Data on the therapeutic efficacy of the treatment given to the patients were collected and analyzed using descriptive statistics of frequency distribution and percentages with statistical package Microsoft SPSS 10.0 window version.

In the economics analysis, Chloroquine/SP, Quinine/SP and Artemisimine/SP had average cost effectiveness ratio (ACER) of 5.19, 3.45 and 1 respectively. The incremental cost effective ratio (ICER) for changing from Chloroquine/SP to Artemisinine was 1 while for changing from Chloroquine/SP to Quinine/SP was 3.68.

The study indicated that ACTs represented by Artemisinine/SP was more cost effective than the non-ACTs represented by Chloroquine/SP and Quinine/SP since the ACER for Artemisinine/SP was the lowest among the three comparators. ACTs was also more clinically effective than the non-ACTs in the treatment of malaria in paediatric patients aged 5years and below ($p<0.05$).

Keywords : ACTs, Non ACTs, Chloroquine, Quinine, Sulphadoxine, Pyrimethamine.

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I. Introduction

Despite the recent introduction of the use of Artemisinine combination therapies (ACTs) in the management of malaria into the National guideline on malaria treatment, most cases of malaria are still treated with old drugs that often fail.(1, 2,3) Most physicians are reluctant to change to ACTs because of the cost. Physicians believed that ACTs is more expensive, (4) therefore cost has proven to be a major obstacle to the widespread use of ACTs. Physician who is the primary decision maker within the health care system have responsibility for controlling health care costs while providing the best possible care for patient.(4) There is need for economic evaluation of pharmacotherapy that will provide prescribers awareness about the costs associated with the treatment they select for their patients.

The pharmacists in a hospital setting are acknowledged as experts on drugs and therefore should maintain a reliable but economic drug delivery system in order to ensure that cost effective drugs are available. (4, 5)

The increase in number of new drugs combined with increase in list of drugs provides a great challenge to manage care organizations (MCOs) as they struggle to deliver quality care while minimizing cost. Pharmacy and therapeutic (P&T) committees are the MCO responsible for evaluating these new drugs and determining their potential value to organization. (5)

Pharmacoeconomics analysis are categorized by the method used to access outcomes. If the outcomes are assumed to be equivalent, the study is called a cost minimization analysis (CMA). If the outcomes are measured in monetary terms such as dollar and naira, the study is called cost benefit analysis (CBA). If the cost are measured in natural unit such as cures, years of life and blood pressure, the study is called a cost effectiveness analysis (CEA) and if the outcomes takes into account patient's preferences such as utilities, the study is called cost utility analysis (CUA). Pharmacoecomics study categorized cost into four. These are direct medical cost, direct non medical cost,

indirect cost and intangible cost. Direct medical cost are the most obvious costs to measure. These are the medically related inputs used directly to provide treatment such as costs associated with pharmaceutical products, physician visits, emergence room visits and hospitalization. Direct non medical costs are cost directly associated with treatment but are not medical in nature. These includes travelling to and from the physician's office, travelling to and from physician's office to hospital, baby sitting for the children of a patient and food and lodging required for the patient and their family during out- of -town treatment.

Indirect costs involve costs that result from the loss of productivity due to illness or death. This economic term "indirect costs" is different from accounting term "indirect costs."

Accounting term indirect costs is used to assign over head. Intangible costs include the costs of pain, suffering, anxiety or fatigue that occur because of an illness or treatment of an illness. It is difficult to assign values to intangible costs. Treatment of illness such as malaria may include all four types of costs. (3, 5)

As used in this study, pharmacoeconomics is the process of identifying, measuring and comparing costs, risks and benefits of programs, services or therapies and determining the alternative that produces the best health outcome for the resources invested based on long term benefits. It involves weighing the cost of providing a practical product or service to determine which alternative yields the optimal outcome for a specific sum and the information obtained can assist clinical decision makers in choosing the most cost effective treatment options. (3, 5)

The objective of this study is to determine the cost effectiveness of artemesinine combination therapies (ACTS) among peadeatric patients attending federal medical center Abeokuta Ogun State in Nigeria with the goal of providing and promoting pharmaceutical care.

II. Patients And Methods

This is a Retrospective pharmacoeconomics analysis in which paedaitric malaria patients who aged 5years and below that attended the Children Outpatient Clinic of Federal Medical Centre, Abeokuta were used for the study. This is a 100-bed tertiary hospital located in South-Western part of Nigeria. It is about 70-kilometers from Lagos. It has 120 medical doctors comprising of Consultants in Paediatrics, Surgery, Family Medicine, Internal Medicine, Cardiology and Gynecology. The outpatient unit of the Pediatrics Department attends to over fifty patients aged 12 years and below daily. The criteria for inclusion were: Patient must be a confirmed malaria patient through symptomatic or diagnostic test and Patient must attend follow up clinic within one week of first visit in order to assess the effectiveness of treatment.

Between the six-month period of April 1st and September 30th 2005, 350 case notes of patients with malaria aged 5 years and below that attended Children Outpatient Clinic were randomly selected from the Medical Record Unit and the mode of treatment with ACTs and non ACTs for each patient was thoroughly studied. Twenty two (22) patients did not meet the inclusion criteria, therefore a total of 328 patients who came back within one week of initial visit for check up were used for the study. This comprise of 153 (46.6%) males and 175 (53.4%) females.

One hundred and twenty seven (127) patients were treated with Chloroquine and Sulphadoxine pyrimethamine (CQ/SP), 113 patients were treated with Quinine, Sulphadoxine and pyrimethamine (QSP), while 88 patients were treated with Artemisinine, Sulphadoxine and Pyrimethamine (ASP).

Clinical success was defined as the complete clinical cure of the malaria confirmed by absence of symptoms or a negative malaria parasite test.

The treatment cost of each comparator included direct medical cost such as drug cost, cost of drug prescribed to treat side effects, cost of laboratory investigation and consultation fee.

The direct non-medical costs were those associated with receiving therapy including follow up visits and the only expenses included under this was the transportation expenses and an estimate of N100 (One Hundred Naira) was used. The average cost effectiveness ratio and the incremental cost effectiveness ratio were then determined using the cost and therapeutic outcomes.

Since only 328 patients (93.72%) reported back within the stipulated one week of treatment for follow-up clinic, the remaining 22 patients (6.28%) that did not come back for follow up clinic may serve as a potential source of bias.

The cost of treating 100 patients was calculated and the percentage effectiveness of the treatment was also calculated.

$$\text{Cost of treating 100 patients} = \text{Unit cost of therapy} \times 100$$

$$\%\text{Effectiveness} = \frac{\text{No of Patients successfully treated}}{\text{No of patient that had the treatment}} \times 100$$

Average cost effectiveness ratio (ACER) was calculated as the average cost per patient treated successfully and done by dividing the total cost and patients successfully treated.

$$ACER = \frac{\text{Healthcare cost (A)}}{\text{Clinical outcome (B)}}$$

Incremental cost effectiveness ratio (ICER) was calculated as the incremental change in cost divided by the incremental change in effectiveness.

$$ICER = \frac{\text{Cost A} - \text{Cost B}}{\text{Outcome A} - \text{Outcome B}}$$

The data obtained were analyzed using SPSS (Statistical Package for Social Science) Version 10.0

1. Descriptive statistics of frequency distribution and percentages were used as appropriate.
2. Chi – square test was used to test hypothesis.

III. Results

Table 1 : The age distribution showed that 22.0% of the patients were less than 1 year old, 29.0% were between 1 & 2 years old, 14.0% were between 2 & 3years old, 15.0% were between 3 & 4 years old while 20.0% were between 4 & 5 years of age.

Table 1 : Patients' Age Distribution

Age	Frequency	Percentage
< 1 YEAR	72	22.0
>1 year- 2 years	95	29.0
>2 years – 3 years	47	14.0
>3 years – 4 years	50	15.0
>4 years – 5years	64	20.0
TOTAL	328	100

Table 2 : The percentage effectiveness of CQ/SP was 11.20%, Quinine/SP was 22.12% and Artemisinine/SP was 97.72%. The average cost effectiveness ratio for CQ/SP was 5.19, Quinine/SP was 3.45 and Artemisinine/SP was 1. The ICER for changing from CQ/SP to Quinine/SP was 3.68, while for changing from CQ/SP to Artemisinine/SP was 1.

Table 2 : Cost Effectiveness Comparison Table

COMPARATORS	COST (A) NAIRA(=N=)	DOLLAR($)	%EFFECTIVENESS (B)
CQ/SP	51000	340.0	11.02
QUININE/SP	68000	453.40	22-12
ARTEMISININE	87000	580.0	97.72
AVERAGE COST EFFECTIVENESS RATIO			
COMPARATOR	AVERAGE COST NAIRA(=N=)	DOLLAR($)	RATIO
CQ/SP	4627.94	30.85	5.19
QUININE/SP	3074.14	20.50	3.45
ARTEMISININE/SP	890.29	5.94	1
INCREMENTAL COST EFFECTIVENESS RATIO			
COMPARATOR	ICER NAIRA(=N=)	DOLLAR($)	RATIO
CQ/SP to QUININE/SP	1531.53	10.2	3.68
CQ/SP to ARTEMISININE/SP	415.22	2.77	1

Table 3 : The drug prices were the local prices in Naira (which was converted to US dollars) paid by patients at the point of purchase at the pharmacy. The adverse effect treatment only covered the price of Chlopheniramine prescribed to treat pruritus in patients.

Table 3 : Treatment Cost Treatment

	CQ/SP NAIRA(N) DOLLAR($)		QUINI/SP NAIRA(N) DOLLAR($)		ARTEMISI/SP NAIRA(N) DOLLAR($)	
Drug Price	100	0.67	270	1.8	570	3.41
Laboratory Test	100	0.67	100	0.67	100	0.67
Consultation	100	0.67	100	0.67	100	0.67
Adverse Effect Treatment	110	0.73	110	0.73	NIL	
Direct Non-Medical Cost (Transport Fare)	100	0.67	100	0.67	100	0.67
Total	510	3.41	680	4.54	870	5.42

Table 4 : The cure rate of Chloroquine, Sulphadoxine and Pyrimethamine was 11.0%, Quinine, Sulphadoxine and pyrimethamine was 22.1% and Artemisinine and Sulphadoxine – pyrimethamine was 97.7%.

Table 4 : Clinical Result Derived From The Treatment

DRUG	CLINICAL OUTCOME SUCCESS	FAILURE	TOTAL
Chloroquine And Sulphadoxine-Pyrimethamine	14 11.0%	113 89%	127 100.0%
Quine And Sulphadoxine-Pyrimethamine	25 22-1%	88 77.9%	113 100.0%
Artemisinine And Sulphadoxine Pyrimethamine	86 97.7%	2 2.3%	88 100.0%
Total	125 38.1%	203 61.9%	328 100.0%

IV. Discussion

The increasing number of therapeutic failure after treatment of *falciparum* malaria with conventional drugs are alarming and there is an urgent need to find effective drugs or drug combination suitable for immediate use when patients first seek medical care. ACTs are more expensive than the other drugs, however, as resitance to these drugs is increasing, there is need to switch to ACTs in all areas with high transmission of drug resistance *P. falciparum.* (6, 7)

The lower the average cost effectiveness ratio (ACER), the less expensive the treatment and the more efficient the therapy. The incremental cost effectiveness ratio (ICER) showed that changing from CQ/SP to Quinine/SP had an ICER of 3.6, while changing from CQ/SP to Artemisinine/SP had 1. The larger the ICER, the more money is required to buy each unit of outcome and intervention becomes less cost effective with increase in the ICER. Although ACTs are more expensive than other malaria drugs which are not ACTs, but in the long term, it proved to be most cost effective option in the treatment of malaria especially multidrug *P.falciparum* infection. (8, 9)

The drug cost analysis would identify CQ/SP as the best alternative based on price, but decisions based solely on these figures are not valid since they do not reflect the true cost picture. In the cost of therapy analysis, Artemisinine/SP emerged as the most cost-effective alternative because of its high clinical success rate. Using the ACER, which is the average cost per patient successfully treated CQ/SP had a ratio of 5.19, Quine/SP had 3.45 and Artemisinine/SP had 1.

This result confirmed the assertion that ACTS are more than 95% likely to be cost effective under most condition. In terms of cost effectiveness, Artemisinine has a potent gametocidal effect on immature early stages of stages I-IV of *falciparum* gametocytes in bone marrow and an effect on complete prevention of gametocytes that have just matured to stage V and this activity which has not been shown in any other antimalarial except Primaquine is significant for the control of infection source and reduction in the transmission of parasites from an infected person to another via mosquito bite. This will in turn reduce the number of people that will get infected with malaria and this markedly reduces the cost of malaria management on the long run. (9, 10, 11, 12)

For each drug in a cost – effectiveness analysis, the major cost factor is not primary success, but primary failure because of the necessity of using two or more treatment regimens. The cost of failure includes the cost of drugs that consist the second and third line drugs, cost of consultation and diagnosis. ACTs will reduce the overall cost of malaria treatment by eliminating initial treatment due to the present high resistance to most of the antimalarials currently in use. The direct non-medical costs include transport expenses and the indirect cost includes the loss of productivity via absence from duty by the patient's parent. Cost of failure also include loss of confidence in public health system since the patient will need to come back to complain about the same ailment. (5)

The clinical result of this study showed that the cure rate for CQ/SP was 11.0%, Quinine/SP was 22.1% and Artemisinine/SP had 97.7% cure rate. This result supports the assertion that Chloroquine containing combinations no longer provide adequate protection against *P.falciparum.* Artemisinine rapidly reduces parasitemia and SP has a long term effect in clearing residual parasites. Owing to the short elimination half life of Artemisinine (1.57hours), the chances of developing resistance appear to be low. While the short half life of Artemisinine necessitates repeated drug administration or combination therapy, it is probably the most crucial pharmacokinetic feature for delaying the development of resistance. (13, 14, 15, 16)

The result also showed that ACTs represented by Artemisinine, Pyrimethamine and Sulphadoxine met the 75% efficacy rate recommended by WHO. This result is consistent with those obtained from previous studies since it was reported from the studies leading to the adoption of ACTs as first line drugs for treatment of malaria covered only children less than 5 years of age and therefore lacked basis for generalization for the whole population.(6,8,17, 18) Artemisinine is an effective partner drug in ACTs because it is a more active therapy than the other antimalarials, reducing the number of parasites by approximately 100, 000 per asexual cycle and therefore reducing the number of parasites that are exposed to the partner drug alone. A report produced by Medicine san Frontier (MSF) strongly endorses the use of ACTS to combat malaria in Africa. (18, 19, 20, 21) Result obtained from the hypothesis test showed that there was a statistically significant difference in therapeutic effectiveness of ACTs and non-ACTs based on the results from the study since p value is less than 0.05. The hypothesis test showed that ACTs represented by Artemisinine is more therapeutically effective than the non-ACTs represented by CQ/SP and Quinine/SP.

V. Conclusion

Pharmacoeconomics evaluation has demonstrated that a more expensive per unit drug can be most effective therapeutic alternative.

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Serum Haptoglobin, Ceruloplasmin and CRP Levels: Markers of Diabetic Retinopathy

By Dr. Satinder Kaur, Parminder Singh, RK Grewal, Navjot kaur & Aman Agarwal

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Abstract - Background : Inflammation has been recognised as a critical contributor to retinal capillary closure, one of the main pathogenic event in diabetic retinopathy. The relationship between acute phase markers of inflammation and diabetic retinopathy was studied. Materials and Methods : 60 Type 2 Diabetes patients attending OPD/IPD of Tertiary care hospital were included. They were divided into three groups of 20 each. Group I: without retinopathy. GroupII: with non proliferative diabetic retinopathy (NPDR). GroupIII: with proliferative diabetic retinopathy (PDR). Results were compared with 20 normal controls. FBS, HbA1c, haptoglobin, ceruloplasmin and CRP were analysed on auto analyzer Hitachi 911(Roche)..

Keywords : Non proliferative diabetic retinopathy (NPDR), proliferative diabetic retinopathy (PDR), acute phase proteins, ceruloplasmin, haptoglobin and CRP.

GJMR-B Classification : WKA, WQ 248, WK 810, WD 200.5.G6

Strictly as per the compliance and regulations of:

Serum Haptoglobin, Ceruloplasmin and CRP Levels: Markers of Diabetic Retinopathy

Dr. Satinder Kaur [α], Parminder Singh [σ], RK Grewal [ρ], Navjot kaur [ω] & Aman Agarwal [¥]

Abstract - Background : Inflammation has been recognised as a critical contributor to retinal capillary closure, one of the main pathogenic event in diabetic retinopathy. The relationship between acute phase markers of inflammation and diabetic retinopathy was studied.

Materials and Methods : 60 Type 2 Diabetes patients attending OPD/IPD of Tertiary care hospital were included. They were divided into three groups of 20 each. Group I: without retinopathy. GroupII: with non proliferative diabetic retinopathy (NPDR). GroupIII: with proliferative diabetic retinopathy (PDR). Results were compared with 20 normal controls. FBS, HbA1c, haptoglobin, ceruloplasmin and CRP were analysed on auto analyzer Hitachi 911(Roche).

Results : Diabetic patients with retinopathy had significantly higher levels of ceruloplasmin compared to normal controls ($p<0.05$ & <0.01 respectively). Diabetic patients with or without retinopathy had significantly raised levels of serum haptoglobin compared to control ($p<0.05$). NPDR patients had significantly raised levels of haptoglobin when compared to group I patients. CRP levels in patients of retinopathy were elevated compared to normal controls and diabetics without retinopathy ($p<0.05$).

Conclusion : Levels of ceruloplasmin, haptoglobin and CRP were significantly increased in diabetic retinopathy as compared to controls and patients without retinopathy. This may point to increase in serum viscosity leading to micro vascular sequelae. These proteins may serve as marker for progression of diabetic retinopathy.

Keywords : Non proliferative diabetic retinopathy (NPDR), proliferative diabetic retinopathy (PDR), acute phase proteins, ceruloplasmin, haptoglobin and CRP.

I. Introduction

Micro vascular complications cause serious morbidity in diabetics. Diabetic retinopathy is the most frequent vision threatening complication in these patients[1]. Understanding the cause and course of diabetic vascular pathology is important. Diabetic retinopathy is multifactorial complication. Persistent hyperglycemia causes metabolic stress (via sorbitol pathway) responsible for early retinal capillary dysfunction and lesions as micro aneurysm, basement membrane thickening, increased permeability and alteration of retinal blood flow[2]. The importance of thrombotic tendency in the aetiology of diabetic retinopathy is widely accepted which in turn may be related to protein composition changes in the plasma.

Haptoglobin, haemoglobin binding protein, plays role in providing protection against heam driven oxidative stress but raised levels as seen in acute phase reaction can increase serum viscosity having important implication in microcirculation pathology[3]. Ceruloplasmin, a copper containing metalloenzyme, possesses antioxidant property (e.g. ferroxidase activity), but elevated levels can promote vasculopathic effect[4]. Systemic inflammation marker CRP is synthesized in hepatocyte in response to cytokines released from site of inflammation. Raised levels of CRP are suggestive of low grade inflammation and are independent marker of vascular disease in diabetes[5]. Chronic inflammation can be potential mediator of diabetic retinopathy and measurement of inflammation markers like CRP, haptoglobin and ceruloplasmin may identify patients at higher risk of progression of disease. Relationship between stages of diabetic retinopathy and inflammation activity was also studied.

II. Material And Methods

60 male and female patients of Type 2 Diabetes (age 40-70 years) visiting Ophthalmology OPD of Tertiary care hospital were included in the study. Patients with previous history of any ocular inflammatory disease or acute inflammatory disease process were excluded from the study. Patients were divided into three groups of 20 each. Group I: without retinopathy, Group II: with NPDR and Group III: with PDR. Results were compared with 20 normal age and sex matched controls (non diabetic)

Overnight fasting blood sample was collected for biochemical investigations. FBS, HbA1c, haptoglobin, ceruloplasmin and CRP were analysed on auto analyzer Hitachi 911(Roche).

The fundus was examined by direct ophthalmoscopy, indirect ophthalmoscopy and/ or slit lamp bio microscopy using +90 D, +78 D lenses. Fundus fluorescein angiography was performed where

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clinically needed. Study protocol was approved by ethical committee of the institution.

III. Statistical Analysis

Mean and standard deviation were computed. The difference between two groups was seen by applying t-test. The level of significance considered was 0.05.

IV. Results

The mean age of patients of PDR (group III) was higher than patients in other two groups. Retinopathy patients had longer duration of diabetes as compared to patients who had no fundus changes (table 1). FBS of all the patients was >140mg/dl and HbA1c was > 7.0 g% showing poor glycemic control.

Diabetic patients with retinopathy (group II&III) had significantly higher levels of ceruloplasmin as compared to normal controls ($p<0.05$ & <0.01 respectively). Diabetic patients with or without retinopathy had significantly raised levels of serum haptoglobin compared to controls ($p<0.05$). NPDR patients had significantly raised levels of haptoglobin when compared to group I patients. CRP levels in patients of retinopathy were elevated as compared to normal controls and diabetics without retinopathy ($p<0.05$) Table II.

V. Discussion

In an attempt to identify the etiological factors and possible risk in the pathogenesis of retinal micro vascular changes, acute phase proteins were studied in various stages of diabetic retinopathy. Its precise cause is uncertain but there is evidence that an imbalance in haemostatic mechanism may be entailed in its initiation and progression. Pathophysiological changes include retinal capillary closure, thrombosis, non-perfusion, capillary leakage and increased serum viscosity[6]. Severity of retinopathy is known to increase with duration of disease as observed in the present study[7]. This may be due to damage caused to retinal vasculature by long standing metabolic abnormality. Excess glucose is metabolised via sorbitol pathway creating metabolic stress in vascular cells, which can impaired cells ability to handle free radicals. Excess glucose can also be channelled to form diacyl glycerol activating protein kinase C pathway and hyperglycemia can cause non enzymatic glycosylation of various proteins making them non functional[8].

There is metabolic and oxidative stress in uncontrolled diabetes, ceruloplasmin is thought to be a scavenger so its levels increase. But high levels of ceruloplasmin can cause vascular injury by generating free radicals and oxidizing LDL making it more atherogenic. ROS disrupt copper binding to ceruloplasmin, thereby impairing its normal protective function as liberated copper may promote oxidative pathology[9]. Ceruloplasmin levels were significantly higher in retinopathy patients as compared to controls. Some studies have shown that it takes part in pathological development of diabetic retinopathy and had a close relation with severity of pathological changes[10-11].

Haptoglobin is a positive acute phase reactant giving protection against Hb induced oxidative stress. Its levels increased in diabetic patients and further elevated in patients with NPDR showing oxidative damage playing role in vascular complication. Surprisingly haptoglobin levels were lower in PDR patients compared to NPDR patients, probable reason may be haptoglobin is getting lost in proteinuria because PDR patients are more likely to have proteinuria as well. This needs further investigation. Other workers have also observed increase in haptoglobin levels in diabetic retinopathy patients. Serum haptoglobin correlates with serum viscosity and it has positive effect on erythrocyte aggregation kinetics[3,12-13]. Hence increased levels may be responsible for development of micro vascular disease.

Increased inflammatory activity in diabetic retinopathy, as reflected by significantly increased levels of CRP, is associated with endothelial dysfunction. CRP is not only an inflammation marker but does contribute in vascular pathogenesis. By triggering complement activation it may exacerbate tissue damage leading to more severe disease. It is one marker which shows significant rise when diabetics start developing vascular complications. Our results are in agreement with other studies[14-15].

VI. Conclusions

Inflammatory pathway plays pivotal role in development and progression of diabetic complications. Elevated concentration of CRP and haptoglobin may be good predictor of onset of micro vascular complications in diabetes. Further studies on CRP as a marker for different stages of retinal vascular disease are needed. So that early diagnosis and treatment can slow progression and prevent blindness.

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Table 1 : Mean age and duration of diabetes in & controls.

Groups	Age in years	Duration in years
Control	45.25±7.33	-
Group I	52.95±11.5	8.5±4.8
Group II	51.1±7.93	10.65±6.75
Group III	58±7.26*	13.9±7.19#

group III VS controls ----- */# p<0.05*
group III VS group I----------#

Table 2 : Mean ceruloplasmin, haptoglobin & CRP levels in diabetic patients with/without retinopathy & controls.

Investigations	Controls	Group I	Group II	Group III
Ceruloplasmin(mg/dl)	65.17±8.4	74.18±19.2	79.48±7.8*	81.01±14.16**
Haptoglobin (mg/dl)	179.4±132.7	274.2±126.4*	401.36±187.7**#	295.24±153.3*
CRP (mg/dl)	2.43±2.9	2.98±4.2	7.49±8.37*#	6.67±4.3*#

group I/group II/group III VS controls ----- */# p<0.05 ** p<0.01*
group II / group III VS group I----------#

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Histochemical Studies On the Peyer's Patches of Sheep *(Ovis aries)*

By N. K. B. Raju, Geetha Ramesh, Sabiha Hayath Basha, S. Ushakumary & R. Suresh kumar

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Abstract - Tissue pieces of jejunum and ileum from different prenatal and postnatal age groups of sheep were collected from Corporation slaughter house, Perambur, Chennai. The goblet cells in the villous epithelium were positive for Periodic acid Schiff (PAS) but the negative reaction was observed in the follicle-associated epithelium as the goblet cells were absent. The follicle-associated epithelium showed positive activity for the alkaline phosphatase. The activity was more intense over the follicle domes in all the prenatal and postnatal age groups. An intense acid phosphatase activity was observed in the follicle-associated epithelium and dome areas of Peyer's patches. A linear activity of the adenosine triphosphatase was observed in the capsule of the ileal follicles and a mild enzyme activity was noticed in the interfollicular region. A reticular pattern of 5'nucleotidase enzyme activity was observed in the follicles of ileal Peyer's patch.

Keywords : *Sheep, Peyer's patches, Histochemistry.*

GJMR-G Classification : *NLMC Code: QT 162,W 20.55.A5, QY 60, QP 82-82.2*

Strictly as per the compliance and regulations of:

Histochemical Studies On the Peyer's Patches of Sheep *(Ovis aries)*

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Abstract - Tissue pieces of jejunum and ileum from different prenatal and postnatal age groups of sheep were collected from Corporation slaughter house, Perambur, Chennai. The goblet cells in the villous epithelium were positive for Periodic acid Schiff (PAS) but the negative reaction was observed in the follicle-associated epithelium as the goblet cells were absent. The follicle-associated epithelium showed positive activity for the alkaline phosphatase. The activity was more intense over the follicle domes in all the prenatal and postnatal age groups. An intense acid phosphatase activity was observed in the follicle-associated epithelium and dome areas of Peyer's patches. A linear activity of the adenosine triphosphatase was observed in the capsule of the ileal follicles and a mild enzyme activity was noticed in the interfollicular region. A reticular pattern of 5'nucleotidase enzyme activity was observed in the follicles of ileal Peyer's patch.

Keywords : *Sheep, Peyer's patches, Histochemistry.*

I. Introduction

The health of the digestive tract is important to receive the food material and for proper digestion and absorption. About 70 per cent of the body immune system is found in the digestive tract. This immune system often referred to as gut-associated lymphoid tissue and works to protect the body from invading pathogens (Ma et al., 2007). Peyer's patches are the aggregations of lymphatic nodules in the mucosa and submucosa of the jejunum and ileum of mice (Rowinski et al., 1984). Peyer's patches are immunocompetent lymphoid organs primarily engaged in immune responses to antigens presented from the intestinal lumen in guinea pig (Jurg et al., 1975).

The secretions from the loops containing Peyer's patches exhibit a stronger early Ig A response to bacteria than the secretions from loops lacking Peyer's patches. The large number of lymphocytes in Peyer's patches increases the probability of an antigen encountering an immunocompetent cell (Keren et al., 1978).

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A thorough knowledge of the histological changes in gut-associated lymphoid tissue (GALT) is very essential to gain a comprehensive knowledge on the gut immunology and to form a basis for the interpretation of various pathological conditions of the gut. Hence, the present work has been undertaken to explore the histochemistry of the GALT in sheep.

II. Materials and Methods

Tissue pieces from the terminal part of jejunum and parts of ileum were collected from sheep. The tissues from six animals each from different age groups viz. three months, four months and five months in prenatal and neonatal (0-2 months), young (3-9 months) and adult (10 months- 2 years) in postnatal groups were procured from the Corporation slaughter house, Perambur, Chennai. The determination of age ascertained as described by Richardson et al. (1976) in prenatal and Noden and de Lahunta (1985) in postnatal age groups.

The routine paraffin sections of 3-5μm thickness were used for carbohydrates. Frozen sections of tissues fixed in chilled formol calcium (4^{o}C) were used for localization of alkaline phosphatase (Singh and Sulochana, 1996), acid phosphatase (Singh and Sulochana, 1996) and 5´Nucleotidase (Bancroft and Gamble, 2003). Frozen sections of fresh unfixed tissues were also used for localization of adenosine triphosphatase (Bancroft and Gamble, 2003). All the frozen sections were cut at 15- 20μm thickness by cryostat.

III. Results and Discussion

a) Carbohydrates

The goblet cells of the villous epithelium were positive for PAS reaction (Fig.1). But, these cells were absent in the follicle-associated epithelium of the ileal Peyer's patches in all the age groups of sheep which is in accordance with Befus et al. (1980) in chicken.

When the combined PAS and alcian blue technique was applied, a blue reaction was observed in the follicle-associated epithelium of the ileal Peyer's patches. However, Burns (1982) observed that when the alcian blue was followed by PAS a purple reaction was observed in the epithelium of Peyer's patches of the domestic fowl. Lalitha (1991) found that, the PAS positive mast cells were observed in the peripheral

cortex of the lymphatic nodule of Peyer's patches in buffaloes.

b) Enzymes

i. *Alkaline phosphatase*

The follicle-associated epithelium showed positive activity for the alkaline phosphatase. The activity was more intense over the follicle domes (Fig.2) which is in agreement with Landsverk (1981) in calves, Bjerknes and Cheng (1981) in rat, Burns (1982) in domestic fowl and Owen and Bhalla (1983) in rat. However, Schmedtje (1965) noted that, the lymphoid follicle domes in rabbit appendix revealed negligible alkaline phosphatase activity over the cytoplasmic band separating intraepithelial lymphocytes from the lumen. Nordstrom et al. (1968), Weiser (1973) reported that the alkaline phosphatase activity increased as maturing epithelial cells migrated upto the villi in rats and human foetuses respectively. Ropke et al. (1972) noted in mouse that the high endothelial postcapillary venules through which lymphocytes entered the lymph nodes and Peyer's patches expressed a weak or no reaction for alkaline phosphatase in contrast to other endothelia. Ono (1975) recorded the alkaline phosphatase activity in tubules and vacuoles of enterocytes overlying the follicle in neonatal rats but not in adult animals. Further, Halleraker et al. (1990) observed in ruminant that, the alkaline phosphatase enzyme activity was shown in the follicle capsule.

A reticular pattern of staining was found in the centre of the follicle, interfollicular area and in the corona. A weak reaction was seen in the dome. Muscularis mucosae was positive for the enzyme. In lamb, the reticular reaction in the dome of the ileal Peyer's patches was absent. Nicander et al. (1991) reported that in sheep and goat foetuses by 90 days of gestation, a positive band of alkaline phosphatase activity was seen at the base of the villi in the follicular area. Weaker staining was observed in stromal elements of subepithelial tissues in both villi and primordial domes.

ii. *Acid phosphatase*

An intense enzyme activity was observed in the follicle associated epithelium and dome areas of ileal Peyer's patches (Fig.3) which is in accordance with Owen et al. (1986) in rat. Halleraker et al. (1990) stated that the cells stained for acid phosphatase were interpreted as macrophages in the follicle and dome in ruminants.

A strong activity in the interfollicular region and a mild activity of the enzyme in the capsule was observed in the ileal Peyer's patches. A pattern of enzyme activity was noticed in the follicles. However, Halleraker et al. (1990) stated that, the smooth muscle cells and reticular cells of the interfollicular tissue reacted strongly. A linear reaction outlined the follicle capsule.

iii. *Adenosine triphosphatase*

A linear activity of the enzyme was observed in the capsule of the ileal follicle (Fig.4). A mild enzyme activity was noticed in the interfollicular region and a weak positive reaction for the enzyme was observed in the follicle. However, Nicander et al. (1991) noticed a stronger activity of enzyme within the follicle and more pronounced activity within the centre of the follicle by 128 days in sheep foetuses. Halleraker et al. (1990) found that in lambs, the reticular cells stained weakly for this enzyme in the T-cell area and in the centre of the follicle. The follicle capsule had a weak, linear and discontinuous staining.

iv. *5'nucleotidase*

A reticular pattern of enzyme activity was observed in the follicles of ileal Peyer's patches (Fig.5). A linear activity of the enzyme was outlined in the capsule of the follicle which is in accordance with Halleraker et al. (1990) in the follicles of jejunal Peyer's patch in ruminants. Nicander et al. (1991) also observed a positive reaction of the enzyme in the capsule by 128 days in sheep foetuses.

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Legends To Figures

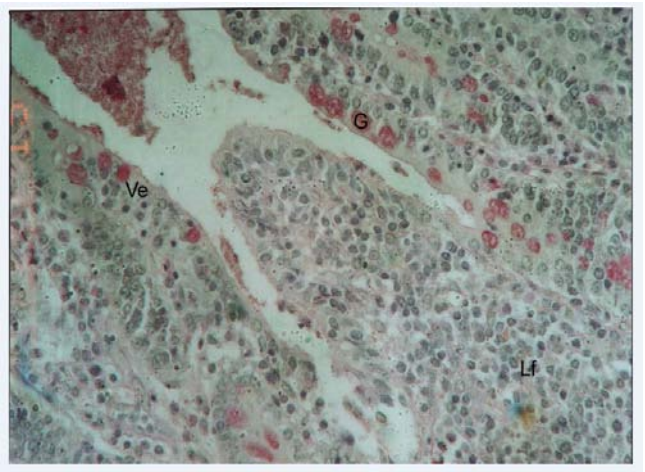

Fig.1 : Photomicrograph of ileal Peyer's patch of two month-old sheep showing PAS negative reaction in lymphoid follicles and positive reaction in goblet cells of the villous epithelium.

Lf- Lymphoid follicle

G- Goblet cells

Ve- Villous epithelium

PAS x 400

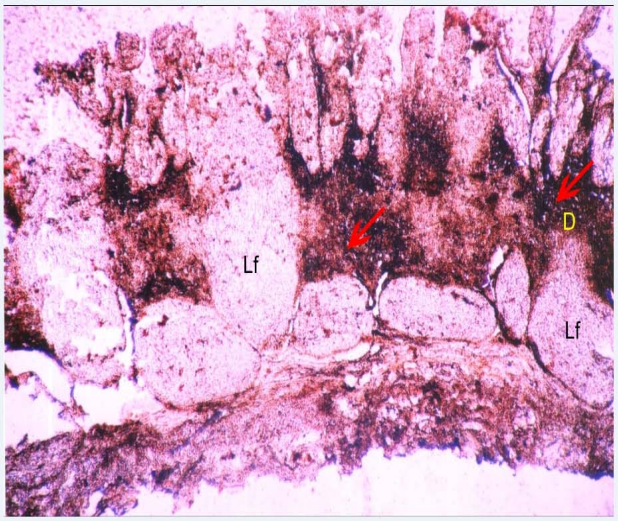

Fig. 2 : Photomicrograph of ileal Peyer's patch of one year-old sheep showing positive alkaline phosphatase activity (arrows) in the domes.

Lf- Lymphoid follicle

D- Dome

Alkaline phosphatase x 40

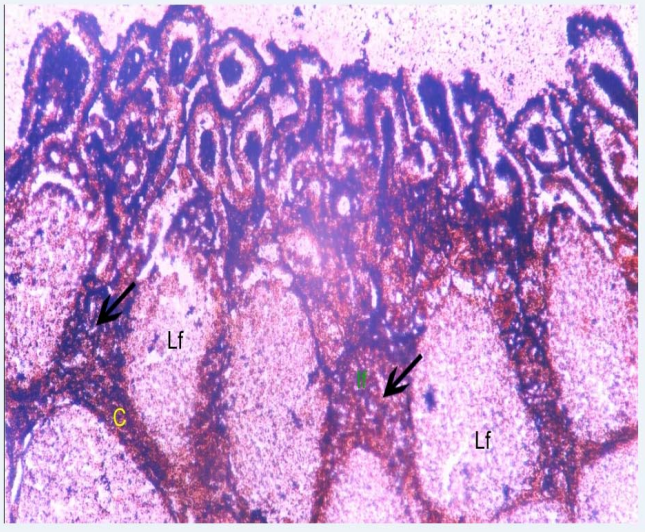

Fig.3 : Photomicrograph of ileal Peyer's patch of five month-old foetus of sheep showing an intense acid phosphatase activity (arrows) in the internodular and a linear activity in the capsule.

Lf- Lymphoid follicle

C- Capsule

If- Interfollicular area

Acid phosphatase x 40

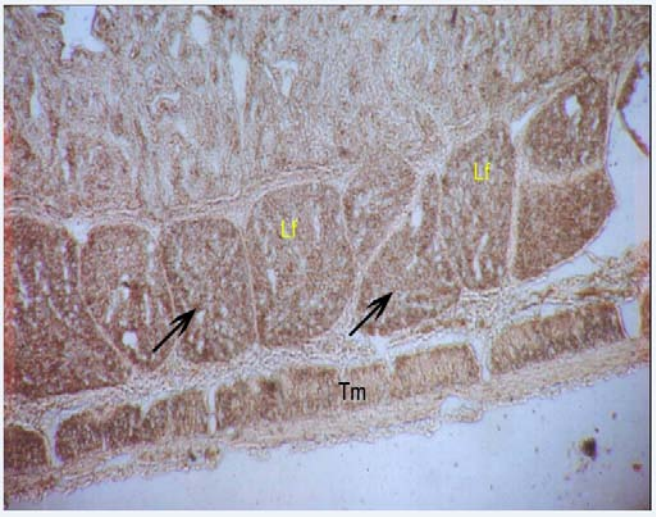

Fig.5 : Photomicrograph of ileal Peyer's patch of eighteen month-old sheep showing a pattern of 5'nucleotidase activity (arrows) in the follicles.

Lf- Lymphoid follicle

Tm- Tunica muscularis

5'nucleotidase x 40

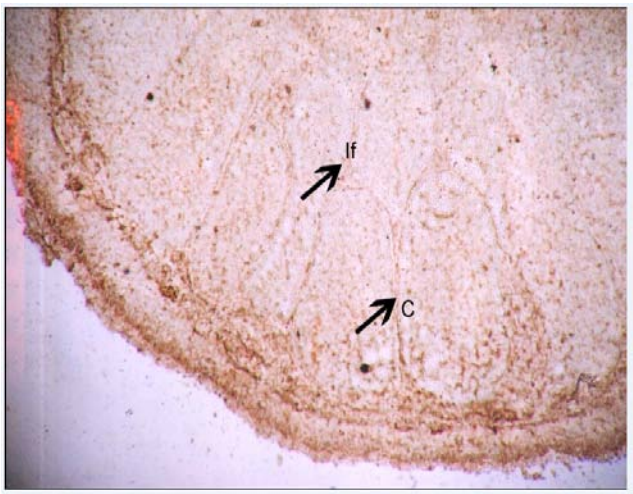

Fig.4 : Photomicrograph of ileal Peyer's patch of six month-old sheep showing a linear activity of the adenosine triphosphatase (arrows) in the capsule and a mild activity in the interfollicular region.

C- Capsule

If- Interfollicular area

Adenosine triphosphatase x 40

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Improving an Ovulation Rate in Women with Polycystic Ovary Syndrome by Using Silymarin

By Mohammed A.Taher, Yaser A. Atia & Manal K. Amin
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Abstract - Polycystic ovary syndrome (PCOS) is a heterogeneous disorder of uncertain etiology, it is the most common endocrinopathy in women and most common cause of anovulatery infertility, characterized by chronic anovulation and hyperandrogenemia. The present study was designed to investigate the effect of silymarin which is known to have antioxidant and insulin sensitivity effects on the levels of glucose, insulin, testosterone, leutinizing hormone(LH) and progesterone. Ovulation rate and Homeostasis Model Assessment of insulin Resistance (HOMA) ratio were determined .A 3-months of treatment were conducted in 60 PCOS patients in three well-matched groups .The first one (n=20),received silymarin (750mg/day) . The second group received metformin (1500mg/day) while the third group treated by combination of metformin (1500mg/day)and silymarin (750mg/day). All these groups had taken the drugs in divided doses. The results showed significant improvement in all parameters at the end of treatment. The percentage of increment in progesterone levels after completion of treatment were 12.12, 15.9, and 17.51 in groups 1, 2, and 3 respectively and the number of patients ovulated after 3 months of treatment were 4, 5, and 10 in groups 1,2, and 3 respectively. However they are more better in group of patients who were treated with combination of silymarin with metformin. In conclusion the addition of silymarin to metformin in treatment of PCOS patients has improving effect on disturbed hormones and ovulation rate.

Keywords : *Polycystic ovary syndrome, silymarin, ovulation rate, metformin.*

GJMR-B Classification : *NLMC Code: WP 540, WP 520*

Strictly as per the compliance and regulations of:

Improving an Ovulation Rate in Women with Polycystic Ovary Syndrome by Using Silymarin

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Abstract - Polycystic ovary syndrome (PCOS) is a heterogeneous disorder of uncertain etiology, it is the most common endocrinopathy in women and most common cause of anovulatery infertility, characterized by chronic anovulation and hyperandrogenemia. The present study was designed to investigate the effect of silymarin which is known to have antioxidant and insulin sensitivity effects on the levels of glucose, insulin, testosterone, leutinizing hormone(LH) and progesterone. Ovulation rate and Homeostasis Model Assessment of insulin Resistance (HOMA) ratio were determined .A 3-months of treatment were conducted in 60 PCOS patients in three well-matched groups .The first one (n=20),received silymarin (750mg/day) . The second group received metformin (1500mg/day) while the third group treated by combination of metformin (1500mg/day)and silymarin (750mg/day). All these groups had taken the drugs in divided doses. The results showed significant improvement in all parameters at the end of treatment. The percentage of increment in progesterone levels after completion of treatment were 12.12, 15.9, and 17.51 in groups 1, 2, and 3 respectively and the number of patients ovulated after 3 months of treatment were 4, 5, and 10 in groups 1,2, and 3 respectively. However they are more better in group of patients who were treated with combination of silymarin with metformin. In conclusion the addition of silymarin to metformin in treatment of PCOS patients has improving effect on disturbed hormones and ovulation rate.

Keywords : Polycystic ovary syndrome, silymarin, ovulation rate, metformin.

I. Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous disorder of uncertain aetiology; it is the most common endocrinopathy in women and most common cause of anovulatory infertility, affecting 5-10% of population of reproductive age.[1] It is characterized by chronic anovulation and hyperandrogenism.[2] Insulin resistance and associated hyperinsulinemia also have been recognized as important pathogenic factors in determining the majority of PCOS women particularly when obesity is present.[3] Most but not all women with PCOS have hyperinsulinemia with insulin resistance.[4] The association between hyperinsulinemic insulin resistance and PCOS well recognized and play an import role in the development of PCOS.[5] Hyperinsulinemia has been shown to reduce sex hormone binding globuline (SHBG) synthesis in liver[6] and insulin has a direct effect on ovarian steroidogenesis in theca cell.[7] Metformin is the oldest and still most insulin sensitizer world wide in the treatment of type 2 diabetes mellitus and PCOS-associated with insulin resistance. It is a biguanide derivative and considered as an insulin sensitizer since it lowers glucose levels without increasing insulin secretion.[8]

Silymarin is an active polyphenolic flavenoid extracted from fruits(seeds) of medicinal plant silybum marianum (milk thistle), extracts were standardized to contain 70-80% silymarin complex which comprised mainly of three major flavolignans, silybinin silychristin and silydianin of which silybinin is the most biological active. Silymarin is considered to be very safe and there are only few reports on its adverse effects, mainly a mild laxative effect has been observed in occasional instances and there are no known contraindications or side effects reported during its regular use.[9] According to the multiple pharmacological actions of silymarin, silybinin have been clinically evaluated in diabetics for their therapeutics value reduces the lipoperoxidation of cell membrane and insulin resistance significantly, decreasing endogenous insulin overproduction and the need for exogenous insulin administration.[10] So this study was designed to evaluate the efficacy of silymarin as insulin sensitizer improving an ovulation rate by treatment of PCOS and consequently its effect on hormonal and biochemical profile of the patients and comparing it with a classical one, metformin.

II. Materials and Methods

a) Patients

This study was conducted into Baghdad city, in al-Elwia maternity teaching hospital from 12/2010-6/2011.The study groups included 80 women selected randomly, 60 patients with PCOS aged (19-39) years with a mean age (27.5) years and 20 healthy control women aged (21-32) years with mean age (24) years. The diagnosis of PCOS was made by the gynaecologists depending on ultrasound examination, clinical features and laboratory tests according to diagnosis criteria of (Rotterdam 2003)[11]. Table-1 shows that the clinical presentations of patients in present study like those reported in other studies of polycystic ovary syndrome in that it is a heterogeneous disorder Investigations included : serum fasting glucose levels,

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fasting insulin levels, serum testosterone, serum progesterone and serum leutinizing hormone (LH).All patients participitated in this study were diagnosed having PCOS and were non-diabetic, not hypertensive, not pregnant , and not taking any medications that affect the reproductive or metabolic functions with 90 days of study. The patients were followed weekly regularly under gynecologist supervision during the period of treatment. The women were grouped into 4 groups as follow:

Group 1: included 20 PCOS patients, with BMI (31.22±1.138 Kg/m2), and age (19-31) years. They received Sylimarin tablets (750mg/day) in 3 divided doses after meals for 3 months.

Group 2: included 20 patients with BMI 30.84±1.23kg/m2) and age (20-35) years. The treatment was including metformin tablets 1500mg/day in 3 divided doses (500mg after meals for 3 months.

Group 3 : included 20 patients with BMI 32.83±1.37 kg/m2), age (22-39) years. The treatment was consisting of combination of 2 drugs (sylimarin 750 mg/day) and metformin (1500 mg /day) in 3 divided doses for 3 months.

Group 4 : included 20 healthy women with BMI 28.4±1.01kg/m2) ,age (21-32) years and these women were with regular cycle (21-32 days) who were taken from outside of the hospital and selected as controls.

b) Sample collection

Venous blood sample withdrew after overnight fasting (at least 12 hours of fasting) from PCOS women and the control group .The samples were taken at 3-5 days after the cycle for determination of serum LH and the sample for progesterone were taken at 21 days of the cycle. The base line samples were taken from the patients and after one month of treatment. Induction of the cycle was done by giving progestin before starting the study.

c) Biochemical analysis

i. *Determination of serum glucose and insulin levels*

Fasting serum glucose and insulin levels were measured by commercial kit obtained from Randox using enzymatic method[12,13].

ii. *Determination of Homeostasis Model Assessment of insulin Resistance (HOMA-IR)*

HOMA - IR was calculated using the following formula[14] :

$$\text{HOMA-IR} = \text{Fasting glucose (mmol/L)} \times \text{Fasting insulin (pmol/ml)}/22.5.$$

Insulin resistance patients were defined as having HOMA>2.7.

iii. *Determination of serum testosterone[15] and LH levels[16]*

Serum testosterone and LH levels were determined by radioimmunoassay(RIA) method using a kit provided by Sigma-Aldrich.

Determination of serum progesterone & Ovulation Rate

Serum progesterone levels were determined using kit obtained from Sigma-Aldrich, using (RIA) method, and the ovulation rate was determined according to mid-luteal phase progesterone level that was equal to or more than 16nmol/L (5ng/ml).[17]

iv. *Determination of body mass index (BMI)*

BMI was calculated using standard formula : BMI= weight (kg)/high (m2).

Obese patients were defined as having MBI > 27kg/m2 [18].

d) Ultrasound study

Transvaginal ultrasound study scan is performed for each patient at about day 12 of the cycle in order to to confirm follicular changes that appear through biochemical and hormonal changes, also it was repeated for each patient who had serum progesterone levels higher than or equal to 16nmol/L in order to confirm improvement of fertility and response of patients to treatment and follow up follicular development.[19]

e) Diagnosis

i. *Hyperandrogenism*

Based on criteria of Androgen Excess Society (AES 2006), which recommended the following diagnostic criteria for PCOS hyperandrogenemia.[20]

1. Hyperandrogenism (hirsutism and/or hyperandrogenemia)
2. Ovarian dysfunction (oligo-anovulation and /or PCOS).
3. Exclusion of related disorders such as hyperprolactenemia and congenital adrenal hyperplasia.

ii. *Hirsutism*

Based on Ferriman-Gallwey score, evaluates nine body sites including the face, chest, areolae, linea alba, upper back, lower back, buttocks, inner thighs and external genetalia.[21]

iii. *Infertility*

Inability of any couple to conceive a child within a 12 months period of unprotected coitus (sexual intercourse).[22]

iv. *Statical analysis*

Student t-test was used to examine the quantitative differences in the mean parameters. The results are expressed as mean±SD and the P-values <0.05were considered statically significant.

III. Results

Table-1 shows that 43.3% of the patients were with hirsutism and 36.6% with acne .Most patients were obese 68.3% and 31.6% were lean. The percentage of infertility among the patients were 31% and only 7% were with regular cycle while the percentage of amenorrhea and oligomenorrhea were 19% and 34% respectively. The percentage of insulin resistance was 78.3%, morover the androgenemia feature was the highest (85%). Table 2 shows a significant elevation ($P<0.05$) in mean serum insulin levels (pmol/L) of base line levels in the three study groups compared with control group and it declined significantly ($p<0.05$) after 1st,2nd and 3rd month of treatment in all groups of patients. There is significant increment ($p<0.05$) in mean serum glucose levels (mmol/L) of base line levels in three groups compared with control group and it declined significantly ($p<0.05$) after 1st, 2nd, and 3rd month of treatment in all groups of patients except in 1st month of first group, it was non-significant ($P>0.05$). Also the same Table illustrated significant increment ($P<0.05$) in mean HOMA-IR of baseline level in 3 PCOS groups compared with control and it declined significantly ($p<0.05$) after 1st, 2nd, and 3rd month of treatment in all groups of patients. There was significant increment ($p<0.05$) in mean serum testosterone levels (nmol/L) of base line levels in 3 groups compared with control group and it declined significantly ($p<0.05$) after 1st, 2nd and 3rd month of treatment in all groups of patients. Mean serum progesterone levels (nmol/L) of baseline levels in three groups decreased significantly ($p<0.05$) compared with control group and elevated significantly ($p<0.05$) after 1st, 2nd, and 3rd month of treatment in all groups of patients except 1st first group, it was non-significant ($p>0.05$). This table also demonstrate significant increase ($p<0.05$) in mean serum LH levels (U/L) of base line levels compared with the control group and it declined significantly ($p<0.05$) after 1st. 2nd. and 3rd month of treatment in all groups of patients except in 1st month of group 1 and 2, it was non-significant ($p>0.05$). Table-3 illustrated that, the percentage of increment in mean serum progesterone levels (nmol/L) was 4.28 %, 8.72 and 12.22 in group 1, also 4.324%, 8.42% and 15.9% in group 2 and 4.179%, 8.79% and 17.51 in group 3 after 1st, 2nd. and 3rd month of treatment for each group respectively. The numbers of women who had ovulated were 4, 5 and 10 in group 1, 2, 3 respectively.

Table 1 : Demographic data of 60 women with polycystic ovary syndrome.

Feature	No. of patients (%)
Hirsutism	26(43.3)
Acne	22(36.6)
Obesity	41(68.3)
Lean	19(31.6)
Infertility	31(51.6)
Amenorrhea	19(31.6)
Oligomenorrhea	34(56.6)
Regular cycle	7(11.6)
Insulin resistance	47(78.3)
Hyperandrogenemia	51(85)

Table 2 : Effect of metformin and /or silymarin on Insulin, glucose, HOMA-IR ratio, total testosterone and progesterone in women with PCOS.

Groups	Analytes	Control	Base line	After 1 M	After 2M	After 3M
1	Insulin(pmol/L)	57.5±0.359	92.18±4.73	89.35±0.35*	85.65±4.28*	81.44±3.66*
	Glucose(mg/dl)	5.1±0.17	5.29± 0.29a	5.01± 0.192NS	4.88±0.128*	4.73±0.128*
	HOMA	2.13±0.015	3.11± 0.244a	2.865±0.233*	2.673±0.178*	2.02±0.178*
	Testosterone(nmol/L)	1.45±0.03	4.59± 0,223a	4.427±0.242*	4.242±0.303*	3.396±0,318
	Progesterone(nmol/L)	17.15±0,02	12.84±0,612a	13.39±0.682NS	13.96±0.804*	14.41±0.942*
	LH(u/L)	5.2±0.365	9.38± 0.317a	9.18± 0.284NS	9±0.245*	8.71±0,376*
2	Insulin(pmol/L)	57.5±0.359	83.7±4.49a	82.1±3.468	80.8±3.01*	74.5±4.73*
	Glucose(mg/dl)	5.1±0.17	5.35± 0.362a	4.49± 0.209*	4.63±0.35*	4.25±0.229*
	HOMA	2.13±0.015	2.68± 0.226a	2.59± 0.212*	2.39±0.199*	2.02±0.178*
	Testosterone(nmol/L)	1.45±0.03	4.07± 0.199a	3.938±0.213*	3.765±0.185	3.9±0.167*

	Progesterone(nmol/L)	17.15±0,02	12.95±0.967a	13.517±0.941*	14.04±1.01*	15.01±1.33*
	LH(u/L)	5.2±0.365	111.13±0.87a	10.56±0.839NS	10.10±0.644*	9.52±0.741*
3	Insulin(pmol/L)	57.5±0.359	106±6.6	94.05±4.26*	84.16±4.50*	77.22±3.09
	Glucose(mg/dl)	5.1±0.17	5.12±0.301a	4.58±0.330*	4.27±0.369*	3.87±0.22*
	HOMA	2.13±0.015	3.55±0.172a	2.75±0.144*	2.298±0.245*	1.91±0.135*
	Testosterone(nmol/L)	1.45±0.03	4.59±0.942a	4.18±0.176*	4.06±0.159*	3.9±0.167*
	Progesterone(nmol/L)	17.15±0,02	13.88±0.875a	14.46±0.792*	15.10±0.673*	16.31±0.916*
	LH(u/L)	5.2±0.365	10.08±0.510a	9.33±0.480*	8.89±4.22*	8.54±0.515*

*Values are Mean±SD, a P< 0.05 for comparison with control group, *P<0.05 for comparison with baseline, NS non-significant P>0.05*

Table 3 : Comparison among mean % of increament in progesterone levels (nmol/L) and number of women who had ovulated during the study in all groups of PCOS patients.

	% of 1st Month	% of 2nd Month	% of 3rd Month	No.of women ovulated
Group1 (sylimarin)	4.28	8.72	12.22	4
Group2 (Metformin)	4.324	8.42	15.9	5
Group 3 (combination)	4.179	8.79	17.51	10

IV. Discussion

The percentage of patients with hirsutism and acne was 43.3% and 36% respectively (table-1) and this finding was consistence with other study performed in diagnosis of PCOS. Cutaneous manifestations of hyperandrogenism in PCOS include hirsutism, acne or combination, and male–pattern hair loss (androgenic alopecia); whereas acanthosis nigrigans is a cutaneous marker of hyperinsulinemia.[23] The study demonstrated that percentage of obese patients was 68.6% while it was 31.6% for lean, this is common in PCOS and it is in line with other studies which demonstrated that 40-60% of women with PCOS are obese (BMI>27 kg/m2).[24,25] The present study showed that (51.6%) of the patients were infertile, 31.6% with amenorrhea, 56% with oligomenorrhea, 11.6% with regular cycle, 78.3% with insulin resistance and 85% with hyperandrogenemia, these results are in agreement partly with other results which demonstrate the presence of infertility by (55-75%), amenorrhea (26-15%), oligomenorrhea (50-90%) regular cycle (22%) and hirsutism (60-90%) in women with PCOS.[24,26] The high levels of androgens lead to chronic anovulation, menstrual disturbances and hirsutism. PCOS patients typically have elevated LH levels and LH:FSH ratios.[27] because hyperandrogenism leads to abnormal folliculogenesis and endomeendometrial development.[28,29]

Hyperandrogenemia is a key feature of the syndrome; but it is not always linked to hyperandrogenic symptoms such as acne or hirsutism; indeed, ethnic groups such as Asian shown insulin resistance and associated hyperinsulinemia are also now recognized as important pathogenic factors in determining hyperandrogenism in the majority of PCOS women, particularly when obesity is present.[30] The present study illustrated a significant (P<0.05) increase in serum insulin and glucose radical quenching enzymes, (glutathion baseline levels and HOMA-IR index baseline value compared with control group, the results were compatible with those observed by Laure C., et al.[31], as characteristic features of women with PCOS. During three months of treatment with metformin and/or silymarin a significant (P<0.05) reduction in these parameters in all groups was observed except the effect of silymarin on glucose levels in the first month, was non-significant (P>0.05), as shown in table (2). Metformin leads to increase glucose utilization, decrease hepatic glucose production, imcrease insulin receptor binding and insulin receptor tyrosin kinase activity, but it has adverse effect on gastrointestinal tract and liver function[32,33], while silymarin, represents a new possibility in the treatment of PCOS, the underlying mechanistic links for this effects may be due to different possible mechanismas; silymarin increases, normalized and stimulated pancreatic activity of antioxidant and free peroxidase, superoxide dismutase and catalase).[34.35] Silymarin may produce its effect on glucose and insulin levels by another mechanism through blockage of TNF-α where that serum TNF-α concentration have been high in normal-weight PCOS women and even higher levels in obese women with PCOS.[36] When combination of silymarine and metformin were used, a powerful synergism effect occurred and led to best results as illustrated in third group, because each drug act by different pharmacological mechanism and different receptor sites which means that they may not compete one with each other to get same response, so that pronounced reduction in glucose, insulin and HOMA-IR values was occurred. The present study

demonstrated a significant ($P<0.05$) increase in baseline testosterone levels compared with control group, this result was compatible with other studies which demonstrate that serum concentration of testosterone and androstenedion are elevated in women with PCOS (the mean concentration are 50%-150% higher than controls).[37] During the 3 months of treatment with metformin and/or sylimarin, a significant reduction ($P<0.05$) from baseline of testosterone was observed (table-2). These results partly in agreement with Velazqwz et al. concerning metformin effect who reported that in an uncontrolled study, treatment with metformin for 8 weeks results in reduction of serum free testosterone in 29 non-diabetic women with PCOS, mostly overweight.[38] Most studies on this subject suggest that insulin lowering agents may affect the entire spectrum of endocrine, metabolic, and reproductive abnormalities in PCOS patients. However not all studies have assessed the effects of metformin in hyperandrogenic women have confirmed these findings. Interestingly, where insulin levels were reduced by treatment, serum androgens were lowered as well.[39] In an uncontrolled trial that assessed 26 obese women with PCOS before and after treatment with 1500 mg metformin/day for 8 weeks, a reduction in insulin concentrations and in serum free testosterone were reported and SHBG increased by 23%[38]. The combination of silymarin and metformin resulted in a more remarkable reduction in testosterone levels than group 1 and 2, this may be contributed to additive effect of these two drugs. It has been reported in this study significant decrease in baseline progesterone levels compared with control group, this result was compatible partly with other study.[40] Treatment with metformin and/or silymarin for 3 months, demonstrated a significant increament ($P<0.05$) in serum progesterone levels in all groups, except in first month of group 1, it was non-significant ($P>0.05$) (table 2). The improvement in ovulation rate (as assessed by measurement of mid-luteal phase progesterone level (>5ng/ml or >16nmol/L) was evaluated according to the percent of increment in baseline progesterone levels and number women who had ovulated, (table 3) which reflect that third group showed highest percentage of increment in progesterone levels and number of women who had ovulated. However, other researchers found a significant enhancement in luteal progesterone levels in PCOS women treated with metformin and they suggested that insulin resistance and hyperinulinemia may be responsible for low progesterone levels during luteal phase in PCOS[41]; therefore the luteal progesterone levels may be enhanced in PCOS by decreasing insulin levels with metformin. It had been reported that an improvement in menstrual pattern or ovulation with only modest improvement in insulin resistance and hyperinsulinemia is sufficient to promote preovulatory follicular maturation.[42] Silymarin was not different entirely from metformin concerning its effect on ovulation rate and progesterone levels as a result of their effect on insulin resistance and hyperinsulinemia. There is a significant negative correlation between insulin and progesterone, and between progesteprogesterone and LH concentration.[41] Therefore it is probaple that effect of silymarin on progesterone levels were consequences of its effect on insulin resistance and hyprinsulinemia. There was remarkable response to combination treatment because each drug act by its own mechanism and higher increment in progesterone and ovulation rate exerted by each drug alone may be enhanced by their combination. The base line LH levels in this work increases significantly ($P<0.05$) compared with control group, and it was compatible with other studies which demonstrate that women with PCOS, have 55-75% of a high LH to FSH ratio due to increased levels of LH than low levels of FSH.[43] Elevated serum concentrations of LH are common in all reported series of women with PCOS.[44] Typically, PCOS associated with increased LH and androgens but with normal or low serum concentrations of FSH. Most investigations have also documented an increased LH pulse amplitude and frequency as characteristic feature of PCOS.[45] During three months of treatment with metformin and/or silymarin a significant reduction ($P<0.05$) in serum LH levels were observed in all groups except in first month of group 1 and 2, it was non-significant ($P>0.05$), (table 2). The reduction of plasma levels of LH are not a primary event in the reduction of hyperandrogenism induced by metformin because many studies have reported a reduction in plasma androgens but not concomitant reduction in LH, indicating that in these cases the reduction of steroid synthesis cannot be secondary to reduced stimulation of LH also. It is possible that spontaneous or induced ovulation or reduction in androgens may lead to a secondary reduction in LH. Therefore androgens returned to pretreatment levels when metformin was suspended and that rise preceded the rise in LH, sustaining the hypothesis that a primary disorder of androgen hypersecretion is the cause of LH hypersecretion.[46] The effect of silymarin can be explained in the same manner, although its action on insulin levels are more pronounced when compared with metformin in current study, however there is a positive correlation between hyperinsulinemia and LH levels[41], the possible effect of silymarin on LH though its action on hyperinsulinemia and insulin resistance, indeed improvement in hyperinsulinemia may lead to decrease response of LH to GnRH. As expected from above mechanism of each drug, a highest reduction in LH levels were observed when combination used, (table 2) which indicates that each drug may improve the other.

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Evaluation of Total Phenolic Contents and Antiulcerogenic Activity of Root Bark of Azadirachta Indica

By M. K iranmai , B. U sha Sri, D. S udharshan Reddy, CB. M ahendra Kumar & Mohammed Ibrahim

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Abstract - The effect of methanol extract of root bark of Azadirachta indica was investigated in mice to evaluate the antiulcerogenic activity. Total phenolics were also determined. The root barks of have been extracted by *Azadirachta indica* successive solvent extraction method. Extracts were subjected to phytochemical analysis and total phenolics were also determined by the modified Folin-Ciocalteu method. The parameters taken to assess anti-ulcer activity were volume of gastric secretion, pH, free acidity, total acidity, and ulcer index and % ulcer protection. The results indicated that the methanol extract significantly ($p < 0.001$) decreases volume of gastric acid secretion, pH, free acidity, total acidity and ulcer index and shows significant ulcer protection compared to standard control. Root bark extracts found to possess significant amount of phenolics and other biologically active constituents based on phytochemical evaluation and shows significant antiulcerogenic activity.

Keywords : *Azadirachta indica, Root bark, Total phenolics, antiulcerogenic activity, Omeprazole, Methanol extract.*

GJMR-I Classification : *NLMC Code: QV 223*

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Evaluation of Total Phenolic Contents and Antiulcerogenic Activity of Root Bark of *Azadirachta Indica*

M. Kiranmai [α], B. Usha Sri [σ], D. Sudharshan Reddy [ρ], CB. Mahendra Kumar [ω] & Mohammed Ibrahim [¥]

Abstract - The effect of methanol extract of root bark of *Azadirachta indica* was investigated in mice to evaluate the antiulcerogenic activity. Total phenolics were also determined. The root barks of *Azadirachta indica* have been extracted by successive solvent extraction method. Extracts were subjected to phytochemical analysis and total phenolics were also determined by the modified Folin-Ciocalteu method. The parameters taken to assess anti-ulcer activity were volume of gastric secretion, pH, free acidity, total acidity, and ulcer index and % ulcer protection. The results indicated that the methanol extract significantly ($p < 0.001$) decreases volume of gastric acid secretion, pH, free acidity, total acidity and ulcer index and shows significant ulcer protection compared to standard control. Root bark extracts found to possess significant amount of phenolics and other biologically active constituents based on phytochemical evaluation and shows significant antiulcerogenic activity.

Keywords : Azadirachta indica, Root bark, Total phenolics, antiulcerogenic activity, Omeprazole, Methanol extract.

I. Introduction

Azadirachta indica (AI) has been advocated for the treatment of disorders like cough, nausea, vomiting, fever, jaundice, gonorrhea, intestinal warm infestation and leprosy in indigenous system of medicine[1] and reported to have antiulcerogenic property.[2-3] The biological, medicinal and industrial uses of various parts of AI and the compounds isolated from it have been reviewed.[4-6] AI barks contained condensed tannins to the extent of 15% along with other non-isoprenoid constituents like flavonoids and phenolics.[7]

Peptic ulcer, one of the most common gastrointestinal disease, is caused by multiple factors including stress, smoking, nutritional deficiencies, noxious agents such as alcohol, NSAID and *Helicobacter pylori* infection, among others.[8-9] Plant extracts are some of the most attractive sources of new drugs and have been shown to produce promising results in the treatment of gastric ulcers.[10] This is an important reason to investigate the antiulcer effect of AI bark extracts that have been used traditionally against gastric diseases.

As to pharmacological effects, different extracts of leaves, seeds and stem barks of AI showed antimicrobial[11-12], antioxidant[13] and antiulcer activities.[14-15] In our previous study, antioxidant effect of hydro alcoholic root bark extract was tested.[16] Given the association between biological constituents present and antiulcerogenic effects of the AI root bark, the present study was carried out by two approaches. First, we performed phytochemical screening of root bark successive solvent extracts. Second, we selected root bark methanol extract to assess its antiulcerogenic activity in ethanol induced gastric ulcer in mice.

II. Materials and Methods

a) Chemicals and reagents

All reagents and chemicals used were of analytical grade. Folin-ciocalteu reagent (Merck Pvt. Ltd. India), Sodium carbonate (Merck Pvt. Ltd. India), standard omeprazole was the kind gift from Aurobindo Pharma Ltd., Hyderabad.

b) Plant material

The root bark of AI was collected from agriculture land of Deshmukhi village of Andhrapradesh, India and the authentication of plant material was done by a botanist at Osmania University, Hyderabad and the voucher no was 0125.

c) Preparation of root bark extracts

Root barks were shade dried and powdered mechanically after cutting into small pieces. The powdered plant material was extracted in a soxhlet extractor by successive soxhlet extraction method based on polarity order of solvents. Solvents employed were pet ether, chloroform, ethyl acetate and methanol. The extracts were cooled at room temperature, filtered and evaporated to dryness under reduced pressure in a rotary evaporator[17].

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d) Phytochemical evaluation

Resultant successive extracts of root barks were subjected to qualitative chemical analysis for the presence of biologically active constituents.[18] Thin layer chromatography was performed for all the extracts by taking Quercetin as biomarker. Mobile phase employed was ethyl acetate: formic acid: glacial acetic acid: water (100: 11: 11: 26).[19]

e) Determination of total phenolics content

The Folin–Ciocalteu reagent (FCR) or Folin's phenol reagent is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolics and polyphenolic antioxidants. It works by measuring the amount of the substance being tested needed to inhibit the oxidation of the reagent. [20, 21] Total phenol contents in the extracts were determined by the modified Folin-Ciocalteu method. An aliquot of the extract was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The tubes were vortexed for 15 sec and allowed to stand for 30 min at 40°C for color development. Absorbance was then measured at 765 nm using the Shimadzu UV-1800 spectrophotometer. Samples of extract were evaluated at a final concentration of 0.1 mg/ml. Total phenolics content were expressed as mg/g tannic acid equivalent using the following equation based on the calibration curve: $y = 0.1216x$, $R^2 = 0.9365$, where x was the absorbance and y was the tannic acid equivalent (mg/g).[22]

f) Animals

Swiss albino mice (24-30 g) of either sex maintained under standard husbandry conditions (temp 23±2°C, relative humidity 55±10% and 12 hours light dark cycle) were used for the screening. Animals were fed with standard laboratory food and ad libitum during the study period. The experimental protocol has been approved by Institutional Animal Ethics Committee (IAEC NO.1330/AC/10/CPCSEA).

g) Ethanol induced gastric ulcer[23,24]

The methanol extract of root bark of AI was selected as it is having significant amount of biologically active constituents (from the results of phytochemical analysis) to evaluate anti ulcer activity by ethanol induced gastric ulcer in albino mice. After 12 hour of fasting Swiss albino mice weighing 24-30 g of either sex were divided into 5 groups, each group consists of 6 animals.

Group 1 served as a control received 1.0 ml/kg p.o 80% Tween 80.

Group 2 served as standard control received 30 mg/kg, p.o Omeprazole.

Group 3 received 100 mg/kg, p.o methanol extract of AI.

Group 4 received 200 mg/kg, p.o methanol extract of AI.

Group 5 received 500 mg/kg, p.o methanol extract of AI.

After 1h all the animals were treated with 0.2 ml of ethanol p.o to induce gastric ulcer. Animals were sacrificed by cervical dislocation one hour after administration of ethanol. The stomach was excised and lesion index was determined by measuring each lesion in mm along its greater length.

h) Determination of gastric parameters

Collection of gastric juice: After post operative period, animals were sacrificed by cervical dislocation and the stomach was dissected out as a whole by passing a ligature at the esophageal end. Gastric content was evacuated into graduated tube by cutting along the greater curvature of the stomach, and was centrifuged at 3000 rpm for 10min.

Volume of gastric juice: The volume of the centrifuged sample was expressed as ml/ 100 g body weight.

pH of gastric juice: pH of gastric juice was measured with the help of pH meter.

Free and total acidity: Gastric juice (1ml) was pipette into a 100ml conical flask and diluted with 9ml distilled water. Two or three drops of Topfer's reagent was then added and titrated with 0.01 N sodium hydroxide until all traces of red colour disappeared and the colour of the solution was yellowish-orange. The volume of alkali added was noted. This volume corresponds to free acidity. Two or three drops of phenolphthalein were then added and the titration was continued until a definite red ring appeared; the volume of alkali added was noted. The volume corresponds to total acidity. The sum of the two titrations was total acidity. Acidity was expressed in terms of mEq/L.

Acidity was expressed as:

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality} \times 100\text{mEq/L/100g}}{0.1}$$

Estimation of gastric ulcerative index changes: The stomach was opened along the greater curvature and it was washed with running tap water. Then the ulcerative area was counted by placing it on a flat wooden plate.

Ulcer Index[25]

The following arbitrary scoring system was used to grade the incidence and severity of lesion.0 = Normal, 1 = Red coloration, 2 = Spot ulcers, 3 = Hemorrhagic streaks, 4 = Ulcers > 3 but < 5 and 5 = Ulcers > 5. Ulcer index and % protection were calculated by following formulas.

$$\text{Ulcer index} = \frac{\text{Arithmetic mean of intensity in group} + \text{Number of ulcer positive animals}}{\text{Total number of animals}} \times 2$$

$$\% \text{ Protection} = \frac{\text{Control mean index} - \text{Test mean index}}{\text{Control mean index}} \times 100$$

III. Results and Discussion

Qualitative chemical analysis results (table-2) were exhibiting the presence of alkaloids, glycosides, flavonoids, tannins, saponins and terpenoids. TLC results (table-1) were qualitatively confirming the presence of flavonoids in successive extracts of root bark by using quercetin as biomarker. Total phenolic contents were quantified by standard procedures and results were given in table 1. Results depicts that phenolic content was significantly found in ethylacetate extract followed by methanol extract.

Table 1 : % Yield, R_f values and total phenolics content of the root bark extracts of AI

Extract ⟹	Petether	Chloroform	Ethylacetate	Methanol	% 80 Ethanol
% Yield w/w	2.50	3.80	1.72	4.70	1.29
R_f Value	0.87	0.89	0.92	0.89	0.89
Total phenolics content, μg/ml	98.19±1.66	19.73±0.41	821.54±2.70	740.10±0.13	380.75±2.78

Total phenolic contents were expressed in Mean±SEM.

Table 2 : Qualitative chemical analysis of root bark extracts of AI

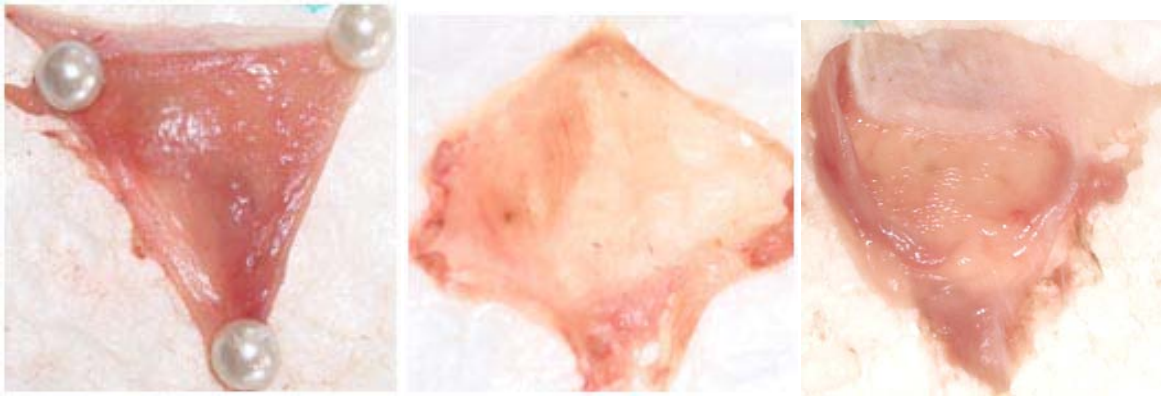
Name of the chemical constituent	PE	$CHCl_3$	EtOAc	MeOH	HA
Alkaloids	-	+++	-	-	-
Carbohydrates	-	+	+	++	++
Glycosides	+	+	-	-	++
Tannins	-	+	-	-	++
Saponins	-	-	-	-	++
Flavonoids	++	++	++	+++	++
Terpenoids	++	++	++	++	+

+++= Three chemical tests are positive, ++= Two chemical tests are positive, += One chemical test is positive, -=not responded. Test results were given in the order they have mentioned in the text.
PE= petether, $CHCl_3$ = Chloroform, EtOAc =Ethyl acetate, MeOH = Methanol, HA = hydroalcoholic (80% ethanol).

The anti-ulcer activity of root bark of AI was evaluated by employing ethanol induced gastric ulcer in mice. Ethanol induced gastric injury is associated with significant production of oxygen free radicals leading to increased lipid peroxidation, which causes damage to cell and cell membrane.[26]

Pretreatment of mice with root bark extracts produced a dose dependent protection in the ethanol induced ulceration model as compared to control group. However the protection was statistically significant reduced the severity of ulcer and caused a significant reduction of ulcer index in this model. Omeprazole produced significant gastric ulcer protection as compared to control group (Table 3). Ethanol damages the plasma membrane and leads to intracellular accumulation of sodium and water by increasing the membrane permeability. These changes ultimately cause cell death and gastric mucosal exfoliation.[27] Ethanol is also known to release the endogenous ulcerogenic mediators. These could precipitate mucosal injury either by causing vascular changes like mucosal edema and increased mucosal permeability or by non-vascular effects like mucus depletion and enzyme release in the stomach.[28] The decrease in volume of gastric juice may also attributed to its anti secretory

potential of the drug. The anti secretory potential may also relate towards gastric juice and interference of gastric blood circulation are responsible for the induction of ulceration.[29] AI root bark methanol extract significantly decreased the gastric juice volume as compared to control. Methanol extract significantly increased p^H as compared to control and nearer to standard. The excessive secretion of hydrochloric acid in the stomach was considered to be an important factor in the formation of peptic ulcer. Hydrochloric acid is known to produce ulceration and digestion of the stomach tissues as well as to reduce the neutralizing capability of the stomach mucus secretions.[30-32] As a measurement of free hydrogen ion, pH indirectly represents the hydrochloric acid concentration in the stomach. Increase in pH is usually affected by either the reduction of the acid secreted in the stomach or the increase in the volume of alkaline and neutral fluids (mucus). The variation in the pH level among the groups shows tendency of protective effects of them towards gastric ulceration. The decrease in acidity was at its maximum level for the reference standard group followed by extract treatment group. The least decrease in acidity was shown by methanol extract treated group at its 500mg/kg dose. Macrocsopic examination of ethanol induced gastric ulcer in mice was shown in figure 1.



(a) (b) (c)

Figure 1 : Macroscopic examination of ethanol induced gastric ulcer in mice
a=control, b= standard control, c=treated control (conc.500mg/kg)

Table 3 : Effect of methanol root bark extract of AI in ethanol induced gastric ulcer

Treatment	Dose (mg/kg)	Vol. of gastric juice(ml)	pH	Free Acidity (mEq/L)	Total Acidity (mEq/L)	Ulcer index	%Protection
Control	-	1.65±0.81	2.9±0.20	31±0.89	74.5±1.87	5.25±0.48	0
Std.control	30	1.48±0.07	3.9±0.13	9.3±0.81	23.3±2.75	1.02±0.2***	75
Treated	100	1.53±0.05	2.77±0.08	28.8±0.98	61.3±1.86	3.16±0.214	21
	250	1.53±0.08	3.22±0.11	16±3.57	42.3±5.68	2.33±1.70	41.75
	500	1.23±0.2	3.70±0.10	11.21±1.23	29.5±6.10	1.58±0.47***	60.50

*Values are expressed in mean±SEM Statistical comparison was performed by using ANOVA coupled with student's' test. *** P<0.001 were consider statistically significant when compared to control group.*

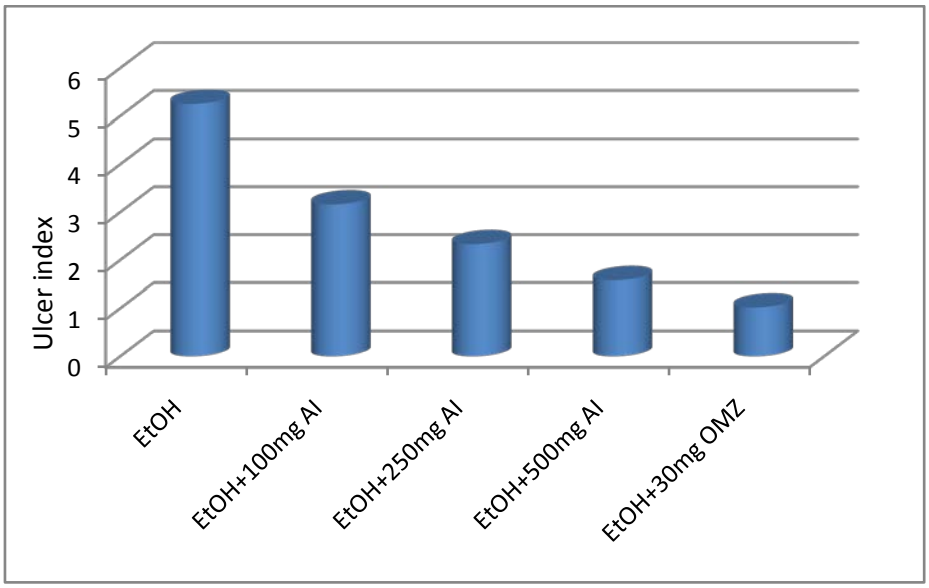


Figure 2 : Dose dependent changes in ulcer index of treated and control group of animals

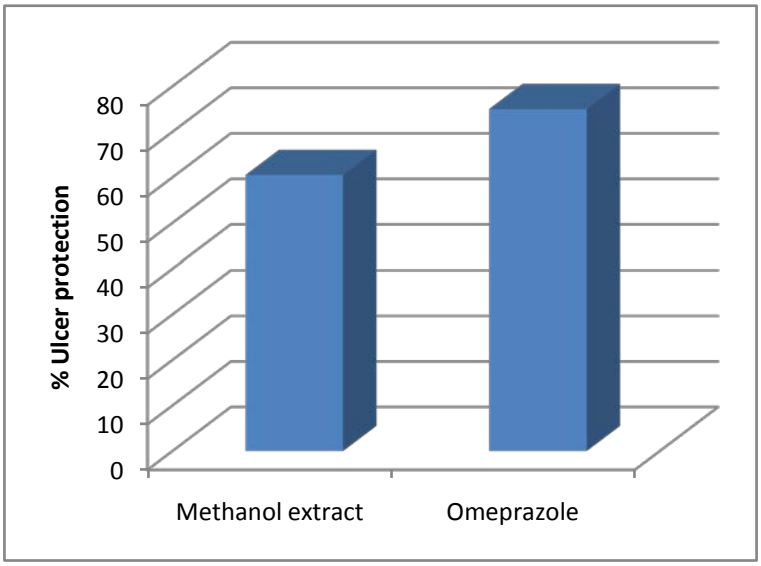


Figure 3 : % Ulcer protection of treatment extract with standard Omeprazole

Barros et al. (2008), report that phenolic compounds have an antiulcerogenic effect related to cytoprotective activity. Moreover, Kahraman et al. (2003) suggest that flavonoid quercetin promotes a decrease in ulcerative lesions due to its antioxidant effect. In addition, a review of antiulcer drugs of plant origin shows that triterpenes, because of their ability to strengthen defensive factors such as stimulation of mucus synthesis or maintenance of the prostaglandin contents of gastric mucosa at high levels, are compounds with potential antiulcerogenic activity (Lewis and Hanson, 1991). Dose dependent ulcer index results were given in figure 2. Comparison of ulcer protection of root bark methanol extract with that of standard control was shown in figure 3. Based on this data, it is suggested that the gastro protection observed in this study could be related to the presence of phenolics and flavonoids in the methanol extract of root bark of Al extract.

IV. Conclusion

In conclusion, the results show that the methanol extract of root bark of *Azadirachta indica* present antiulcer activity, as evidenced by ethanol induced gastric ulcer model in albino mice. Results suggest that the effectiveness of the extract as anti ulcerogenic agent may be due to presence of flavonoids and phenolics compounds. The results of this study showed that the root bark of *Azadirachta indica* contains appreciable amount of phenolic contents along with other biologically active constituents.

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Study of Growth of Pre - School Children in Urban Slums of Bijapur City

By Sharanagouda M.Patil, C.M.Kulkarni, Manjunath Aithala & R.T.Kashinath

BLDE University

Abstract - A randomized study was carried out to determine the changes during the growth period in 1-6 years groups. The study consists of 350 children from different slum in Bijapur city. The different parameters were measured and compared with national centre for Chronic Disease prevention and health promotion Committee 2000(CDC) standards. Most of the parameters like Height, Weight and Head circumference were reduced significantly in Study groups in comparison to CDC standards in both sexes. The Significant change in the values of different parameters in Study group may be due to illiteracy, poor sanitation and nutrition etc.

Keywords : *Growth Period, Illiteracy, Poor sanitation, Nutrition, CDC.*

GJMR-I Classification : *NLMC Code: QT 200, WA 19*

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Study of Growth of Pre - School Children in Urban Slums of Bijapur City

Sharanagouda M.Patil [α], C.M.Kulkarni [σ], Manjunath Aithala [ρ] & R.T.Kashinath [ω]

Abstract - A randomized study was carried out to determine the changes during the growth period in 1-6 years groups. The study consists of 350 children from different slum in Bijapur city. The different parameters were measured and compared with national centre for Chronic Disease prevention and health promotion Committee 2000(CDC) standards. Most of the parameters like Height, Weight and Head circumference were reduced significantly in Study groups in comparison to CDC standards in both sexes. The Significant change in the values of different parameters in Study group may be due to illiteracy, poor sanitation and nutrition etc.

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I. Introduction

Growth is a complex phenomenon that is normally accompanied by an orderly sequence of maturation changes. It involves accretion of protein and increase in length and size, not just an increase in weight, which may be due to formation of fat or retention of salt and water [4]. Growth and development begin at conception and end at maturity. They are characteristic of children and any obstacle in this process at any stage can possibly result in aberration of growth and development [8].

Studies on growth and physical development of infants and children are important as they provide determinants of a nation's health. Measurements of height and weight are still the simplest and one of the reliable means by which the progress of a normal child is evaluated and gross abnormalities detected even when no other clinical sign of illness is detected [5].

It is difficult to derive norms of Indian children due to wide variation in socioeconomic status, nutrition conditions, and ethnical and regional differences in India[1]. A number of workers in India have studied the nutritional anthropometry of urban school children in the past (Udani [12] 1963, Currimbhoy [3] 1963, Mukerjee and Kaul [7] 1970). With the accent on community pediatrics and realizing the vast potentials of research in rural areas, some samples have been surveyed in rural areas too (Prabhakar A. and Naik et al [10] 1975, Indirabai et al [6] 1979, Rao et al [11] 1984).

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Numerous attempts were made in the last four decades or more to utilize the data on body measurements for the evaluation of nutritional status and the general health of children in this country. But no concerted efforts were made to establish norms of height, weight and other anthropometric measurements. Certain isolated observations were on record, but their significance was difficult to judge for lack of accurate background information and uniformity in the methods adopted in obtaining data. Hence a study was undertaken to access the growth pattern of preschool children of certain slums in Bijapur urban area and the results were compared with standard growth pattern.

II. Materials and Methods

A cross sectional study was conducted in preschool children from 3 different slums situated in Bijapur urban area 3 slums selected are

1. Sanjay Gandhi slums.
2. Minimadar Oni.
3. Sunagar Galli.

Total population of these slums is about 2900 and preschool children are about 368 i.e. 12% of population. The children having any evidence of chronic infections, congenital anomalies, tuberculosis are excluded from the studies.

Following physical measurements were carried out on selected preschool children of these slum areas:

1. Height (in cm.)
2. Weight (in Kg.)
3. Head Circumference (in cm.)

III. Statistical Analysis

Statistical analysis was done using software SPSS version 9. The z score worked out for both boys and girls of different age groups and the score obtained have been more than the tabled value at 0.05 level of significance indicating the observed results were significant.

V. Results

Results of present study are narrated in table 1 to 3 and graphs 1 to 6.

Table -1 and graphs 1 as well as 2 depicts the height in centimeters of preschool children (boys and

girls) of the selected slum areas and corresponding CDC reference values in the age group of 1- 2 years, 2-3 years, 3-4 years, 4-5 years and 5-6 years. As evident from the table that the observed values are significantly lower in both boys and girls of all the age groups included in the present study as compared to CDC reference values.

Table -2 and graphs 3 as well as 4 shows the weight in kgs of preschool children (boys and girls) of the selected slum areas and corresponding CDC reference values in the age group of 1- 2 years,2-3 years,3-4 years,4-5 years and 5-6 years. As seen from the table the observed values are significantly lower in both boys and girls of all the age groups included in the present study as compared to CDC reference values.

Table -3 as well as graphs 5 and 6 gives the head circumferences of preschool children (boys and girls) of the selected slum areas and corresponding CDC reference values in the different age groups included in the present study : 1- 2 years, 2-3 years, 3-4 years, 4-5 years and 5-6 years. As evident from the table the observed values are significantly lower in both boys and girls of all the age groups included in the present study as compared to CDC reference values.

VI. Discussion

There is great paucity of literature in our country on growth and development in preschool children. It is only in recent years that Udani [12] first discussed the effect of different socio – economic factors on various parameters of growth in children, from birth to eleven years. Later I.C.M.R (Indian Council for Medical Research)[5] also gave a statistical report of data on growth from 0 -21 years of age.

Recent work tends to suggest that environmental influences, especially nutrition, are of greater importance than genetic background or other biological factors. Physical dimensions of the body are much influenced by nutrition, particularly in the rapidly growing period of early childhood.

In a vast country like India where people have different ethnic, religious, social and cultural back-ground and a variety of customs and dietary habits, one can expect to find influence of these factors on growth and physical development of children. So, the result of anthropometric surveys should be expressed in relation to local standards that have been constructed from measurement of apparently healthy subjects of same ethnic group.

Bijapur city is included in urban area. It is a city situated in North Karnataka. Present cross sectional study is an effort to study the growth of preschool children selected randomly from three different slums in urban area.

Observations of ICMR (1972) [5] and Phadake [9] (1968) indicate the average values of growth of city children are better than those of children from villages. It is difficult to say which of the various environmental factors, such as illiteracy, state of hygiene, wearing habits, and understanding of nutritional requirements may be responsible for this difference between the rural and urban children.

Our observation shows a significant decrease of approximate 5% in heights in different age groups and both sexes, as compared to CDC standards (Table – I, Graphs 1 & 2). This observed decrease in height may be due to the same factors as that suggested by ICMR & Phadake studies. Further our study indicates, there is significant decrease of approximate 10% in weight in different age groups and in both sexes (Table 2, Graphs 3 & 4) as compared to CDC standards.

According to ICMR,[5] the head circumference at the age 1 year is 44.4cms and 43.5cms in boys and girls respectively. At the age of 5 years, it is 48.5cms and 47.8cms in boys and girls respectively. The total increase in the head circumference was 4.1cms and 4.2cms in boys and girls respectively. Therefore, it appears that there is definite reduction in head circumference. In our study group, mean Head circumference is measured in different age groups and both sexes separately (Table – 4, Graphs7 & 8). The mean head circumference in boys has increased by 2.07cm between 1 – 6 years. This increase is less as compared to CDC standards of 4.92cms in the same age group in boys. It is observed in girls that the mean head circumference has increased by 4.59cms between 1 – 6 years. This increase is less as compared to CDC standards of 5.80cms in the same age group in girls.

The results of present study show significant decrease in different parameters including head circumference as compared to American standards also.[2] Hence the observed difference may be due to racial factors and also due to the improved standards of nutrition and environment in the later.

V. Conclusion

In conclusion decrease in height , weight and head circumference of both sexes of preschool children of selected Bijapur slum areas in all the age groups is suggestive of chronic malnutrition may be due to over population, illiteracy, poor nutrition, poor sanitation and poor socioeconomic status.

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Table 1 : Table showing height in centimeter of preschool children of different age groups with approved CDC reference values

Age group	BOYS					GIRLS				
	Frequ e-ncy	Observed data		CDC Standards	Z–score	Freq u-ency	Observed data		CDC Standards	Z–score
		Mean	STD. DEV.				Mean	STD. DEV.		
1 to 2 Yrs	09	71.16	1.16	76.1	12.77	06	72.00	4.6	74.4	1.27
2 to 3 Yrs	39	82.54	6.13	87.7	5.26	20	79.75	5.7	86.2	5.06
3 to 4 Yrs	34	91.14	11.19	96.1	2.58	33	91.09	6.9	95.0	3.26
4 to 5 Yrs	34	100.8	15.43	102.5	0.64	30	96.95	5.3	101.0	4.19
5 to 6 Yrs	109	103.79	9.81	109.2	5.76	36	101.2	5.5	108.0	7.39

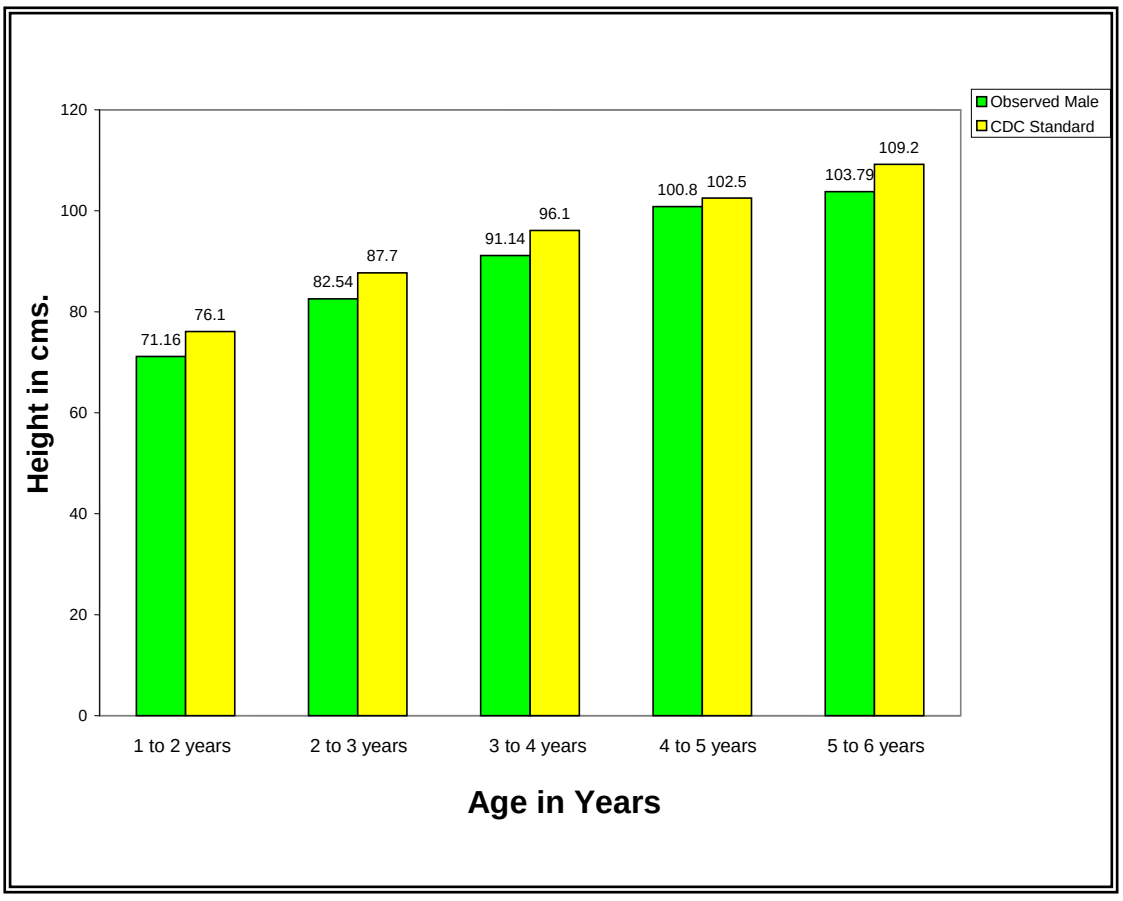
Table 2 : Table-2 showing weight in kg's of preschool children of the different age groups with approved CDC reference values.

Age group	BOYS					GIRLS				
	Freq u-ency	Observed data		CDC Standards	Z–score	Freq u-ency	Observed data		CDC Standards	Z–score
		Mean	STD. DEV.				Mean	STD. DEV.		
1 to 2 Yrs	09	09	0.89	10.46	4.92	06	9.16	1.7	9.67	0.73
2 to 3 Yrs	39	12.39	1.42	13.74	5.94	20	11.59	1.91	13.2	3.77
3 to 4 Yrs	34	13.93	2.26	15.33	3.61	33	13.28	2.12	14.3	2.76
4 to 5 Yrs	34	15.43	5.07	17.3	2.15	30	13.73	1.94	15.4	4.71
5 to 6 Yrs	109	16.46	2.53	18.5	8.42	36	15.4	1.66	17.6	7.95

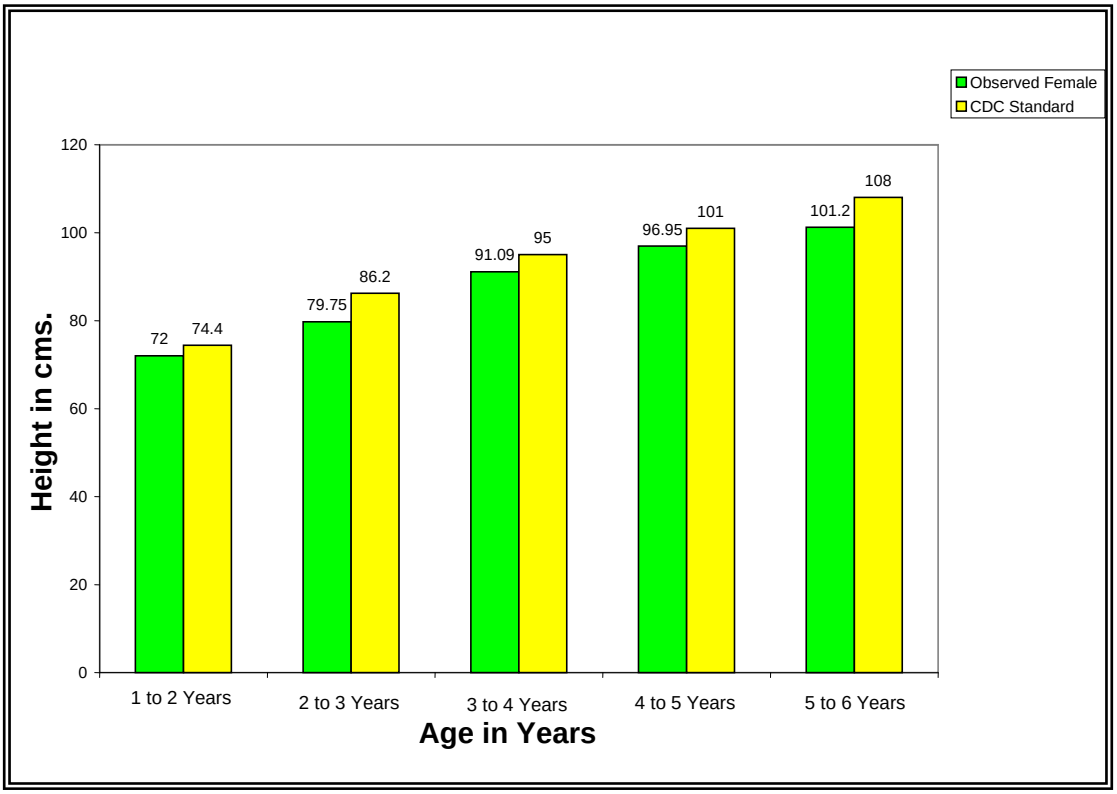
Table 3 : Table showing head circumference of preschool children of different age groups with CDC reference values.

Age group	Boys					Girls				
	Frequ e-ncy	Observed data		CDC Standard s	Z–score	Freq u-ency	Observed data		CDC Standard s	Z–score
		Mean	STD. DEV.				Mean	STD. DEV.		
1 to 2 Yrs	09	46.5	1.22	46.5	0	06	43.16	7.46	45.2	0.66
2 to 3 Yrs	39	47.88	2.18	48.7	2.92	20	45.55	4.98	47.5	1.75
3 to 4 Yrs	34	48.51	1.71	49.7	4.06	33	47.04	4.76	48.6	1.88
4 to 5 Yrs	34	48.5	1.89	50.5	6.17	30	47.36	2.63	49.7	4.87
5 to 6 Yrs	109	48.57	4.26	51.2	6.45	36	47.75	2.29	51.0	8.52

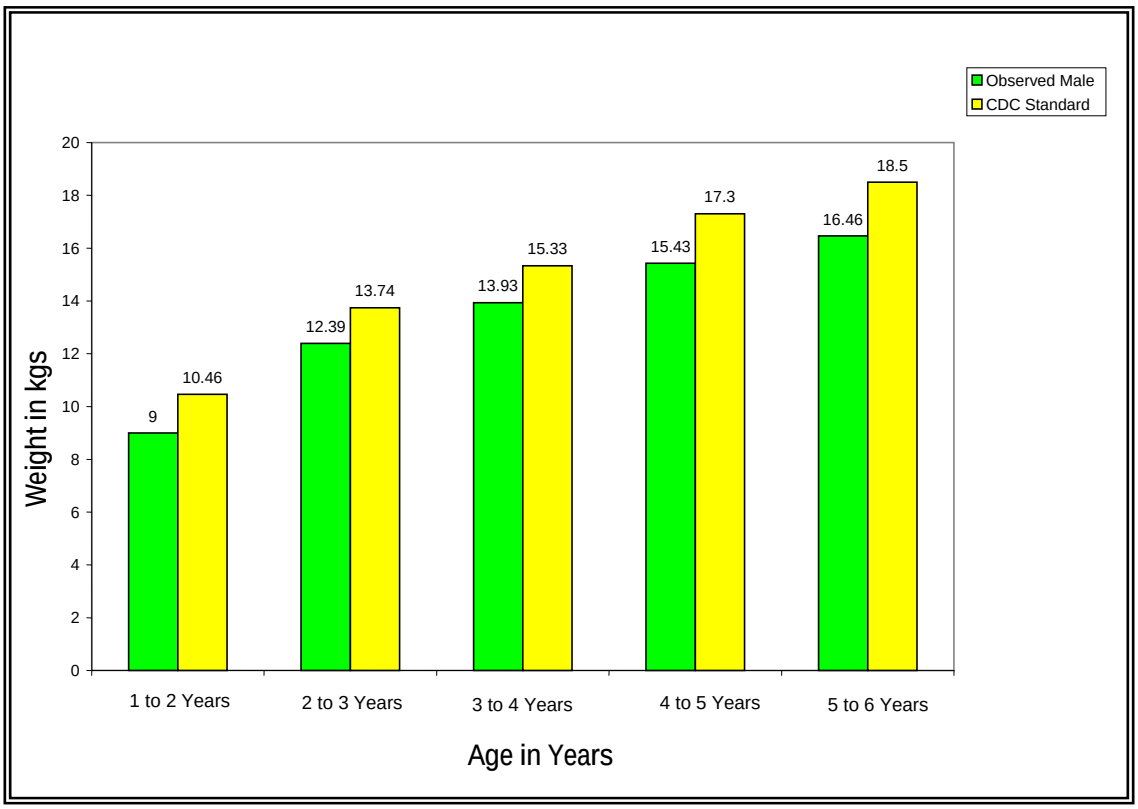
Graph 1 : Graph showing height in centimeters of preschool children (boys) with CDC reference values.



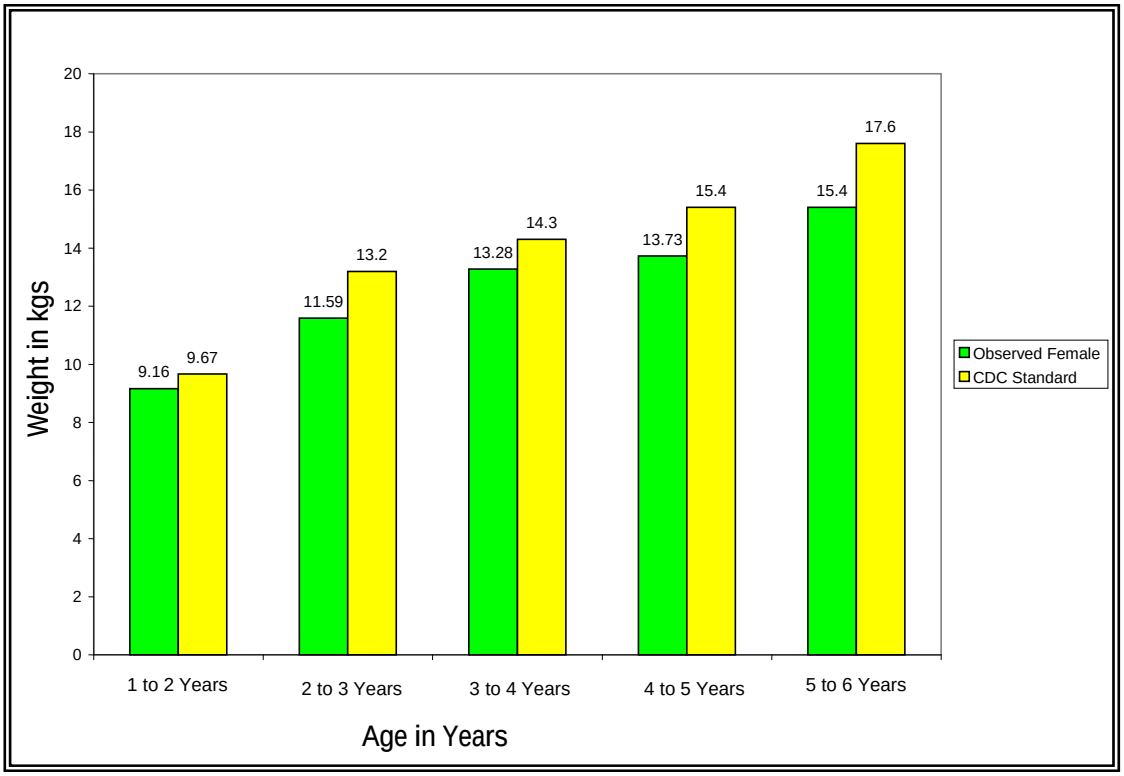
Graph 2 : Graph showing height in centimeters of preschool children (girls) with CDC reference values.



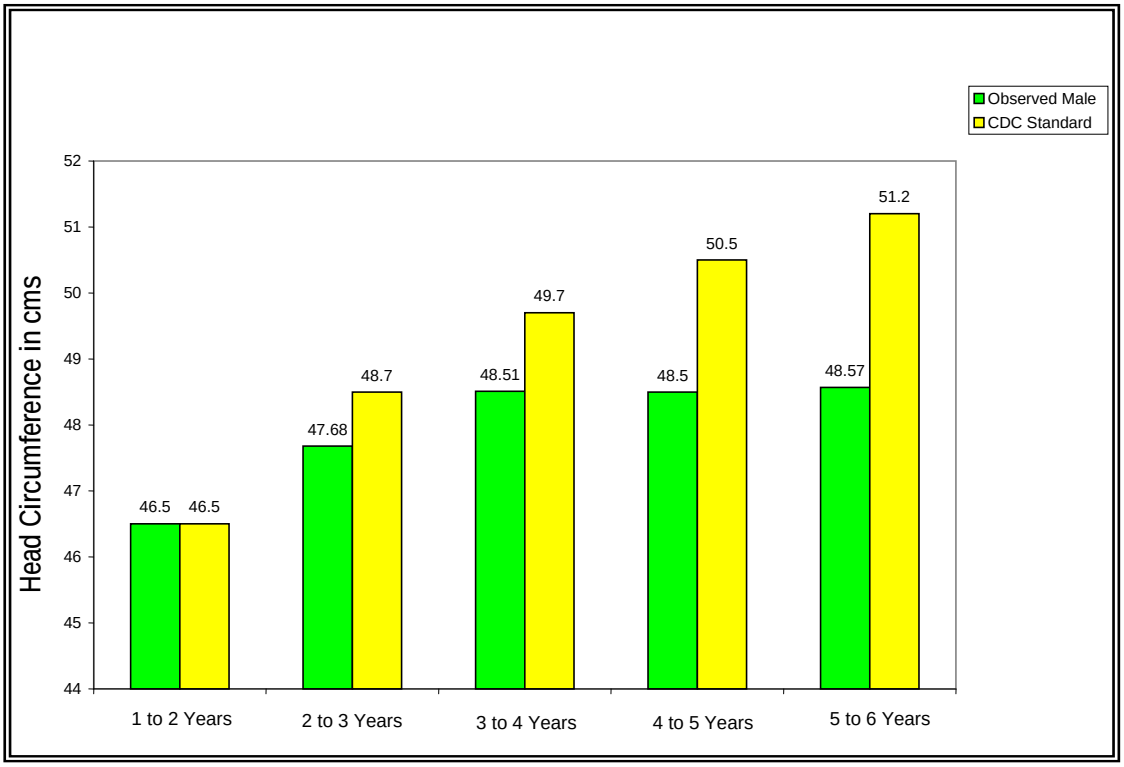
Graph 3 : Graph showing weight in kg of preschool children (boys) with CDC reference values.



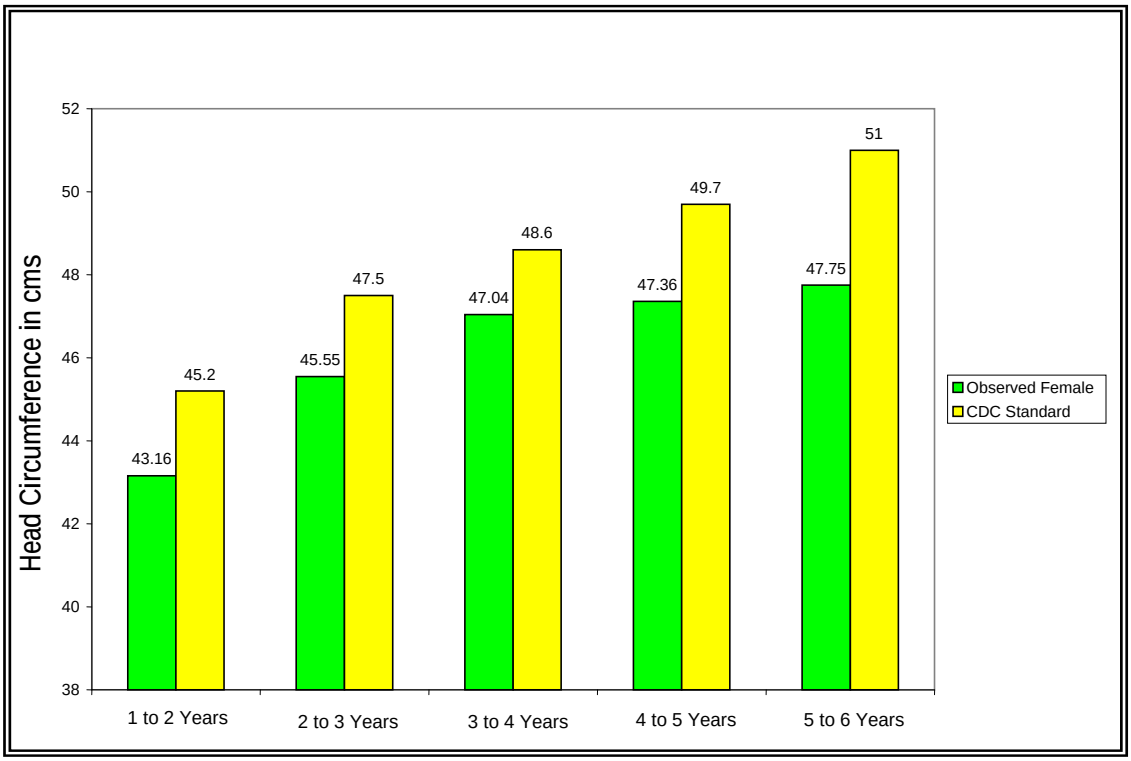
Graph 4 : Graph showing weight in kg of preschool children (girls) with CDC reference values.



Graph 5 : Graph showing head circumference of preschool children (boys) with CDC reference values.



Graph 6 : Graph showing head circumference of preschool children (girls) with CDC reference values.



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Hepatoprotective Activity of Phyllanthus Amarus Seeds Extracts in CCl4 Treated Rats: In Vitro & In Vivo

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Abstract - Aim - To study the hepatoprotective activity of Phyllanthus amarus seeds extracts in CCl4 treated rats. Methods - The crushed and dried seeds of Phyllanthus amarus were divided into two parts; one part was extracted successively with petroleum ether, benzene, chloroform and finally with methanol by soxhlet apparatus and concentrated by rotary vacuum. The other part is extracted by cold maceration process for aqueous extraction. The extracts were used for In-vitro and In-vivo studies to analyze the reparative activity of liver injury due to CCl4 in rats. Results - In-vitro models like Reducing power, Superoxide anion scavenging activity, Hydroxyl radical scavenging activity and Nitric oxide radical scavenging activity were carried out with methanolic extract of Phyllanthus amarus seeds for its antioxidant properties. A protective activity could be demonstrated in the CCl4 induced liver damage in rats. In-vivo methanolic and aqueous extracts of the seeds of Phyllanthus amarus 250mg/kg were found to have protective properties in rats with CCl4 induced liver damage as judged from serum biochemical enzyme marker activities and histopathological studies..

Keywords : *Hepatoprotective activity, Phyllanthus amarus seeds, CCl4 and Methanolic extract.*

GJMR-G Classification : *NLMC Code: WI 740,QY 60.R6, W 20.55.A5*

Strictly as per the compliance and regulations of:

Hepatoprotective Activity of *Phyllanthus Amarus* Seeds Extracts in CCl_4 Treated Rats: *In Vitro* & *In Vivo*

Syed Asad B [α], Iqbal MM [α], Kiranmai M [α] & Ibrahim M [α σ]

Abstract - Aim - To study the hepatoprotective activity of *Phyllanthus amarus* seeds extracts in CCl_4 treated rats.

Methods - The crushed and dried seeds of *Phyllanthus amarus* were divided into two parts; one part was extracted successively with petroleum ether, benzene, chloroform and finally with methanol by soxhlet apparatus and concentrated by rotary vacuum. The other part is extracted by cold maceration process for aqueous extraction. The extracts were used for *In-vitro* and *In-vivo* studies to analyze the reparative activity of liver injury due to CCl_4 in rats.

Results - *In-vitro* models like Reducing power, Superoxide anion scavenging activity, Hydroxyl radical scavenging activity and Nitric oxide radical scavenging activity were carried out with methanolic extract of *Phyllanthus amarus* seeds for its antioxidant properties. A protective activity could be demonstrated in the CCl_4 induced liver damage in rats. *In-vivo* methanolic and aqueous extracts of the seeds of *Phyllanthus amarus* 250mg/kg were found to have protective properties in rats with CCl_4 induced liver damage as judged from serum biochemical enzyme marker activities and histopathological studies.

Conclusion - The methanolic and aqueous extracts of *Phyllanthus amarus* seeds do have a protective capacity both *In-vitro* and *In-vivo* models of CCl_4 mediated liver injury.

Keywords : Hepatoprotective activity, Phyllanthus amarus seeds, CCl_4 and Methanolic extract.

I. Introduction

The liver is an organ of paramount importance not only for its metabolism of various xenobiotics and environmental pollutants [1] but for its unique and considerable regenerative capacity, even a moderate cell injury is not reflected by measurable change in its metabolic functions. However, some of its functions are so sensitive that abnormalities start appearing depending upon the nature and degree of its initial damage [2].

Reactive Oxygen Species (such as H_2O_2, O_2^{2-}, and OH^-, collectively known as ROS) play important physiological functions and can also cause extensive cellular damage. Cells are provided with efficient molecular strategies to control strictly the intra cellular ROS level and to maintain the balance between oxidant and antioxidant molecules. Oxidative stress, resulting from an imbalance between the generation of ROS and the antioxidant defense capacity of the cell [3], effects major cellular components including lipids, proteins and DNA. This phenomenon is closely associated with a number of human disorders such as many degenerative diseases including cardiovascular disease, diabetes, cancer, neurodegenerative disorders [4,5] and with almost all liver pathologies [6-8]. All these conditions appear mostly related to chronic oxidative stress. However, the acute exposure to high levels of ROS seems to be responsible for the development of different damages such as during ischemis/reperfusion in liver [9-10]. Carbon tetrachloride (CCl_4) (2 mg / kg, i.p) is acute hepatotoxic agent, which induces peroxidative degeneration of membrane lipids causing hypo perfusion of the membrane. Cytosolic enzyme like SGPT, SGOT and ALP.

A number of medicinal plants are used in traditional system of medicinal for the management of liver disorders. Nature has given us a large number of medicinal plants, some of which are yet to be explored and validated for their medicinal value. The 21st century has seen a paradigm shift toward therapeutic evaluation of herbal products in liver diseases, carefully synergizing the strengths of traditional medicine with the modern concept of evidence based medical evaluation, standardization and randomized placebo controlled clinical trials to support clinical efficacy. Several herbs are known to possess antioxidant properties and may be useful as liver protective agents [10].

The herbs containing antioxidant principles are reported to be highly effective in preventing or curing the liver toxicities due to above mentioned challenges. In the present study, the herb *Phyllanthus amarus* containing polyphenolic compounds is selected to assess hepatoprotective activity [11].

II. Materials and Methods

a) Plant Material

The seeds of *Phyllanthus amarus* were collected from local gardens of Tirupathi. The plant was

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identified and authenticated by Dr. Madava Chetty, Assosiate Professor, Department of Botony, S.V.University, Tirupathi, AP, India.

b) Preparation of Extracts

The crushed and dried seeds of *Phyllanthus amarus* were divided into two parts; one part was extracted successively with petroleum ether, benzene, chloroform and finally with methanol by soxhlet extraction and concentrated by rotary vacuum [12]. The other part is extracted by cold maceration process for aqueous extraction [13]. The obtained extracts were dried by evaporation. The yield 30% w/w and 15.6% w/w were stored in refrigerator and weighed quantities were suspended in tween 80 and 2% tragacanth solution respectively for the experiment. The extracts were used for In-vitro and In-vivo studies to analyze the reparative activity of liver injury due to CCl_4 treated rats.

c) Experimental Animals

Male albino rats weighing 130-160g were obtained from the animal house of Nizam Institute of Pharmacy, Hyderabad and housed in Polycarbonate cages. The rats had free access to standard pellet chow and water *ad libitum* throughout the experiment with the exception of some experiments (see below) in which the animals were deprived of food, but not water, for 18-24 hr. before the experiments were performed. After procurement, all the animals were divided into different groups and were left for one week for acclimatization to experimentation room and were maintained on standard conditions (23°c, 60%-70% relatively humidity and 12 hr. photo period). There were five animals in each group for observational screening. All experimental protocols described below were approved by the ethical board.

d) Acute Oral Toxicity Studies

The acute oral toxicity study is determined according to the guidelines of Organization for Economic Co-operation & Development (OECD) following the up & down method (OECD guideline No. 423). Based on the method, a limit test was performed to categorize the toxicity class of the compound and then main test was performed on three female rats to estimate the exact LD50. The animals were fasted overnight with free access to water, weighed and a single dose of the test substance was administered. Animals were observed individually during first 30 min, periodically during 48 h with special attention given during first 4 h (short-term toxicity) and daily, thereafter for total of 14 days (short-term toxicity). LD50 was found to be greater than 2500 mg/kg, in limit test. The test substance could be classified in the hazard classification as Class 5 - 2000 mg/kg <LD50 <5000 mg/kg in the globally harmonized system (GSH). LD50 of test drug was found to be 2500mg/kg from main test[14].

e) Hepatotoxins

It is emphasized that hepatotoxin that cause acute hepatitis should have close resemblance with the viral hepatitis, clinically, biochemically and histopathologically. Certain drugs are also responsible for many hepatic diseases, such as chronic hepatitis, fatty liver, cirrhosis and certain vascular lesions of liver. In many instances drug induced hepatitis is distinguishable from viral hepatitis chemical injured viral hepatitis for experimental studies should be severe enough to cause cell death or to modify hepatic functions. The mechanism of acute hepatic injury depends upon the chemical compound and the species of animal used. We have studied the hepatoprotective activity against CCl_4 induced hepatotoxicity.

CCl_4 is one of the most powerful hepatotoxin in terms of severity of injury it causes toxic necrosis leading to biochemical changes having clinical features similar to those of acute viral hepatitis [15], liver injury was produced by administration of CCl_4 mixed with tween 80. Animals were given single doses of CCl_4 2 mg/kg, i.p per day throughout the experimental setup. Control animals received an equal volume of tween 80.

III. Methods for the Hepatoprotective Evaluation

a) In-vitro antioxidant activity

In-vitro models were carried out to evaluate antioxidant activity are Reducing power, Superoxide anion scavenging activity, Hydroxyl radical scavenging activity and Nitric oxide radical scavenging activity.

1. *Reducing power*

The reducing power of methanolic and aqueous extracts of *Phyllanthus amarus* seeds were determined according to the method of Oyaizu [16]

Procedure: Extracts of *Phyllanthus amarus* seeds were mixed in 1ml of distilled water so as to get 20µg, 40µg, 60µg, 80µg, and 100µg concentration. This was mixed with phosphate buffer (2.5ml, 0.2M, pH 6.6) and potassium ferricyanide (2.5ml, 1%). The mixture was incubated at 50°C for 20 minutes. A portion (2.5ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and $FeCl_3$ (0.5ml, 0.1%), and the absorbance (OD) was measured at 700nm.

Increased absorbance of the reaction mixture indicates increased in reducing power.

The percent reducing power was calculated by using the formula:

$$\% \text{ increase in absorbance} = \frac{\text{Control OD-Test OD}}{\text{Control OD}} \times 100$$

2. *Superoxide anion scavenging activity*

Oxygen is essential for the survival of aerobic cells, but it has long been known to be toxic to them

when supplied at concentration greater than those in normal air. The biochemical mechanisms responsible for oxygen toxicity include lipid peroxidation and the generation of $H_2O_2^+$ the superoxide radical, O_2^+. This superoxide radical can inhibit or propagate the process of lipid peroxidation. Measurement of superoxide anion scavenging activity of *Phyllanthus amarus* seeds was done by using the method explained by Nishimiki [17] and modified by Ilhami et al.,

Procedure: About 1ml of nitroblue tetrazolium (NBT) solution (156μM NBT in 100mM phosphate buffer, pH 7.4), and 0.1ml of sample solution of methanolic extract of *Phyllanthus amarus* seeds and standard in water was mixed. The reaction was started by adding 100μl of phenazine methosulphate (PMS) solution (60μM PMS in 100mM phosphate buffer, pH7.4) to the mixture. The reaction mixture was incubated at 25°C for 5 minutes, and the absorbance at 560nm was measured against blank.

Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. % inhibition of OD was calculated by using the formula mentioned earlier.

3. *Hydroxyl radical scavenging activity*

In biochemical systems, superoxide radical and H_2O_2 react together to form the hydroxyl radical which can attack and destroy almost all known biochemical [18]

Phenylhydrazine when added to erythrocyte hosts cause peroxidation of endogenous lipids and alteration of membrane fluidity. This peroxidation damage to erythrocytes is probably initiated by active oxygen species like $O_2^{\bullet}$, $OH^{\bullet}$ and H_2O_2 which are generated in solution from auto-oxidation of phenyl hydrazine. This forms the basis of this experiment.

Procedure: Hydroxyl radical generation by phenyl hydrazine has been measured by the 2-deoxyribose degradation, assay of Halliwell and Gutteridge[19] In 50mM phosphate buffer (pH 7.4), 1mM deoxyribose and 0.4ml of methanolic extract of *Phyllanthus amarus* seeds and standard were taken. 0.2ml phosphate buffer was added to make reaction solution 1.6ml. after 10 min incubation 0.4ml of o.2mM phenyl hydrazine was added. Incubation was terminated after 1hr and 4 hrs and 1ml each of 2.8% TCA and 1% (w/v) thiobarbituric acid were added to the reaction mixture and heated for 10 minutes in a boiling water bath. The tubes were cooled and absorbance was taken at 532nm.

Decreased in absorbance is indicating the hydroxyl free radical scavenging activity. The % reduction in the OD is calculated.

4. *Nitric oxide radical scavenging activity*

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons etc. involved in the regulation of various physiological processes. Excess concentration of NO is associated with several diseases oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions, which acts as free radicals. This forms the basis of this experiment.

Procedure: Nitric oxide (NO) radial were generated from sodium nitroprusside solution of physiological pH[20]. Sodium nitroprusside (1ml of 10mM) were mixed with 1ml of methanolic extract of *Phyllanthus amarus* seeds of different concentration (20-100μg/ml) in phosphate buffer (pH 7.4). The mixture of incubated at 25°C for 150 min. To 1ml of incubated solution, 1ml Griess's reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) was added. Absorbance was read at 546nm. % inhibition of OD was calculated by using the formula mentioned earlier.

b) In- vivo antioxidant activity

The Wister rats were divided into 5 groups of 6 individuals each [21] for 5days study.

Group I	Negative Control	Tween 80 1ml/kg I.P.
Group II	Positive Control	CCl_4 2mg/kg I.P.
Group III	Standard	Liv 52 100mg/kg p.o.
Group IV	Methanolic Extract	250mg/kg p.o.
Group V	Aqueous Extract	250mg/kg p.o.

Table: 1

Group I received Tween 80 1ml/kg I.P., on 2nd and 3rd day. Group II, III, IV and V received CCl_4 2mg/kg I.P., on 2nd, 3rd, days. The Group III, IV, V, received Liv 52 100mg/kg p.o., Methanolic Extract 250mg/kg p.o., Aqueous Extract 250mg/kg p.o., before 30min of toxicant respectively. Animals were sacrificed on the 5th day under mild ether anesthesia.

The blood samples were collected from retro orbital plexus for evaluating the serum biochemical parameters and liver was dissected out, blotted off blood, washed with saline and stored in 10% formalin and preceded for histopathology to evaluate the details of hepatic architecture in each group microscopically.

c) Statistical analysis

The statistical analysis was carried out by one-way analysis of variance (ANOVA). The values are represented as mean ± SE. Comparison of mean values of different groups treated with different dose levels of extracts and positive controls were estimated by Tukey's Multiple Comparison Test. $P < 0.05$ was considered significant.

IV. Results

In vitro: It was observed that methanolic extract demonstrated dose dependent increase in the reducing property. 25mcg sodium metabisulphate (Std.) showed 73.09% reducing property. Methanolic extract at 100mcg had more reducing property than 25mcg

sodium metabisulphate i.e. 88.88%.

There was percentage increase in the superoxide anion scavenging activity. Methanolic extract showed lesser activity than standard i.e. 70.64%.

It was observed that methanolic extract demonstrated dose dependant percentage increase in the hydroxyl radical scavenging activity in case of 1 hr. incubation period i.e. 64.20% But, showed higher in case of 4 hrs incubation period i.e. 55.55%

It was observed that that methanolic extract demonstrated dose dependant percentage increase in the nitric acid radical scavenging activity. 25mcg sodium metabisulphate (Std.) showed 70.52% activity. Methanolic extract had more nitric oxide radical scavenging activity at 100 mcg than compared to 25 mcg sodium metabisulphate. i.e. 71.78%.

In vivo: There was increased level of SGPT in CCl4 treated group 312.420U/L. The SGPT level was restored to 77.84 U/L by 250mg/kg methanolic extract of the seeds which was near to effect of 100mg/kg Liv.52 i.e. 65.395 U/L.

SGOT levels increased significantly in CCl4 treated group i.e. 318.412 U/L. methanolic extract of the seeds reduced the elevated level of SGOT to 72.69 U/L, which was very near to100mg/kg Liv.52 i.e. 71.212 U/L.

In case of bilirubin, methanolic extract reduced the level of bilirubin by 4.892mg/dl to 1.20mg/dl.

There was increase in ALP level observed in CCl4 treated group (235.86 IU/L). ALP level was restored to 97.44 IU/L by methanolic extract of the seeds which was near to effect of 100mg/kg Liv.52 i.e. 95.68 IU/L.

There was no significant rise in total cholesterol and triglycerides levels in CCl4 treated group. Significant effect was observed with in methanolic extract and aqueous extract was comparable with 100mg/kg Liv.52.

Liver section of control rat showed normal hepatocytes and normal architecture (Figure 1A). Liver sections from CCl4 treated rats demonstrated the destruction of architectural pattern, nodule formation in the lobular zone, inflamed periportal zone, and moderate inflammation of portal area (Figure 1B). Liver sections from Liv 52 treated rats showed regeneration of normal hepatocytes (Figure 1C). Liver sections from a methanolic extract of *Phyllanthus amarus* seeds treated rat showing normal lobular architecture (Figure 1D). Liver section from an aqueous extract of *Phyllanthus amarus* seeds treated rat showing normal lobular architecture no necrosis or fatty changes or any inflammatory reaction can be seen. (Figure 1E). These histopathological findings demonstrate a hepatoprotective effect of the extracts against CCl4-mediated liver damage.

VI. Discussion

The purpose of this study was to explore the hepatoprotective effect of extracts of *Phyllanthus amarus* seeds in the hepatic damage caused by CCl_4. Administration of CCl_4 to normal rats increased serum levels of AST, ALT, ALP, and bilirubin. The enzymes leaking out from damaged liver cells into circulating blood represent the damage to hepatic cells. It is well established that the toxic metabolite of CCl_4, a free radical CCl_3 is responsible for damage to liver cells. *In-vitro* models like Reducing power, Superoxide anion scavenging activity, Hydroxyl radical scavenging activity and Nitric oxide radical scavenging activity were carried out with methanolic extract of *Phyllanthus amarus* seeds for its antioxidant properties. A protective activity could be demonstrated in the CCl_4 induced liver damage in rats. *In-vivo* methanolic and aqueous extracts of the seeds of *Phyllanthus amarus* 250mg/kg were found to have protective properties in rats with CCl_4 induced liver damage and caused statistically significant decrease in all the above parameters.

Liver section of control rat showed normal hepatocytes and normal architecture (Figure 1A). Liver sections from CCl4 treated rats demonstrated the destruction of architectural pattern, nodule formation in the lobular zone, inflamed periportal zone, and moderate inflammation of portal area (Figure 1B). Liver sections from Liv 52 treated rats showed regeneration of normal hepatocytes (Figure 1C). Liver sections from a methanolic extract of *Phyllanthus amarus* seeds treated rat showing normal lobular architecture (Figure 1D). Liver section from an aqueous extract of *Phyllanthus amarus* seeds treated rat showing normal lobular architecture no necrosis or fatty changes or any inflammatory reaction can be seen. (Figure 1E). These histopathological findings demonstrate a hepatoprotective effect of the extracts against CCl4-mediated liver damage. The methanolic and aqueous extracts of *Phyllanthus amarus* seeds do have a protective capacity both *In-vitro* and *In-vivo* models of CCl_4 mediated liver injury

VII. Histopathological Studies in ccl4 Induced Hepatotoxicity

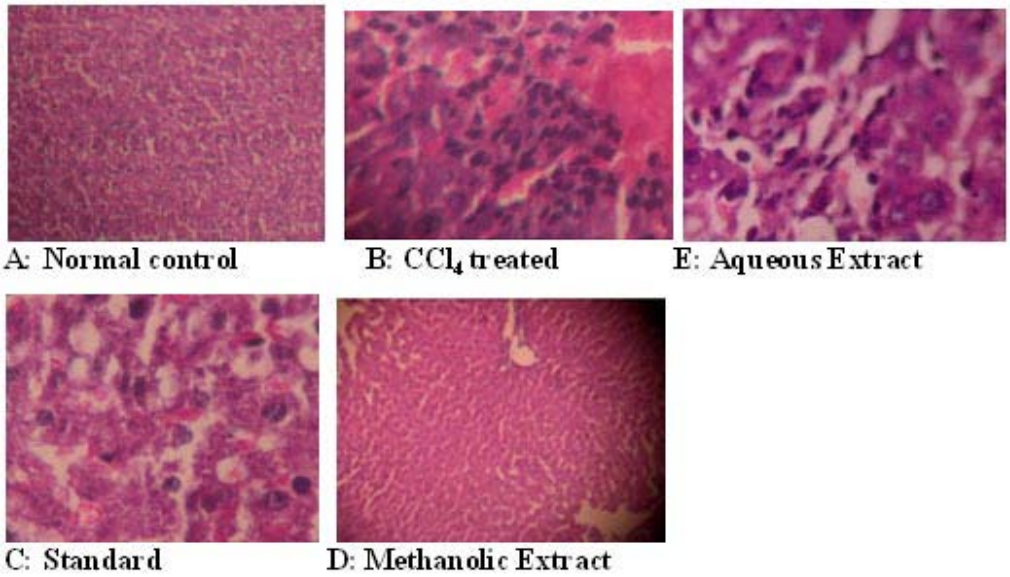


Figure 1: Photograph of rat liver shows (HE, × 100). A: Liver of a control rat showing normal hepatocytes and normal architecture; B: Liver section from a CCl4 treated rat demonstrating the destruction of architectural pattern, nodule formation in the lobular zone, inflamed periportal zone, moderate inflammation of portal area; C: Liver section from a Liv 52 treated rat showing regeneration of normal hepatocytes; D: Liver section from a methanolic extract of *Phyllanthus amarus* seeds treated rat showing normal lobular architecture; E: Liver section from a aqueous extract of *Phyllanthus amarus* seeds treated rat showing normal lobular architecture no necrosis or fatty changes or any inflammatory reaction can be seen.

Table 2 : Reducing power activity of methanolic extract of *Phyllanthus amarus* seeds

Group	Absorbance Mean±SEM	% Increase
Control	0.171±0.001	---
Control + Std 25μg	0.296±0.002***	73.09
Control + methanolic extract 20μg	0.182±0.001*	6.43
Control + methanolic extract 40μg	0.216±0.002***	26.31
Control + methanolic extract 60μg	0.259±0.002***	51.46
Control + methanolic extract 80μg	0.283±0.002***	65.49
Control + methanolic extract 100μg	0.323±0.002***	88.88

Values are the mean ± S.E.M., n=3

*Significance***P<0.001 and * P<0.05 compared to control. Std: Sodium metabisulphate*

Figure : 2

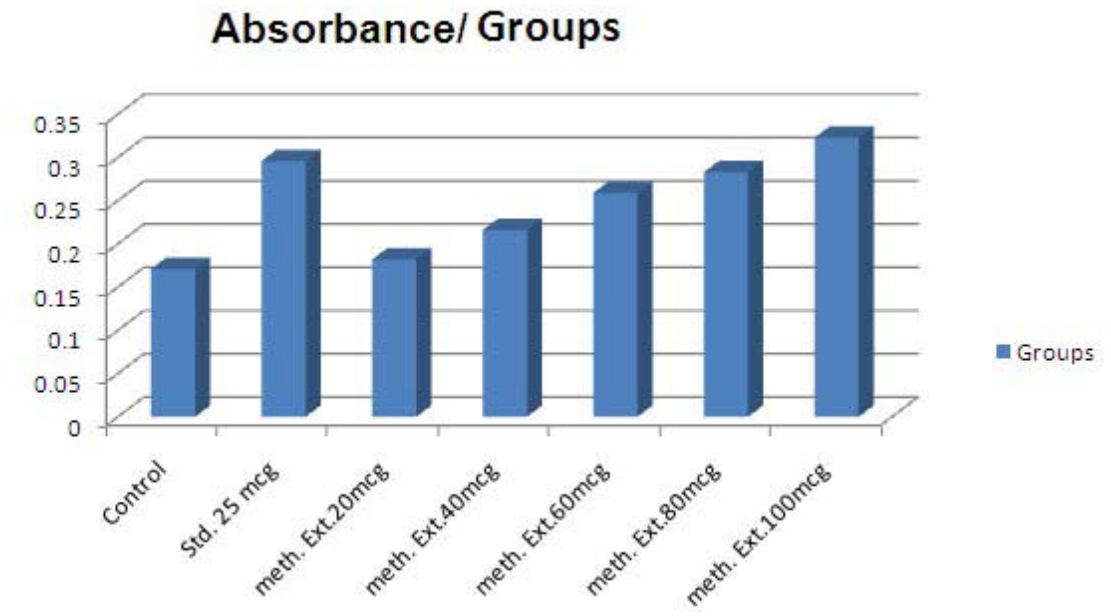


Table 3 : Superoxide anion scavenging activity of methanolic extract of *Phyllanthus amarus* seeds

Group	Absorbance Mean±SEM	% Increase
Control	0.862±0.020	---
Control + Std 25μg	0.225±0.001***	73.89
Control + methanolic extract 20μg	0.469±0.001***	45.59
Control + methanolic extract 40μg	0.422±0.002***	51.04
Control + methanolic extract 60μg	0.377±0.002***	56.26
Control + methanolic extract 80μg	0.319±0.002***	62.99
Control + methanolic extract 100μg	0.253±0.002***	70.64

Values are the mean ± S.E.M., n=3
*Significance***P<0.001 compared to control. Std: Sodium metabisulphate*

Figure : 3

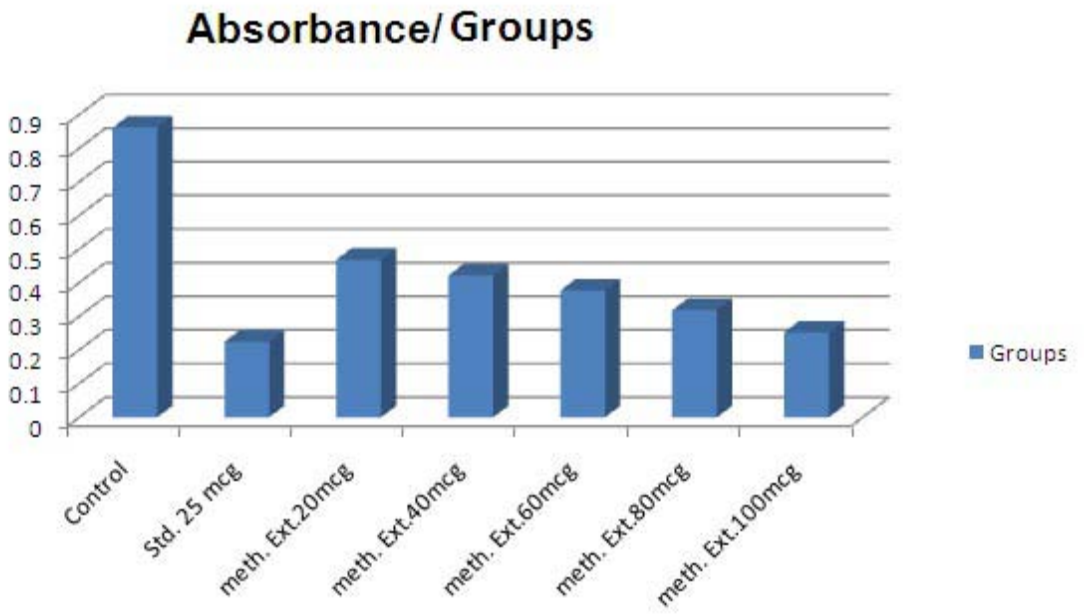


Table 4 : Hydroxyl radical scavenging activity of methanolic extract of *Phyllanthus amarus* seeds

Group	Absorbance Mean±SEM	% Inhibition	Absorbance Mean±SEM	% Inhibition
Control	0.352±0.0005	---	0.405±0.0020	---
Control + Std 25µg	0.118±0.0037***	66.47	0.182±0.0040***	55.06
Control + methanolic extract 20µg	0.296±0.0028***	15.90	0.331±0.003***	18.27
Control + methanolic extract 40µg	0.243±0.0020***	30.96	0.253±0.001***	28.14
Control + methanolic extract 60µg	0.195±0.0030***	46.59	0.206±0.002***	37.53
Control + methanolic extract 80µg	0.151±0.002***	57.10	0.180±0.003***	49.13
Control + methanolic extact 100µg	0.126±0.0026***	64.20	0.159±0.0041***	55.55

Values are the mean ± S.E.M., n=3

*Significance***P<0.001 compared to control. Std: Sodium metabisulphate*

Figure : 4a

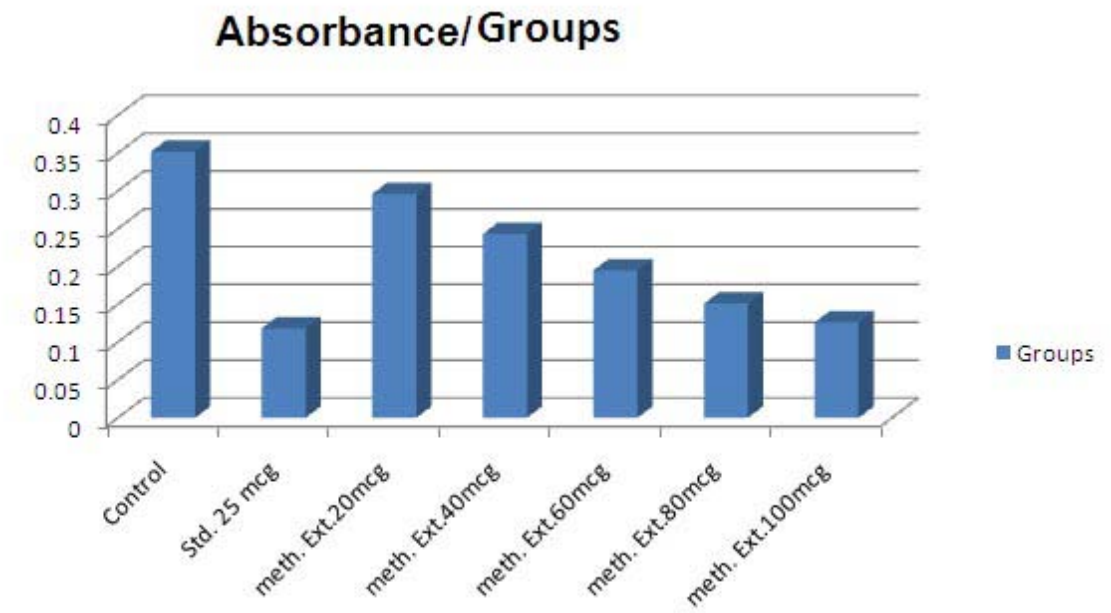


Figure: 4b

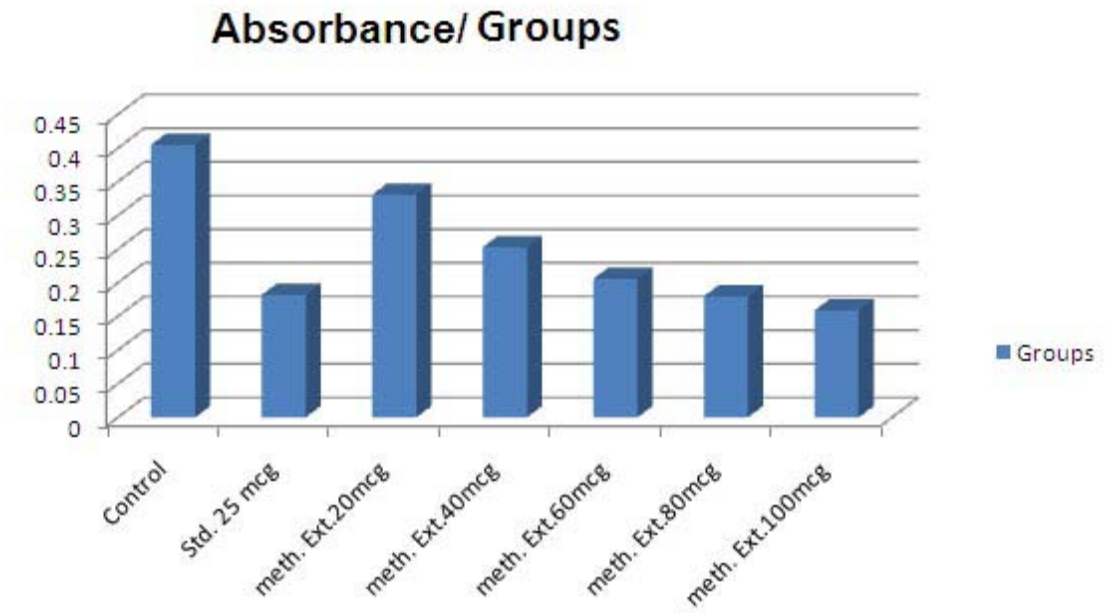


Table 5 : Nitric oxide radical scavenging activity of methanolic extract of *Phyllanthus amarus* seeds

Group	Absorbance Mean±SEM	% Increase
Control	0.397±0.0015	---
Control + Std 25μg	0.117±0.0015***	70.52
Control + methanolic extract 20μg	0.242±0.0008***	39.04
Control + methanolic extract 40μg	0.218±0.0017***	45.08
Control + methanolic extract 60μg	0.186±0.0017***	53.14
Control + methanolic extract 80μg	0.142±0.0026***	64.23
Control + methanolic extract 100μg	0.112±0.0028***	71.78

Values are the mean ± S.E.M., n=3

*Significance***P<0.001 compared to control. Std : Sodium metabisulphate*

Figure : 5

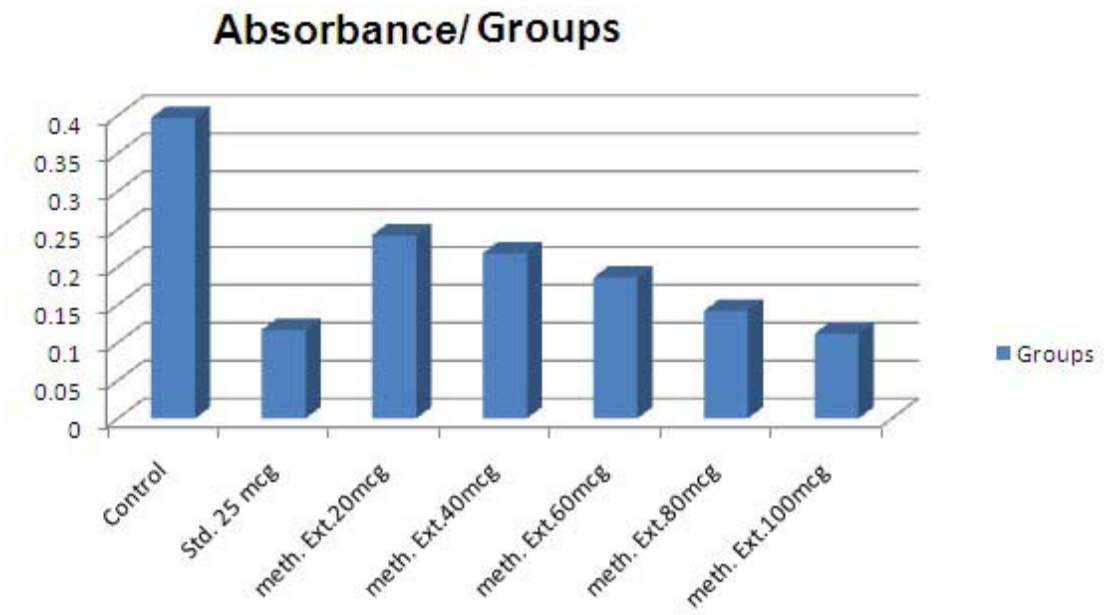


Table 6 : Effect of methanolic & aqueous extracts of *Phyllanthus amarus* seeds on biochemical markers in CCl4 induced hepatotoxicity

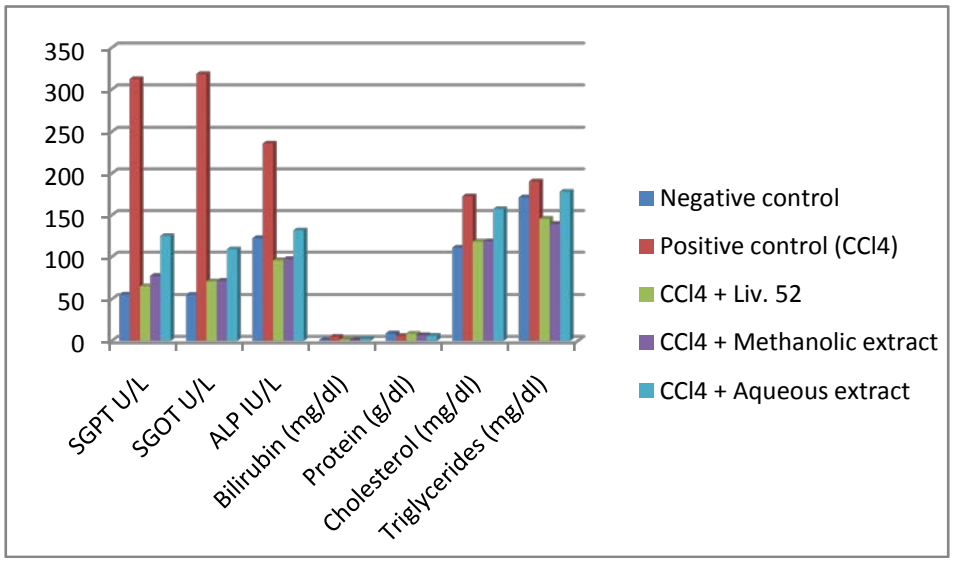
Treatment	Biochemical parameters Mean±SEM						
	SGPT U/L	SGOT U/L	ALP IU/L	Bilirubin (mg/dl)	Protein (g/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Negative control 1ml/kg Tween 80	55.558 ± 3.331	55.216 ± 5.617	122.29 ± 6.486	0.926 ± 0.029	8.88 ± 0.34	110.88 ± 10.771	171.22 ± 7.198
Positive control CCl4 treated 2ml/kg	312.42 ± 14.275	318.412 ± 13.543	235.86 ± 8.207	4.892 ± 0.451	5.85 ± 0.40	172.62 ± 10.522	190.36 ± 7.516
CCl4 + Liv. 52 2ml/kg + 100mg/kg	65.395 ±	71.212 ±	95.68 ±	1.546 ±	8.46 ±	118.25 ±	145.48 ±

	8.700***	6.843***	9.485***	0.301***	0.24**	8.980**	8.253***
CCl4 + Methanolic extract 2ml/kg + 250mg/kg	77.88 ± 3.083***	72.04 ± 5.352***	97.44 ± 7.425***	1.204 ± 0.190***	6.74 ± 0.56*	118.53 ± 5.185**	139.39 ± 4.406***
CCl4 + Aqueous extract 2ml/kg + 250mg/kg	124.77 ± 5.319***	108.96 ± 2.597***	131.62 ± 10.462***	2.475 ± 0.188***	5.92 ± 0.33	157.11 ± 9.494	177.99 ± 6.207

Values are the mean ± S.E.M of six rats / treatment
*Significance *P<0.05, **P<0.01 and ***P<0.001, compared to CCl4 treatment.*

Effect of methanolic & aqueous extracts of *Phyllanthus amarus* seeds on biochemical markers in CCl4 induced hepatotoxicity

Figure No. 6



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Application of Value Stream Mapping to Eliminate Waste in an Emergency Room

By Preetinder Singh Gill
Eastern Michigan University

Abstract - Value Stream Mapping (VSM) is a lean/quality management tool which assists in establishing the current state of a process while aiding to uncover opportunities for improvement vis-à-vis the seven sources of waste. This research effort involves a review of existing literature pertaining to application of the VSM tool in hospital emergency rooms/departments. The paper will present the potential benefits emanating from application of VSM along with assessing its effectiveness in scenarios where it has been implemented already. Furthermore, challenges faced in implementation of the VSM tools are collated. Various solutions to address these challenges have been presented in the light of tribulations faced by today's healthcare industry.

GJMR-I Classification : NLMC Code: WA 778, WA 780

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Application of Value Stream Mapping to Eliminate Waste in an Emergency Room

Preetinder Singh Gill

Abstract - Value Stream Mapping (VSM) is a lean/quality management tool which assists in establishing the current state of a process while aiding to uncover opportunities for improvement vis-à-vis the seven sources of waste. This research effort involves a review of existing literature pertaining to application of the VSM tool in hospital emergency rooms/departments. The paper will present the potential benefits emanating from application of VSM along with assessing its effectiveness in scenarios where it has been implemented already. Furthermore, challenges faced in implementation of the VSM tools are collated. Various solutions to address these challenges have been presented in the light of tribulations faced by today's healthcare industry.

I. Introduction

Lean principles originated with objectives of reducing waste, decreasing inventory and operating costs, improving product quality, increasing productivity, and ensuring job satisfaction (Womack et al., 1990). Lean took roots at Toyota Motor Company's shop floor in Japan about 50 decades. Over time the approach has spread into many organizations world wide regardless of their core businesses

The lean approach includes many principles and activities. These are shown in the figure 1. Essence of the lean approach, according to Burgess and Radnor (2008), involves: 1) identifying what creates value for customers and understanding how those requirements can be met, 2) developing a value stream for each product or process family and identifying waste, 3) maintaining a continuous flow by standardizing processes, practices, and procedures, 4) creating a pull system at all steps where continuous flow is not possible and 5) managing perfection by removing or eliminating non-value added activities.

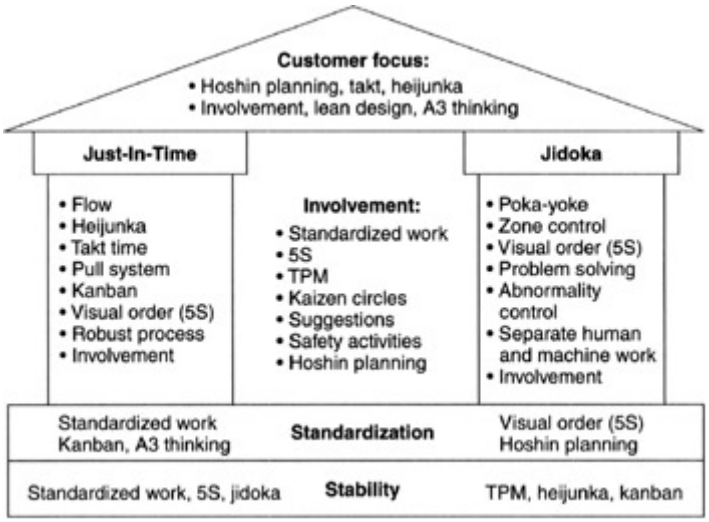

Figure 1 : Lean Principles and Activities (Pascal, 2007)

Value Stream Mapping - VSM - is a lean tool which assists establishing the current state of a process while aiding to uncover opportunities for improvement. The value stream mapping process could be divided into four steps: 1) identify a product or service family, 2) create current value stream to determine the current problem from the standpoint of the organization and customer, 3) create the ideal future state map, 4) identify corrective actions needed to close the gap between the current state and the ideal future state, 5) implement the corrective actions, 6) create a new current state map to verify if problems identified in step 2 have been eliminated/mitigated. Importance of keenly vested cross-functional team work is indispensable to success of VSM process.

Keyte and Locher (2004) explain that the VSM approach, which has been traditionally used in manufacturing settings, could be applied to service settings, including administrative processes, as well. Kim et al. (2006) conclude that despite inherent challenges in implementing lean principles, they can help deliver "…high¯quality and efficient care to

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patients…" in turn the health care sector could "…anticipate the same high level of success that the manufacturing and service industries have achieved using this approach" (p.199). In service settings, creating a current or a future state value stream map for a specific process could involve following steps: 1) determine the start and end points of the process, 2) identify all stakeholders, 3) identify metric which could be used as a stand-in for the value flowing through the process, 4) create a process flow by identifying all preceding and succeeding steps to a specific step, 5) quantify in terms of the metric identified in step 3 amount of useful and wasteful work, 6) identify opportunities for improvement and, 7) identify improvement actions to address opportunities for improvement. Some examples of the metrics include time and, monetary load. Furthermore, a set of icons and symbols may have to be defined to concisely depict the process and the flow of value.

While making a quantitative assessment, in terms of the identified metric, following evaluation criteria could be employed to determine whether each process step is:

Valuable, meaning whether it actually creates value from the standpoint of the customer... Capable, meaning the degree to which a good quality result is achieved every time ... Available, meaning the degree to which the step is able to operate when it is needed ... Adequate, meaning the degree to which capacity is in place to respond to customer orders as needed ... Flexible, means the degree to which a process step can switch over quickly and at low cost from one member of a product family to another. (Womack, 2006, p.150-151)

A value stream map is illustrated in figure 2. Typical VSM symbols as shown in figure 3. Software packages such as Microsoft Visio, eVSM, iGrafx and, Edraw Max could be used to draw value stream maps.

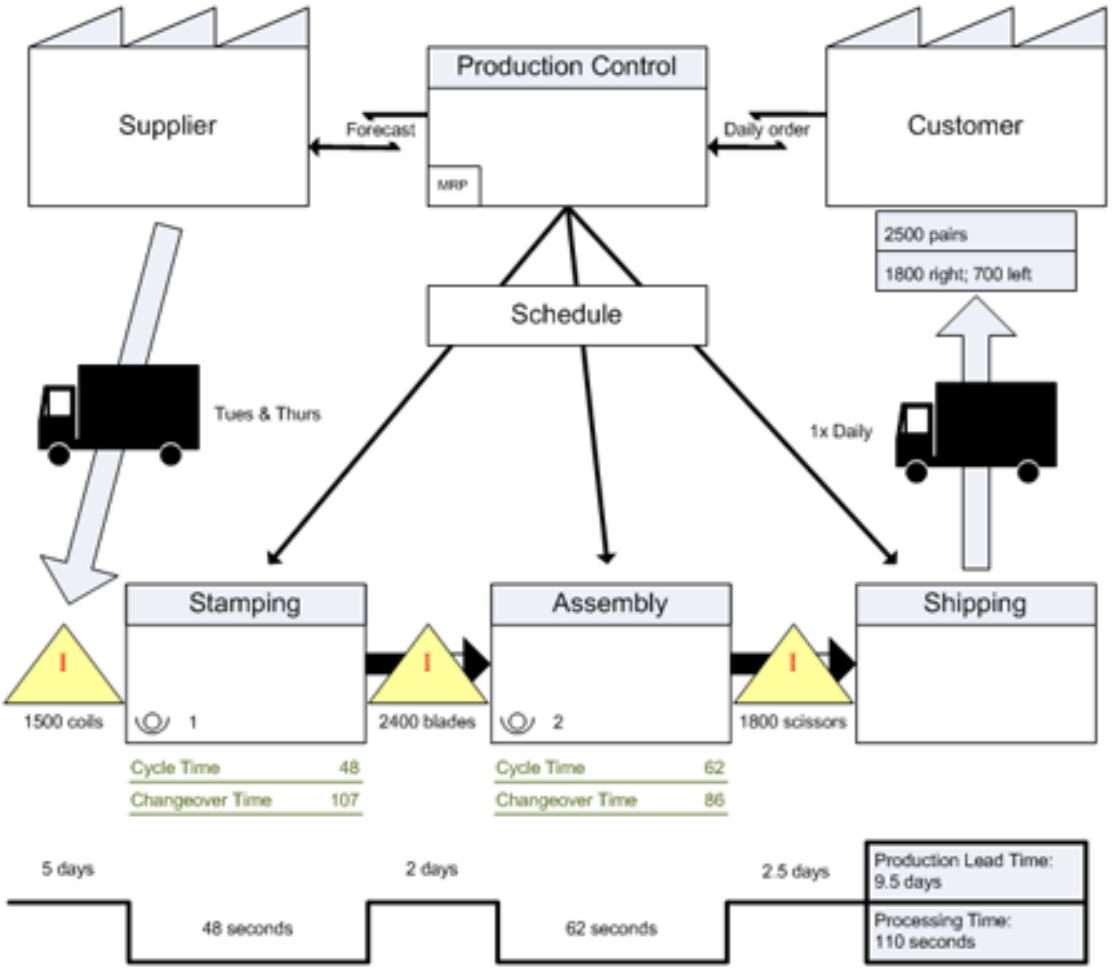

Figure 2 : An example value stream map (Microsoft, 2012)

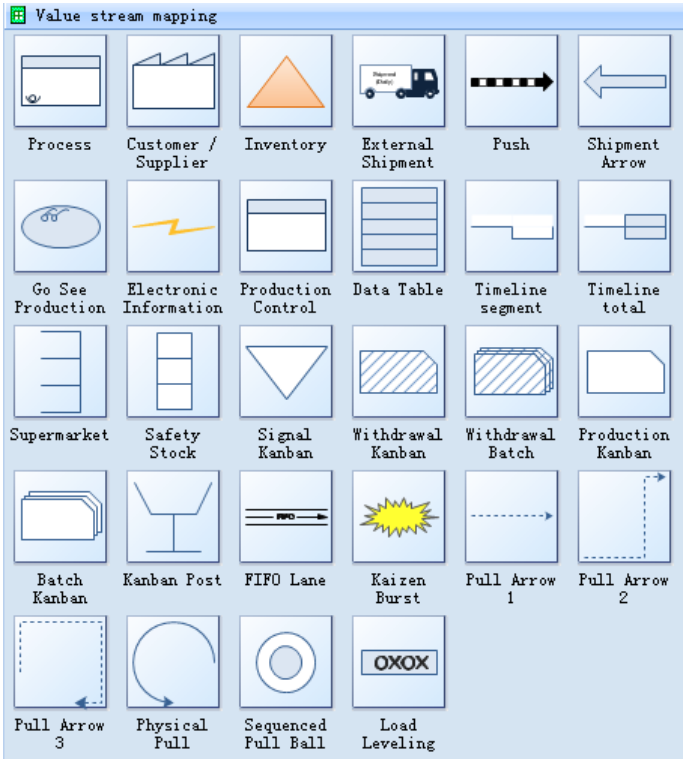

Figure 3 : Typical VSM symbols (EdrawSoft, 2012)

II. Health Care and Lean Practices

Rother and Shook (2003) suggest that value stream encompasses all actions, value added and non-value added, from concept to production and beyond, required in taking a product or service to customers. They further explicate that value stream mapping entails following the production path of a test piece in order to draw a visual representation of every involved process including material and information flow. Values stream mapping can help to identify issues associated with seven types of waste (Hines and Rich, 1997) in the flow of value. Additionally, the value stream mapping assists in establishing effective solutions to minimize the non value added steps thereby creating a lean system.

A 2009 report published by Thomson Reuters that U.S. healthcare system wastes $700 billion a year. Similarly The Boston Globe, in 2008, reported that waste in health care was close to $760 billion a year. The waste among other factors has been attributed to 1) misuse, overuse, underuse, of services and equipment, 2) errors and mistakes along with associated rework, 3) lack of communication and coordination, 4) other special cause variation. These wastes fall in alignment with the seven wastes described by Pascal (2007).

Emergency rooms/departments are integral to modern health care system. Merrian-Webster define emergency room as a hospital room or area staffed and equipped for the reception and treatment of persons with conditions (as illness or trauma) requiring immediate medical care. Much like the entire health care system the emergency rooms are rife with waste. Khurma et al. (2008) conclude that lean methodologies can be successfully implemented to address wastes associated with an emergency room including but not limited to transportation, over-processing waiting and motion. Similar conclusions were also drawn by Bush (2007) and Van Den Heuvel et al., (2006). Pursuant to the preceding discussion it can hence be argued that lean principles including VSM can be employed to systematically and systemically eliminate these sources of waste in the health care industry.

III. Implementation of Vsm in Emergency Room

Koelling et al. (2005) describe in detail the process of performing a VSM in an emergency room environment. They provide a theoretical and procedural basis of the VSM process which included emphasis on; 1) identifying the target system – services/process families – which needs to be improved, 2) constructing current and future state VSMs by using standard set of icons, symbols and arrows. The researchers constructed a process flow chart to properly chalk out the scope of their VSM related activities. The flow chart is supplemented with brief description of activities happening at each station. The researchers elucidate the methods to calculate cycle time, change over time, inventory time. The researchers acknowledge

challenges presented by health care sector might be different than manufacturing. However, they do assert that there is high probability of successfully leveraging VSM in a health care (emergency room) environment. Akin to Koelling et al., Manos et al., (2006) explain that VSM in health care should be associated with a continuous Plan-Do-Check-Act (PDCA) cycle.

Willoughby et al., (2010) studied wait time and service time for patients visiting emergency rooms. It was found the about half of the five hours spent by a patient in the emergency room was spent waiting. Using the VSM methodology the researchers calculated the value added, non value added and lead times for treating patients. Subsequently by applying the PDCA process the researchers inculcated many simple yet effective improvements. The researchers noted major challenges they faced included: 1) maintaining a smooth pace of lean activity during their project, 2) finding required human resources leading intermittent contribution to the PDCA, 3) lack of conviction on the part of workforce, 4) lack of expertise and training of the workforce with regards to assigned tasks and responsibilities and lean methodologies including VSM, 5) arbitrarily varying patient influx – lack of production leveling and 6) cost intensive data collection due to lack of automation. This research study puts forth a gamut of challenges which can make benefits realized by the researcher temporary. Eller (2009) although used different key performance indicators (KPI) has had similar success as Willoughby et al. However, Eller seems to treat the lean tool/VSM for singular use only. Clearly, without complete involvement of the workforce kaizen - continuous improvement - is not possible. Furthermore, VSM based activities might have identified the issues at a given time. Value stream improvement is not an event but it is an ongoing incrementally constructive process.

King et al. (2006) mapped the patient flow in a teaching general hospital with an objective to minimize "… complex queuing…" (p.396) and reduce waiting time in the emergency rooms/departments. The researchers leverage the VSM methodology, supplemented with staff experienced in performing their tasks, to identify possible improvements. Like some other researchers included in this review, King et al. also focused on the triage process. The triage process involves sorting and prioritizing the patients based on the severity of their illness. Experience of the medical staff was used to categorize the patients who were then treated under redesigned procedures/lines specific to their category.

The researchers identified that they felt initial resistance. However, actively involving the workforce and the customers in their improvement initiatives proved to very effective. Additionally, it seemed that the changes made were permanent and other departments which the hospital organization may soon follow suit. This specific team adapted application of the methodology according to their requirements. The lessons learned from this study underscore the importance of the human involvement in the success of any lean initiative.

Similar to King et al., Kaale et al. (2005) adapted the VSM methodology to match their specific healthcare setting. This group of researchers aimed at reducing the wait time in the triage process. A key difference between King et al. and Kale et al. is the amount of decision making during the triage process. At least one of the improvement actions instituted by Kaale et al. (2005) seem to reduce the triage time by pushing the work to the next silo. Specifically, they determined that "…efforts should be implemented to minimize triage nursing evaluation time of critical patients with expedited room placement" (p.108). This approach could be a major issue in effectively implementing VSM and other lean principles across the organization. Lean approach needs to be all inclusive and not à-la-carte.

Studies by Dickson, Singh, Cheung et al. (2009) and Dickson, Anguelov, Vetterick et al. (2009) have been reviewed in conjunction. Both research papers and associated activities commenced their kaizen by drawing out the current state VSMs. This in turn lead to a through review and subsequent redesign of the process focused on reducing waste. The reduced of the non value added activities/inventory time involved usage both the PDCA and empowering the frontline workers. Furthermore, the future state VSM, based on improvement actions suggested by frontline workers, was successfully used to reduce patient length of stay and improve customer satisfaction. The researchers seem to be committed to continuous improvements. They kept improving at every iteration of VSM. The author highlighted the importance of the role played by the management. Two keys points which could prove debilitating to VSM lean activities, as pointed out by the researchers, include: 1) failing to make many small incremental improvements in the hope of waiting for big breakthrough and 2) not adapting the lean principles to local cultures.

Holden (2010) lists VSM as a significant lean tool. More importantly the researcher also lists nine key suggestions for useful lean implementation in an emergency room. These include: 1) entire organization should recognize that a problem exists and a solution is needed, 2) lean approach should be human-centered, 3) expertise in application of lean principles and work responsibilities is an absolute must, 4) top management should support the lean activities and should allocate any needed resources, 5) install lean champions who well respected socially and who can motivate others to join in, 6) adapt lean to local context and culture, 7) learn for past experiences, 8) focus on continuous improvement – lean is a never ending process not an event and 9) lean cannot be implemented in pockets – it

requires an holistic systems approach. These nine suggestions provide an important insight about challenges which an emergency room could face in effort to implement VSM and in turn becoming lean. If an emergency room (organization) fails to follow even one of the suggestions above results of a VSM activity, or any other lean principle for that matter, would be futile.

IV. Discussion

The preceding discussion establishes that VSM could be applied successfully to an emergency room setting albeit some challenges. The challenges could be divided into two groups – intrinsic and extrinsic

Intrinsic to the workgroup: VSM facilitators should possess exhaustive knowledge of the conventions, symbols and lean philosophy. The knowledge increases with experience and broadening of perspective. A VSM facilitator should be adept in moderation and consensus building skills. Furthermore, work group team members in a VSM should have a working knowledge of the method. This can be achieved by introductory training. Team members need to have full confidence in the process for it to be successful. More importantly team members should comprehensively know the emergency room processes being evaluated. VSM like any other lean tool is human centered. Constructive feedback and open-mindedness towards incremental success would help the team in identifying the waste and subsequently eliminating it. VSM is a continuous improvement tool based on PDCA cycle. VSM practitioners should thus iteratively look for sources of waste in an effort to minimize it. Lastly, the workgroup should communicate their achievements to other colleague in order to inspire them.

Extrinsic to the workgroup: Management support to should ensure that the VSM workgroup comprises of employees who are closest to the problem. In an emergency room setting it could involve doctors, nurse, para-medical and other staff, security. Furthermore, the management should empower the employees with authority and responsibility to make the necessary changes in a timely manner. Management should support lean principles across the organization instead of cherry picking the tools and/or departments for implementation. Traditional VSM in an emergency room should be modified and enhanced to meet the unique circumstances. This could include adding more icons and/or usage of swim lanes to describe various stakeholders.

In conclusion, VSM could prove to be an invaluable tool in eliminating waste from an emergency room. However, the practitioners and their sponsors must ensure that the VSM should be used to: identify waste, plan waste reduction, revaluate the level of waste in the improved process, in an organization wide, perpetual cycle.

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Effect of Cigarette Smoking on Blood Lipids – A Study in Belgaum, Northern Karnataka, India

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Abstract - Cigarette smoking is an important and independent risk factor of atherosclerosis, coronary artery disease and peripheral vascular disorders. Apart from active smokers, passive-smokers are also prone for the development of smoking related disorders. Smoking adversely affects the concentration of the plasma lipids and lipoprotein levels. The lipid profile was measured from 100 selected smokers and nonsmokers and the study shows that as the intensity and duration of smoking increases a significant increase in the levels of very low density lipoprotein -cholesterol, low density lipoprotein -cholesterol, triglyceride and total cholesterol are noted in almost all groups of cigarette smokers as compared to nonsmokers. Simultaneously a significant reduction in the level of High density lipoprotein-cholesterol is observed in cigarette smokers as the intensity and duration is increased. These findings add another health enhancing benefit by the cessation of smoking.

Keywords : *Cigarette smoking, Triglyceride, low density lipoprotein cholesterol, very low density lipoprotein cholesterol, High Density lipoprotein cholesterol.*

GJMR-I Classification : *NLMC Code: QV 665, QV 664, QV 662*

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Effect of Cigarette Smoking on Blood Lipids – A Study in Belgaum, Northern Karnataka, India

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Abstract - Cigarette smoking is an important and independent risk factor of atherosclerosis, coronary artery disease and peripheral vascular disorders. Apart from active smokers, passive-smokers are also prone for the development of smoking related disorders.

Smoking adversely affects the concentration of the plasma lipids and lipoprotein levels. The lipid profile was measured from 100 selected smokers and nonsmokers and the study shows that as the intensity and duration of smoking increases a significant increase in the levels of very low density lipoprotein -cholesterol, low density lipoprotein -cholesterol, triglyceride and total cholesterol are noted in almost all groups of cigarette smokers as compared to nonsmokers. Simultaneously a significant reduction in the level of High density lipoprotein-cholesterol is observed in cigarette smokers as the intensity and duration is increased. These findings add another health enhancing benefit by the cessation of smoking.

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I. Introduction

Lipids play an important role virtually in all aspects of biological life. Some of these roles include serving as hormones or hormone precursors, helping in digestion, providing energy, storage function and metabolic fuels; acting as functional and structural compounds in biomembranes and forming insulation to allow nerve conduction or to prevent heat loss (1).

Cigarette smoking is an important and independent risk factor for atherosclerosis, coronary artery disease and peripheral vascular disorders. (2). There is a dose response relationship between the number of cigarettes smoked per day and cardiovascular morbidity and mortality (3). Long delay between smoking and onset of smoking related diseases resulted in the ignorance of ill effects of smoking (4). On an average smoker lose more than a day of their life span for every week of smoking. Smoking kills more than one in three regular smokers (5). In India consumers not paid much attention to the tobacco smoking related diseases. (6). Qualities of Indian cigarettes are far away from western standards (7). India is the 3rd largest producer and exporter of tobacco in the world. About 550 million kgs of tobacco is grown in 4.2 lakh hectares of land and 250 million kgs of tobacco is released for local consumption (8). In India 337 million people above 10 years of age consume tobacco. Every year 1 million people die prematurely due to tobacco smoking related diseases (9).

The mechanism by which smoking increases the cardiovascular diseases are unclear. Recently it has been suggested that smoking adversely affects the concentration of plasma lipids and lipoprotein levels. However studies to date have revealed incomplete, inconclusive or conflicting results about the association of smoking on the plasma lipids and lipoproteins (10). It has been estimated that 1% increase in plasma concentration is associated with a 2.7% increase in risk (11).

As tobacco is grown more in northern Karnataka and also due to paucity of work done in this part the present study was undertaken. The present study provides a detailed profile of the plasma lipid and lipoprotein levels depending on duration and intensity of smoking.

II. Materials and Methods

The present study composed of 100 selected age and sex matched smokers and non-smokers between the age group of 20 to 60 years. All the subjects were consuming vegetarian diet and few of them were taking non-vegetarian diet occasionally, and belonging to different walks of the community. The subjects were volunteer participants in the study and gave informed consent.

All subjects were evaluated and selected by detailed medical history, physical examination, systemic examination and routine investigations to rule out any underlying diseases. Subjects having diseases, which are known to influence the blood lipids or patients on lipid lowering drugs or a diet restriction for any reason and persons chewing tobacco, ex-smokers, obese persons, alcoholics and having risk factors like hypertension, diabetes mellitus were excluded from the present study. Each patient gave informed consent and the study was approved by ethical and research committee of J.N. Medical College, Belgaum.

The present study comprises of 2 groups

Group I- Non smokers (Control) n=25

Group II – Cigarette smokers n = 75

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Cigarette smokers (Group –II) were divided into 3 subgroups depending upon duration and intensity of smoking.

Each group comprises about 25 volunteers

Group II A - Mild smokers (n =25)

(Duration - 1 to 5 years, smoking 10- 15 cigarettes / day)

Group II B - Moderate smokers (n=25)

(Duration 6 to 10 years, smoking 16-20 cigarettes / day)

Group II C - Heavy smokers (n= 25)

(Duration - more than 10 years, smoking >20 cigarettes/day)

In order to ensure accurate and reproducible results overnight 12-14 hours fasting blood samples were collected from these subjects. Serum was separated by centrifugation at 3600 rpm for six minutes. The clear serum samples were employed for the estimation of total cholesterol (12), Triglycerides (13) and HDL-Cholesterol (14). The levels of LDL cholesterol and VLDL cholesterol were calculated by using Friedewalds formula (15).

LDL Cholesterol (mg%) = Total Cholesterol – (HDL cholesterol + TG/5)

VLDL Cholesterol (mg%) = TG/5

The significance level of different parameters between the study groups were carried out using students "t" test.

III. Results

The present study was composed of 25 healthy non-smokers as controls and 75 active smokers between the age group of 20 to 60 years as the test group. Depending upon the duration and intensity of smoking, cigarette smokers were divided into 3 sub groups of mild, moderate and heavy smokers, as stated above.

The results of the present study are given in table-1 and graph-1. Table-1 gives the levels of total cholesterol, TG, HDL-C, LDL-C and VLDL-C in normal (Group- I), in mild smokers (Group-II A), in moderate smokers (Group –II B) and in heavy smokers (Group –II C). Graph-I depicts the comparison of the parameters in different test groups (Group- IIA, II B and IIC).

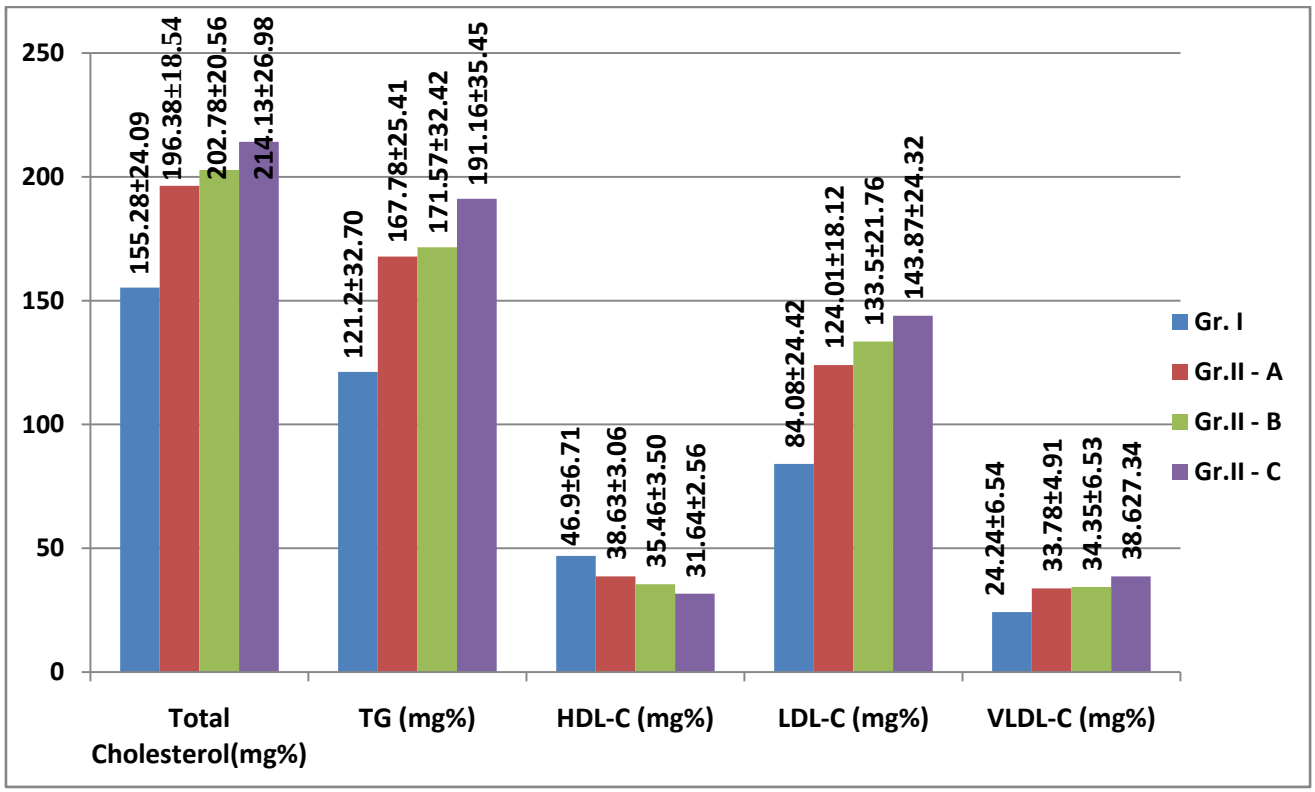
As it is evident from the table-1 and graph-1, the intensity and duration of smoking shows a significant increase in levels of cholesterol, triglyceride, LDL-C, VLDL-C in almost all the groups of cigarette smokers as compared to non smokers. Simultaneously a significant reduction in level of HDL-C is observed in cigarette smokers as compared to non-smokers and a parallel increase in these parameters with the increase in intensity and duration of smoking.

Table 1 : Table showing serum lipid profile in cigarette smokers and non-smokers in relation to duration and intensity of smoking.

PARAMETERS	NONSMOKERS	CIGARETTE SMOKERS		
	GROUP – I (n=25)	GROUP –II A (n=25)	GROUP –II B (n=25)	GROUP –II C (n=25)
Total Cholesterol (mg%)	155.28 ± 24.09	196.38 ±18.54 P<0.001	202.78 ± 20.56 P<0.001	214.13 ± 26.98 P<0.001
Triglycerides (mg%)	121.20 ± 32.70	167.78 ± 25.41 P<0.001	171.57± 32.42 P<0.001	191.16 ± 35.45 P<0.001
HDL-Cholesterol (mg%)	46.90 ± 6.71	38.63 ± 3.06 P<0.001	35.46 ± 3.50 P<0.001	31.64 ± 2.56 P<0.001
LDL-Cholesterol (mg%)	84.08 ± 24.42	124.01 ± 18.12 P<0.001	133.50 ± 21.76 P<0.001	143.87 ± 24.32 P<0.001
VLDL-Cholesterol (mg%)	24.24 ± 6.54	33.78 ± 4.91 P<0.001	34.35 ± 6.53 P<0.001	38.62 ± 7.34 P<0.001

Values are expressed as Mean ± SD. All P values are in comparison with nonsmokers.

Graph 1 : Graph showing Lipid profile in cigarette smokers and non-smokers in relation to duration and intensity of smoking.



IV. Discussion

Cigarette smokers have a high risk of coronary heart disease than nonsmokers. Several possible explanations have been offered for this association altered blood coagulation, impaired integrity of the arterial walls, changes in the blood lipid and lipoprotein concentration.

Smoking promotes CHD and atherosclerosis. This may be due to nicotine in cigarette smoke causes an increase in myocardial oxygen requirement by increasing the use of free fatty acid and also smoking by an unknown mechanism lowers the antiatherogenic factor HDL– C, remains a significant independent predictor of coronary artery disease.

In our study the mean value of serum total cholesterol in cigarette smokers is significantly higher ($P<0.001$) as compared to nonsmokers (refer Table -1). It is observed that cholesterol levels are raised in all groups of cigarette smokers but the risk is more in heavy smokers.

Analyzing the results with regard to the duration of smoking it is observed that on the whole there is a significant increase in the level of serum cholesterol with regard to an increase in duration and intensity of cigarette smoking.

The mean values of serum LDL-C and VLDL-C are observed to be significantly high ($P < 0.001$) in all groups of cigarette smokers (refer Table -1).

The present study also showed a significant increase ($P<0.001$) in serum triglycerides in cigarette smokers as compared to non-smokers (refer Table -1.)

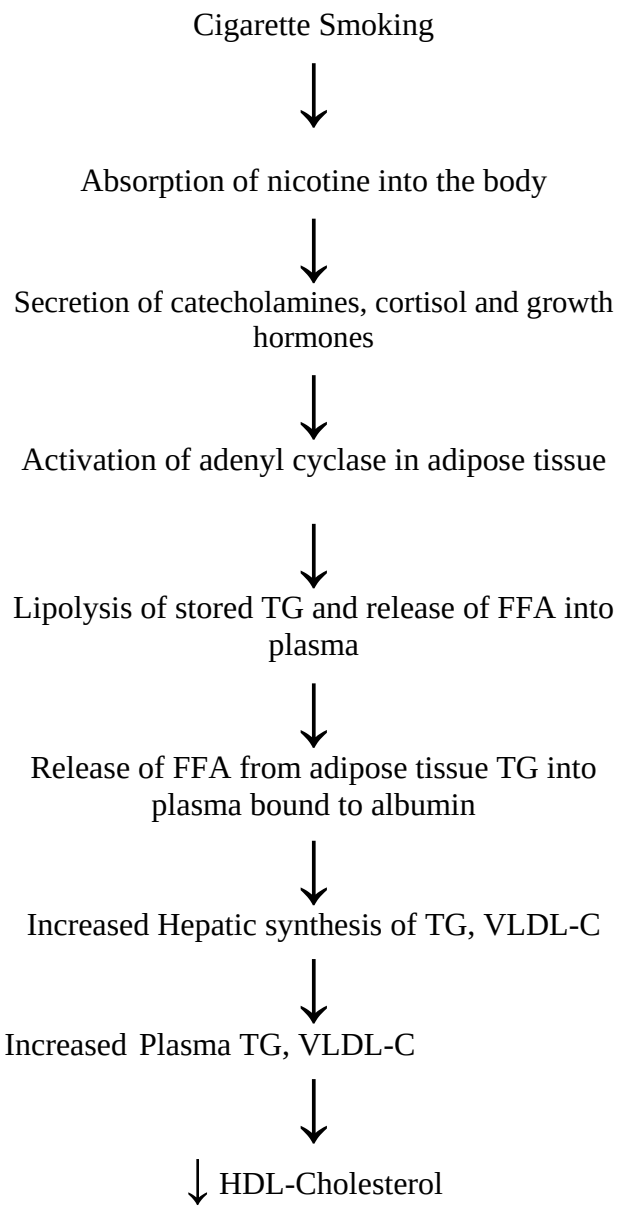
The P values obtained with regards to all fractions of serum lipid profile are found to be highly significant in smokers who smoked more number of cigarettes as compared to nonsmokers.

The same characteristics are analyzed with regard to duration of smoking again a significant increase in VLDL-C, LDL-C, triglyceride and total cholesterol is noted in almost all groups of cigarette smokers as compared to nonsmokers. On the whole, a significant reduction in the level of HDL-C is observed in cigarette smokers smoking for longer duration.

There are contradicting and varying results regarding total cholesterol, TG, LDL-C and VLDL-C in smokers. An increase in the total cholesterol, TG, LDL-C and VLDL-C and a significant decrease in HDL-C found in the present study in smokers as compared to nonsmokers agrees with earlier reports (16-20). Further parallel increase is seen in these parameters in mild to heavy smokers (refer table-I). The rise in blood lipid levels in smokers may be through catecholamine and adenyl cyclase axis induced tissue lipolysis as suggested in chart -1.

Majos O. D. et al. (21) in their study reported that there is significant decrease in HDL-C, but there is no change in total cholesterol and triglycerides in cigarette smokers as compared to non-smokers. The above findings, except for decrease in HDL-C are contradictory to our findings.

Chart -1 : Chart showing a possible mechanism by which nicotine absorbed from cigarette smoke may elevate plasma lipids and lipoproteins.



V. Conclusion

Our study clearly shows a strong relationship between elevation of serum lipids and cigarette smoking. It also emphasis that the changes in the serum lipids tends to be high with the increase in duration and intensity of smoking. The risk of increase in serum cholesterol with an increase in LDL-C and decrease in HDL-C assume a great significance since this has been the pattern associated with CHD.

The low level of HDL-C in cigarette smokers and the increased exposure of the vascular endothelium to potentially atherogenic lipoproteins as a consequence of impaired clearance of triglyceride rich lipoproteins may provide a mechanism whereby smoking predisposes to greater risk of developing atherosclerotic plaques and CHD.

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Technological Innovation and Public Health: A Descriptive Exploratory Investigation of Relationship between Technological Innovation Indicators and Public Health Indicators in the United States from 2003 to 2007

By Preetinder Singh Gill
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Abstract - Technological innovation and public health are vital for prosperity. This study quantitatively explored and described the relationship between these constructs. Indicators representing technological innovation and public health were identified. Data associated with the indicators were collected from various U.S. federal governmental sources for the four U.S. Census regions. The four U.S. Census regions were then compared in terms of the indicators. Power law regression equations were developed for each combination of technological innovation and public health indicators. Additionally, the relationship between technological innovation and public health was described using the structural equation modeling - SEM - technique. It was found that the four regions ranked differently in terms of both technological innovation indicators and public health indicators. The results of the study showed that better technological innovation indictor scores were associated with better public health indicator scores. Results of the SEM provided preliminary evidence that technological innovation shares causal relation with public health.

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I. Introduction

Viability of an organization or a geographical region is contingent upon its ability to stay competitive. Innovation and market competition share an inverted U relationship (Aghion et al., 2005). This means that a high amount of innovation is accompanied by reduced market competition. Thus, by being and staying highly innovative, geographical regions can establish and cement their leadership position. Schumpeter (1942) asserted that innovation is stochastically propelled by the temporal, incremental, monopolistic incentives to the innovators. It was argued that new consumer goods provide perpetual impetus for economic progress. Simply put, innovation ensures economic success.

Stewart et al. (2003) showed that US employers spend billions of dollars every year in health related expenses. Multiple studies have confirmed that better health ensures lower absenteeism and higher productivity (Wojick, 2009, Suhrcke et al., 2006 and Fuchs, 1966). Furthermore, Romer (1990) showed that increased human capital would increase growth. It could be argued that better public health ensures enhanced and enriched human capital, which in turn, ensures sustainability of an economically competitive region. The United Nations human development index presents the clearest evidence that year-over-year countries with stronger economies tend to have better public health and advanced technological accomplishments (UNDP, 2009 and WEF, 2010). Thus, it can be argued that both technological innovation and public health are critical to economic success.

II. Importance of the Research Study

Dreyfuss (2011) noted that it is challenging to measure value of knowledge and impact of knowledge generated by technological innovation. Technological assessment is widely used method for measuring the impact technological innovation. Goodman (2004) suggested that technology assessment – TA – involves appreciation of the critical role of technology in modern society and its potential for unintended, and sometimes harmful, consequences. Generally speaking, TA is a cause and effect analysis which establishes a one-dimensional relationship between a single new technology and its possible effects. Health technology assessment – HTA – is the systematic evaluation of properties, effects or other impacts of health related technologies with an objective of achieving informed policy making (Goodman, 2004). Banta (2002) compared and contrasted the development and the deployment of HTA across various nations. Perry & Thamer (1997) performed and compared HTA for the U.S. and other countries. Abelson et al. (2007) studied public involvement and accountability mechanisms in HTA. Furthermore, they distinguish specific roles for the public, and relate them to several layers of policy analysis and policy making. Researchers like Royle & Oliver (2004), Oliver et al., (2009) and Cohen et al., (2004) studied socioeconomic factors in relation to health technologies. However, the use of HTA seems to

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be limited to assessment of specific health related technologies and not on the complete spectrum of technological development. Furthermore, the relationship between socioeconomic factors and technological innovation was not qualified by any of these researchers.

The classical innovation diffusion theory (Rogers, 1995) asserts that relative advantage, complexity, compatibility, observability and trialability determine the success of an innovative endeavor. These measures are commonly used to assess specific attributes of a technological innovation, in relation to socioeconomic factors, to establish the possibility of its successful adoption. Although Rogers (1995) held that the construct of technological innovation is dynamic and iterative which propagates under myriad of socioeconomic forces the theory doesn't address the collective impact of technological innovation on the society. Furthermore, Gelijns and Rosenberg (1994) showed that medical technology innovation is not necessarily linear as it progresses from basic research to market application. Berwick (2003) observed that policy makers need to better understand impact of innovation since the "…processes of innovation and dissemination have their own rules, their own pace, and their own, multilayered forms of search and imagining" (p.1974). Hence, it is pertinent to study how technological innovation, at a macro level, impacts the broad socioeconomic systems including the public health.

The World Health Organization's Commission on Intellectual Property Rights, Innovation and Public Health (2006) in its report about innovation and health products asserted that research institutes and universities should maintain research priorities relevant to health concerns. Ridley (2010) reported that the WHO is promoting technological innovation as a key to improving health and well-being. However, it must be noted that the WHO's focus is limited to health care related technologies only. Türmen & Clift (2006) reported that organizations and policies outside health care sector impact technological innovation. They also maintained that without access to products of technological innovation there is no public health benefit. Nevertheless, the relationship between technological innovation and public health was not quantified.

Getz (2011) and Feller, Finnegan & Nilsson (2011) made a case for propagating open innovation to negate effects of silo-ed approach to innovation and to improve effectively of the underlying research. Wild and Langer (2008) emphasized the need to address the blank spots in the prioritization process associated implementing health related technologies. Moniruzzaman and Anderson (2008) showed that unique inverted U shaped relationship exists between injury, mortality and the economy in different countries. They stress the need to understand the relationship between innovation and health care. Hughes (2011) argued that value created by a technological innovation goes beyond the preconceived specific service specifications. Greenberg (2006) presented multiple instances where technologies not directly geared toward healthcare had substantive public health effect. Additionally, Varey (2011) argued that governments should take a holistic approach to innovation. Varey (2011) also held that policy makers can increase the efficiency of the tight budgets by investing in a boarder range of technologies beyond the ones which are exclusively linked to health care.

III. Problem Statement and Objective of the Study

The relationship between technological indicators and innovation indicators has not been adequately studied. In order to address this gap, the study explored the relationship between technological innovation indicators & public health indicators for the four U.S. Census regions over a period of five years. This in turn involved descriptive and inferential data analyses with regards to selected technological innovation indicators and public health indicators to address specific research questions. The research questions are listed in the following section.

Benefits of this study include: 1) better understanding of the relationship between technological innovation and public health, 2) creation of a knowledge base to provide opportunities for informed decision making by policy makers and 3) comparing public health and technological innovation in the four U.S. Census regions and over five years.

IV. Research Questions

1) Is there a statistically significant difference between median values of technological innovation indicators or median values of public health indicators for the four U.S. Census regions?

2a) What relationship, if any, exists between technological innovation indicators and public health indicators in the Midwest U.S. Census region?

2b) What relationship, if any, exists between technological innovation indicators and public health indicators in the Northeast U.S. Census region?

2c) What relationship, if any, exists between technological innovation indicators and public health indicators in the South U.S. Census region?

2d) What relationship, if any, exists between technological innovation indicators and public health indicators in the West U.S. Census regions?

V. Hypothesis

The hypotheses associated with this study were tested at a significance level with p value ≤ 0.05. The hypotheses tested were:

1) There is no statistically significant difference between median values of technological innovation indicators or median values of public health indicators associated with the four U.S. Census regions.

2a) There is no statistically significant relationship between any technological innovation indicator and any public health indicator in the Midwest U.S. Census region.

2b) There is no statistically significant relationship between any technological innovation indicator and any public health indicator in the Northeast U.S. Census region.

2c) There is no statistically significant relationship between any technological innovation indicator and any public health indicator in the South U.S. Census region.

2d) There is no statistically significant relationship between any technological innovation indicator and any public health indicator in the West U.S. Census region.

VI. Delimitations/Limitations

Delimitation: Data from the District of Columbia and other U.S. territories were not included in the study.

Delimitation: The smallest geographical unit included in the study was a single U.S. Census region (U.S. Census Bureau, 2000)

Delimitation: Chatterjee and Sorenesen (1998) provided evidence for the application of the Pareto principle in regression analyses. Accordingly, it can be argued that a relatively small number of indicators can represent a given construct. Hutton (2000) Cummings (2004), Mackay (2007) and Mizell (2009) also underscore that a small number of indicators can help in effectively describing a construct.

Delimitation: The public health indicators, for this study, were selected from the 26 leading health indicators tracked by the U.S. Department of Health and Human Services (2012).

Delimitation: The technological innovation indicators, for this study, were selected from list of innovation indicators tracked by Organization for Economic Co-operation (2012) and studied by Reffitt & Sorenson (2007) for Michigan Department of Labor & Economic Growth.

Delimitation: Six technological innovation indicators and five public health indicators were selected by the author in consensus with the research adviser.

Delimitation: Effectiveness of individual technological innovations and significance specific health issues were not evaluated.

Delimitation: The study did not quantify the resources, time, effort and knowledge needed to generate technological innovation or alleviate public health problems.

Limitation: The data were collected from publicly available sources commissioned by governmental agencies and/or organizations.

Limitation: 2003-2007 is the only contagious period for which data are available for the selected technological innovation indicators and public health indicators. Availability of data influenced selection of the indicators.

Limitation: The data collected for the study were limited to the fifty U.S. states.

Limitation: Constructs of technological innovation and public health are defined in terms of the respective indicators. Hence, formative models were used for structural equation modeling (Henseler, Ringle, & Sinkovics, 2009).

VII. Definition of Terms

Government Agency: An administrative unit of government authorized by law or regulation to perform a specific function (Princeton University, 2012) and Rutgers University, 2003).

Pareto Principle: The concept that most of a given set of results are due to a small number of causal factors e.g., 80 percent of the results can be explained by 20 percent of the causes (Food and Agriculture Organization of United Nations, 2010).

Indicator Score: Indicator score signifies desirability. In case of technological innovation indicators higher absolute value signifies better indicator score. In case of public health indicators lower absolute value signifies better indicator score. Poor indicator score signifies undesirable level of an indicator. Best indicator score signifies desirable level of an indicator. Fair indicator score signifies a value between poor and best indicator scores.

Power Law: A power law is a special kind of mathematical relationship between two quantities. When the number or frequency of an object or event varies as a power of some attribute of that object, the number or frequency is said to follow a power law. A power law could be expressed as $f(x) = \alpha * x^{\beta} + \varepsilon$ where α is a constant, β is the scaling factor and ε is the error term (Katz, 2006). Power laws are scale invariant. In other words, if x is scaled by a constant, γ, then f(x) would be scaled by a constant γ^{β}.

Public Health Indicator: A public health indicator is "…a variable with characteristics of quality, quantity and time used to measure, directly or indirectly …" an aspect of public health (Gruskin & Ferguson, 2009, p. 714)

- Health status indicator is the percent of people reporting that their general health is fair or poor in

the annual behavioral risk factor surveillance survey conducted by the U.S. Centers for Disease Control and Prevention.

- Insurance indicator is the percent of people reporting that they have any kind of health care coverage in the annual behavioral risk factor surveillance survey conducted by the U.S. Centers for Disease Control and Prevention.
- Obesity and overweight rate indicator is the percent of people reporting that their weight classification by body mass index is overweight or obese in the annual behavioral risk factor surveillance survey conducted by the U.S. Centers for Disease Control and Prevention.
- Preterm birth rate indicator is the ratio of the births before 36 weeks of gestation to the total number of births as reported on U.S. Centers for Disease Control and Prevention's Natality public-use data on CDC WONDER Online Database.
- Suicide rate indicator is the ratio of suicide deaths per capita of population as reported by on U.S. Centers for Disease Control and Prevention's National Center for Injury Prevention and Control.
- Tobacco use indicator: is the percent of people reporting that they are current smokers in the annual behavioral risk factor surveillance survey conducted by the U.S. Centers for Disease Control and Prevention.

U.S. Census Region: A grouping of 50 federated states into four groups by the U.S. Census Bureau (2000)

- Midwest region consists of Indiana, Illinois, Michigan, Ohio, Wisconsin, Iowa, Nebraska, Kansas, North Dakota, Minnesota, South Dakota and, Missouri
- Northeast region consists of Connecticut, Maine, Massachusetts, New Hampshire, Rhode Island, Vermont, New Jersey, New York and, Pennsylvania
- South region consists of Delaware, Florida, Georgia, Maryland, North Carolina, South Carolina, Virginia, West Virginia, Alabama, Kentucky , Mississippi, Tennessee, Arkansas, Louisiana, Oklahoma and, Texas
- West region consists of Arizona, Colorado, Idaho, New Mexico, Montana, Utah, Nevada, Wyoming, Alaska, California, Hawaii, Oregon, Washington

Sub-linear Relation: A sub-linear relationship is quantified by $\beta < 1$ in the power law equation. Negative sub-linear relation signifies $-1 < \beta < 0$.

Super-linear Relation: A super-linear relationship is quantified by $\beta > 1$ in the power law equation.

Technological Innovation Indicator: A technological innovation indicator is "…a variable with characteristics of quality, quantity and time used to measure, directly or indirectly …" an aspect of technological innovation (Gruskin & Ferguson, 2009, p. 714)

- Articles per 1000 capita indicator is the number of articles per 1000 people in a state - reported by U.S. National Science Foundation's National Center for Science and Engineering Statistics.
- Patents per 1000 capita indicator is the number of patents per 1000 people in a state – reported by U.S. Patents and Trademarks Office.
- Percentage of workforce in scientist and engineer occupation indicator is the number of workers who work as scientists and engineers expressed as a percentage of the total work force in a state - reported by U.S. National Science Foundation's National Center for Science and Engineering Statistics.
- Value of R&D performed as percent of GDP indicator is the volume research and development investment expressed as a percentage of the state GDP - reported by U.S. National Science Foundation's National Center for Science and Engineering Statistics.
- Venture capital per $1000 of GDP indicator is the volume of venture capital investment in a state per $1000 of the state GDP - as reported by U.S. National Science Foundation's National Center for Science and Engineering Statistics.

VIII. Assumptions

It was assumed that the data collected by the governmental agencies is an accurate representation of the underlying population. It was assumed that no bias exists in the process of data collection and reporting on the behalf of the governmental agencies. It was assumed that the governmental agencies ensured safety, confidentiality and anonymity of human subjects when publishing the data. Nevertheless, the data obtained from the governmental agencies was free of any and all type of personal identification information. Furthermore, the indicators and associated PLS SEM formative models selected by the author were assumed to be substantively representative of the technological innovation and public health, albeit to varying extents. It was assumed that algorithms and outputs from Statgraphics Centurion XV® version 15.2.14 and SmartPLS 2.0 M3 provide an accurate descriptive analysis of the data.

IX. Methodology

The research study commenced with identifying governmental sources of the technological innovation and public health indicators. The author and the research adviser selected a set of 5 technological

innovation indicators and a set of 6 public health indicators. Kruskal-Wallis median comparison tests were performed to assess whether there were significant differences between indicator scores from the four U.S. Census regions.

The study then followed a correlational and inferential design. The correlational portion of the study involved forming single variable power law regression equations. The technological innovation indicators served as the independent variables. The public health indicators served as the dependent variables. As a result of this exercise 30 power law regression equations were generated for each U.S. Census region. The scaling factors were examined to establish if the independent and dependent variables fit sub-linear or super-linear relationships. The α coefficients were calculated to ascertain slopes of the power law regression equations. The inferential portion of the study involved performing the formative structural equation modeling to study causal relations between innovation inputs, innovation outputs and health outcomes.

X. Population and Sample

All fifty U.S. states formed the population for this study. Data categories included: 1) census of the population for suicide, preterm births, articles, patents, workforce, R&D and, venture capital investment indicators and 2) sampling of the population for health status, insurance, obesity and tobacco use indicators.

XI. Data Collection

Data collection involved downloading indicator data from various governmental agencies. The governmental agencies were short-listed by the author based on their mandated objectives with regards to technological innovation, intellectual property and, public health. Data sources for various indicators are listed in table 1. This exercise had dual focal points: 1) creating a list of potential indicators which were subsequently narrowed-down by consensus between the author and research adviser and, 2) collecting and preparing the data for analyses in subsequent phases of the research project. The state of Hawaii had data missing for the year 2004. Hence, a total of 249 data points were collected for each indicator instead the maximum possible 250 data points - 5 years times 50 states. Data were prepared for analyses by tabulation into a single table arranged by year and region. The indicators in the study were coded for ease of use in the statistical analysis software. A legend showing the cross-reference between indicators and the codes used is presented in table 8 in Appendix A.

Data	Data type	Source
State population data	Census	U.S. Census Bureau
Health status indicator	Sample	U.S. Center of Disease Control Behavioral Risk Factor Surveillance
Insurance indicator	Sample	U.S. Center of Disease Control Behavioral Risk Factor Surveillance
Obesity and overweight rate indicator	Sample	U.S. Center of Disease Control Behavioral Risk Factor Surveillance
Preterm birth rate indicator	Census	U.S. Center of Disease Control Natality Data
Suicide rate indicator	Census	U.S. Center of Disease Control - National Center for Injury Prevention
Tobacco use indicator	Sample	U.S. Center of Disease Control Behavioral Risk Factor Surveillance
Articles per 1000 capita indicator	Census	U.S.Patent and Trademark Office
Patents per 1000 capita indicator	Census	U.S. National Science Foundation
Percentage of workforce in science and engineer occupation indicator	Census	U.S. National Science Foundation
Value of R&D performed as percent of GDP indicator	Census	U.S. National Science Foundation
Venture capital per $1000 of GDP indicator	Census	U.S. National Science Foundation

Table 1 : Indicator data sample type and sources

XII. Data Analysis

The research project involved quantitative data. Descriptive and inferential statistics were used for data analysis. Significance level of $p \leq 0.05$ was used to test the null hypotheses. The kurtosis and skewness values for the indicators are presented in table 2. These values are outside the range of -2 to +2. This indicates a significant departure from normality. In other words the underlying distribution of none of the indicators is normal. A natural logarithm transformation improved the kurtosis and skewness values. The results presented in table 3. It can hence be concluded, Pearson product moment correlation values could be satisfactorily calculated after applying that the natural logarithm transformation. Pearson product moment correlations and the associated p values are presented in table 4. In case of the public health indicators the highest Pearson product moment correlation, 0.711, is observed between *health status* indicator and *preterm birth rate* indicator. *Suicide rate* indicator is the only indicator which shows a low level or insignificant of correlation with other indicators. All other public health indicators share a statistically significant Pearson product moment correlation with each other. In the case of technological innovation indicators the highest Pearson product moment correlation, 0.778, is observed between *percentage of workforce in scientist and engineer occupation* indicator and *value of R&D performed as percent of GDP* indicator. All technological innovation indicators share a statistically significant Pearson product moment correlation with each other.

	HSG_EVGG	T_Y	OW_Ob	PTB_R	I_Y	S_R	Pat_PGC	Art_PGC	VC_GGDP	GERD	SE_PWF
Average	15.452	21.095	61.009	0.125	14.951	12.540	0.251	0.492	1.034	2.214	3.442
Standard deviation	3.303	3.288	3.202	0.018	4.200	3.477	0.205	0.224	1.648	1.565	1.024
Stnd. skewness	4.764	-1.176	-2.832	4.978	2.544	3.814	13.728	12.502	21.422	10.048	5.581
Stnd. kurtosis	-0.452	2.522	1.085	3.929	-0.360	1.146	21.500	21.830	40.955	8.963	1.482

Table 2 : Raw indicator data description

	ln(HSG_EVGG)	ln(T_Y)	ln(OW_Ob)	ln(PTB_R)	ln(I_Y)	ln(S_R)	ln(Pat_PGC)	ln(Art_PGC)	ln(VC_GGDP)	ln(GERD)	ln(SE_PWF)
Average	2.732	3.032	4.107	-2.083	2.664	2.463	-1.613	-0.788	-0.712	0.633	1.206
Standard deviation	0.205	0.171	0.054	0.144	0.294	0.269	0.739	0.422	1.422	0.669	0.293
Stnd. skewness	1.562	-2.099	-1.509	1.180	-1.930	-2.038	-0.316	0.236	-1.981	-0.606	0.611
Stnd. kurtosis	-1.980	1.909	1.977	0.922	-0.387	0.628	-1.543	1.225	-0.539	-1.466	-1.243

Table 3 : Transformed indicator data description

	ln(HSG_EVGG)	ln(T_Y)	ln(OW_Ob)	ln(PTB_R)	ln(I_Y)	ln(S_R)
ln(HSG_EVGG)		0.519	0.485	0.711	0.619	0.067
p-value		<0.001	<0.001	<0.001	<0.001	0.294
ln(T_Y)	0.519		0.478	0.505	0.234	0.172
p-value	<0.001		<0.001	<0.001	<0.001	0.007
ln(OW_Ob)	0.485	0.478		0.527	0.218	0.127
p-value	<0.001	<0.001		<0.001	0.001	0.046
ln(PTB_R)	0.711	0.505	0.527		0.425	0.041
p-value	<0.001	<0.001	<0.001		<0.001	0.525
ln(I_Y)	0.619	0.234	0.218	0.425		0.476
p-value	<0.001	<0.001	<0.001	<0.001		<0.001
ln(S_R)	0.067	0.172	0.127	0.041	0.476	
p-value	0.294	0.007	0.046	0.525	<0.001	

	log(Pat_PGC)	log(Art_PGC)	log(VC_GGDP)	log(GERD)	log(SE_PWF)
log(Pat_PGC)		0.490	0.566	0.668	0.692
		<0.001	<0.001	<0.001	<0.001
log(Art_PGC)	0.490		0.498	0.625	0.651
	<0.001		<0.001	<0.001	<0.001
log(VC_GGDP)	0.566	0.498		0.576	0.666
	<0.001	<0.001		<0.001	<0.001
log(GERD)	0.668	0.625	0.576		0.778
	<0.001	<0.001	<0.001		<0.001
log(SE_PWF)	0.692	0.651	0.666	0.778	
	<0.001	<0.001	<0.001	<0.001	

Table 4 : Pearson correlations and the associated p values for transformed indicator data

In order to test hypothesis 1 single factor ANOVA was performed for every transformed indicator with regards to the four U.S. Census regions. Statistically significant difference at 95% confidence interval is highlighted with an asterisk symbol in figure 5 in Appendix A. Furthermore, the Kruskal-Wallis median comparison tests were also performed on the untransformed data to confirm the results of the single factor ANOVA. The p-values are presented in table 5. Since all values are below 0.05 it can be concluded that there is a significant difference between the medians of the indicators for the four U.S. Census regions.

		K-W Median Test p-value
Public Health Indicators	HSG_EVGG	<0.0001
	T_Y	<0.0001
	OW_Ob	<0.0001
	PTB_R	<0.0001
	I_Y	<0.0001
	S_R	<0.0001
Technological Innovation Indicators	Pat_PGC	<0.0001
	Art_PGC	<0.0001
	VC_GGDP	<0.0001
	GERD	<0.0001
	SE_PWF	<0.0001

Table 5 : p Values of Kruskal-Wallis median comparison tests for various indicators

Box whisker plots associated with the Kruskal-Wallis median comparison tests for each indicator with regards to the four U.S. Census regions are presented in figure 6 in Appendix B. The consolidated results of the Kruskal-Wallis median comparison tests are presented in table 6. A region's performance with regards to each indicator is categorically ranked in terms of codes B, F or P. B represents the best indicator scores, P represents the poor indicator scores and F represents mid-level indicator scores. The results of the ANOVA and K-W test showed that there are statistically significant differences in both the technological innovation indicator scores and public health indicator scores with regards to the four U.S. Census regions.

		Midwest	North East	South	West
Public Health Indicators	HSG_EVGG	B	B	P	B
	T_Y	F	B	P	B
	OW_Ob	P	B	P	B
	PTB_R	F	B	P	F
	I_Y	B	B	P	P
	S_R	F	B	F	P
Technological Innovation Indicators	Pat_PGC	F	B	P	F
	Art_PGC	F	B	P	P
	VC_GGDP	P	B	P	F
	GERD	F	B	P	F
	SE_PWF	F	B	P	F
Total	B	2	11	0	3
	F	7	0	1	5
	P	2	0	10	3

Table 6 : Ranked comparison of U.S. Census regions with regards to various indicators

Data analysis showed that the South is the only U.S. Census region which has poor *health status* indicator scores. In fact the South U.S. Census region had the poorest scores for all public health and technological indicators. On the other hand the Northeast U.S. Census region had the best scores for all public health and technological indicators. The Midwest and the West U.S. Census regions did not have the best scores for any of the technological innovation indicators. The Midwest U.S. Census region had the best scores for *health status* indicator and *insurance* indicator. This region had poor scores for *obesity and overweight rate* indicator and *venture capital per $1000 of GDP* indicator. The West U.S. Census region had the best scores for *health status* indicator, *tobacco use* indicator and, *obesity and overweight rate* indicator. This region had poor scores for *insurance rate* indicator, *suicide rate* indicator and, *articles per 1000 capita* indicator.

Hence, we can reject the null hypothesis 1 based on the results of the data analysis presented above. In other words no evidence was found which supports the hypothesis that there is no statistically significant difference between median values of technological innovation indicators or median values of public health indicators for the four U.S. Census regions at the 0.05 level of significance.

The technological innovation systems and public health systems are complex (Baranger, 2001) and dynamic in nature. Hence, they exhibit scaling properties (Amaral and Ottino, 2004). The signature of a scaling property is a power law correlation between variables of the system (Katz, 2006). Mitzenmacher (2002) and Newman (2005) also asserted that power laws can be applied to a wide variety of complex phenomena. Furthermore, power law relationships "…are readily identifiable when they are plotted on a log–log scale because they appear linear" (Katz, 2005, p.896). This identifier was observed for each technological health indicator and public health indicator combination used in this study. Hence, in order to test hypotheses 2a, 2b, 2c and 2d power law regression analysis was performed on the data associated with various indicators included in the study.

The alpha (α) and beta (β) values are tabulated in table 7. The slopes, alpha (α), signify the proportionality – direct or inverse – between the dependent and independent variables. The scaling factors, beta (β), signify sub-linear or super-linear relationships between the independent and dependent variables. Natural logarithm transformation was applied to the data to normalize the wide range in the data values. The significance level, p and goodness to fit indicator R^2 for each equation are also shown in table 7. It was found that for the Midwest U.S. Census region 10 of the possible 30 combinations between technological health indicators and public health indicators share a statistically significant relationship. For the Northeast U.S. Census region 21 of the possible 30 combinations between technological health indicators and public health indicators share a statistically significant relationship. For the South U.S. Census region 28 of the possible 30 combinations between technological health indicators and public health indicators share a statistically significant relationship. For the West U.S.

Census region 17 of the possible 30 combinations between technological health indicators and public health indicators share a statistically significant relationship.

Midwest	Pat_PGC				Art_PGC				VC_GGDP				GERD				SE_PWF			
	α	β	R^2	p	α	β	R^2	p	α	β	R^2	p	α	β	R^2	p	α	β	R^2	p
HSG_EVGG	2.559	-0.032	1.387	0.370	2.648	0.051	1.151	0.415	2.615	-0.015	0.927	0.502	2.572	0.070	6.378	0.052	2.634	-0.019	0.066	0.846
T_Y	3.086	0.012	0.345	0.656	3.087	0.028	0.645	0.542	3.072	-0.003	0.080	0.844	3.045	0.038	3.405	0.158	3.189	-0.105	3.628	0.145
OW_Ob	4.106	-0.016	11.834	**0.007**	4.131	-0.001	0.034	0.890	4.122	-0.005	4.908	0.118	4.136	-0.008	2.697	0.210	4.156	-0.021	2.876	0.195
PTB_R	2.192	-0.048	12.776	**0.005**	-2.099	0.020	0.734	0.515	-2.128	-0.016	4.387	0.140	-2.118	0.009	0.461	0.606	-2.027	-0.073	4.086	0.121
I_Y	2.179	-0.183	28.367	**<0.001**	2.474	-0.010	0.030	0.896	2.405	-0.064	10.024	**0.024**	2.501	-0.036	1.034	0.440	2.816	-0.286	9.308	**0.018**
S_R	2.202	-0.146	24.925	**<0.001**	2.283	-0.224	19.041	**0.001**	2.345	-0.066	19.382	**0.001**	2.521	-0.138	21.357	**<0.001**	2.892	-0.385	23.186	**<0.001**

NorthEast	Pat_PGC				Art_PGC				VC_GGDP				GERD				SE_PWF			
	α	β	R^2	p	α	β	R^2	p	α	β	R^2	p	α	β	R^2	p	α	β	R^2	p
HSG_EVGG	2.435	-0.161	34.237	**<0.001**	2.570	-0.092	10.375	**0.031**	2.617	-0.016	1.659	0.399	2.729	-0.112	16.468	**0.006**	2.897	-0.213	12.330	**0.018**
T_Y	2.865	-0.095	24.177	**0.001**	2.937	-0.067	11.282	**0.024**	2.973	-0.022	6.782	0.084	3.059	-0.088	20.501	**0.002**	3.313	-0.260	37.322	**<0.001**
OW_Ob	4.016	-0.048	38.256	**<0.001**	4.053	-0.036	19.122	**0.003**	4.071	-0.008	5.984	0.105	4.095	-0.025	9.992	**0.034**	4.167	-0.074	18.277	**0.003**
PTB_R	-2.299	-0.079	16.422	**0.006**	-2.210	0.001	0.004	0.969	-2.214	0.021	5.994	0.105	-2.221	0.009	0.235	0.752	-2.215	0.003	0.006	0.959
I_Y	2.334	-0.090	8.304	0.055	2.341	-0.189	34.027	**<0.001**	2.439	-0.042	9.260	**0.042**	2.599	-0.163	27.095	**<0.001**	2.928	-0.374	29.636	**<0.001**
S_R	2.103	-0.066	1.655	0.400	2.086	-0.184	11.926	**0.020**	2.191	-0.112	24.797	**0.001**	2.371	-0.192	13.927	**0.012**	2.851	-0.511	20.522	**0.002**

South	Pat_PGC				Art_PGC				VC_GGDP				GERD				SE_PWF			
	α	β	R^2	p	α	β	R^2	p	α	β	R^2	p	α	β	R^2	p	α	β	R^2	p
HSG_EVGG	2.419	-0.223	47.411	**<0.001**	2.568	-0.340	44.094	**<0.001**	2.832	-0.056	17.427	**<0.001**	2.955	-0.208	40.149	**<0.001**	3.401	-0.463	66.460	**<0.001**
T_Y	2.906	-0.108	27.619	**<0.001**	2.971	-0.173	28.055	**<0.001**	3.078	-0.048	29.580	**<0.001**	3.170	-0.112	28.651	**<0.001**	3.409	-0.249	47.400	**<0.001**
OW_Ob	4.074	-0.033	25.936	**<0.001**	4.114	-0.032	10.018	**0.004**	4.133	-0.009	11.863	**<0.001**	4.150	-0.020	9.057	**0.007**	4.204	-0.054	22.449	**<0.001**
PTB_R	-2.137	-0.089	27.765	**<0.001**	-2.001	-0.055	4.221	0.068	-1.997	-0.042	33.815	**<0.001**	-1.935	-0.045	6.859	**0.019**	-1.767	-0.165	31.059	**<0.001**
I_Y	2.404	-0.191	16.978	**<0.001**	2.424	-0.404	30.394	**<0.001**	2.779	-0.034	3.371	0.112	2.901	-0.307	42.406	**<0.001**	3.317	-0.461	32.204	**<0.001**
S_R	2.209	-0.131	32.864	**<0.001**	2.219	-0.280	60.234	**<0.001**	2.444	-0.033	12.374	**0.002**	2.535	-0.159	47.025	**<0.001**	2.782	-0.269	44.929	**<0.001**

West	Pat_PGC				Art_PGC				VC_GGDP				GERD				SE_PWF			
	α	β	R^2	p	α	β	R^2	p	α	β	R^2	p	α	β	R^2	p	α	β	R^2	p
HSG_EVGG	3.601	-0.330	9.209	**0.014**	2.654	-0.026	0.723	0.501	2.584	-0.121	2.116	0.248	2.719	-0.020	0.814	0.517	2.681	0.021	0.391	0.621
T_Y	4.410	0.014	1.784	0.293	4.443	-0.004	1.640	0.313	4.469	0.023	9.446	**0.014**	4.444	0.002	0.677	0.558	4.453	-0.009	8.187	**0.022**
OW_Ob	1.750	0.427	27.736	**<0.001**	2.803	-0.084	13.969	**0.002**	2.619	-0.343	33.097	**<0.001**	2.880	-0.063	19.389	**0.001**	2.978	-0.084	11.643	**0.006**
PTB_R	3.893	0.065	9.322	**0.014**	4.067	-0.004	0.498	0.579	4.014	-0.065	17.100	**0.001**	4.065	-0.002	0.405	0.651	4.078	-0.008	1.602	0.319
I_Y	-2.484	0.121	9.388	**0.013**	-2.208	-0.038	11.495	**0.006**	-2.254	-0.114	14.326	**0.002**	-2.154	-0.042	28.100	**<0.001**	-2.130	-0.035	8.251	**0.020**
S_R	1.929	0.332	6.864	**0.035**	2.877	0.023	0.400	0.617	2.618	-0.250	6.653	**0.038**	2.840	-0.004	0.027	0.906	2.834	0.015	0.144	0.764

Table 7 : Power law regression equation parameters for technological innovation indicators and public health indicators

Every combination between technological health indicators and public health indicators in every U.S. Census region which shares a statistically significant relationship the scaling factor β is sub-linear in nature. For a majority of combinations the scaling factor β is negative, indicating the existence of negative sub-linear relationships. This implies that as the technological innovation indicator scores move from poor to best the public health indicator scores also move from poor to best.

Based on the results of the data analysis presented above we can reject the null hypotheses 2a, 2b, 2c and 2d. In other words no evidence was found which supports the hypotheses that there is no statistically significant relationship between any technological innovation indicator and any public health indicator combination for any of the four U.S. Census regions at the 0.05 level of significance.

XIII. Structural Equation Modeling

It must also be noted R^2 values range from low to moderate for all combination between technological health indicators and public health indicators in every U.S. Census region which shares a statistically significant relationship. The highest R^2 values were 66.460 and 60.234 for *scientist and engineer occupation* indicator - *health status* indicator combination and *articles per 1000 capita* indicator – *suicide rate* indicator combination respectively in the South U.S. Census region. Relative weakness of R^2 values for individual combinations prompted a need to further explore the relationship between the two types of indicators. Structural equation modeling – SEM was employed to study linkages between the constructs of public health and technological innovation. The SEM structural model can be used to describe the causal relationships among the latent variables/constructs (Anderson and Gerbing, 1982).

SEM models consist of observed variables (also called manifest or measured, MV for short) and unobserved variables (also called underlying or latent, LV for short) that can be independent (exogenous) or dependent (endogenous)\ in nature. LVs are hypothetical constructs that cannot be directly measured, and in SEM are typically represented by multiple MVs that serve as indicators of the underlying constructs. The SEM model is an a priori hypothesis about a pattern of linear relationships among a set of observed and unobserved variables (Shah & Goldstein, 2006, p. 149)

For this research effort the covariance based partial least square – PLS technique for SEM was used to explore the relationship between public health and technological innovation.

PLS path models are formally defined by two sets of linear equations: the inner model and the outer model. The inner model specifies the relationships between unobserved or latent variables, whereas the outer model specifies the relationships between a latent variable and its observed or manifest variables. (Henseler, Ringle, & Sinkovics, 2009, p. 284)

The nonparametric bootstrap procedure can be used in PLS path modeling to provide confidence intervals for all parameter estimates, building the basis for statistical inference … The PLS results for all bootstrap samples provide the mean value and standard error for each path model coefficient. This information permits a student's t-test to be performed for the significance of path model relationships. (Henseler, Ringle, & Sinkovics, 2009, p. 305-306).

For the purposes of this research effort the constructs of technological innovation and public health have been defined in terms various indicators. Henseler, Ringle, & Sinkovics (2009) asserted that a formative measurement model "…is adequate when a construct is defined as a combination of its indicators" (p. 289). Furthermore, the PLS bootstrap path modeling algorithm allows for the computation of cause effect relationship models that employ both reflective and formative measurement models (Diamantopoulos & Winklhofer, 2001). Green & Ryans (1990), Johansson & Yip (1994), Birkinshaw, Morrison, & Hull (1995), Venaik, Midgley, & Devinney (2005), Julien & Ramangalahy (2003) and, Nijssen & Douglas (2008) maintained that the PLS could be used for data with any type of distribution and in cases with large or small sample sizes. It could hence, be inferred that the PLS SEM formative models will be adequate for data associated with this research study.

In order to setup the PLS cause effect diagrams the constructs of public health and technological innovation were described in terms of following latent variables: health outcomes, innovation input and innovation output. The technological innovation indicators formed the exogenous variables. The public health indicators formed the endogenous variables. The variables, t values and the SEM for the Midwest U.S. Census region is shown in figure 1. The variables, t values and the SEM for the Northeast U.S. Census region is shown in figure 2. The variables, t values and the SEM for the South U.S. Census region is shown in figure 3. The variables, t values and the SEM for the West U.S. Census region is shown in figure 4. For data samples with degrees of freedom ≥ 60, statistical significance is demonstrated at 95%, two sided, confidence intervals if the t values ≥2.

Factor loadings for each indicator are also shown these figures. Discussion of results of factor analyses associated with PLS SEM is beyond the scope of this paper. However, detailed results of the PLS SEM are presented in Appendix C.

For the Midwest U.S. Census region the paths from innovation input to innovation output and from innovation output to health outcomes have t values greater than 2. For the Northeast U.S. Census region the paths from innovation input to innovation output, from innovation input to health outcomes and, from innovation output to health outcomes have t values greater than 2.

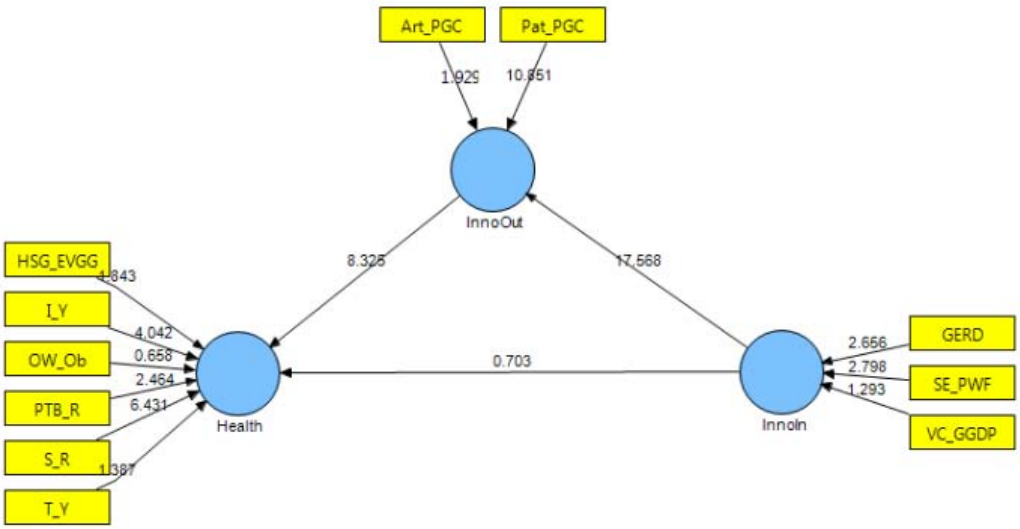

Figure 1 : SEM for Midwest U.S. Census region - Bootstrap sample rate 300

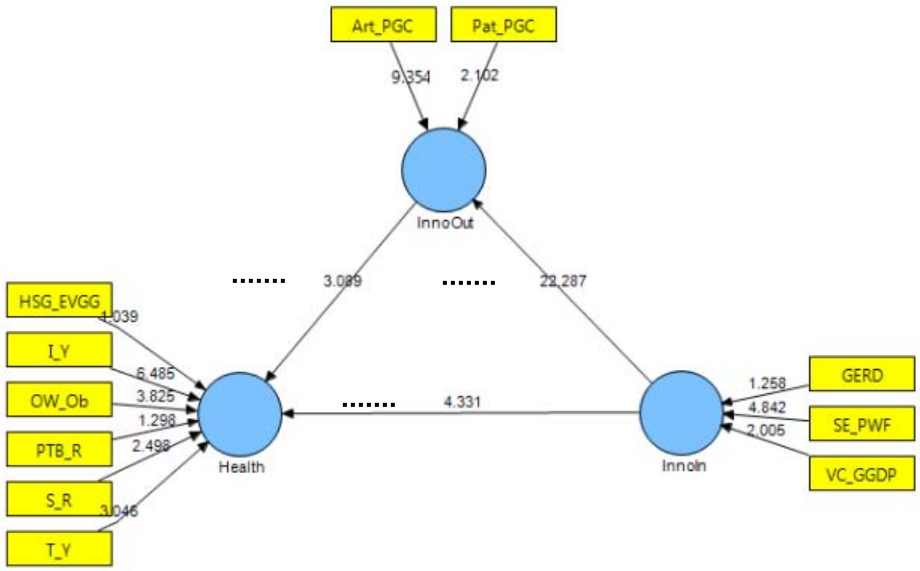

Figure 2 : SEM for Northeast U.S. Census region - Bootstrap sample rate 300

For the South U.S. Census region the paths from innovation input to innovation output, from innovation input to health outcomes and, from innovation output to health outcomes have t values greater than 2. For the West U.S. Census region the paths from innovation input to innovation output and from innovation output to health outcomes have t values greater than 2.

The results of PLS SEM provide evidence that there could be a causal relation between innovation outputs and health outcomes for all four U.S. Census regions. Additionally, the results of PLS SEM also provide evidence that there could be causal relation between innovation inputs and health outcomes for the South and Northeast regions of the U.S. Census regions.

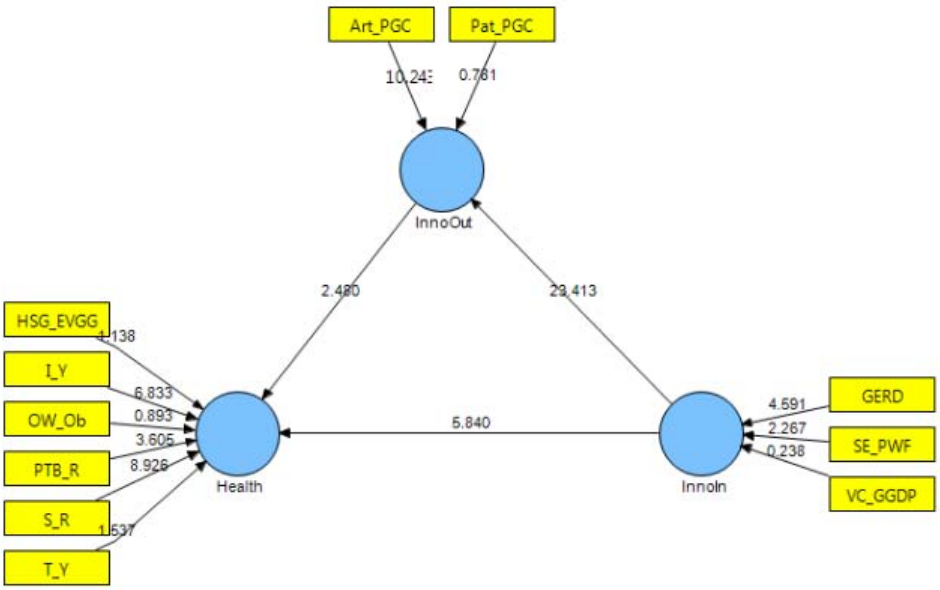

Figure 3 : SEM for South U.S. Census region - Bootstrap sample rate 300

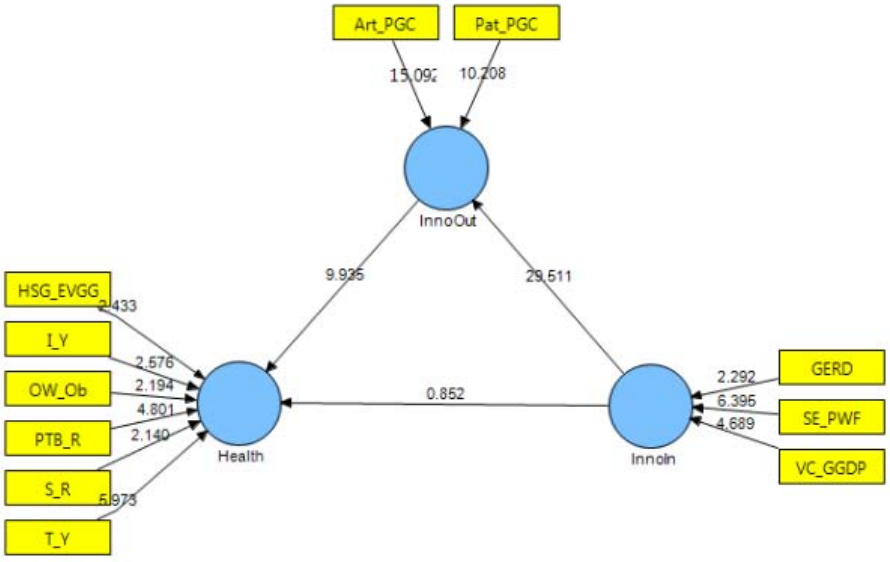

Figure 4 : SEM for West U.S. Census region - Bootstrap sample rate 300

XIV. Discussion/Conclusion

The results of the data analyses show that various U.S. Census regions fare differently in terms of technological innovation and public health. In other words technological innovation scores and public health indicator scores were at different levels for the four U.S. Census regions. It was found that the South region lagged behind other regions for both sets of indicator scores. Additionally, the Northeast regions led other regions for both sets of indicator scores. Further research should focus on studying the reasons behind this disparity between the four regions.

The relationships between the technological innovation indicators and public health indicators were quantified in terms of power law regression equations. It was found that technological innovation and public health generally share a sub-linear relation. For multiple technological innovation indicator and public health indicator combinations the relationship was negative sub-linear. Hence, it could be argued that better technological innovation is linked with better public health. The power law regression equations could serve as predictive models which could be used to calculate projected improvement in the public health indicators given a specific improvement in the technological indicators. Future studies should explore the relationship between technological innovation and public health in terms indicators not included in this study. The study should be repeated for longer periods of time to improve validity of the results. Future research could also focus on identifying the specific dimensions of technological innovation which directly impact the public health.

The results of SEM data analyses provided evidence that high levels of technological innovation were associated with better public health. Additional research, including experimental studies, is needed to confirm the causal effect of technological innovation on public health. If such a casual relation is confirmed policy makers could, for example, focus on enhancing the numbers of scientists and engineers in the work force. The scientists and engineers would in turn generate more patents and articles which in turn could lead to better public health.

The results of the data analyses also build the case that that policy makers should focus on development of the broad spectrum of technologies rather than solely focusing on health related technologies to improve public health. Additionally, this research study could serve as a guideline to compare various geographical regions - countries, states, counties - in terms of public health and technological innovation. Such a comparison provides a methodology to uncover areas in need of improvement. The methodology used in this study could be used to benchmark geographical regions with successful and synergetic technological innovation public health systems.

XV. Acknowledgment

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Appendix A

Coding for indicators

Indicator type	Indicator	Indicator code
Public Health Indicators	Health status	HSG_EVGG
	Insurance	I_Y
	Obesity and overweight	OW_Ob
	Preterm birth rate	PTB_R
	Suicide rate	S_R
	Tobacco use	T_Y
Technological Innovation Indicators	Articles per 1000 capita	Art_PGC
	Patents per 1000 capita	Pat_PGC
	Percentage of workforce in science and engineering occupation	SE_PWF
	Value of R&D performed as percent of GDP	GERD
	Venture capital per $1000 of GDP	VC_GGDP

Table 8 : Indicator and associated codes

Multiple Range Tests

HSG_EVGG

Contrast	Sig.
M - N	
M - S	*
M - W	
N - S	*
N - W	
S - W	*

T_Y

Contrast	Sig.
M - N	*
M - S	*
M - W	*
N - S	*
N - W	
S - W	*

OW_Ob

Contrast	Sig.
M - N	*
M - S	
M - W	*
N - S	*
N - W	
S - W	*

PTB_R

Contrast	Sig.
M - N	*
M - S	*
M - W	*
N - S	*
N - W	*
S - W	*

I_Y

Contrast	Sig.
M - N	
M - S	*
M - W	*
N - S	*
N - W	*
S - W	

S_R

Contrast	Sig.
M - N	*
M - S	
M - W	*
N - S	*
N - W	*
S - W	*

Pat_PGC

Contrast	Sig.
M - N	*
M - S	*
M - W	
N - S	*
N - W	*
S - W	*

Art_PGC

Contrast	Sig.
M - N	*
M - S	*
M - W	*
N - S	*
N - W	*
S - W	

VC_GGDP

Contrast	Sig.
M - N	*
M - S	
M - W	*
N - S	*
N - W	
S - W	*

GERD

Contrast	Sig.
M - N	*
M - S	
M - W	*
N - S	*
N - W	*
S - W	*

SE_PWF

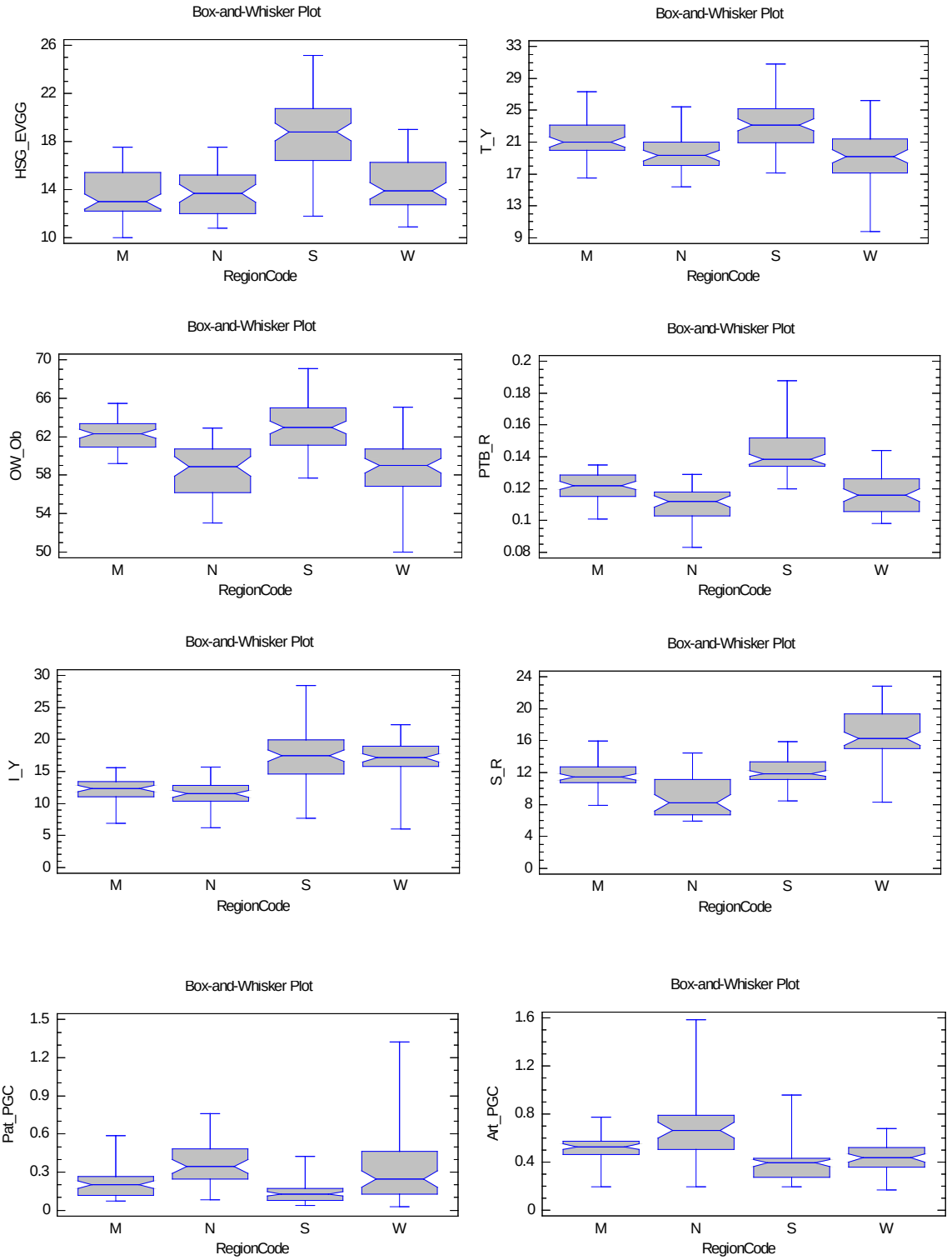
Contrast	Sig.
M - N	*
M - S	
M - W	
N - S	*
N - W	
S - W	*

* denotes a statistically significant difference in means

Figure 5 : ANOVA for indicator data from various U.S. Census regions

Appendix B

Kruskal -Wallis median comparison per indicator by Region



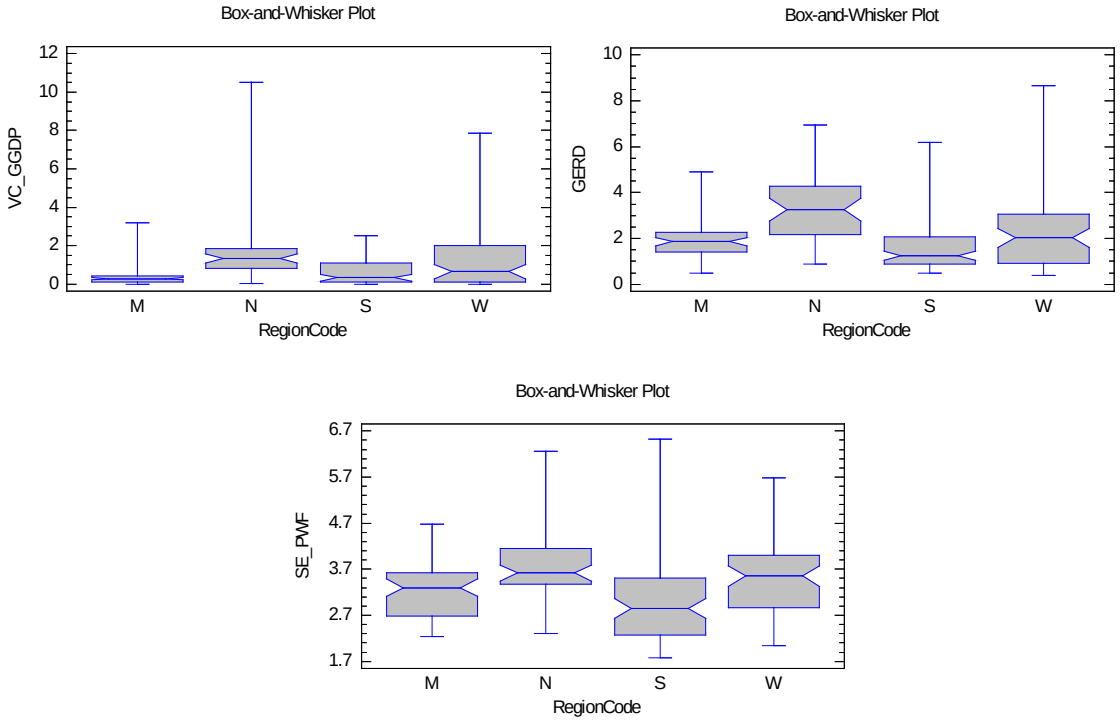


Figure 6 : Box whisker plots associated with the Kruskal-Wallis median comparison tests for the four U.S. Census regions with regards to each indicator

Appendix C

PLS SEM results

Table 9 shows path coefficients for the Midwest U.S. Census region. Table 10 shows path coefficients for the Northeast U.S. Census region. Table 11 shows path coefficients for the South U.S. Census region. Table 12 shows path coefficients for the West U.S. Census region.

	Original Sample (O)	Sample Mean (M)	Standard Deviation (STDEV)	Standard Error (STERR)	T Statistics (|O/STERR|)
InnoIn -> Health	0.1058	0.1288	0.1417	0.1417	0.7465
InnoIn -> InnoOut	0.7591	0.7771	0.0421	0.0421	18.0246
InnoOut -> Health	-0.9126	-0.9358	0.1073	0.1073	8.5055

Table 9 : PLS SEM path coefficients for Midwest U.S. Census region – Bootstrap sample rate 200

	Original Sample (O)	Sample Mean (M)	Standard Deviation (STDEV)	Standard Error (STERR)	T Statistics (|O/STERR|)
InnoIn -> Health	-0.5196	-0.5296	0.1315	0.1315	3.9518
InnoIn -> InnoOut	0.8626	0.8558	0.0381	0.0381	22.6555
InnoOut -> Health	-0.3893	-0.3818	0.1366	0.1366	2.8493

Table 10 : PLS SEM path coefficients for Northeast U.S. Census region – Bootstrap sample rate 200

	Original Sample (O)	Sample Mean (M)	Standard Deviation (STDEV)	Standard Error (STERR)	T Statistics (|O/STERR|)
InnoIn -> Health	-0.6302	-0.6403	0.0948	0.0948	6.6459
InnoIn -> InnoOut	0.8496	0.8494	0.0366	0.0366	23.2259
InnoOut -> Health	-0.281	-0.2735	0.1004	0.1004	2.7991

Table 11 : PLS SEM path coefficients for South U.S. Census region – Bootstrap sample rate 200

	Original Sample (O)	Sample Mean (M)	Standard Deviation (STDEV)	Standard Error (STERR)	T Statistics (|O/STERR|)
InnoIn -> Health	0.1041	0.0906	0.1247	0.1247	0.8346
InnoIn -> InnoOut	0.769	0.775	0.0266	0.0266	28.8883
InnoOut -> Health	-0.9474	-0.9436	0.0961	0.0961	9.8603

Table 12 : PLS SEM path coefficients for South U.S. Census region – Bootstrap sample rate 200

It must be noted that PLS SEM was run at 4 sample rate vis-à-vis the bootstrap algorithm: 200, 300, 500 and, 800 samples. The resultant t values for each sample rate point to the same conclusions described in the Structural Equation Modeling section.

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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.

Administration Rules Listed Before Submitting Your Research Paper to Global Journals Inc. (US)

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals Inc. (US):

Segment Draft and Final Research Paper: You have to strictly follow the template of research paper. If it is not done your paper may get rejected.

- 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.

Criterion for Grading a Research Paper (Compilation) by Global Journals Inc. (US)

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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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