Online ISSN: 2249-4618
Print ISSN: 0975-5888

Global Journal

of Medical Research: B

Pharma, Drug Discovery, Toxicology and Medicine

Assessment of Adherence

Evaluation of Anti-Angiogenic

Highlights

Comparative Antimicrobial

Anti-Hypertensive Medication

Discovering Thoughts, Inventing Future

VOLUME 15 ISSUE 1 VERSION 1.0

Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology and Medicine

Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology and Medicine
Volume 15 Issue 1 (Ver. 1.0)

Open Association of Research Society

Incorporation No.: 0423089
License No.: 42125/022010/1186
Registration No.: 430374
Import-Export Code: 1109007027
Employer Identification Number (EIN):
USA Tax ID: 98-0673427

Global Journals Inc.

*(A Delaware USA Incorporation with "Good Standing"; **Reg. Number: 0423089**)*

Sponsors: Open Association of Research Society
Open Scientific Standards

Publisher's Headquarters office

Global Journals Headquarters
301st Edgewater Place Suite, 100 Edgewater Dr.-Pl,
Wakefield MASSACHUSETTS, Pin: 01880,
United States of America
USA Toll Free: +001-888-839-7392
USA Toll Free Fax: +001-888-839-7392

Offset Typesetting

Global Journals Incorporated
2nd, Lansdowne, Lansdowne Rd., Croydon-Surrey,
Pin: CR9 2ER, United Kingdom

Packaging & Continental Dispatching

Global Journals
E-3130 Sudama Nagar, Near Gopur Square,
Indore, M.P., Pin:452009, India

Find a correspondence nodal officer near you

To find nodal officer of your country, please email us at *local@globaljournals.org*

eContacts

Press Inquiries: *press@globaljournals.org*
Investor Inquiries: *investors@globaljournals.org*
Technical Support: *technology@globaljournals.org*
Media & Releases: *media@globaljournals.org*

Pricing (Including by Air Parcel Charges):

For Authors:
22 USD (B/W) & 50 USD (Color)
Yearly Subscription (Personal & Institutional):
200 USD (B/W) & 250 USD (Color)

Integrated Editorial Board
(Computer Science, Engineering, Medical, Management, Natural Science, Social Science)

Dr. Bart Lambrecht
Director of Research in Accounting and FinanceProfessor of Finance
Lancaster University Management School
BA (Antwerp); MPhil, MA, PhD (Cambridge)

Dr. Carlos García Pont
Associate Professor of Marketing
IESE Business School, University of Navarra
Doctor of Philosophy (Management), Massachusetts Institute of Technology (MIT)
Master in Business Administration, IESE, University of Navarra
Degree in Industrial Engineering, Universitat Politècnica de Catalunya

Dr. Fotini Labropulu
Mathematics - Luther College
University of ReginaPh.D., M.Sc. in Mathematics
B.A. (Honors) in Mathematics
University of Windso

Dr. Lynn Lim
Reader in Business and Marketing
Roehampton University, London
BCom, PGDip, MBA (Distinction), PhD, FHEA

Dr. Mihaly Mezei
ASSOCIATE PROFESSOR
Department of Structural and Chemical Biology, Mount Sinai School of Medical Center
Ph.D., Etvs Lornd University
Postdoctoral Training,
New York University

Dr. Söhnke M. Bartram
Department of Accounting and FinanceLancaster University Management SchoolPh.D. (WHU Koblenz)
MBA/BBA (University of Saarbrücken)

Dr. Miguel Angel Ariño
Professor of Decision Sciences
IESE Business School
Barcelona, Spain (Universidad de Navarra)
CEIBS (China Europe International Business School).
Beijing, Shanghai and Shenzhen
Ph.D. in Mathematics
University of Barcelona
BA in Mathematics (Licenciatura)
University of Barcelona

Philip G. Moscoso
Technology and Operations Management
IESE Business School, University of Navarra
Ph.D in Industrial Engineering and Management, ETH Zurich
M.Sc. in Chemical Engineering, ETH Zurich

Dr. Sanjay Dixit, M.D.
Director, EP Laboratories, Philadelphia VA Medical Center
Cardiovascular Medicine - Cardiac Arrhythmia
Univ of Penn School of Medicine

Dr. Han-Xiang Deng
MD., Ph.D
Associate Professor and Research
Department Division of Neuromuscular Medicine
Davee Department of Neurology and Clinical NeuroscienceNorthwestern University
Feinberg School of Medicine

Dr. Pina C. Sanelli
Associate Professor of Public Health
Weill Cornell Medical College
Associate Attending Radiologist
NewYork-Presbyterian Hospital
MRI, MRA, CT, and CTA
Neuroradiology and Diagnostic Radiology
M.D., State University of New York at Buffalo,School of Medicine and Biomedical Sciences

Dr. Roberto Sanchez
Associate Professor
Department of Structural and Chemical Biology
Mount Sinai School of Medicine
Ph.D., The Rockefeller University

Dr. Wen-Yih Sun
Professor of Earth and Atmospheric SciencesPurdue University Director
National Center for Typhoon and Flooding Research, Taiwan
University Chair Professor
Department of Atmospheric Sciences, National Central University, Chung-Li, TaiwanUniversity Chair Professor
Institute of Environmental Engineering, National Chiao Tung University, Hsin-chu, Taiwan.Ph.D., MS The University of Chicago, Geophysical Sciences
BS National Taiwan University, Atmospheric Sciences
Associate Professor of Radiology

Dr. Michael R. Rudnick
M.D., FACP
Associate Professor of Medicine
Chief, Renal Electrolyte and Hypertension Division (PMC)
Penn Medicine, University of Pennsylvania
Presbyterian Medical Center, Philadelphia
Nephrology and Internal Medicine
Certified by the American Board of Internal Medicine

Dr. Bassey Benjamin Esu
B.Sc. Marketing; MBA Marketing; Ph.D Marketing
Lecturer, Department of Marketing, University of Calabar
Tourism Consultant, Cross River State Tourism Development Department
Co-ordinator , Sustainable Tourism Initiative, Calabar, Nigeria

Dr. Aziz M. Barbar, Ph.D.
IEEE Senior Member
Chairperson, Department of Computer Science
AUST - American University of Science & Technology
Alfred Naccash Avenue – Ashrafieh

Contents of the Issue

Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology and Medicine
Volume 15 Issue 1 Version 1.0 Year 2015
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Antibiotics Utilization and their Cost in Ayder Referral Hospital, Mekelle, Ethiopia

By Solomon Abrha, Rahel Assefa, Fantahun Molla, Wondim Melkam, Admassu Assen, Anwar Mulugeta, Abrham Wondimu, Jemal Mohammed & Birhanetensae Masresha

Mekelle University, Ethiopia

Abstract- Inappropriate use of antibiotics can potentially lead to antimicrobial resistance and increase the necessity to use more expensive antibiotics to treat common and life threatening infections. The major goal of this research was to determine antibiotics utilization and their cost among in-patients treated in Ayder Referral Hospital. An institution based cross-sectional study was conducted in medical, gynecology and obstetrics, and surgical wards from September to December, 2012. The prevalence of antibiotics use was 35.5%. Antibiotic therapy was found to be inappropriate in 137 patients (80.6%).

Keywords: irrational drug use, antibiotics, ayder referral hospital, cost, inpatients.

GJMR-B Classification : NLMC Code: QV 252

Strictly as per the compliance and regulations of:

Antibiotics Utilization and their Cost in Ayder Referral Hospital, Mekelle, Ethiopia

Solomon Abrha [α], Rahel Assefa [σ], Fantahun Molla [ρ], Wondim Melkam [ω], Admassu Assen [¥], Anwar Mulugeta [§], Abrham Wondimu [χ], Jemal Mohammed [ν] & Birhanetensae Masresha [Θ]

Abstract- Inappropriate use of antibiotics can potentially lead to antimicrobial resistance and increase the necessity to use more expensive antibiotics to treat common and life threatening infections. The major goal of this research was to determine antibiotics utilization and their cost among in-patients treated in Ayder Referral Hospital. An institution based cross-sectional study was conducted in medical, gynecology and obstetrics, and surgical wards from September to December, 2012. The prevalence of antibiotics use was 35.5%. Antibiotic therapy was found to be inappropriate in 137 patients (80.6%). The most common reason for inappropriateness was the inappropriate duration of treatment 65(47.4%). Cephalosporins were the most commonly used antibiotics which accounted for 32.7% of the total number. The most common disease for which antibiotics were prescribed was respiratory tract infections 69(37.1%) followed by genitourinary infections 45(24.2%). Many patients were given intravenously administered antibiotics which comprise 71(55.9%) prescribed antibiotics. In this study, antibiotics were used inappropriately and the patients were incurred to high cost due to irrational drug use. It is recommended that the hospital should develop specific formularies, treatment guidelines and antimicrobial policies so as to standardize the treatment protocols which are used for infectious diseases and to promote rational use of antibiotics.

Keywords: irrational drug use, antibiotics, ayder referral hospital, cost, inpatients.

I. Introduction

Antimicrobials are agents that can suppress the growth of pathogens or destroy them. Use of these drugs in clini¬cal practice has changed the natural course and improved the prognosis of infectious diseases. Appropriate antibiotic use is one of the main goals of the medical community.1, 2 They can be used as prophylactic and therapeutic agents, but their increasing and in¬discriminate uses are the main contributors to the emergence of resistant microbial strains.2 The issues of antimicrobial misuse are of global concern, not only because of the development and spreading of antimicrobial antibiotics resistant bacteria, but also due to escalating health care costs that cause severe financial hardship for the poor in developing countries.[3]

The primary objective of drug utilization studies is to enhance the rational use of drugs in populations. For the individual patient, the rational use of antibiotics implies the use of the right drug with the right dose at the right interval and at the right duration together with the correct information, at an affordable price.4 When the use of drugs is not in accordance with the above definition, there are often undesirable health and/or economic problems, such as insufficient therapeutic effect, adverse drug reactions, preventable side effects, interactions of drugs and the worst of all is increasing resistance of bacteria to antimicrobial medicines which in turn results in increased, prolonged and expensive hospital admission.[5]

Hence, drug utilization research can increase our understanding of how drugs are being used and pave the way to manage undesirable health and/or economic problems resulted from inappropriate use. Several reports have investigated the antibiotic utilization pattern in various hospitals around the world.1-5 These studies have reported concern about the continuous, indiscriminate, and excessive use of antimicrobial agents that promote the emergence of antibiotic-resistant organisms.6 More than 80% of the most common bacteria, Staphylococcus aureus are now resistant to penicillin such as ampicillin.7 Given the fact that the rule and regulation of drug control in developing courtiers is not as firm as developed countries, high level and irrational antimicrobial use, in fact, would aggravate the emergency of antimicrobial resistance.[8]

Monitoring antimicrobial use as well as evaluating prescription habits are among the strategies recommended to contain resistance to antimicrobials in hospitalized patients. Antimicrobial resistance substantially raises already-rising health care costs and ultimately increases patient morbidity and mortality.[9] Hence, the present study was conducted to determine antibiotic utilization among inpatients at ARH in Mekelle, Ethiopia.

II. Methodology

a) Study Area

The study was conducted in Ayder referral and teaching hospital (ARH), in Mekelle town which is

Author α : Department of Pharmacy, College of Health Sciences, Mekelle University, Mekelle, Ethiopia.
e-mails: abrha.solomon13@gmail.com
Author σ ρ¥ χ: Pharmaceutics Course and Research Unit, Department of Pharmacy, College Health Sciences, Mekelle University.
e-mails: fantahunmolla@gmail.com, admassuhassen77@gmail.com, rhichail@gmail.com
Author ω ν Θ: Pharmacology and Toxicology Course and Research Unit, Department of Pharmacy, College Health Sciences, Mekelle University. e-mail: melkamwondin@gmail.com
Author §: Department of Pharmacology, Faculty of Medicine, Addis Ababa University.

located 783 km away from Addis Ababa, capital city of Ethiopia. ARH provides its medical services to around 8 million populations in its catchment areas of Tigray, Afar and South-eastern parts of the Amhara Regional States. It has a total capacity of 500 beds in four major departments and other specialty units along with six other affiliated hospitals in the Tigray regional state. The hospital has more than 45 specialists in the various areas of medical specializations and adequate number of other health professionals which constitute the health care team.

b) Study design, study population and sampling

An institution based cross-sectional study was conducted in medical, gynecology and obstetrics, and surgical wards from September to December, 2012. The study population was all admitted patients in the aforementioned wards within the study period. The sample size was calculated by considering 95% confidence interval, 5% margin of error and 10% contingency for loss. The calculated sample size was 170. Samples were selected using simple random sampling within their respective wards and the samples were allocated to each ward proportionally.

c) Data collection and analysis

The medical record (chart) of each patient was reviewed and information pertinent to the objective of the study was collected. Medication data including name of the antibiotics prescribed, dosage regimen (dosage form, dose, route, frequency and duration of administration) and use of antibiotic combination were noted.

Data were coded, checked for completeness and consistency. Data processing and analyzing were done by using statistical package for social sciences (SPSS) version 20 and Microsoft excel 2010. Descriptive statistics such as frequency and proportion for categorical variables including cross-tabulations were used for data summarization. The local prices of antibiotics were based on the respective hospital's pharmacy prices. All p values were two tailed with the significance level set at 0.05. Use of antibiotics was measured based on the standard treatment guideline (STGs) prepared by FMOH and distributed to different hospitals including ARH.

d) Ethical consideration

The study was approved by the health research ethics review committee (HRERC) of college of health sciences, Mekelle University. The purpose of the study was explained to respective departments and letter of permeation was sought from the heads of departments of Internal medicine, Surgery and Gynecology and obstetrics.

III. Results

a) Demographic and clinical characteristics of in-patients

Medical records of 722 patients were reviewed in order to identify the use of antibiotics during their hospital stay. Among these, 170 patient's medical records with complete information were found to be eligible for further study. The demographic characteristics of the patients are shown in Table 1. About 53.3% of them were female, and three quarter of patients belonged to the 53-92 year age group. The patients were admitted in three different wards i.e. surgical, internal medicine and gynecology and obstetrics ward with gynecology being the highest with 87 (51.2%) admissions. Among the total, 255 patients received antibiotics and the percentage of antibiotics utilization was calculated to be 35.6%.

Table 1 : Age, sex, and ward distribution of patients prescribed with antibiotics in ARH, Mekelle, Ethiopia, 2012 (n=170)

Variables	Frequency	Percentage (%)
Gender		
Male	79	46.5
Female	91	53.5
Age group		
19-52	127	74.7
53-92	43	25.3
Ward		
Internal Medicine	87	51.2
Surgery	47	27.6
Gynecology and obstetrics	36	21.2

Patients admitted in three wards with antibiotics regimens included infections of different organ systems. Most patients were diagnosed with respiratory tract infections (RTI) 69(37.1%), followed by genitourinary infections (GUTI) 45(24.2%) (Figure 1).

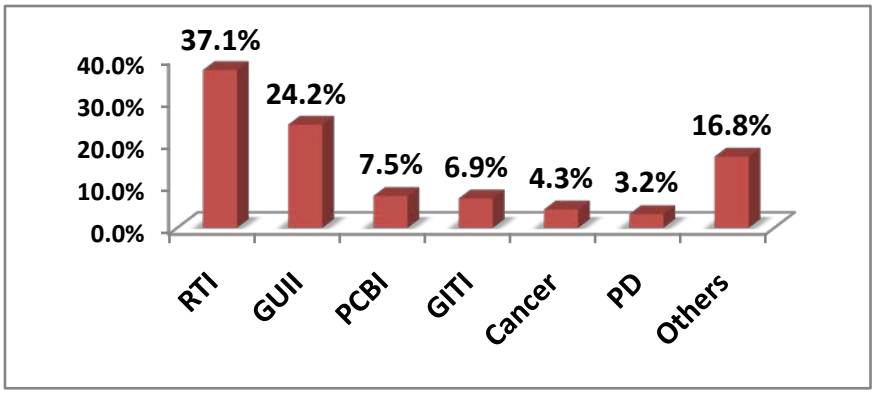


Figure 1 : Diagnosis of patients prescribed with antibiotics in ARH, Mekelle, Ethiopia, 2012 (n=170) (RTI: respiratory tract infection, GUTI: genitourinary infection, PCBI: pregnancy and childbirth infections, GITI: gastrointestinal infections, PD: parasitic diseases)

As it is indicated in Figure below, 41.2% of the patients received single antibiotics, whereas 58.8% received more than two antibiotics per prescription during their hospital stay.

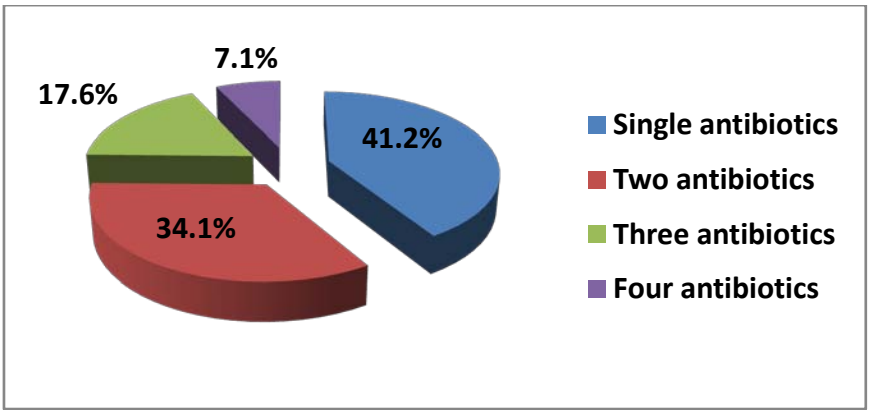


Figure 2 : Number of antibiotics prescribed for patients per prescription in ARH, Mekelle, Ethiopia, 2012 (n=170)

b) Antibiotics use in selected wards during the study period

A total of eleven different antibiotic groups and 306 single antibiotics were used in all wards (Table 2). Among these, 100(32.7%) antibiotics were prescribed from cephalosporins followed by 48(15.7%) from nitro immidazoles (metronidazole) and 46(15%) from penicillins. Surprisingly, only 2(0.7%) aminoglycosides was prescribed during the study period.

Table 2 : Antibiotic groups used for patients in ARH, Mekelle, Ethiopia, 2012 (n=306)

Antibiotics Group[a]	Frequency	Percentage (%)
Cephalosporins	100	32.7
Nitro immidazoles	48	15.7
Penicillins	46	15
Anti-TB[b]	32	10.4
Quinolones	31	10.1
Macrolides	26	8.5
Tetracyclines	18	5.9
Aminoglycosides	2	0.7
Other antibiotics[c]	3	1

[a] *Grouping was based on 'Anatomical Therapeutic Chemical Classification System'*
[b] *Isoniazid, ethambutal, rifampicin, streptomycin*
[c] *Other antibiotics Included Clindamycin, Vancomycin*

The frequency of single antibiotics use is shown in Table 3. A total of 306 single antibiotics were used in all wards with ceftriaxone being the most commonly used antibiotics 88(28.7%) followed by metronidazole and penicillin with 48(15.7%) and 46(15%), respectively.

Table 3 : Most frequently used single antibiotics for patients in ARH, Mekelle, Ethiopia, 2012 (n=306)

Single Antibiotics	Frequency	Percentage (%)
Ceftriaxone	88	28.7
Metronidazole	48	15.7
Anti-TB[a]	32	10.4
Clarithromycin	22	7.1
Ciprofloxacin	19	6.2
Ampicillin	18	5.9
Doxycycline	17	6
Amoxicillin	12	3.9
Cloxacillin	12	3.9
Norfloxacillin	12	3.9
Cephalexin	10	3.2
Other antibiotics[b]	16	5.2

[a] *Isoniazid, ethambutal, rifampicin, streptomycin*

[b] *Included Erythromycin, Gentamicin, Vancomycin, Augmentin, C.penicillin, Clindamycin, Azythromycin, Ceftazidime, Cefuroxime, TTCeyeointment*

From the total single antibiotics, the majority of the antibiotics were given intravenously 171(55.9%) followed by oral administration 134(43.8%) (Figure 3).

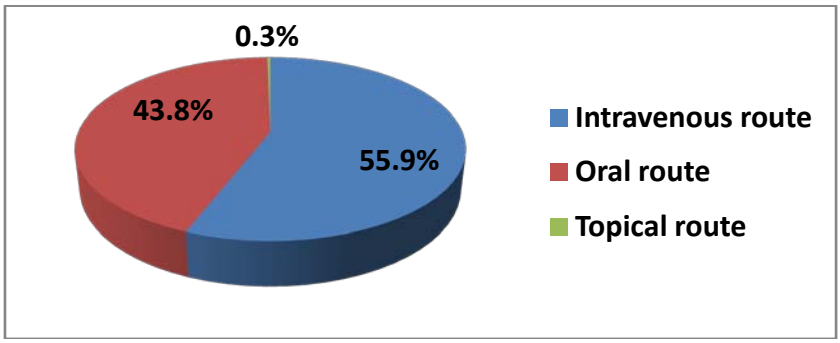


Figure 3 : Route of administration of antibiotics in ARH, Mekelle, Ethiopia, 2012 (n=306)

c) Rationality of antibiotic use

Antibiotic therapy was found to be inappropriate in 137 patients (80.6%). The most common reason for inappropriateness was improper duration of treatment (DOT) 65(47.4%) followed by improper drug regimen 52(38%) and unjustified use 20(14.6%) (Table 4). All of the reasons for inappropriateness were found to be statistically significant.

Table 4: Reasons for inappropriateness of antibiotic therapies in ARH, Mekelle, Ethiopia, 2012 (n=137)

Reason for inappropriateness	Frequency	Percentage (%)	*p*-value	OR (95% CI)
DOT	65	47.4	0.011	4.725 (1.425- 15.671)
Regimen*	52	38	0.009	15.900 (2.025- 124.850)
Unjustified use	20	14.6	0.024	3.814 (1.198- 12.148)

**Improper dose and frequency*

d) Cost of antibiotics

The local price and total cost expenditure of antibiotics during the study period is shown in Table 5. A total of 55125 (3243 USD) Ethiopian birr was spent to purchase 306 antibiotics during the study period.

Table 5 : Total cost of antibiotics used in ARH, Mekelle, Ethiopia, 2012 (n=306)

Antibiotics prescribed	Unit	Frequency	Average DOT[a]	Total Qty prescribed	Unit price		Total cost		Percent (%)
					Br	Ce	Br	Ce	
Ceftriaxone	Vial	Bid	9 days	88	9	90	15681	60	28.4
Metronidazole	Vial	Tid	11 days	48	18	00	28512	00	51.7
Clarithromycin	Tab	Bid	9 days	22	5	40	2138	40	3.88
Ciprofloxacin	Tab	Bid	8 days	19	1	00	304	00	0.55
Ampicillin	vial	Qid	7 days	18	1	56	786	24	1.4
Doxycycline	Cap	Bid	8 days	17	0	28	76	16	0.14
Amoxicillin	Cap	Tid	13 days	12	0	88	411	84	0.7
Cloxacillin	Vial	Qid	9 days	12	2	90	1252	80	2.27
Norfloxacin	Tab	Bid	8 days	12	0	59	113	28	0.21
Cephalexin	Cap	Qid	7 days	10	2	50	700	00	1.27
Erythromycin	Tab	Qid	12 days	2	0	80	76	80	0.14
Vancomycin	vial	Bid	5 days	2	142	00	2840	00	5.15
Gentamycin	Amp	Bid	7 days	2	1	56	43	68	0.08
Augmentin	Tab	Tid	9 days	3	3	70	299	70	0.54
C.penicillin	Vial	Q4hr	19 days	2	4	40	1003	20	1.82
Clindamycin	Tab	Tid	11 days	1	9	40	310	20	0.56
Azithromycin	Tab	Qd	3 days	1	26	13	78	39	0.14
Ceftazidime	Vial	bid	10 days	1	7	00	140	00	0.25
Cefuroxime	cap	Bid	7 days	1	23	20	324	80	0.59
TTCeyeoint	tube	bid	5 days	1	3	25	32	50	0.06
		Total					55125	59	

**The local prices of antibiotics were based on the respective hospital pharmacy's prices*

**1USD=17 ETB (exchange rate when the study was conducted)*

a DOT: duration of treatment

IV. Discussion

Appropriate antibiotic use has both clinical and economic significance to any health system and should be given adequate attention. Inappropriate use of antibiotics can potentially lead to antimicrobial resistance and increase the necessity to use more expensive antibiotics to treat common and life threatening infections.10 The finding of the present study revealed the presence of high levels (80.6%) of the inappropriate use of antibiotics in the study area. This result is more or less similar to the findings of studies done in Thailand (80.9%) 11 and Japan (73.3%) 6; higher than study conducted in Sudan (60%) 3 and Gondar, Ethiopia (70.8%) 12; and much higher than the study conducted in Canada (13.8%) 13 and Turkey (35.6%) 14. From this trend it can easily be understood that the principles of rational use of antimicrobial have been well established in developed countries whereas their inappropriate use is still out of control, in developing countries. For that matter, a selective restriction policy of antibiotic use with the aid of agreed guidelines can lower the rate of inappropriate use of antibiotics in developing country including Ethiopia. Besides, using an antibiotic order form for restricted antibiotics and audited by pharmacists could enhance a more appropriate use of the antibiotics.

The possible reasons for irrational use of antibiotics were also assessed in this study. Duration of treatment (47.4%), regimen (38%), and unjustified use (14.6%) were found to be significantly associated with inappropriate use of antibiotics in this hospital ($p<0.05$). Similar reasons were also forwarded in the results of the study done by Baktygul K and his co-workers. 6 Besides, drug that did not follow the specified indications, no dosage adjustment in patients with renal impairment, improper dose, improper dosing interval were reported as reasons for irrational antibiotics use in the study conducted in teaching hospital, Thailand.11

The group of drugs mostly used in the study site included cephalosporins , nitro immidazoles and penicillins. The same group of antibiotics but different in proportion with the current study was also reported by Katakam P et al., 2012.9 In addition, penicillins, aminoglycosides, and cephalosporins were demonstrated as the most frequently used antibiotic group in the result of the study done in Japan.6 From the preceding it is understood that penicillins and cephalosporins have been continued to be a mainstay of therapy in hospitals because of their broad spectrum of activity, clinical efficacy and favorable tolerability profiles.6 However, studies in hospitals of Jordan15 and Estonia16 reported different groups of antibiotics as compared to the aforementioned studies. For instance, fluoroquinolones,

penicillins, and aminoglycosides were the most commonly used antibiotics in Jordan whereas tetracyclines and aminoglycosides were the antibiotics used most commonly in Tartu hospital, Estonia. In general, the wards of similar medical specialties used similar groups of antibiotics.

The most frequently used single antibiotics in the current hospital were ceftriaxone (28.7%), followed by metronidazole (15.7%), anti-TB drugs and clarithromycin (7.1%), whereas study conducted in Libya 9 reported amoxicillin+clavulanic, ceftriaxone and metronidazole were the commonly utilized antibiotics with proportion of 31.3, 26.6 and 13.3%, respectively. Another study from Japan indicated that penicillin G , gentamicin and metronidazole were the most frequently used antibiotics of which Penicillin G was the most prevalent with 24.5% as opposed to 15.9 % for gentamicin and 15.4% for metronidazole6.The variation observed could be due to differences in disease pattern and drug availability in different countries.

It is generally preferable to keep the number of antibiotics per prescription as low as possible to minimize the risk of drug interaction, development of bacterial resistance and hospital cost, and to enhance patient compliance. 17 In this study, a considerable number of patients received as many as 3 to 4 antibiotics in a single encounter, whereas majority received only 1 or 2 antibiotics. The average number of antibiotics used per encounter in this study was more or less similar to what was obtained in teaching hospitals of Southern Nigeria 4 and northwest Ethiopia. 12 However, there was polypharmacy in more than half of (59.8%) the encounters which is far from WHO recommended value (1.6-1.8) in this study. This might be due to empirical use of antibiotics as infectious diseases are prevalent in Ethiopia.

Respiratory tract infections (37.1%), genitourinary infection (24.2%), infections related to pregnancy and childbirth (7.5%), gastrointestinal infections (6.9%), cancer (4.3%) and parasitic diseases (3.2%) were the conditions for which patients were admitted with antibiotics regimens in the hospital. These findings are in agreement with other local studies 12 as well as studies in Africa 9 and Asia. 6, 11 However, recent survey in South Nigeria found that the conditions for which antibiotics were prescribed included trauma (14.3%), malaria fever (14.1%), cardiovascular diseases (13.5%), retroviral disease (11.8%), and central nervous system disorders (6.1%).17

Administering antibiotics using intravenous (IV) route is appropriate when oral route is not effective, rapid response is needed, and large doses are required which is not feasible with the oral route.18 In this study, 55.9% of the antibiotics were administered through IV route while 43.8% were administered orally. Similar findings were also revealed in the studies conducted in hospitals of Nigeria 17 and Japan 6. Changing route of administration from intravenous to oral route has been studied and shown to save costs, shorten the length of hospital stays, and decrease the adverse reactions of intravenous administration, all with equal therapeutic outcome.6

The cost of antibiotics used by the patients during the study period was also investigated in the current study. A total of 3243 USD (55125 ETB) was spent by the patients for antibiotics which is 0.5% of the annual budget of the hospital for medications. This finding is in accordance with the study done by Erah PO and his co-workers in Nigeria. 17 In their result, the authors pointed out that many antibiotics in Nigeria are too expensive for the patients to purchase and the possible reasons mentioned by the authors includes high cost of transportation and many local taxes. All in all, where possible, nearly all patients would prefer to receive treatment with minimum cost. However, due to irrational drug use and high resistance of many microorganisms to many other antibiotics, the prices being paid by patients for medicines are major concern in health care delivery in developing countries. In our setting, for instance, there was a high use of intravenous antibiotic. This could be raised as one reason for high cost incurred by the patients in the study period as intravenous antibiotics account for the most expensive category of antibiotics in hospitalized patients.19

V. Conclusions and Recommendations

The prevalence of inappropriate use of antibiotics in Ayder referral hospital was 80.6%. The major reasons for inappropriate use of antibiotics were found to be duration of treatment, regimen and unjustified use. Respiratory tract infections (RTI) and genitourinary infection (GUTI) were the two most commonly reported diagnosis for which patients received antibiotics in the current study. Cephalosporins , nitro immidazoles, and penicillins were mostly used groups of drugs, whereas ceftriaxone, metronidazole and anti-TB drugs were the most frequently used single antibiotics in the hospital. Parenteral route of administration was the most common route of administration. Moreover, relatively large amount of money (3243 USD) was spent by the patients for antibiotics. Hence, the following specific recommendations have been made based on the finding of the study in order to give a clue about the possible direction to follow and focus to alleviate the problems of antibiotics resistance occurred due to drug misuse. It is known that cost is an important factor governing access to and use of medicines in developing countries and irrational use of antibiotics could significant results in an increased cost. Therefore, many healthcare institutions should introduce programmmes aiming at reducing the expenditure by

improving rational antibiotic use, initiating education campaigns, regulating drug auditing practices, restricting dispensing techniques and controls. Furthermore, patients on intravenous therapy often has prolonged hospital stay to complete antibiotic treatment, a switch from intravenous to oral therapy could favor an earlier discharge and directly save health care costs.

References Références Referencias

1. Bantar C, Sartori B, Vesco E, Heft C, Saúl M, Salamone Fet, Oliva ME, A Hospital wide Intervention Program to Optimize the Quality of Antibiotic Use: impact on prescribing practice, antibiotic consumption, cost savings, and bacterial resistance, Clin Infect Dis., 37, 2003, 180-186.
2. Marcelo C, Tanise F, Marina B, Bruna EK, Antibiotic prescription in a teaching hospital: a brief assessment, Rev. Assoc. Med. Bras., 57, 2011, 414-417
3. Elsiddi HAA, Irrational use of antibiotics among people residing in Almamoura, Sud J Pub health., 5, 2010, 50-55.
4. Kayode OM, Olabisi ME, Assessment Of Rational Use Of Drugs Among Patients With Chronic Heart Failure (CHF) Using Case Notes Of Patients At The Tertiary Health Care Institution In South West Nigeria, J Pharm Biomed Sci., 22, 2012,:1-8.\
5. Ministry of health (MOH) of Pakistan, Rational antibiotic utilization in selected pediatric condition.
6. Baktygul K, Marat B, Ashirali Z, Harun-or-rashid MD, Sakamoto J, An assessment of antibiotics prescribed at the secondary health-care level in the Kyrgyz Republic, Nagoya J. Med. Sci. 73, 2011, 157 -168.
7. Kayode OM, Michael AA, A Study of Rational Prescriptions of Penicillin and Cephalosporin Antibiotics in a Secondary Health Care Facility in South West Nigeria, Global J Med Res, 12, 2012,1-7.
8. Nouwen JL, Controlling Antibiotic Use and Resistance, CID, 42, 2006, 776–777.
9. Katakam P, Elfituri AA, Ramadan ZH, Abadi OG, A Retrospective Study on Antibiotic Use in different Clinical Departments of a Teaching Hospital in Zawiya, Libya, Ibnosina J Med BS, 4, 2012, 13-19.
10. Tamuno I, Fadare JO, Drug Prescription Pattern in a Nigerian Tertiary Hospital, Trop J Pharm Res, 11, 2012, 146-152.
11. Ayuthya SKN, Matangkasombut OP, Sirinavin S, Malathum K, Sathapatayavongs B, Utilization of restricted antibiotics in a university hospital in Thailand, Southeast Asian J Trop Med Public Health, 34, 2003,179-186.
12. Abula T, Kedir M, The pattern of antibiotic usage in surgical in-patients of a teaching hospital, Northwest Ethiopia, Ethiop. J Health Dev, 18, 2004, 35-39.
13. Boucher M, Vaillancourt R, McCarthy A, Drug use evaluation of oral antibiotics prescribed in the ambulatory care settings in the Canadian armed forces, 10, 2003,5-10.
14. Erbay A, Colpan A, Bodur H, Cevik MA, Samore MH, Ergönül O, Evaluation of antibiotic use in a hospital with an antibiotic restriction policy, Int J Antimicrob Agents, 21, 2003, 308-312.
15. Al-Niemat SI, Bloukh DT, Al-Harasis MD, Al-FanekAF, Salah RK, Drug use evaluation of antibiotics prescribed in a Jordanian hospital outpatient and emergency clinics using WHO prescribing indicators, Saudi Med J, 29, 2008, 743-748.
16. Kiivet RA, Dahl ML, Adrian L , Maimets M, Wettermark B, Berecz R, Antibiotic Use in 3 European University Hospitals, Scand J Infect Dis, 30, 1998, 277-280.
17. Erah PO, Ehiagwina MO, Assessment of rational prescribing and relative cost of antibiotics for in-patients treated in selected tertiary health care facilities in southern Nigeria, Int J Pharma Bio Sci, 1, 2010, 1-15.
18. Tessema Z, Teshale C, Hawaze S, A retrospective review of antibiotic utilization in adult medical wards of a primary care hospital in Ethiopia, Int J Pharm, 4, 2014, 56-62.
19. Aleykutty NA, Mathews SM, effect of drug utilization evaluation on the pharmacoeconomics of antibiotics in hospitalized patients, Int J Drug Formul Res, 3, 2012,:82-86.

This page is intentionally left blank

Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology and Medicine
Volume 15 Issue 1 Version 1.0 Year 2015
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Evaluation of Anti-Angiogenic Effect of Naturally Occurring Compound from Ficus.Bengalensis on Regeneration & Development of Zebra-Fish Fin

By Uday P kundap, Dr. Sadhana Sathaye, Rachana D Sarawade, Dr. Ketan Patel & Pankaj D Jain

Mumbai University, India

Abstract- Angiogenesis is the process that leads to the formation of new blood vessels or neovascularisation. Angiogenesis inhibitors are designed to prevent the formation of new blood vessels. The main aim of this study is to evaluate Anthocyanin obtained from medicinal plant *Ficus.bengalensis* for Anti-angiogenesis & to standardize a method for the study of angiogenesis. A variety of animal models have been described to provide more quantitative analysis of *in vivo* angiogenesis and to characterize pro- and anti Angiogenic molecules.

Keywords: anthocyanin, regenerative angiogenesis, vegf (vascular endothelial growth factor), zebrafish embryos (larvae).

GJMR-B Classification : NLMC Code: QV 745

Strictly as per the compliance and regulations of:

Evaluation of Anti-Angiogenic Effect of Naturally Occurring Compound from Ficus.Bengalensis on Regeneration & Development of Zebra-Fish Fin

Uday P kundap [α], Dr. Sadhana Sathaye [σ], Rachana D Sarawade [ρ], Dr. Ketan Patel [ω] & Pankaj D Jain [¥]

Abstract- Angiogenesis is the process that leads to the formation of new blood vessels or neovascularisation. Angiogenesis inhibitors are designed to prevent the formation of new blood vessels. The main aim of this study is to evaluate Anthocyanin obtained from medicinal plant *Ficus.bengalensis* for Anti-angiogenesis & to standardize a method for the study of angiogenesis. A variety of animal models have been described to provide more quantitative analysis of *in vivo* angiogenesis and to characterize pro- and anti Angiogenic molecules. However, it is still necessary to establish a quantitative, reproducible and specific method for studies of angiogenesis factors and inhibitors. *In-vivo* animal models were used, Anti-Angiogenic effects on Zebrafish fin regeneration. Parameters observed were Regenerative angiogenesis & variation in Zebrafish fin. In regenerative angiogenesis, test drug shows inhibition of fin regeneration as compared to Control Group. We demonstrate that the zebrafish is a viable model for screening small molecules that can inhibit angiogenesis. corroborating to the validity of the standardized method, Studies support and accelerate the application of Zebrafish & model that predict the anti-Angiogenic potential value of compound.

Keywords: anthocyanin, regenerative angiogenesis, vegf (vascular endothelial growth factor), zebrafish embryos (larvae).

I. Introduction

Angiogenesis, the process that leads to the formation of new blood vessels or neovascularisation, which is highly important during development but is largely not observed in the adult, except physiological exceptions in which angiogenesis occurs under tight regulation found in the female reproductive system and during wound healing. (Malin Dollinger et al.) In pathological situations, however, angiogenesis may be turned on, which contribute to the onset and progression of most severe human pathologies characterized by high mortality, including cancer, diabetes, obesity and retinopathies. Thus, angiogenesis is one of the largest and fastest evolving areas of research today, the knowledge of the molecular mechanisms that regulate neovascularisation continues to emerge, and there is increasing hope for the new discoveries that will lead to newer therapies targeting angiogenesis. (Uday P Kundap et al 2013) Angiogenesis is the physiological process through which new blood vessels develop from pre-existing vessels. This is distinct from vasculogenesis, which is the *de novo* formation of endothelial cells from mesoderm cell precursors. Anti-angiogenesis is a form of targeted therapy that uses drugs or other substances to stop tumors from making new blood vessels. Without a blood supply, tumors can't grow. Anti-angiogenesis research began more than 35 years ago with the work of the late Judah Folkman, MD. (Bagchi. D et al 2004, Han-Chung Wul et al) Anthocyanin are the flavonoid compounds that produce plant colours ranging from orange and red to various shades of blue and purple. Anthocyanin are members of the flavonoid group of phytochemicals, which is a group predominant in teas, honey, wines, fruits, vegetables, nuts, olive oil, cocoa and cereals. The flavonoids are thought to be perhaps the most important single group of phenolic in food. (Gael McGill et al.) Adult zebrafish have a remarkable regenerative capability. Many tissues which may not be regenerated in mammals are quickly regenerated in zebrafish. Among these are the heart, retina, maxillary barbell and fins importantly, as they regenerate, new blood and lymph vessels grow into the regenerating tissue – which enables studies on regenerative angiogenesis. One commonly used assay in the adult zebrafish, based on this principle is the regenerating tail fin. After amputation, the tail fin will re-grow and after approximately 1 month, the fin is back to its original size.

Author α: M – Pharmacy (Pharmacology) Dr. L. H. Hiranandani College of Pharmacy, CHM campus, opposite to railway station, Ulhasnagar-03, e-mail: uday.kundap@yahoo.com
Author σ: Associate Professor in Pharmacy (Pharmacology) Department of Pharmacology, Institute of Chemical Technology, Mumbai, India e-mail: sadhanasathaye@hotmail.com
Author ρ: Asst. Porf (HOD) Pharmacology, Dr. L. H. Hiranandani College of Pharmacy, CHM campus, opposite to Ulhasnagar railway station Ulhasnagar 03, Thane, India. e-mail: kamble.rachana@gmail.com
Author ω: Post-doctoral fellow, Department of Pharmaceutics, Florida A&M University, 021, Dyson Pharmacy, Tallahasse, FL,32307 e-mail: ketan.uict@gmail.com
Author ¥: PhD Research Fellowship Department of Pharmacology, Institute of Chemical Technology, Mumbai, India e-mail: pdjain23@gmail.com

The zebrafish embryo has become an important vertebrate model for assessing drug effects. It is well suited for studies in genetics, embryology, development, and cell biology. (L. D. Jensen1, et al) Zebrafish embryos exhibit unique characteristics, including ease of maintenance and drug administration, short reproductive cycle, and transparency that permits visual assessment of developing cells and organs. Because of these advantages, zebrafish bioassays are cheaper and faster than mouse assays, and are suitable for large-scale drug screening. The main aim of this study is to evaluate Anthocyanin obtained from medicinal plant Ficus.bengalensis for Anti-angiogenesis & to standardize a method for the study of angiogenesis. A variety of animal models have been described to provide more quantitative analysis of in vivo angiogenesis and to characterize pro- and anti Angiogenic molecules. However, it is still necessary to establish a quantitative, reproducible and specific.

II. Material & Methods

a) Collection of plant

The dried stem bark of Plant *Ficus.bengalensis Linn*. Was collected from Uran region of Navi-Mumbai Maharashtra India & were authenticated from Agarkar Research Institute, G. G. Agarkar Road, Pune, Sample deposited on 13/9/2012 & voucher number allotted is S/B-110.

b) Extraction of Anthocyanin

This unit describes methods for extraction, isolation, and purification of anthocyanin pigments from plant tissues. The choice of extraction method should maximize pigment recovery with a minimal amount of adjuncts and minimal degradation or alteration of the natural state.

Basic Protocol 1 describes the extraction of Anthocyanin with acetone and their partition with chloroform. Basic Protocol 2 describes a simple, fast, and effective method for purification of Anthocyanin from polyphenol compounds, sugars, and organic acids using solid-phase adsorption. This produces a uniform composite sample with a high surface area, which allows for efficient pigment extraction. (Oszmianski and Lee, 1990, Sheikh Anis, et al. 2012)

Basic Protocol – 1

Acetone Extraction & Chloroform Partition of Anthocyanin: In this method, acetone extracts the Anthocyanin from the plant material and chloroforms partitioning further isolates and partially purifies the pigments. The addition of chloroform results in phase separation between the aqueous portion (which contains the anthocyanin, phenolics, sugars, organic acids, and other water-soluble compounds) and the bulk phase (which contains the immiscible organic solvents, lipids, carotenoids, chlorophyll pigments, and other nonpolar compounds). This method has the advantage of producing an extract with no lipophilic contaminants. The absence of a concentration step minimizes the risk of acid-dependent pigment degradation. (Oszmianski and Lee, 1990)

Materials: Powdered plant material,Frozen Acetone 70% (v/v) aqueous acetone or aqueous acidified acetone: 70% aqueous acetone with 0.01% HCl, Chloroform, Acidified water: 0.01% (v/v) HCl in deionized, distilled water,Waring Blender with stainless steel container (Waring) or general-purpose homogenizer, Whatman no. 1 filter paper, Buchner funnel, Separatory funnel, 500-ml boiling flask

Rotary evaporator with vacuum pump or water aspirator, 40°C

Basic protocol – 2

Anthocyanin Purification: Purification of anthocyanin-containing extracts is often necessary, as the solvent systems commonly used for extraction are not specific for anthocyanin. Considerable amounts of accompanying materials may be extracted and concentrated in the coloured extracts, which can influence the stability and/or analysis of these pigments (Jackman and Smith, 1996). Anthocyanin purification using solid-phase extraction (Figure) permits the removal of several interfering compounds present in the crude extracts. Mini-columns containing silica gel 60 chains bonded on silica retain hydrophobic organic compounds (e.g., anthocyanin, phenolics), while allowing matrix interferences such as sugars and acids to pass through to waste. Washing the retained pigments with ethyl acetate will further remove phenolic compounds other than anthocyanin. (Oszmianski and Lee, 1990)

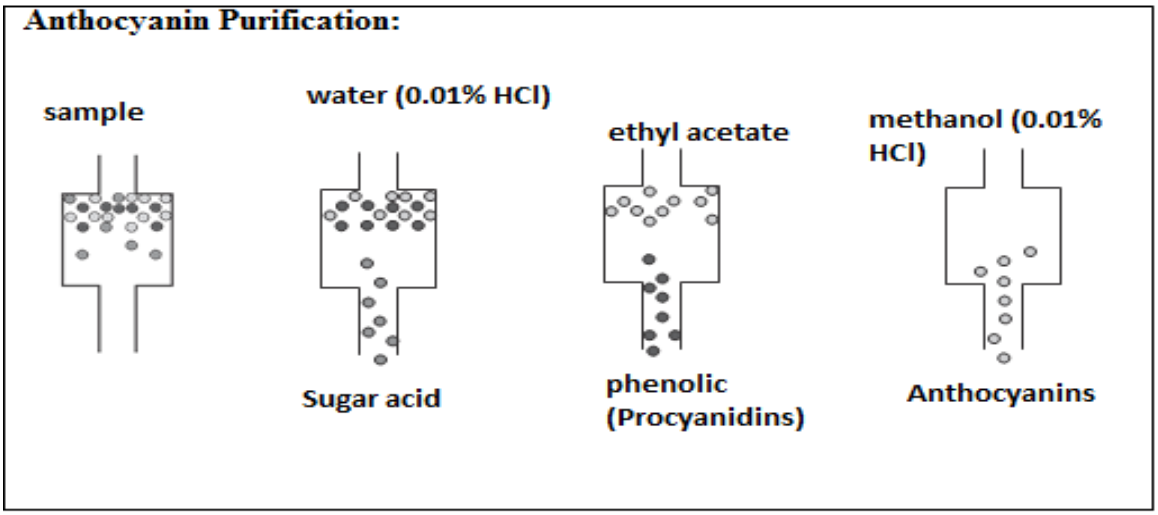


Figure 1 : Anthocyanin Purification

Materials: Methanol, Acidified water: 0.01% (v/v) HCl in deionized, distilled water, Aqueous anthocyanin extract (see Basic Protocol 1), Ethyl acetate, Silica gel 60, Acidified methanol: 0.01% (v/v) HCl in methanol, 50- to 100-ml boiling flask, Rotary evaporator with vacuum pump or water aspirator, 40°C, Freeze-resistant container (optional).

c) Animals

Animals were obtained from the Animal House of H(S)NCB's facility for Breeding & Experimentation, Dr. L.H. Hiranandani college of Pharmacy, opposite to Ulhasnagar station, CHM Campus, Ulhasnagar-03, maintained in an animal holding room (Protocol Registration no. 879/ac/05/CPCSEA). The experimental protocol was approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Efforts were made to minimize animal suffering and to reduce the number of animals used.The experiments were performed after the approval by the Institutional Animal Ethics Committee (IAEC) and were carried out in accordance with the current guidelines for the care of laboratory animals. Efforts were made to minimize animal suffering and to reduce the number of animals used.

Zebrafish: Danio rerio (wild type) were obtained from local suppliers and maintained at 280C on a 14 hours light/10 hour dark cycle in 40 L glass tanks with four females and eight males in separate tanks. PH of breeding water was maintain at 6.6 - 6.8, temperature of 78-82 0 F. Embryos were collected by natural spawning with 2:1 male to female ratio and staged according to Kimmel et al. Embryo stage is denoted as hours post-fertilization (hpf). (Amy L Rubinstein.2003, Christian Lawrence. 2000).

d) In-vivo Model Method

Anti-Angiogenic effects on Zebrafish fin regeneration assay: (John N et al, Kimmel CB 1995 et al)

i. *Setting up the tank:* For Setting up the tank 5 .5 gallon tank was taken, 25 watt submersible heater, air stone supplied with air from a vibrator pump and enough marbles to place on the bottom of the tank to a depth of 2 .5 inches deep across the whole tank bed. For conditioning, feeder with variety of frozen and flake food 3-5 times, PH was set at 6.6 with a water temperature of 780 F. (Christian Lawrence. 2007)

ii. *Amputation of Zebrafish Fin:* Eight Zebrafish were transfer into each beaker. Tip of zebrafish fin was cut to initiate angiogenesis in the model. Percent Regeneration of the Zebrafish was calculated at 7th day to determine the anti-Angiogenic activity.

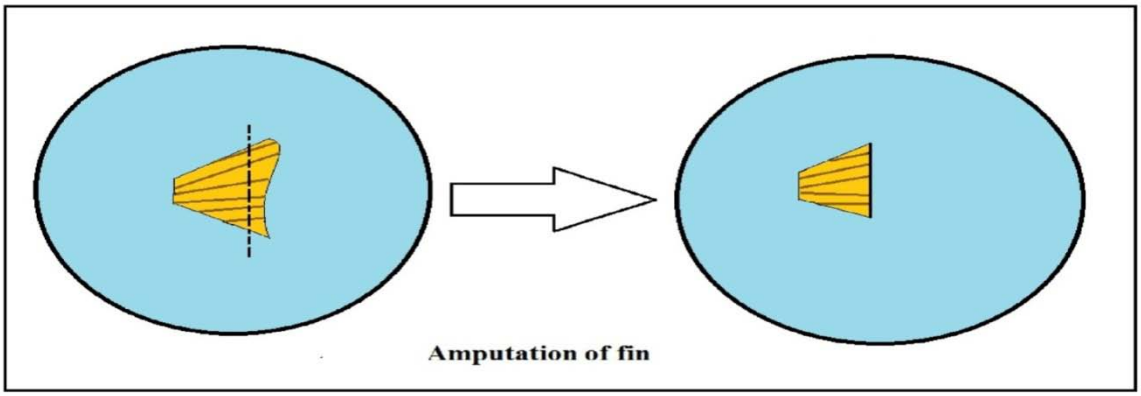


Figure 2 : Amputation of Zebrafish fin

e) Drug treatment

i. *Tank A:* 600ml of water with fish feed (Three times a day)

ii. *Tank B:* Vehicle control group: 200µl methanol & 200µl PEG-400; Dose added i: e 80µl in 600ml water.

iii. *Tank C:* Standard drug: 1ngm of Std. drug in 200µl of PEG-400. 1000ng = 1µgm; Dose added i: e 80µl in 600ml of water = 0.5ppm concentration of drug in 600ml water (paclitaxel), 1000ηgm of Paclitaxel = 0.5ppm = in 600ml water

iv. *Tank D:* Test drug: 1mg of drug in 200µl methanol & 200µl PEG-400; Dose added i: e 80µl in 600ml water = 3.3ppm concentration of drug in 600ml water. 1mg of test drug = 3.3ppm = in 600ml water.

III. Results

a) Anti-Angiogenic effects on Zebrafish (Danio rerio) fin regeneration assay

Adult Zebrafish have a remarkable regenerative capability; many tissues which may not be regenerated in mammals are quickly regenerated in Zebrafish. As they regenerate, new blood & lymph vessels grow into the regenerating tissue - which enables studies on regenerative angiogenesis.

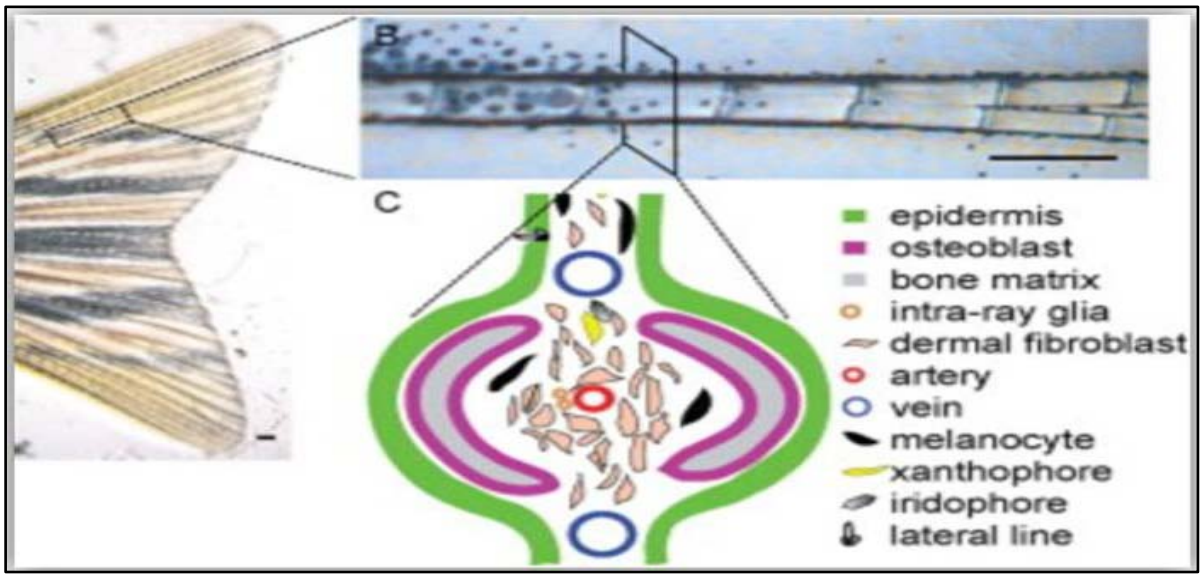


Figure : 3 a

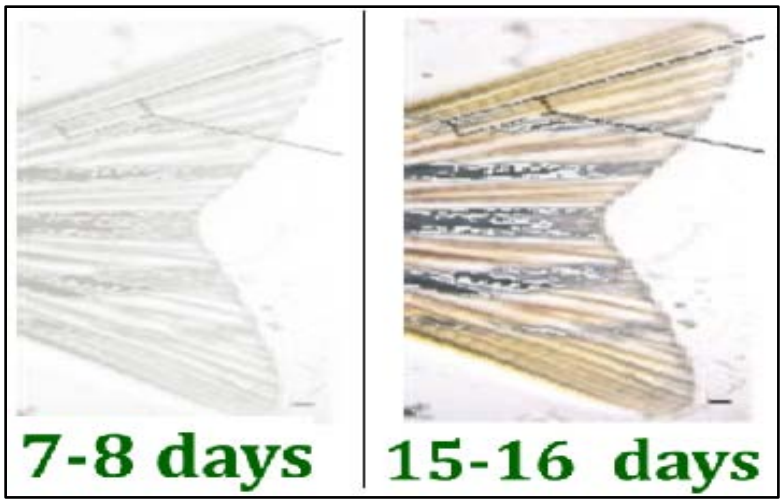


Figure : 3 b

Figure 3 a : TS of Zebrafish fin under microscope shows the presence of artery & vein, which indicates the presence of various number of blood vessels in fin,

Figure 3 b : Zebrafish fin requires approx. 7-8 days for regeneration & 15-16 days for maturation) One commonly used assay in the adult Zebrafish, based on this principle is the regenerating tail fin. After amputation, the tail fin re-grows & after approximately 1month, the fin is back to its original size. The process of Fin regeneration encompasses many of the same mechanisms as in human wound healing & regeneration & is therefore a good model of regenerative angiogenesis. The vasculature is remarkably simple & as the fin grows back various levels of vascular remodeling can be observed & therefore studied in detail. This regeneration model is probably the most commonly used, adult Zebrafish angiogenesis model, & is considered to be complementary to developmental angiogenesis.

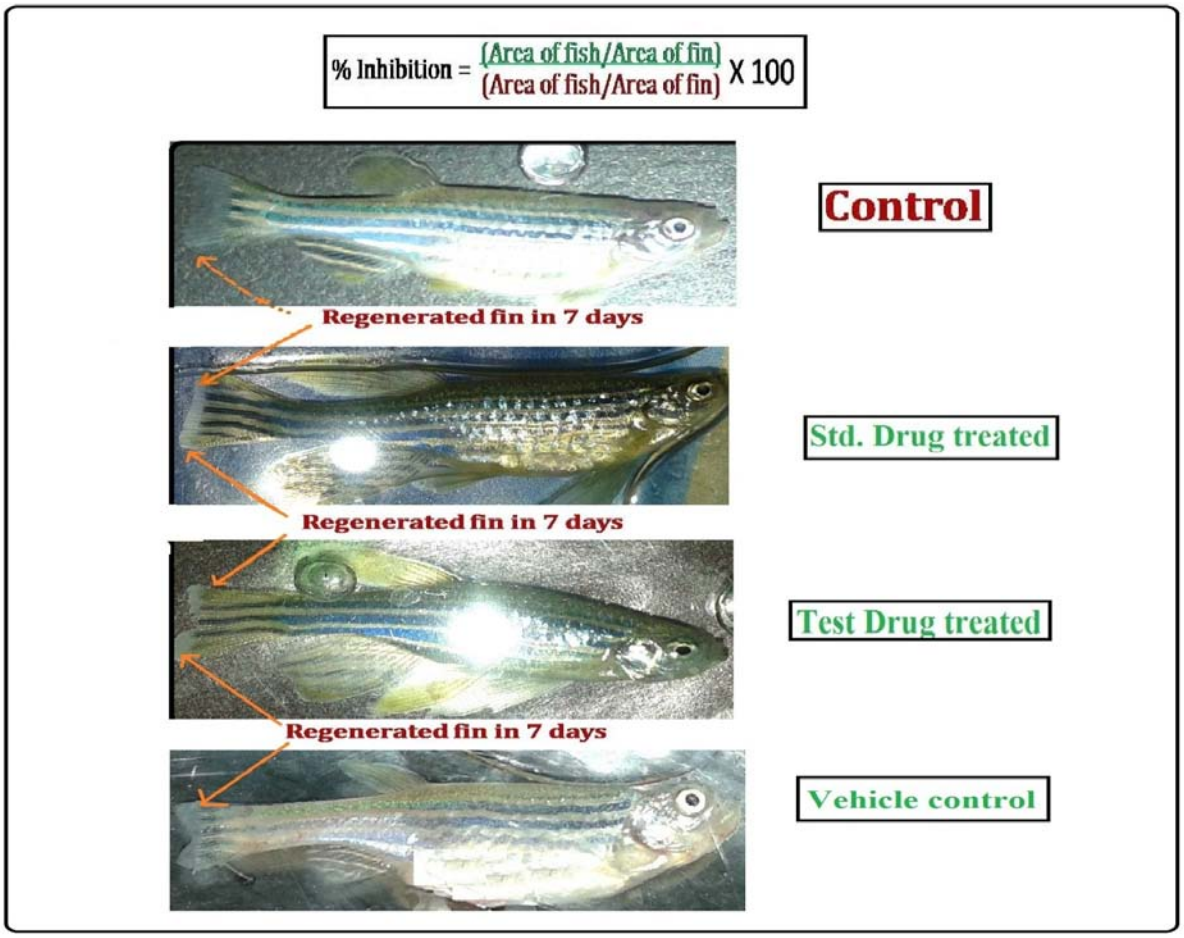


Figure 4 : Control group & vehicle control group shows the maximum regeneration of Zebrafish fin & blood vessels, Standard drug Group & test drug group shows uneven, stunted growth & irregular of regeneration Zebrafish fin).

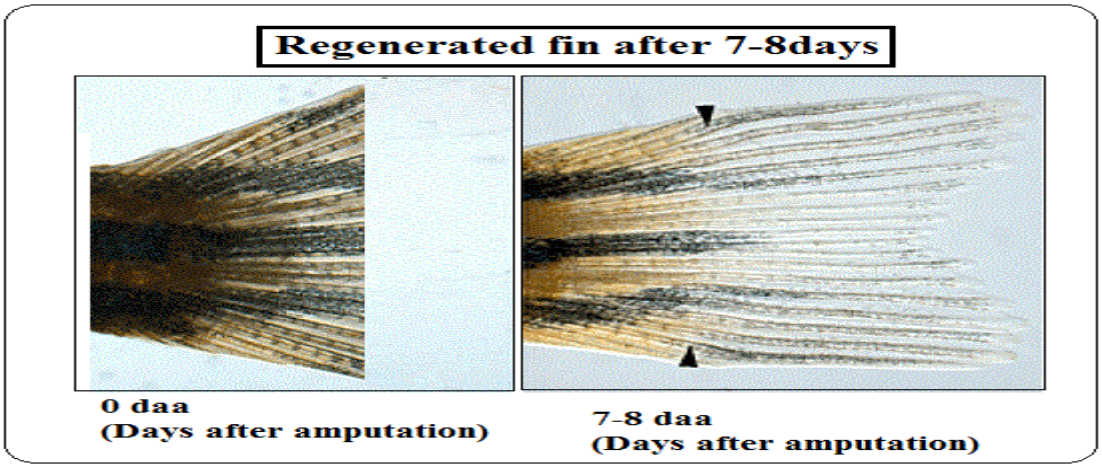


Figure 5 : Regenerated fin after 7-8days

Microscopic view of regenerated fin

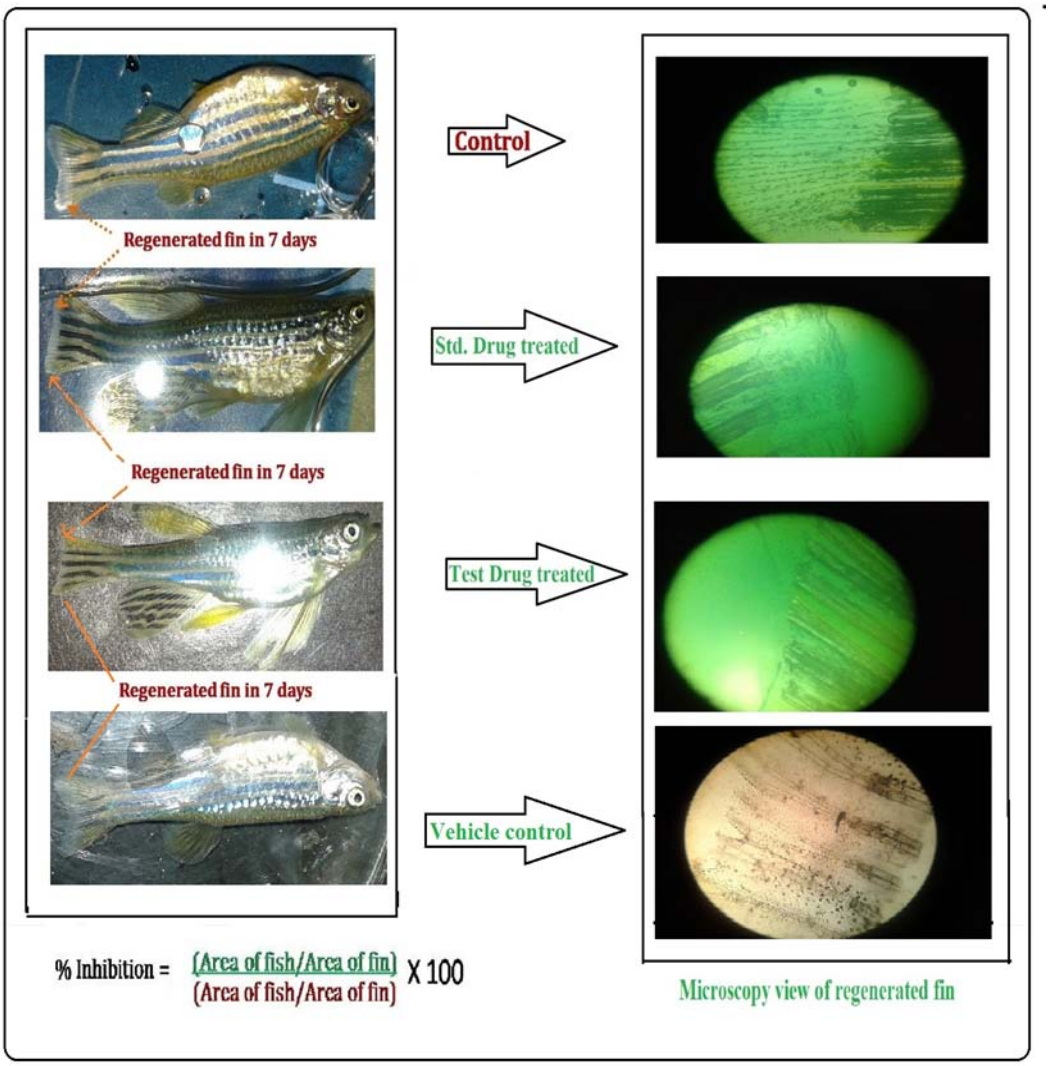
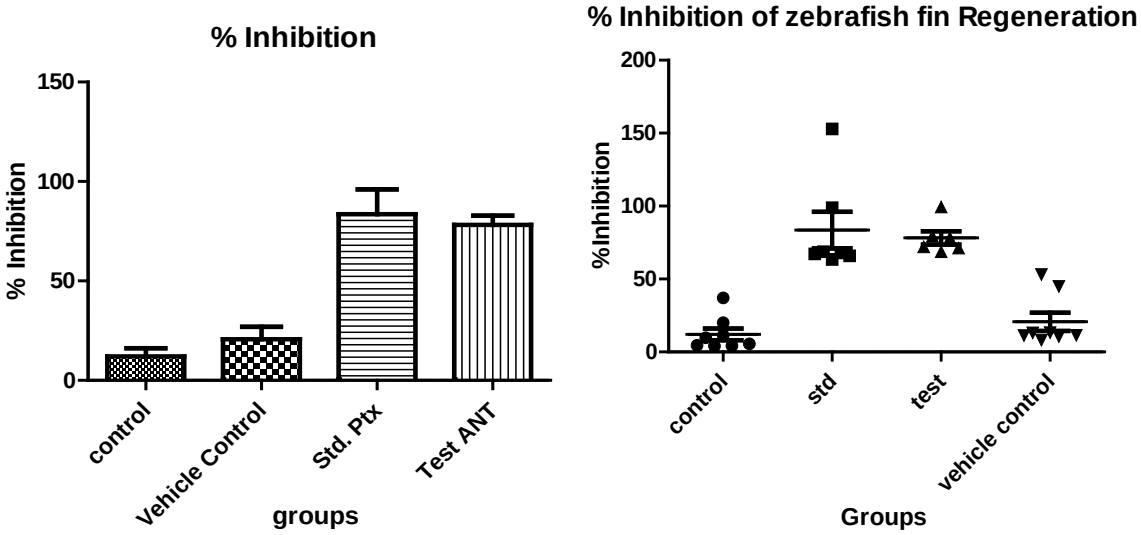


Figure 6 : (Microscopic view of Regenerated fin after 7 days: *Control group* shows the normal growth of blood vessels, *standard drug group* shows the stunted growth of blood vessels, *Test drug group* shows insufficient growth as compared to control group, *Vehicle control group* shows normal growth as control group.)

Graph for % Inhibition of Zebrafish fin regeneration assay



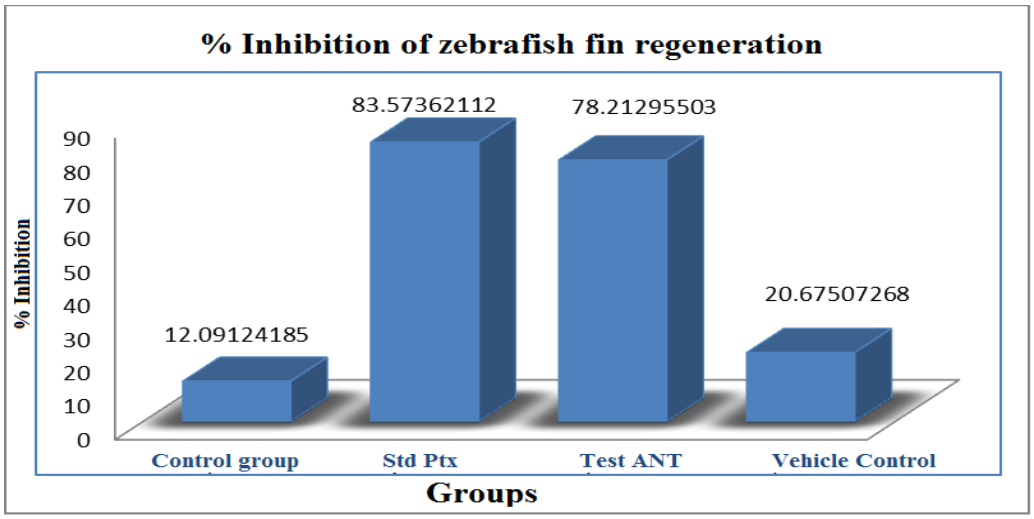


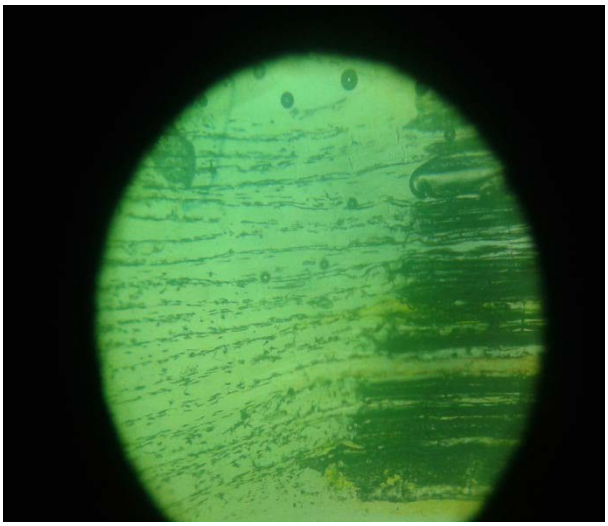
Table 2 : Statistical analysis table for Zebrafish fin regeneration assay

Group	Macroscopic score
Control	12.09± 4.045
Standard group(0.5mg/ml)	83.57±12.43***
Test group (1 mg/ml)	78.21±4.57***
Vehicle Control	20.68±6.246

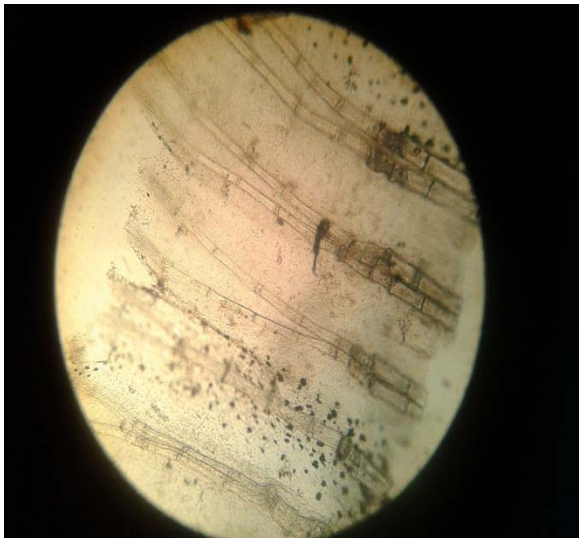
Mean± S. E : *= P<0.05; **= P<0.01; ***=P<0.0001

f) Microscopic Images

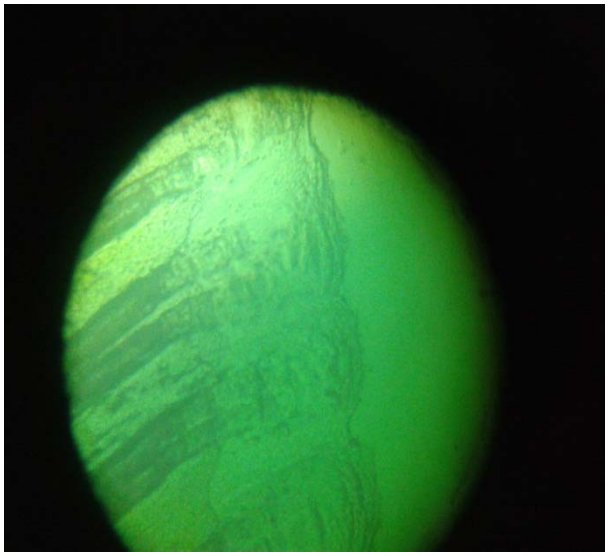
Regenerated Zebrafish fin was observed under the microscope after 7-8 days of regeneration, fish from control group shows the normal & complete growth of blood vessels in fin. Vehicle control group also shows the complete & normal growth of blood capillary bloodvessels in regenerated fin. Unique observation was noticed in standard drug group, the growth of capillary blood vessels in fin was stunted, the growth of blood vessels was blocked in standard drug paclitaxal (0.5ppm conc). Fishes in the test drug shows the simillar features as standard drug group. The growth of the blood vessels was also blocked by the test compound.



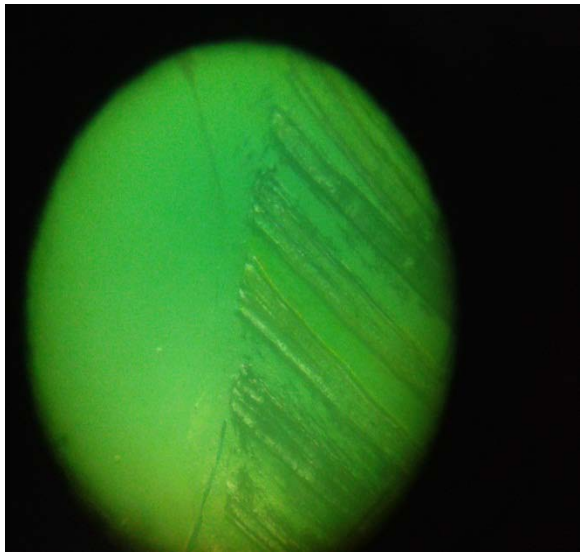
Control Group



Vehicle Control Group



Strandard Drug Group



Test Drug Group

IV. Discussion

a) *Anti-Angiogenic effects on zebrafish (Danio rerio) fin regeneration assay*

Adult zebrafish have a remarkable regenerative capability. Many tissues which may not be regenerated in mammals are quickly regenerated in zebrafish. Among these are the heart, retina, maxillary barbell and fins importantly, as they regenerate, new blood and lymph vessels grow into the regenerating tissue – which enables studies on regenerative angiogenesis. One commonly used assay in the adult zebrafish, based on this principle is the regenerating tail fin. After amputation, the tail fin will re-grow and after approximately 1 month, the fin is back to its original size. Angiogenesis process was activated in zebrafish by cutting there fin. Test compound (1mg/300ml of water) was added in the water. Comparison of test drug & Standard Drug effect was done with Control & vehicle control for regeneration of tail fin as a parameter for studying regenerative anti_angiogenesis. Control group was kept to know the normal growth of fin. (Amy L Rubinstein, 2003, Westerfield M 2000) The test (Anthocyanin extract) & Standard (Paclitaxel) compound were evaluated for Anti- Angiogenic activity after 7 days of amputation. Standard drug showed 83.57± 12.43*** Test drug showed 78.21 ± 4.57*** angiogenesis inhibition, control group 12.09 ± 4.04, Vehicle control 20.67± 6.24 of angiogenesis inhibition which is significant according to statistical analysis (one-way annova). Regenerated Zebrafish fin was observed under the microscope after 7-8 days of regeneration, fish from control group shows the normal & complete growth of blood vessels in fin. Vehicle control group also shows the complete & normal growth of capillary blood vessels in regenerated fin. Unique observation was noticed in

standard drug group, the growth of capillary blood vessels in fin was stunted, and the growth of blood vessels was blocked in standard drug paclitaxel (0.5ppm conc). Fishes in the test drug (3.3ppm) shows the similar features as standard drug group. The growth of the blood vessels was also blocked by the test compound which shows 78.21% inhibition of angiogenesis.

v. Conclusion

Design and development of small molecule therapeutics to inhibit angiogenesis has gained considerable importance in anti-angiogenesis research. We demonstrate that the zebrafish is a viable model for screening small molecules that can inhibit angiogenesis. The mechanism of Anthocyanin action is not yet known, but hypotheses include decreased levels of tumor necrosis factor alpha (TNF-a)-induced VEGF expression), inhibition of H2O2- and as well as through the inhibition of VEGF and VEGF receptor expressionThus, our study suggests that even mill molar concentration of test drug (Anthocyanin) could be an effective drug for in vivo inhibition of angiogenesis and thus might gain significance in future therapeutics. (SimonaLucioli 2012, L. D. Jensen1,et al)

vi. Acknowledgement

Dr Radhikanand Dhavale, PhD in Spiritual Literature, Siddhayog Ashram, Pune, Mrs Rachana D Sarawade, HOD Prof. Department of Pharmacology, Dr L. H. Hiranandani College of Pharmacy, Ulhasnagar, Dr. Sadhana Sathaye, Department of Pharmacology, Institute of Chemical Technology, Matunga, Dr Ketan Patel, Post-doctoral fellow, Department of Pharmaceutics, Florida A&M University, 021, Dyson pharmacy, Tallahasse, FL USA, Pankaj Jain, Devang. S for their valuable guidance and support

References Références Referencias

1. Amy L Rubinstein. “Zebrafish: From disease modeling to drug discovery”. Zygogen LLC 520 Kell Hall 24 Peachtree Center Avenue Atlanta GA 30303 USA e-mail: amy@zygogen.com Current Opinion in Drug Discovery & Development 2003 6(2): 218-223 Current Drugs ISSN 1367-6733.
2. Bagchi. D , C. K. Sen, M. Bagchi, M. Atalay Anti-Angiogenic, Antioxidant, and Anti-carcinogenic Properties of a Novel Anthocyanin-Rich Berry Extract Formula, Kluwer Academic Publishers- Plenum Publishers, Biochemistry (Moscow) Volume 69, Issue 1 , pp 75-80, 2004-01-01
3. Barcz E, Sommer E, Janik P, Marianowski L, Skopinska-Rózewska E. Adenosinereceptor antagonism causes inhibition of Angiogenic activity of human ovariancancer cells. Oncol Rep 2000;7(6):1285–91.
4. Christian Lawrence, “The husbandry of zebrafish (Danio rerio): A review”. Brigham and Women's Hospital, Karp Family Research Laboratories, 06-004B, One Blackfan Circle, Boston, Massachusetts, 02115, U.S.A. Received 16 January 2007; received in revised form 24 April 2007; accepted 25 April 2007
5. Fraysse B, Mons R, Garric J. Development of a zebrafish 4-day embryolarval bioassay to assess toxicity of chemicals. Ecotoxicol Environ Saf2006;63:253–67.
6. Fishman MC. Zebrafish genetics: the enigma of arrival. ProcNatlAcadSci U S A 1999;96:10554–6.
7. Gael McGill & David E.Fisher. Apoptosis in Tumorigenesis & Cancer therapy, Division of Pediatric Hematology/oncology, Dana Farbe Cancer Institute & Children Hospital, Harvard Medical School, 44 Binney St, Boston, Ma 02115
8. Han-Chung Wul, De-kaun Chang & chia-Ting, & Chia-ting Huang, Targeted Therapy for Cancer, Institute of Cellular & Organismic Biology, Acadamiasinica, Taipei, Taiwan.
9. H. Gerhard Vogel, “Drug Discovery and Evaluation Pharmacological Assays” Co-Editors: Wolfgang H.VogelBernward A. Schölkens Jürgen Sandow Günter Müller Wolfgang F. Vogel Second Edition, ISBN 3-540-42396-6 Springer-Verlag Berlin Heidelberg New York
10. H. Gerhard Vogel, “Drug Discovery and Evaluation Pharmacological Assays” Co-Editors: Wolfgang H.VogelBernward A. Schölkens Jürgen Sandow Günter Müller Wolfgang F. Vogel Second Edition, ISBN 3-540-42396-6 Springer-Verlag Berlin Heidelberg New York.
11. John N. Abelson and AelvinI. Simon, “METHODS IN ENZYMOLOGY”. Division of Biology California Institute of Technology Pasadena, California, Khandelwal KR, Practical pharmacognosy, Niraliprakashan, March 2008:149-153.
12. Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. Stages of embryonic development of the zebrafish, DevDyn 1995;203:253–310
13. L. D. Jensen1,2 et al. “Animal Models of Angiogenesis and Lymphangiogenesis”. 1Department of Microbiology, Tumor and Cell Biology, The Karolinska Institute, Stockholm, 2Institution of Medicine and Health, Linköping University, Linköping, SwedenNeeharika V. Vamsi KR. Reddy B.
14. Malin Dollinger, M.D, Ernest H. Rosenbaum, Margaret Tempero, MD, and Sean Mulvihill, MD Everyone’s guide to CANCER THERAPY, Canadian Medical Association.
15. Oszmianski and Lee, 1990. See above. “A description of methods for isolating polyphenolics and Anthocyanin from grapes by solid-phase extraction”.

16. SimonaLucioli, "Anthocyanin: Mechanism of action and therapeutic efficacy". Centro di Ricerca per la Frutticoltura, CRA-Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Via di Fioranello, 52 - 00134 Roma, Italy. 2012: 27-57 ISBN: 978-81-308-0509-2.
17. Sheikh Anis, Sameer singh, MandoriaNarendra, "A Review on Phytopharmacological Aspects of Ficus bengalensis, Institute of Pharmacy, ujjain, IJPRD;Vol 4(06): Aug-2012 (018-022). Seng WL, Kurt Eng, Lee J, McGrath P. Use of a monoclonal antibody specific for activated endothelial cells to quantitate angiogenesis in vivo in zebrafish after drug treatment. Angiogenesis 2004;7:243–53
18. Uday P Kundap*, RachanaSarawade. "Recent Potent Molecular Targets for Cancer Treatment – A Review" International Journal of Pharmaceutical & Biological Archives 2013; 4(4): 790 – 808.Received 08 Jun 2013; Revised 18 Sep 2013; Accepted 27 Sep 2013. Available Online at www.ijpba.info
19. Westerfield M. The Zebrafish Book, Guide for the Laboratory Use of Zebrafish (Danio rerio). 4th Ed Eugene: University of Oregon Press; 2000
20. Wrolstad, R.E., Skrede, G., Lea, P., and Enersen, G. "Extraction, Isolation, and Purification of Anthocyanin". Contributed by Luis E. Rodriguez-Saona University of Maryland and Joint Institute for Food Safety and Applied Nutrition Washington, D.C.

This page is intentionally left blank

Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology and Medicine
Volume 15 Issue 1 Version 1.0 Year 2015
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Comparative Antimicrobial Activity of Ethanol and Hexane Leaf Extracts of *Ficus Exasperata* on Five Microbial Isolates

By Godwill Azeh Engwa, Richard N. Tagbo, Chinedu Chukwuekezie, Marcellus Unaegbu & Marian N. Unachukwu

Godfrey Okoye University, Nigeria

Abstract- This study evaluated the antimicrobial activity of *Ficus exasperata* and compared the efficacy of ethanol and hexane extracts on five microbial isolates. To ascertain the set objective, after extraction with ethanol and hexane, the disk and well diffusion agar methods were employed to investigate the antimicrobial activity of the extracts and its minimum inhibitory and bactericidal concentrations. A phytochemical screening was done for the confirmation of the result and the data was statistically analysed.

Keywords: ficus exasperata, antimicrobial activity, ethanol, hexane, extract, phytochemicals, microbial isolates.

GJMR-B Classification : NLMC Code: QV 752

Strictly as per the compliance and regulations of:

Comparative Antimicrobial Activity of Ethanol and Hexane Leaf Extracts of *Ficus Exasperata* on Five Microbial Isolates

Godwill Azeh Engwa [α], Richard N. Tagbo [σ], Chinedu Chukwuekezie [ρ], Marcellus Unaegbu [ω], & Marian N. Unachukwu [¥]

Abstract- This study evaluated the antimicrobial activity of *Ficus exasperata* and compared the efficacy of ethanol and hexane extracts on five microbial isolates. To ascertain the set objective, after extraction with ethanol and hexane, the disk and well diffusion agar methods were employed to investigate the antimicrobial activity of the extracts and its minimum inhibitory and bactericidal concentrations. A phytochemical screening was done for the confirmation of the result and the data was statistically analysed. *Ficus exasperata* extracts inhibited the growth of all the five microbial isolates i.e *Salmonella typhi, Staphylococcus aureus*, *Escherichia coli, Klebsiella pneumoniae* and *Candida albican*. The greatest antimicrobial activity was found with *Salmonella typhi* and the least with *Candida albican.* The 200mg/ml extract was the most effective in inhibiting microbial growth while the 25mg/ml was the least. The minimum inhibitory concentration and the minimum bactericidal concentrations of *Ficus exasperata* were 100 and 200mg/ml respectively. Ethanol extract was more effective than hexane for all the different concentrations in inhibiting microbial isolates which was significant ($p < 0.05$) for the 150, 100 and 25mg/ml concentrations. A phytochemical screening showed the presence of tannin, saponin, alkaloid, flavanoid and glycosides in the ethanol extract against tannin, alkaloid and flavanoid for hexane extract thus confirming the effectiveness of ethanol use in extraction. *Ficus exasperata* possesses a broad spectrum of antimicrobial activities and thus is a potential candidate for prospective antimicrobial treatment with activities that will be at its best if ethanol is used in extraction to recover a wide range of phytochemicals.

Keywords: ficus exasperata, antimicrobial activity, ethanol, hexane, extract, phytochemicals, microbial isolates.

I. Introduction

Infectious diseases are the number one cause of death due to illnesses across the world and account for approximately one-half of all deaths in tropical countries. According to World Health Organisation (WHO) report, about 15 million (>25%) of 57 million annual deaths worldwide are the direct result of infectious diseases [1]. Of these infectious diseases, microorganisms are the commonest organisms responsible for morbidity and mortality [2, 3]. As such, bacterial and fungal diseases continue to remain a major public health problem [4].

Efforts in the management of bacterial and fungal infections had been very effective for long with the use of antibiotics till the emergence of antimicrobial drug resistance in the past two decades [5, 6]. Since then, the use of conventional drugs have been challenging in the treatment and management of these diseases and the quest for alternative solutions have been a major global concern to WHO and other public health institutions and organizations.

In recent time, there is so much concern on the use of plants and their constituents for treatment as have extensively been used in folk medicine for the treatment of many ailments [7, 8]. So many plants have been shown to have medicinal properties against microbial and fungal infections [9, 10]. One of such plant is *Ficus exasperata*, locally known as "sand paper plant" and "Ewe ipin" in the Yoruba language of Western Nigeria. Different parts of the plant are locally used for treating various infectious diseases such as eye-sores, ring worm, stomach pains and leprosy etc. [11-13]. The leaf extract of *Ficus exasperata* has been reported for the treatment of various diseases including coughs, intestinal pains, colics, bleeding, ulcer, wounds, bacterial, fungal infections etc. [13-20]. Various pharmacological actions such as anti-hypertensive, antioxidant, anti-inflammatory, anti-ulcer, anti-lipidic, anti-bacterial and anti-fungal activities have been described for *Ficus Exasperata* [16-22].

These pharmacological activities are attributed to the presence of certain bioactive components in the plant [23] which have been identified and characterized and are now the basis for new therapies. Synergism is reported to be the most probably mechanism of action responsible for the overall pharmacological activity of medicinal plants [24, 25]. Synergic effect depends on the photochemical load; that is, the number of various types of phytochemicals extracted which is determined by the extraction method employed. Polar solvent have shown to recover more bioactive components from plants than non_polar solvents hence, a greater

Author α σ ρω : Biochemistry, Department of Chemical Sciences, Faculty of Natural and Applied Sciences, Godfrey Okoye University, P.M.B 01014 Thinkers Corner, Enugu Nigeria.
e-mail: engwagodwill@gmail.com
Author ¥ : Microbiology, Department of Biological Sciences, Faculty of Natural and Applied Sciences, Godfrey Okoye University, P.M.B 01014 Thinkers Corner, Enugu Nigeria.

phytochemical load [26, 27]. In this study, we compared the effect of ethanol (polar) and hexane (non-polar) extracts of *Ficus exasperata* on five microbial isolates. The crude extracts were further subjected to phytochemical screening to evaluate the phytochemical load.

II. Materials and Methods

a) Plant material

Fresh leaves of *Ficus exasperata* were collected from Enuguagu, Achi, Orji-river local Government, Enugu and transported to the Chemistry Laboratory of Godfrey Okoye University, Enugu State of Nigeria.

b) Preparation of ethanol and hexane extracts

The leaves of *Ficus exasperata* were air dried for two weeks and ground to fine powder with a Binatone blender (Model BLG-401). Extraction was done as described by Adebayo and Ishola using a soxhlet extractor [28]. 70 g of each portion of the leaf powder were dissolved in Hexane and Ethanol and the mixture was transferred in the extractor separately. Hexane extraction was done at 69°c adding 0.080g of anti-bulbing chips to speed up the boiling point while ethanol extraction was done at 50°C with 0.070g of anti-bulbing chips.

c) Specimen collection and culture

Clinical isolates of *Salmonella typhi, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia and Cadida albicans* were obtained from the Microbiology Laboratory of Godfrey Okoye University. The isolates were tested for viability by resuscitating them in buffered peptone water and only the viable isolates were sub-cultured. Plates containing 15ml of sterile nutrient agar (Oxoid, England) after autoclaving each were inoculated with the viable isolates by aseptic streaking and cultured at 37°C for 24 hours.

d) Evaluation of antimicrobial activity

In vitro antimicrobial activity was evaluated by the agar well and disc diffusion methods.

i. *Agar well Diffusion method*

Agar well diffusion technique as described by Adeniyi et al. was adopted for the study [29]. Using sterile pasteur pipette, 5mm diameter wells were created at the centre of each plate and 1ml of the various concentrations of the plant extracts were dispensed into each well. The extracts were allowed to diffuse into the medium for 1hour pre-diffusion at room temperature after which the plates were incubated at 37°c for 24 hours and the zones of growth inhibition measured in millimetre (mm).

ii. *Disc diffusion method*

In this method, 0.5mm sterile filter paper disc was soaked in extract solution for 2 hours then placed on the surface of the agar plate. The plates were kept at room temperature for 2 hours pre-diffusion at room temperature and incubated at 37°c for 24 hours.

Also, a standard antibiotics, chloramphenicol of 250mg was dissolved in 2.5ml of distilled water to obtain a concentration of 100mg/ml. A twofold serial dilution was done trice to obtain the following concentrations; 50, 25, and 12.5 mg/ml. These four concentrations of chloramphenicol which served as positive control were impregnated on filter papers disc and placed alongside the ethanol plant extract (100mg/ml) filter paper disc on the surface of the agar plate. The plates were kept at room temperature for 2 hours pre-diffusion at room temperature and incubated at 37°c for 24 hours.

e) Minimum inhibitory concentration (MIC)

The minimum inhibition concentration was determined using the dilution method as described by Robbers et al. [30] which made use of a twofold dilution assay. The extract was diluted with distilled water trice at a ratio of 1:2 to obtain concentrations of 200, 100, 50, and 25ug/ml. Nutrient agar broth was prepared according to manufacturer's instruction and dispensed into separate test tubes. 1ml each of the four extract dilutions was added in order of decreasing concentrations to the broth and incubated at 37°c for 24 hours.

f) Minimum Bactericidal Concentration (MBC)

The minimum bacterial concentration was determined as described by Robbers et al. [30]. The broths from the MIC assay were streaked on a solid nutrient agar plate and incubated at 37°c for 24 hours. Various dilutions of the plant extracts were impregnated on sterile filter papers and placed on the surface of the solid dry agar plate. After pre-diffusion of the plate at room temperature for 2 hours, they were incubated at 37°c for 24 hours.

g) Phytochemical screening

Phytochemical analysis of both the hexane and ethanol extract was carried out for tannins, glycosides, saponin, flavonoids, alkaloids and steroids using standard methods of Sofowora, Trease and Evans, and Harbon [31, 32, 33].

III. Data Analysis

The zone of inhibition was considered as the distance of the clear zones that showed no growth on the surface of agar plate after culture and measured in millimetres (mm) using a ruler. The lowest concentration of extract which showed no turbidity in the broth culture was recorded as the MIC and the concentration that exhibited no bacterial growth after culture was considered as the MBC value.

The data was expressed as Mean ± SEM. The differences between the groups was compared using the analysis of variance method (ANOVA) followed by

the Post-Hoc multiple comparison within group test. Differences were considered significant at $P \leq 0.05$.

IV. Results and Discussion

Extraction is a key step for the recovery and isolation of bioactive phytochemicals from plant materials. The pharmacological activities of plants greatly depend on the extraction method being employed [34]. Solvent extraction has widely been used to recover and isolate bioactive molecules as well as in the evaluation of their in vitro activity [35, 36]. In this study, ethanol and hexane were used in the extraction to investigate the activity of *Ficus exasperata* against microbial isolates. The percentage yield after extraction was higher in ethanol compared to hexane. Ethanol extraction recovered 10. 2g of extract with a percentage yield of 14.6% compared to 7.5g of hexane extract with a yield of 10.6% (Table 1).

Table 1 : Percentage yield for ethanol and hexane extracts of *Ficus exasperata*

Extraction Method	Initial weight of plant (g)	Weight of plant extract (g)	Percentage Yield (%)
Ethanol	70	10.2	14.6
Hexane	70	7.5	10.6

After extraction, the activity of *Ficus exasperata* was evaluated on five microbial isolates; *Salmonella typhi, Staphylococcus aureus*, *Escherichia coli, Klebsiella pneumoniae* and *Candida alblican*. Antimicrobial screening using the disk and well diffusion methods showed *Ficus exasperata* extracts (ethanol and hexane) to possess antibacterial and antifungal activities as were able to inhibit the growth of all the microbial isolates.

In the disk diffusion method, the zone of inhibition (mm) ranged from 2.5 to 18.5 and the highest inhibition was recorded for the ethanol extract while the lowest was for the hexane extract. Inhibition was concentration dependent that is; the 200mg/ml extract concentration showed the highest inhibition while the 25mg/ml showed the least microbial growth inhibition. Comparing the inhibition profile for the various concentrations and microbial isolates, ethanol extract showed a greater inhibition than hexane extract and was significantly different ($p < 0.05$) for the 150 and 25mg/ml concentrations. The inhibition pattern varied in respect to the concentration for all the microbial isolates with the greatest inhibition recorded on *Salmonella typhi* followed by *Klebsiella pneumoniae, Staphylococcus aureus*, and *Escherichia coli* in decreasing order while *Candida alblican* showed the least inhibition (Table 2).

Table 2 : Antimicrobial profile for ethanol and hexane extracts of *Ficus exasperata* by the disc diffusion method

Concentration of Extract (mg/ml)	Extraction method	Zone of Inhibition (mm)					Mean±SEM	p-value
		ST	SA	EC	KP	CA		
200	EE	18.5	12.2	18.3	11.5	10.3	14.16±1.76	
	HE	15.0	10.2	10.3	16.1	8.2	11.96±1.52	0.377
150	EE	14.5	10.0	8.3	12.4	9.2	10.88±1.13	
	HE	12.2	10.3	6.2	10.0	4.5	08.02±0.55	0.047
100	EE	10.0	8.0	7.0	8.1	7.0	06.60±1.12	
	HE	10.0	8.1	4.5	6.4	4.0	08.64±1.42	0.089
50	EE	6.0	5.5	5.2	5.5	5.0	05.44±0.17	
	HE	5.0	5.0	5.0	5.0	5.0	05.00±0.00	0.060
25	EE	4.0	3.8	3.7	3.8	2.8	03.62±0.21	
	HE	4.0	3.0	3.0	3.0	2.5	03.10±0.25	0.031

Legend: ST: Salmonella typhi, SA: Staphylococcus aureus, EC: Escherichia coli, KP: Klebsiella pneumoniae CA: Candida alblican, EE: Ethanol Extract, HE: Hexane Extract

The well diffusion method also showed antimicrobial activity on all the microbial isolates but not as effective as the disk diffusion method. The zone of inhibition ranged from 2.0 to 15.5mm and the highest inhibition was recorded for the ethanol extract while the lowest was observed for the hexane extract. Similarly, the inhibition was concentration depedent as the highest extract concentration (200mg/ml) showed the greatest inhibition that reduced with decreasing concentration. Comparatively, ethanol extract showed a greater antimicrobial activity than hexane having a greater zone of inhibition for all the different concentrations and isolates and the difference was significant for the 100 and 25mg/ml concentrations ($p < 0.05$). Also, the microbial inhibition profile was similar to that of the disk diffusion method with *Salmonella typhi* having the highest inhibition followed by *Klebsiella pneumoniae Staphylococcus aureus*, *Escherichia coli* and *Candida alblican* in decreasing order (Table 3).

Table 3 : Antimicrobial profile for ethanol and hexane extracts of *Ficus exasperata* by the well diffusion method

Concentration of Extract (mg/m)	Extraction method	Zone of Inhibition (mm)					Mean±SEM	p-value
		ST	SA	EC	KP	CA		
200	EE	15.3	10.5	7.5	12.2	9.0	10.90±1.35	
	HE	12.1	9.2	8.2	13.1	6.1	09.74±1.28	0.250
150	EE	10.2	8.3	7.5	8.0	7.5	08.30±0.49	
	HE	10.1	8.2	5.3	7.2	4.1	06.98±1.06	0.111
100	EE	8.4	6.4	5.0	6.2	5.2	06.24±0.21	
	HE	8.0	5.4	3.8	4.3	3.5	05.00±0.82	0.010
50	EE	3.9	3.5	3.1	3.0	3.1	03.32±0.17	
	HE	4.2	3.1	3.5	3.8	3.0	03.52±0.22	0.389
25	EE	3.5	3.0	3.0	2.8	2.0	02.86±0.24	
	HE	3.0	2.5	2.5	2.5	2.0	02.50±0.16	0.021

The minimum inhibitory concentration (MIC) is the least concentartion of a plant extract that shows no growth of microbial isolates in broth. In this study, the MIC was the 100mg/ml concentration and was observed for both the ethanol and hexane extracts. The ethanol concentration inhibited the growth for all the isolates except *Candida alblican* while growth was observed for *Klebsiella pneumoniae and Candida alblican* with the hexane extract (Table 4).On the other hand, the minimum bactericidal concentration (MBC) which is the least concentration that will completely kill a particular microorganism was the 200mg/dl concentration. This concentration; both for ethanol and hexane extracts was effective in killing microbial isolates. For the ethanol extract, this concentration was sensitive in killing *Salmonella typhi, Staphylococcus aureus*, and *Escherichia coli* while only *Salmonella typhi and Staphylococcus aureus isolates* were killed by the hexane extract concentration (Table 5).

Table 4 : Minimum Inhibitory Concentration (MIC) for ethanol and hexane extracts of *Ficus exasperata*

Concentration of Extract (mg/ml)	Extraction method	Microbial growth profile				
		ST	SA	EC	KP	CA
200	EE	–	–	–	–	–
	HE	–	–	–	–	–
100	EE	–	–	–	–	+
	HE	–	–	–	+	+
50	EE	+	+	+	+	+
	HE	+	+	+	+	+
25	EE	+	+	+	+	+
	HE	+	+	+	+	+

Legend: + Growth; –No growth

Table 5 : Minimum Bactericidal Concentration (MBC) for ethanol and hexane extracts of *Ficus exasperata*

Concentration of Extract (mg/ml)	Extraction method	Microbial growth profile				
		ST	SA	EC	KP	CA
200	EE	–	–	–	+	+
	HE	–	–	+	+	+
100	EE	+	+	+	+	+
	HE	+	+	+	+	+
50	EE	+	+	+	+	+
	HE	+	+	+	+	+
25	EE	+	+	+	+	+
	HE	+	+	+	+	+

Ficus exasperata extract was effective against all the different microbial isolates. When the activity of the plant extract was compared to various concentrations of the standard drug chloramphenicol, it was shown to be similar to that of the 100mg/ml exthanol plant extract concentration (Table 6). Previous studies have shown *Ficus exasperata* to possess antimicrobial activities against several microbial species [37-39, 28]. However, the extracts had the least activity on *Candida alblican* suggesting that *ficus exasperata* is

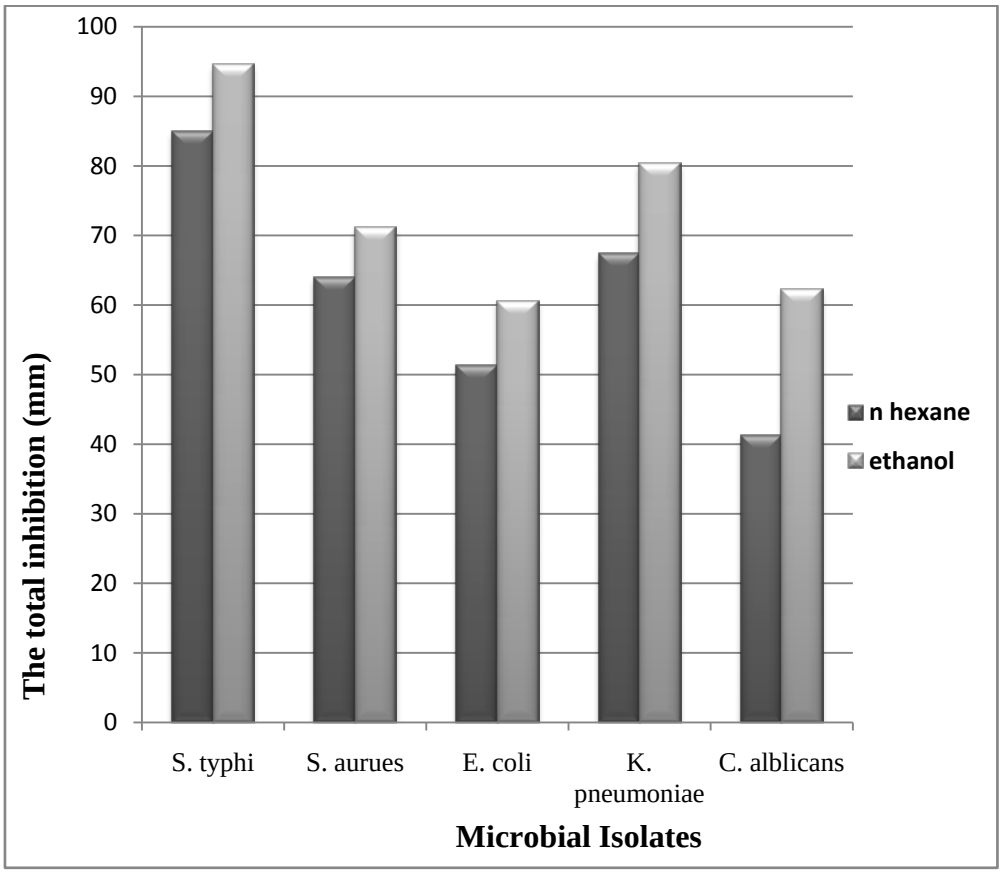
more effective on bacterial species especially gram negative bacteria such as *Salmonella typhi* and *Escherichia coli* which showed the highest inhibition pattern as well as the gram positive species than fungal species. This result confirms the local use of *Ficus exasperata* for medicinal purposes in treating infectious diseases caused by gram negative bacteria such as gastro intestinal infections, diarrhoea, typhoid etc [10-12]. The ability of the extracts to inhibit the growth of several bacterial and fungal species is an indication of the broad spectrum antimicrobial potential of *Ficus exasperata* which makes it a potential candidate for a prospective antimicrobial drug.

Table 6 : Antimicrobial profile for *Ficus exasperata* plant extract compared to standard drug

Chloramphenicol (mg/ml)	100	50	25	12.5	Ethanol plant extract (100mg/ml)
Zone of Inhibition (mm)	14.60	7.01	3.55	1.78	12

In all, ethanol extract of *Ficus exasperata* showed an overall better inhibition pattern against microbial isolates than hexane though both were effective in inhibiting microbial growth (Figure 1). These results confirm the findings of previous studies which have also shown ethanol extract to possess the strongest antimicrobial activity and most effective in in vitro studies compared to other solvents used for extration [40]. The reason for a greater activity of ethanol over the hexane extract could be attributed to the polarity of the solvent which has earlier been reported to be responsible for the extraction of a wide range of phytochemicals that potentiates the pharmacological activity of plant extracts [40-42]. The polarity of ethanol gives it the ability to penetrate cell membrane to extract intracellular ingredients from plant and also, since most phytochemicals are mostly aromatic or saturated compounds which are uncharge, they can easily be extracted by charge or polar solvents [43]. As such, ethanol; a polar solvent will yield more phytotochemicals which in synergy will generate a greater pharmacological activity than hexane which is non polar. Thus, the greater the phytochemical load, the greater the activity of a plant extract.

Figure 1 : Comparison of the total Inhibition profile for ethanol and hexane extracts of *Ficus exasperate*



To ascertain the above hypothesis, a qualitative phytochemical screening was done to evaluate the phytochemical load for both solvents used in extraction. The result confirmed ethanol extraction to be a better

and more effective solvent compared to hexane as it recovered a greater number (load) of phytochemicals. Out of the seven phytochemicals screened, five; tannin, saponin, alkaloid, flavanoid and glycosides were identified in the ethanol extract against three; tannin, alkaloid and flavanoid for hexane extract (Table 7).

Table 7 : Phytochemical profile for ethanol and hexane extracts of *Ficus exasperata*

Screened Phytochemicals	Tannins	Saponins	Alkaloids	Flavonoids	Steroids	Glycosides	Anthraquinones
Ethanol Extract	+	+	+	+	–	+	–
Hexane extract	+	–	+	+	–	–	–

Phytochemicals are plant molecules that are not directly involve in plant's growth but for other secondary activities such as protection against pest, pigmentation, abiotic stress etc. [44]. These chemicals have been reported in several studies to be responsible for the healing potentials of medicinal plants [45]. In this study, a wide range of phytochemicals; tannin, saponin, alkaloid, flavanoid, glycosides were recovered which have been report for antimicrobial activities through different mechanisms of action [45]. Hence, the collaborative or synergic action of these phytochemicals is responsible for the antimicrobial activity of *Ficus exasperata*.

V. Conclusion

Ficus exasperata possess a broad spectrum of antimicrobial activities and thus, a potential candidate for a prospective antimicrobial treatment whose activity will be at its best if ethanol is used for extraction to recover a wide range of phytochemicals. Due to its broad spectrum of activity, the local use of *Ficus exasperata* for various medicinal purposes is therefore encouraged.

VI. Acknowledgement

We wish to acknowledge the management of Godfrey Okoye University Nigeria for providing materials, reagents, equipment and laboratories for analysis. Special thanks go to the Vice Chancellor, Rev. Fr. Prof. Christian Anieke for financial assistance and moral support and to the staff and final year students of Chemical and Biological Sciences Departments for their technical assistance in carrying out this project.

VII. Conflict of Interest

The authors declare no conflict of interest in conducting this research.

References Références Referencias

1. Pinner, RS., Teutsch L., Simonsen, L., Klug, J., Graber, M., and Berkelman, R. 1996. Trends in infectious diseases mortality in the United States. J Am Med Assoc 275:189-193
2. Murry, CJ., Lopez, AD. 1997. Mortality by cause for eight regions of the world: global burden of disease study. Lancet 349:1269-1276.
3. Shira, S. 2008. An introduction to infectious diseases, 24 January 2008.
4. Kenrad, EN. 2014. Epidermiology of Infectious disease: General principles, Jones and Bartlett publishers. Online Accession 08/10/2014.
5. El-Mahmood, AM., Doughari, JH., Ladan, N. 2008. Antimicrobial screening of stem bark extracts of Vitellaria paradoxa against some enteric pathogenic micro-organisms. Afr J Pharm Pharmacol 2 (5): 89-94
6. WHO. 2013. Antimicrobial drug resistance. World Health Organisation secretariat.
7. Sanjoy, KP., 2003. Yogeshwer S. Herbal Medicine: Current Status and the Future. Asian Pacific J Cancer Prev 4: 281-288
8. Rivera, JO., Loya, AM., Ceballos, R. 2013. Use of Herbal Medicines and Implications for Conventional Drug Therapy Medical Sciences. Alternative and Integrative Medicine, Altern Integ Med 2:6
9. Mshana, NR., Abbiw, DK., Addae-Mensah, I., Adjanohoun, E., Ahyi MRA, et al. 2014. Traditional Medicine and Pharmacopoeia: Contribution to the Revision ethanobotanical and floristic studies in Ghana. 1st edition, Organization of African Unity/Scientific, Technical and Research Commission, Accra, Ghana, ISBN: 978-2453-66-2. Accessed online 2014.
10. Akah, PA., Orisakwe, OE., Gamaniel, KS., Shittu A. 1998. Evaluation of Nigerian traditional medicines: II. Effects of some folk remedies on peptic ulcer. J Ethanopharm 62: 123-127
11. Bouquet, AJ., 1969. Natural products as an alternative remedy. 24th Ed. Royal Botanic Gardens, Kew, London, UK.
12. Abbiw, T. 1990. Study of Tropical shrubs and plant. J. Biogeorge; 23: 591-602.
13. Burkill, HM. 1997. The useful plants of tropical West Africa. 43rd Ed. Royal Botanic Gardens Kew, London, UK,
14. Woode, E., Poku, RA., Abotsi, WKM. 2011. Anticonvulsant effects of a leaf extract of Ficus exasperata Vahl (Moraceae) in Mice. Int J Pharm 7(3): 405-409.
15. Abotsi, WMK., Woode, E., Ainooson, A., Amo-Baromah, A., Boakye-Gyasi, E. 2010. Antiathritic and antioxidant effects of the leaf extract of Ficus

exasperata P. Beauv (Moraceae). Pharmacognosy Res 2: 89-97.

16. Ayinde, BA., Mogbai, EK., Amaechina, FC. 2007. Pharmacognosy and hypotensive evaluation of Ficus exasperata Vahl (Moraceae) leaf. Acta Pol Pharm 64: 543-546.
17. Castel-Branco, MM., Alves, GL., Figueiredo, IV., Falcao, AC., Caramona, MM. 2009. The maximal electroshock seizure (MES) model in the preclinical assessment of potential new antiepileptic drugs. Methods, Find. Exp Clin Pharm 31: 101-116.
18. Ijeh, II., Ukweni, AI. 2007. Acute effect of administration of ethanol extracts of Ficus exasperata Vahl (Moraceae) on kidney function in Albino rats. J Med Plant Res 1: 27-29.
19. Woode, E., Poku, RA., Ainooson, GK., Boakye-Gyasi, E., Abotsi, WKM., Mensah, TL., Amo-Baromah AK. 2009. An evaluation of the anti-inflammatory, anti-pyretic, and antinociceptive effects of Ficus exasperata Vahl leaf extract. J Pharm Toxicol 4: 138-151.
20. Odunbaku, OA., Ilusanya, OA., Akasoro, KS. 2008. Antibacterial activity of ethanolic leaf extract of Ficus exasperata on Escherichia coli and Staphylococcus albus. Scientific Res Essay 3 (11): 562-564.
21. Buniyamin, AA., Eric, KIQ., Fabian, CA. 2007. Pharmacognosy and Hypotensive evaluation of Ficus exasperata Vahl (moraceae) leave. Acta Poloniae Pharmaceutica-Drug Research 64(6) : 543-546
22. Sonibare, MO., Isiaka, AO., Taruka, MW., Williams, NS., Soladoye, M., Emmanuel, O. 2006. Constituents of Ficus exasperata leaves. Natural product communications 23-26.
23. Williamson, EM. 2001. Synergy and other interactions in phytomedicines. Phytomed 8: 401–409.
24. Berenbaum, MC. 1989. What is synergy? Pharmacol. Rev.; 41: 93–141.
25. Engwa, AG., Nnamdi, P., Nnadi, JC., Offor, TI., Eze, BC. 2013. Comparative Qualitative Analysis of the Phytochemical Load of Water, Methanol, Ethyl Acetate and Hexane Extracts of Six Selected Medicinal Plants. Int J Pharmacog Phytochem Res 5(3); 164-167
26. Lapornik, B., Prosek, M., Wondra, AG. 2005. Comparison of extracts prepared from plant by products using different solvents and extraction time. Journal of Food Engineering; 71: 214–222.
27. Adebayo, E. and Ishiola. 2009. Evaluations of the methanolic extract of Ficus exasperata stem, bark, leaf and root for phytochemical analysis and antimicrobial activities. Afr. J. Plant Sci 3 (12): 283-287
28. Adeniyi, BA., Odelola, HA., Oso, BA. 1996. Antimicrobial potential of Diospyros mesiliforus (Ebenaceae) Afr. J. Med. Sci 255: 221-224.
29. Robbers, J., Speedie, M., Tyler, V. 1996. Pharmacognosy and Pharmacobiotechnology. Williams and Wilkins, Baltimore 1-14
30. Sofawa, AE. 1993. Medicinal And traditional medicine in Africa. Vol. 2 Spectrum Books Ltd. 142-146.
31. Trease, GE. and Evans, WC. 1989. Pharmacognosy (11th edition). Braille TRida Canada Macmillan Publishers, Canada 257.
32. Harbone JB (ed.). 1973. Methods of plants analysis. Phytochemical Methods Chapman and Hall London
33. Mbakwem-Aniebo, C., Onianwa, O., Okonko, IO. 2012. Effects of *Ficus Exasperata* Vahl on Common Dermatophytes and Causative Agent of Pityriasis Versicolor in Rivers State, Nigeria. Am J Dermatol and Venereol 1(1): 1-5
34. Ncube, NS., Afolayan, AJ., Okoh, AI. 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. Afr J Biotech 7 (12): 1797-1806.
35. Das, K., Tiwari, RKS., Shrivastava, DK. 2010. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. J Med Plants Res 4(2): 104-111.
36. Chinedu, FA., Uyai, U., Emezie, AU., Utoh-Nedosa, AU. 2012. Anti-diarrhoeal, antispasmodic and phytochemical properties of ethanol extract of the leaves of Ficus exasperate. Asian J. Res. Pharm. Sci 2 (1): 26-32
37. Lawal, IO., Borokini, TI., Oyeleye, A., Williams, OA., Olayemi, JO. 2012. Evaluation of Extract of Ficus Exasperata Vahl Root Bark for Antimicrobial Activities Against Some Strains of Clinical Isolates of Bacteria and Fungi. Int J Modern Botany; 2(1): 6-12
38. Igbinosa1, OO., Igbinosa, EO. and Aiyegoro, OA. 2009. Antimicrobial activity and phytochemical screening of stem bark extracts from Jatropha curcas (Linn). Afr J Pharm and Pharmacol 3(2): 058-062.
39. Bimakr, M. 2010. Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (Mentha spicata L.) leaves. Food Bioprod Process 1-6.
40. Eloff, JN. 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants. J Ethnopharm 60: 1–8.
41. Handa, SS., Khanuja, SPS., Longo, G., Rakesh, DD. 2008. Extraction Technologies for Medicinal and Aromatic Plants. International centre for science and high technology. Trieste; 21-25.
42. Wang, GX. 2010. In vivo anthelmintic activity of five alkaloids from Macleaya microcarpa (Maxim) Fedde

against Dactylogyrus intermedius in Carassius auratus. Veterinary Parasitol 171: 305–313.
43. Cowan, MM. 1999. Plant products as antimicrobial agents. Clin microbiol reviews 12(4): 564-582.
44. Prashant, Ti., Bimlesh, K., Mandeep, K., Gurpreet, K., Harleen, K. 2011. Phytochemical screening and Extraction: A Review. Int Pharm Sci 1 (1):98-106

Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology and Medicine
Volume 15 Issue 1 Version 1.0 Year 2015
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

An Assessment of Adherence of Patients to Anti-Hypertensive Medication and Factors for Non-Adherence in Oromia Region Adama Referral Hospital, Ethiopia

By Remedan Anuwer, Muluneh Fromsa Seifu, Belayneh Kefale Gelaw, Thirumurgan Gunasekaran, Esayas Tadesse Gebremariam & Mustefa Ahmed Mohammed

Ambo University, Ethiopia

Abstract- *Background:* Hypertension (HTN), or high blood pressure (systolic blood pressure ≥140 mmHg and diastolic blood pressure ≥90 mmHg) is an overwhelming global challenge which ranks third as a cause of disability-adjusted life-year. Hypertension causes 7.1 million premature deaths each year worldwide and accounts for 13% of all deaths globally. There are effective medical therapies for hypertension management; but the problem of non-adherence to medical treatment remains a challenge for the medical professionals and social scientists. The objective of this study is to assess the magnitude of adherence and factors for non adherence related to anti-hypertensive treatments in hypertensive patients visiting Adama Referral Hospital.

Keywords: *hypertensive patients, adherence, adama, prescribed medication.*

GJMR-B Classification : *NLMC Code: QV 151*

Strictly as per the compliance and regulations of:

An Assessment of Adherence of Patients to Anti-Hypertensive Medication and Factors for Non-Adherence in Oromia Region Adama Referral Hospital, Ethiopia

Remedan Anuwer [α], Muluneh Fromsa Seifu [σ], Belayneh Kefale Gelaw [ρ], Thirumurgan Gunasekaran [ω], Esayas Tadesse Gebremariam [¥] & Mustefa Ahmed Mohammed [§]

Abstract- ***Background:*** Hypertension (HTN), or high blood pressure (systolic blood pressure ≥140 mmHg and diastolic blood pressure ≥90 mmHg) is an overwhelming global challenge which ranks third as a cause of disability-adjusted life-year. Hypertension causes 7.1 million premature deaths each year worldwide and accounts for 13% of all deaths globally. There are effective medical therapies for hypertension management; but the problem of non-adherence to medical treatment remains a challenge for the medical professionals and social scientists. The objective of this study is to assess the magnitude of adherence and factors for non adherence related to anti-hypertensive treatments in hypertensive patients visiting Adama Referral Hospital.

Methodology: An institution based cross-sectional study was conducted at Adama Referral Hospital to assess patient adherence towards antihypertensive therapy. The data was collected by using a pre-tested structured questionnaire after translated to local language, in exit interview method immediately after the patients have got the service, and the collected data was processed and analyzed by using SPSS version 20 computer software.

Result: There were 96 respondents during the study period, from 96 respondents 42(43.75%) were males and the rest were females. Among the respondents 33.34% were > 64 years old and 20.83%% were employed. Regarding the educational level larger proportions of respondents can read and write. Among the study population 45.83% of the respondents were orthodox and only 3.12% were others. 29.16% of the respondents had an income of 100-800 Ethiopian birr per month. Majority of respondents were non adherent due to lack of money. It was found that the level of education does not significantly affect knowledge towards adherence. Also majority of patients >64 ages were non adherents to their medication.

Conclusion: The overall research finding shows that among 96 respondents of hypertensive patients in Adama Referral Hospital 44 (45.8%) were non adherent to the prescribed medication. It was recommended that health professionals must educate hypertensive patients about their disease with specific emphasis on its causes, the severity of the disease, their medications and the consequences of non-adherence with treatment.

Keywords: hypertensive patients, adherence, adama, prescribed medication.

I. Introduction

a) Background Information

Hypertension has no cure therefore; patients are expected to take medications for life. Drug treatments of hypertension demands that patients comply with their medications as prescribed and they should return for a refill when medications are exhausted. They should honor their appointments for follow up visits with clinician and adopt health actions that are recommended to lower their blood pressure (1).

Medication adherence has been defined in terms of an agreement between the patient's behavior of taking medications and the clinical prescription (2). Faulty adherence or non-adherence with medications may include errors of purpose, timing or dosage as well as total or partial omission, or use of inadvertent combinations. Non-adherence with medications is one of the major factors in the failure of therapeutic programs in patients having a chronic disease (2).

In the available literature, the magnitude of non-adherence with medications prescribed for patients with hypertension was 16.7% (3). Generally, the adherence of patient's decreases with time and it is lower in long-term medications than in short-term medications. In depressive patients, adherence was shown to be 68% after 3 weeks of treatment, but this percentage decreased after 6, 9 and 12 weeks to 63%, 50% and 40% respectively (4). An adherence study conducted with short-term medications revealed an overall incidence of non-adherence of 26% (5). Ensuring patients' adherence with antihypertension medications and lifestyle modifications to prevent complications of hypertension remains a major challenge to public health in many developing countries.

Non-adherence with treatment is the most important single reason for uncontrolled hypertension.

Author α σ ρ Ω ¥ § : Ambo University, College Of Medicine and Health Sciences, Department Of Pharmacy.
e-mail: b.pharm2006@yahoo.com, belayneh.kefale@yahoo.com, esayastadesse23@yahoo.com, mustebatie@yahoo.com, bkefale5@gmail.com

Several factors, which may be patient or health system related, continue to militate against adherence behavior. Thus it is essential to identify such factors and develop strategies to improve adherence. It is true that the possible factors of non-adherence may vary from country to country and may contribute to the variations that exist among the reported values of non-adherence. With regard to the possible factors of non-adherence that are related to the patient, the disease, the drugs prescribed the physician and the treatment environment (6, 7).

b) Statement of the Problem

In line with the global realities, Hypertension sufferers' non adherence to their pharmacological regimen and frequent lifestyle changes result in uncontrolled hypertension that leads to different life threatening organ complications such as cardiovascular, renal and cerebro-vascular diseases (8). In order to mitigate the effects of the disease in populations, it is essential to improve adherence among sufferers of the disease by identifying underlying factors in order to mitigating against adherence behavior and developing effective interventions to overcome identified factors (9). Factors affecting adherence behavior are unique to individuals and specific, and also, studies done in other countries were not applied to the circumstances surrounding the Adama Referral Hospital. However, these studies attempted to identify factors affecting drug treatment and lifestyle modification adherence and provide possible recommended strategies that could improve adherence for both drug treatment and lifestyle modifications with involving hypertension patients those who visit Adama Referral Hospital. Therefore, there is a great need of organized research that is closely linked to the patient compliance towards their anti-hypertensive treatment to improve the adherence to therapy and healthy lifestyle modification.

Concerning Client adherence towards antihypertensive treatment, specific studies do almost not exist in our local setting. Taking this into consideration this study has attempted to answer the following question: What are the reasons for non-adherence with the drugs among hypertensive patients visiting Adama Referral Hospital.

c) Significance of the study

The results of the study may contribute to increase the awareness of health care providers particularly physicians on the issue of adherence and may aid to develop strategies for improvement of adherence.

This study was also sought to examine various factors responsible for adherence and non-adherence in the research context and elucidate relationships existing between them. Such information would assist health care professionals to manage hypertension appropriately. It would also assist policy makers in developing context specific and relevant policies capable of improving the management of hypertension in the clinics. Ultimately, it is envisioned that the implementation of effective strategies would lead to improved adherence, increased levels of controlled blood pressure and reduced occurrences of complications.

The main aim of this study is to add to the existing body of knowledge about factors affecting adherence to hypertension medication and lifestyle modifications necessary to maintain hypertension control, and to propose strategies that was assist policy makers and clinicians with hypertension management decisions in ARH, Ethiopia.

d) Objectives of the Study

- *General Objective*
  - ✓ The general objective of this study will be to assess the magnitude of adherence and factors related to anti-hypertensive treatments in hypertensive patient Adama Referral Hospital.
- *Specific Objective*
  - To describe the magnitudes of adherence and non-adherence of hypertensive patient towards antihypertensive drugs and life style modifying regimens
  - To assess the factors related to adherence and non-adherence of anti-hypertensive drugs and life style modifying regimen

II. Method and Materials

a) Study Area

Adama is one of the towns of Oromia regional state, East Shewa zone and located 99Km far from Addis Ababa. There are different governmental and non-governmental institution in the town such as 8 health Center, one referral hospital, one general hospital, 50 private clinics, and 105 pharmacies.

The study was conducted in Adama hospital medical college on patients who have hypertension cases. Adama hospital medical college was established & started its full function in 1965. The hospital is now providing several health service including diabetes and hypertension for the community

b) Study design and period

In this research, the study design was an institution based cross-sectional study and the study was conducted from March 19th to May 23rd, 2014.

c) Population

i. *Source Population*

The source populations for the study was all hypertensive patients attending at Adama Referral Hospital of medical OPD for treatment follow up & life modifying services during the study period.

ii. *Study Population*

The study populations were those adult hypertensive patients who are on anti-hypertensive treatment and life style modifying service follow up, strong enough and willing to respond or impartial to respond during their exit (exit interview) from outpatient department of Adama Referral Hospital during the study period.

iii. *Inclusion and Exclusion criteria*

All hypertensive people who were attending medical OPD during the study period and volunteer will be included. People who are unable to give response for an informed consent will be excluded; like all pediatric age groups less than fifteen (< 15) years, all critically sick, all groups who will not committed to respond (refusals), all groups who have weak perception to express and all mentally ill or psychiatric patients.

d) Simple Size Determination

In this research, a convenience sampling techniques was used to select the study population. The sample was selected because of their convenience. The data was conducted in short period of time for around two month with exit interview method from accessible population who are used the service.

III. Study Variable

a) Independent Variable

Socio demographic characteristics, such as:

- Age
- Sex
- Occupational status
- Educational status
- Religion
- Ethnicity
- Income
- Marital Status
- Address /accessibility/

b) Dependent Variable

- Adherence towards antihypertensive treatment and lifestyle modifying service
- Non-adherence towards antihypertensive treatment and lifestyle modifying service

c) Data Collection Procedure and Instrument

i. *Data collection instruments*

Data was collected through standard or structured questionnaire. Before starting the data collection the questionnaire was be translated to local language Amharic and pre-tested. The data was collected by 1 BSc Graduating pharmacy students by using a pre-tested structured interviewer administered questionnaire consisting both open & close ended questionnaires prepared to address adherence and factors related to anti-hypertensive drugs. The questionnaire was administered to all volunteer hypertensive people who fulfill the inclusion criteria while they are at medical OPD in Adama referral hospital. The hypertensive people were contacted by the assigned data collectors. The respondents were encouraged to answer the questions within the time they devoted as much as possible. And the data was collected through strict supervision and daily follow up or cross check up by the researchers.

ii. *Data Collection Procedure*

At each health unit selected interviewee was identified who fulfill the criteria while they exit after they get service. Every effort was made to choose a site for interviewing that allow the interviewer to be seat out of sight and at a sufficient distance from the health institute to avoid interviews being over heard each other. Interviewers was instructed to select the next exiting patient following the completion of each interview in order to avoid introducing bias by selecting only patient willing to wait for all interviews.

iii. *Data Processing and Analysis*

After data were collected, the patient responses were cleared, entered in a computer and relevantly organized and made ratio and percentage with computer and present in table and chart. Statistical significance test was applied to reflect the association between the variables by using statistical package for social science version 20 (SPSS version-20), Chi-square and P-Value. Further interpretation was made with the context of the study objectives. Based on the results, conclusion and recommendation were made.

d) Ethical Issues Consideration

Letter of Permission was obtained from Ambo University, college of Health science, school of pharmacy before data collection and was given to the study area, Adama Referral hospital administration. Brief explanation was also given on the objectives as well as the benefit of the study to the concerned officials and their verbal consent was obtained. Each respondent who was interview is asked to give their consent after explaining the purpose, objective, and benefit of the study. Confidentiality and privacy of every respondent's information was ensured. The finding was availed to the concerned bodies up on the final result.

e) Operational Definition

i. *Adherence:* is defined as "the extent to which a person's behavior (taking medicines or executing lifestyle changes) coincides with medical or health advice".

ii. *Non-adherence:* any form of deviation from adherence like losing one appointment, missing doses, etc.

iii. *Hypertension:* is defined as the persistent systolic blood pressure equal to and greater than 140 mmHg and/or persistent diastolic blood pressure equal to and greater than 90 mmHg.

iv. *Hypertensive patient:* refers to a person diagnosed with hypertension as defined above.
v. *Factors:* are conditions of hypertensive patients that influence the development and course of the disease.
vi. *Lifestyle:* attitudes, habits and behaviors of hypertension patients.

IV. Results

There were 96 respondents during the study period, among 96 respondents 42(43.75%) were males and the rest were females. Among the respondents 33.34% were >64 years old and only 9.37% were between 15-24 years and both farmers and daily labors were 10.42% each. Regarding the educational level larger proportions of respondents can read and write (28.13%), and only 12.5% of the study populations were at primary level. Among the study population 45.83% of the respondents were orthodox and only 3.12% were catholic. 29.16% of the respondents had an income of 100-800 birr per month and 18.75% had an income of >1200 birr per month.

Table 1 : Socio demographic characteristics of respondents in ARH Adama, Ethiopia, June, 2014

Variable		Sex		
		Female (%)	Male (%)	Total (%)
Age	15-24	5(5.2%)	4(4.17%)	9(9.37%)
	25-49	14(14.58%)	11(11.46%)	25(26.04%)
	50-64	16(16.67%)	14(14.58%)	30(31.25%)
	>64	19(19.8%)	13(13.54%)	32(33.34%)
	Total	54(56.25%)	42(43.75%)	96(100%)
Marital status	Married	20(20.83%)	14(14.58%)	34(35.41%)
	Single	9(9.38%)	11(11.46%)	20(20.84%)
	Divorced	8(8.33%)	7(7.29%)	15(15.62%)
	Widowed	17(17.71%)	10(10.42%)	27(28.13%)
	Total	54(56.25%)	42(43.75%)	96(100%0
Religion	Protestant	9(9.36%)	8(8.34%)	17(17.72%)
	Orthodox	26(27.08%)	18(18.75%)	44(45.83%)
	Muslim	18(18.75%)	14(14.58%)	32(33.3%)
	Catholic	1(1.04%)	2(2.08%)	3(3.12%)
	Total	54(56.25%)	42(43.75%)	96(100%)
Educational level	Illiterate	8(8.33%)	7(7.3%)	15(15.63%)
	Read and Write	17(17.71%)	10(10.42%)	27(28.13%)
	Primary	6(6.25%)	6(6.25%)	12(12.5%)
	Secondary	12(12.5%)	5(5.2%)	17(17.7%)
	Above secondary	11(11.46%)	14(14.58%)	25(26.04%)
Occupation	Housewife	13(13.54%)	-	13(13.54%)
	Employed	8(8.33%)	12(12.5%)	20(20.83%)
	Farmer	3(3.13%)	7(7.3%)	10(10.43%)
	Daily labor	4(4.17%)	6(6.25%)	10(10.42%)
	Merchant	9(9.38%)	9(9.37%)	18(18.75%)
	other	17(17.7%)	8(8.33%)	25(26%)
	Total	54(56.25%)	42(43.75%)	96(100%)
Ethnicity	Oromo	18(18.75%)	16(16.67%)	34(35.42%)
	Amhara	22(22.91%)	18(18.75%)	40(41.66%)
	Gurage	10(10.42%)	5(5.21%)	15(15.63%)
	Other	4(4.17%)	3(3.12%)	7(7.29%)
	Total	54(56.25%)	42(43.75%)	96(100%)
Address place of residence	Adama town	36(37.5%)	29(30.21%)	65(67.71%)
	Walanchit	2(2.08%)	1(1.04%)	3(3.12%)
	other urban area	4(4.17%)	4(4.17%)	8(8.34%)
	rural area	12(12.5%)	8(8.33%)	20(20.83%)
	Total	54(56.25%0	42(43.75%)	96(100%)
Monthly income	<100	16(16.67%)	9(9.38%)	25(26.05%)
	100-800	20(20.83%)	8.33%)	28(29.16%)
	801-1200	11(11.46%)	14(14.58%)	25(26.04%)
	>1200	7(7.29%)	11(11.46%)	18(18.75%)
	Total	54(56.25%)	42(43.75%)	96(100%)

In this study 35.41% of respondents were married and 15.62% were divorced. Most of the respondents (67.71%) came from Adama town and minors were from Walanchit town (3.12%). Among the

study population the number of social drugs users were higher for alcohol than for others which includes khat and cigarette. In this study number of non adherent was higher for cigarette because from 10 users of alcohol 8 was non adherent as we compare with other social drug users.

Table 2 : Number of respondents that uses social drug and non adherent in ARH, Adama, Ethiopia, June, 2014.

Social drugs	No (%)	No of non adherent
Alcohol	30(31.25%)	15
Khat	18(18.75%)	8
Cigarette	10(10.42%)	8

Table 3 : Number of respondent that uses contraindicated substance and engage in exercise and non adherent in ARH, Adama, Ethiopia, June, 2014

Contraindicated substance	No (%)	No of non adherent
Animal fat	16(16.3%)	8(8.3%)
Salt intake	19(19.7%)	10(10.3%)
Exercise	23(24%)	6(6.2%)

From the study population the number of salt intakers was higher than those eating a meal high in animal fat. The number of respondent engaged in exercise was 24%.

There were also some problems of patients which contribute to non-adherence of the patients. These were lack of money, use of traditional medicine, negligence and forgetfulness.

Table 4 : Some perceived problems of respondents which lead to the non adherents of the respondents, ARH, Adama, Ethiopia, June, 2014

Perceived problems	No of respondents that were non-adherence due
Lack of money	38
Negligence	13
Forgetfulness	19
Use of traditional medicine	7

The number of patients that were non adherents due to lack of money, forgetfulness, negligence, and use of traditional medicine were 38, 19, 13, and 7, respectively. Thus, majority of respondents were non adherent due to lack of money.

Table 5 : Association of educational level and lack of sufficient information on knowledge of adherence in ARH, Adama, Ethiopia, June, 2014

Educational level	Total number	Lack of sufficient information	
		Yes	No
Illiterate	15	3	12
Read and write	27	6	21
Primary	12	2	10
Secondary	16	2	14
Above secondary	26	2	24

P-value= 0.623

The statistical association between educational level and lack of sufficient information on knowledge of adherence was (p=0.623). Majority of respondents 84.6% were sufficient knowledge on adherence and15.4% were lack of sufficient information on knowledge of adherence. The number of patients that lack sufficient knowledge on adherents 3(3.1%), 2(2.1%), 2(2.1%), and 2(2.1%) were illiterate, primary, secondary, and above secondary levels, respectively.

Table 6 : Association between socio-demographic data and non-adherent in ARH, Adama, Ethiopia, June, 2014.

Age	Total number	Respondents that were Non-adherent
14-25	9	0
26-49	25	9
50-64	30	16
>64	32	19
P-value=0.02		
Monthly income	**Total number**	**Respondents that were non-adherent**
<100	27	21
100-800	25	11
801-1200	26	11
>1200	18	1
P-value= 0.001		

In this study there is significant association between monthly income and adherence because they had p-values less than 0.05. Majority of respondents under low monthly income were non adherent to their medication. In this study also there were an association between age and adherence (p=0.02). Also majority of patients >64 ages were non adherents to their medication.

Table 7 : Treatment related factor of respondent which lead to the non adherents of the respondents in ARH, Adama, Ethiopia, June, 2014.

Treatment related factor	Number of respondents that were non adherence
Adverse effect of the drug	18
Different kind of medicine	9
Prolonged duration of treatment	21
Lack of the role health worker	4

From the study population the prolonged duration of the treatment is major treatment related factor that lead to non adherent of the respondents. Number of respondents that were non adherents due to treatment related factors such as adverse effect of the drug, different kind of medicine, and lack of the role health worker were 18, 9, and 4, respectively

V. Discussion

This study showed the magnitude of treatment related factor of non adherence with adverse effect of the drug , prolonged duration of treatment , different kind of medicine, and lack of role of health worker in describing about the drug were 18.8%, 21.8%, 9.3%, and 4.1% , respectively. Comparable findings were reported. The study conducted in Seychelles showed the magnitude of non adherence with medication side effect, use of alternative remedies and ineffective medication were (9.9%), (12.87%), and (5.49%)(10), respectively. Also study in Finland reported that adverse effect led to non adherence was 33 %(11).

In this study the number of respondents from social drugs user such as alcohol(31.25%), smoke cigarette(10.42%), and chew chat(18.75%) number of non adherents were (15.6%), (8.3%), and (8.3%) respectively. Also this study suggests that the number patients take contraindicated substance such as animal fat and salt (8.3%), and (10.3%) were non adherent respectively. Additionally from number of respondents that engaged in physical exercise 6.2% was non adherent to their medication. In line with this study in Seychelles showed the magnitudes of non adherence to engage in physical exercise (50%), stop smoke (15.84%), stop alcohol drink (21.57%), reduce salt intake (24.51%), and stop eating a meal high in animal fat (28.35%) (10). When we compare this study with study in seychelles different finding were reported In this study about 9.4% of male and 1% female were current cigarette smoking. Similar findings were obtained from study in Addis Ababa. It was reported 13.5% of males and less than 1% of females were current cigarettes smoking (12).

This study also showed age of the patient had significant association with respondents that were non adherent (p=0.02) this study shows that 36.5% patients whose age was >50 years were non adherent. Age of the patient was one of significantly associated factor with adherence.

There is one study that was conducted in Pakistan and 75% of adherent is due to increasing age which disagreed with finding of this study (13). In this study number of non adherent was increased as age of patient increased. This might be due to the reason that most patients does not know the disease that they acquired earlier due to different factors.

However, in this study there was no significant association between educational level and having information on knowledge of adherence for hypertension. 84.6 % of respondents had knowledge on adherence of antihypertensive medication. Similar finding was obtained from a study conducted in Gondar that majority of respondents (76.8%) were knowledgeable about HTN and its treatment (14). The overall level of awareness about hypertension and its treatment was very low. Higher awareness among ability of writing and reading and at above secondary education level is associated with higher adherence.

Another relevant point was economic factor. According to this study majority of the respondents were under low economic status. Greater than 50% of the respondents had an income of less than 800 birr per month. Comparatively Similar study conducted in Yirgalem showed that 83% lack of money was a major factor associated with treatment adherence (15). In this study also there were an association between monthly income and adherence (P=0.001). Therefore the price for treatment of the disease was the major factor for non adherence of the respondents.

In this study the total non adherence of respondents were 45.8% mostly due to 39.6% economic problem of the patient contributed to the non adherence of the respondents. In contrary to this the study conducted in Scotland showed 91% of the populations were non adherent to their medication (16). Also study conducted in Gondar showed that 35.2% were non adherent to their medication (14). Similar finding were reported from study conducted in Malaysia in which 44.2% of the populations were non adherent (17).

VI. Conclusion and Recommendation

a) Conclusion

The study showed that from 96 respondent of hypertensive patients in Adama Referral Hospital 45.8%was non adherent to the prescribed medication. There were a number of perceived problems of patients with hypertension .This include forgetfulness, negligence, adverse effect of medication and old age or disability, economic problem, and use of social drugs of stopping of medication.

From the above factors economical problem and negligence were the major obstacles for the patients to be non adherent. Related to perceived problem of respondents on the health care system, there was old age of >50 on 36.5% of the respondents were non adherent and 44.9% of patients had problem due to cost of the medication since no free service was available for special cases in the hospital.

b) Recommendation

- Health professionals must educate hypertensive patients about their disease with specific emphasis on its causes, the severity of the disease, their medications and the consequences of non-adherence with treatment.
- Education may be transmitted by preparing leaflet for educated patients and verbally for illiterate one.
- And also the disadvantage of non adherence should be told to the patient always and other problem on the patient's side that could affect adherence should also be told.
- The hospital should have free service for special case like for very poor patients and for very old patients who had no sufficient income for they and the hospital should prepare normal schedule for BP measurement follow up for the respondent.

Abbreviation

ARH: Adama Referral Hospital

AU: Ambo University

BSc: Bachelor of Science

CBE: community based education

CMHS: Collage of Medicine and Health Sciences

FMOH – Federal Ministry of Health

HTN: Hypertension

OPD: outpatient department

SES – Socio Economic Status

TPA: Total Physical Activity

UNICEF - United Nations International Children's Fund

WHO: World Health Organization

VII. Acknowledgements

We are very grateful to our college staff members for unreserved guidance and constructive suggestions and comments from the stage of proposal development to this end. We would like to thank Ambo University for supporting the budget which required for this research. Finally our deepest gratitude goes to Adama referral hospital staff workers who helped and allowed us in collecting and gathering data from the hospital.

a) *Funding:* Ambo University

b) *Conflict of interest:* None declared

c) *Ethical approval:* Approval and permission was sought from Ethical Review Board of College of Medicine and Health Sciences of Ambo University

References Références Referencias

1. Greeff, D. 2006. An approach to preventing and treating hypertension through Lifestyle modification. Professional Nursing Today 10(5): 8-22.
2. Sackett DL. The magnitude of compliance and non-compliance. In: Sackett DL, Haynes RB.(eds.).

Compliance with therapeutic re-gimens. Johns Hopkins University Press, Baltimore, 1976; 9-25.
3. Contreras EM, Main CG, etal. Observancia terapeutica enla hipertension arterial. Atencion Primaria 1995; 16(8):496-500.
4. Myers ED, Branthwaite A. Out-patient compliance with antidepressant medications Br J Psychiatry 1992; 160:83-86.
5. Abula T. Patient non-compliance with therapeutic regimens and factors of non-compliance in Gondar. Ethiopia J Health Dev. 2000;14(1):1-6.
6. Benet LZ. Principles of prescription order writing and patient compliance instructions. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW. (Eds.). The pharmacological basis of therapeutics. MC. Graw-hill companies, INC., New York, 1996; 1697-1706.
7. Griffith S. A review of the factors associated with patient compliance and the taking of prescribed medicines. Br J General Practice 1990; 40:114-116.
8. Psaty, BM, Lumley, T, Furberg, CD, Schellenbaum, G, Pahor, M, Alderman, MH & Weiss, NS. 2003. Health outcomes associated with various antihypertensive therapies used as first-line agents: a networkmeta- analysis. Journal of American Medical Association 289:2534.
9. Thrall, G, Lip, GYH & Lane, D. 2004. Compliance with pharmacological therapy in hypertension: can we do better, and how? Journal of Human Hypertension 18: 595e
10. Thomas AE, Master of public health, University of South Africa, 2009. Factors affecting compliance with antihypertensive drug treatment and required lifestyle modifications among hypertensive patients on Praslin Island, Seychelles 108-114.
11. Jokisalo, E, Kumpusalo, E, Enlund, H, Halonen, P & Takala, J. 2002. Factors related to non – compliance with antihypertensive drug therapy. Journal of Human Hypertension 16 (8): 577- 583.
12. Fikiru T, Peter B, Stig W: Population based prevalence of high blood pressure among Adults in Addis Ababa: uncovering a silent epidemic. BMC Cardiovascular Disorders 2009, 9:39.
13. Hashmi SK, Afridi MB, Abbas K, Sajwani RA, Saleheen D, Frossard PM, Ishaq M, Ambreen A, Ahmad U: Factors associated with adherence to anti-hypertensive treatment in Pakistan. PLoS One 2007, 2(3):e280.
14. Abere DA, Getahun A, Solomon MW, Zelalem BM, 2012. Adherence to Antihypertensive treatments and associated factors among patients on follow up at University of Gondar Hospital, Northwest Ethiopia. BMC Public Health 12:282.
15. Alexander T, Enguday S, Haregwoin A, Hiwot A, Tariku B 2006. Compliance and Factors related to treatment of chronic illness in adult population visiting Yirgalem Hospital: 13-14.
16. Rm Y: Il M: Patterns and determinants of treatment compliance among Hypertensive patients. East Mediterr Health J 2002, 8(4–5):579-592.
17. Morisky DE, Green LW, Levine DM: Concurrent and predictive validity of a self- Reported measure of medication adherence. Med Care 1986, 24(1):67-74.

Global Journals Inc. (US) Guidelines Handbook 2015

www.GlobalJournals.org

FELLOWS

FELLOW OF ASSOCIATION OF RESEARCH SOCIETY IN MEDICAL (FARSM)

Global Journals Incorporate (USA) is accredited by Open Association of Research Society (OARS), U.S.A and in turn, awards "FARSM" title to individuals.The 'FARSM' title is accorded to a selected professional after the approval of the Editor-in-Chief/Editorial Board Members/Dean.

- The "FARSM" is a dignified title which is accorded to a person's name viz. Dr. John E. Hall,Ph.D., FARSS or William Walldroff, M.S., FARSM.

FARSM accrediting is an honor. It authenticates your research activities. After recognition as FARSM, you can add 'FARSM' title with your name as you use this recognition as additional suffix to your status. This will definitely enhance and add more value and repute to your name. You may use it on your professional Counseling Materials such as CV, Resume, and Visiting Card etc.

The following benefits can be availed by you only for next three years from the date of certification:

FARSM designated members are entitled to avail a 40% discount while publishing their research papers (of a single author) with Global Journals Incorporation (USA), if the same is accepted by Editorial Board/Peer Reviewers. If you are a main author or co-author in case of multiple authors, you will be entitled to avail discount of 10%.

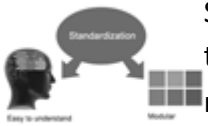
Once FARSM title is accorded, the Fellow is authorized to organize a symposium/seminar/conference on behalf of Global Journal Incorporation (USA). The Fellow can also participate in conference/seminar/symposium organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent.

You may join as member of the Editorial Board of Global Journals Incorporation (USA) after successful completion of three years as Fellow and as Peer Reviewer. In addition, it is also desirable that you should organize seminar/symposium/conference at least once.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time.This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

The FARSM can go through standards of OARS. You can also play vital role if you have any suggestions so that proper amendment can take place to improve the same for the benefit of entire research community.

As FARSM, you will be given a renowned, secure and free professional email addres with 100 GB of space e.g. johnhall@globaljournals.org. This will include Webmail, Spam Assassin, Email Forwarders,Auto-Responders, Email Delivery Route tracing, etc.



The FARSM will be eligible for a free application of standardization of their researches. Standardization of research will be subject to acceptability within stipulated norms as the next step after publishing in a journal. We shall depute a team of specialized research professionals who will render their services for elevating your researches to next higher level, which is worldwide open standardization.

The FARSM member can apply for grading and certification of standards of their educational and Institutional Degrees to Open Association of Research, Society U.S.A. Once you are designated as FARSM, you may send us a scanned copy of all of you credentials. OARS will verify, grade and certify them. This will be based on your academic records, quality of research papers published by you, and some more criteria. After certification of all your credentials by OARS, they will be published on your Fellow Profile link on website https://associationofresearch.org which will be helpful to upgrade the dignity.

The FARSM members can avail the benefits of free research podcasting in Global Research Radio with their research documents. After publishing the work, (including published elsewhere worldwide with proper authorization) you can upload your research paper with your recorded voice or you can utilize chargeable services of our professional RJs to record your paper in their voice on request.

The FARSM member also entitled to get the benefits of free research podcasting of their research documents through video clips. We can also streamline your conference videos and display your slides/ online slides and online research video clips at reasonable charges, on request.

The FARSM is eligible to earn from sales proceeds of his/her researches/reference/review Books or literature, while publishing with Global Journals. The FARSS can decide whether he/she would like to publish his/her research in a closed manner. In this case, whenever readers purchase that individual research paper for reading, maximum 60% of its profit earned as royalty by Global Journals, will be credited to his/her bank account. The entire entitled amount will be credited to his/her bank account exceeding limit of minimum fixed balance. There is no minimum time limit for collection. The FARSM member can decide its price and we can help in making the right decision.

The FARSM member is eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get remuneration of 15% of author fees, taken from the author of a respective paper. After reviewing 5 or more papers you can request to transfer the amount to your bank account.

MEMBER OF ASSOCIATION OF RESEARCH SOCIETY IN MEDICAL (MARSM)

The ' MARSM ' title is accorded to a selected professional after the approval of the Editor-in-Chief / Editorial Board Members/Dean.

The "MARSM" is a dignified ornament which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., MARSM or William Walldroff, M.S., MARSM.

MARSM accrediting is an honor. It authenticates your research activities. Afterbecoming MARSM,you can add 'MARSM' title with your name as you use this recognition as additional suffix to your status. This will definitely enhance and add more value and repute to your name. You may use it on your professional Counseling Materials such as CV, Resume, Visiting Card and Name Plate etc.

The following benefitscan be availed by you only for next three years from the date of certification.

MARSM designated members are entitled to avail a 25% discount while publishing their research papers (of a single author) in Global Journals Inc., if the same is accepted by our Editorial Board and Peer Reviewers. If you are a main author or co-author of a group of authors, you will get discount of 10%.

As MARSM, you willbe given a renowned, secure and free professional email address with 30 GB of space e.g. johnhall@globaljournals.org. This will include Webmail, Spam Assassin, Email Forwarders,Auto-Responders, Email Delivery Route tracing, etc.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time.This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

The MARSM member can apply for approval, grading and certification of standards of their educational and Institutional Degrees to Open Association of Research, Society U.S.A.

Once you are designated as MARSM, you may send us a scanned copy of all of your credentials. OARS will verify, grade and certify them. This will be based on your academic records, quality of research papers published by you, and some more criteria.

It is mandatory to read all terms and conditions carefully.

AUXILIARY MEMBERSHIPS

Institutional Fellow of Open Association of Research Society (USA) - OARS (USA)

Global Journals Incorporation (USA) is accredited by Open Association of Research Society, U.S.A (OARS) and in turn, affiliates research institutions as "Institutional Fellow of Open Association of Research Society" (IFOARS).

The "FARSC" is a dignified title which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., FARSC or William Walldroff, M.S., FARSC.

The IFOARS institution is entitled to form a Board comprised of one Chairperson and three to five board members preferably from different streams. The Board will be recognized as "Institutional Board of Open Association of Research Society"-(IBOARS).

The Institute will be entitled to following benefits:

The IBOARS can initially review research papers of their institute and recommend them to publish with respective journal of Global Journals. It can also review the papers of other institutions after obtaining our consent. The second review will be done by peer reviewer of Global Journals Incorporation (USA) The Board is at liberty to appoint a peer reviewer with the approval of chairperson after consulting us.

The author fees of such paper may be waived off up to 40%.

The Global Journals Incorporation (USA) at its discretion can also refer double blind peer reviewed paper at their end to the board for the verification and to get recommendation for final stage of acceptance of publication.

The IBOARS can organize symposium/seminar/conference in their country on behalf of Global Journals Incorporation (USA)-OARS (USA). The terms and conditions can be discussed separately.

The Board can also play vital role by exploring and giving valuable suggestions regarding the Standards of "Open Association of Research Society, U.S.A (OARS)" so that proper amendment can take place for the benefit of entire research community. We shall provide details of particular standard only on receipt of request from the Board.

The board members can also join us as Individual Fellow with 40% discount on total fees applicable to Individual Fellow. They will be entitled to avail all the benefits as declared. Please visit Individual Fellow-sub menu of GlobalJournals.org to have more relevant details.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time. This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

After nomination of your institution as "Institutional Fellow" and constantly functioning successfully for one year, we can consider giving recognition to your institute to function as Regional/Zonal office on our behalf.
The board can also take up the additional allied activities for betterment after our consultation.

The following entitlements are applicable to individual Fellows:

Open Association of Research Society, U.S.A (OARS) By-laws states that an individual Fellow may use the designations as applicable, or the corresponding initials. The Credentials of individual Fellow and Associate designations signify that the individual has gained knowledge of the fundamental concepts. One is magnanimous and proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice.

Open Association of Research Society (US)/ Global Journals Incorporation (USA), as described in Corporate Statements, are educational, research publishing and professional membership organizations. Achieving our individual Fellow or Associate status is based mainly on meeting stated educational research requirements.

Disbursement of 40% Royalty earned through Global Journals : Researcher = 50%, Peer Reviewer = 37.50%, Institution = 12.50% E.g. Out of 40%, the 20% benefit should be passed on to researcher, 15 % benefit towards remuneration should be given to a reviewer and remaining 5% is to be retained by the institution.

We shall provide print version of 12 issues of any three journals [as per your requirement] out of our 38 journals worth $ 2376 USD.

Other:

The individual Fellow and Associate designations accredited by Open Association of Research Society (US) credentials signify guarantees following achievements:

- The professional accredited with Fellow honor, is entitled to various benefits viz. name, fame, honor, regular flow of income, secured bright future, social status etc.

- In addition to above, if one is single author, then entitled to 40% discount on publishing research paper and can get 10%discount if one is co-author or main author among group of authors.
- The Fellow can organize symposium/seminar/conference on behalf of Global Journals Incorporation (USA) and he/she can also attend the same organized by other institutes on behalf of Global Journals.
- The Fellow can become member of Editorial Board Member after completing 3yrs.
- The Fellow can earn 60% of sales proceeds from the sale of reference/review books/literature/publishing of research paper.
- Fellow can also join as paid peer reviewer and earn 15% remuneration of author charges and can also get an opportunity to join as member of the Editorial Board of Global Journals Incorporation (USA)
- • This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in–depth understanding of the application of suitable techniques to a particular area of research practice.

Note :

"

- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone.

"

Process of submission of Research Paper

The Area or field of specialization may or may not be of any category as mentioned in 'Scope of Journal' menu of the GlobalJournals.org website. There are 37 Research Journal categorized with Six parental Journals GJCST, GJMR, GJRE, GJMBR, GJSFR, GJHSS. For Authors should prefer the mentioned categories. There are three widely used systems UDC, DDC and LCC. The details are available as 'Knowledge Abstract' at Home page. The major advantage of this coding is that, the research work will be exposed to and shared with all over the world as we are being abstracted and indexed worldwide.

The paper should be in proper format. The format can be downloaded from first page of 'Author Guideline' Menu. The Author is expected to follow the general rules as mentioned in this menu. The paper should be written in MS-Word Format (*.DOC,*.DOCX).

The Author can submit the paper either online or offline. The authors should prefer online submission.Online Submission: There are three ways to submit your paper:

(A) (I) First, register yourself using top right corner of Home page then Login. If you are already registered, then login using your username and password.

(II) Choose corresponding Journal.

(III) Click 'Submit Manuscript'. Fill required information and Upload the paper.

(B) If you are using Internet Explorer, then Direct Submission through Homepage is also available.

(C) If these two are not conveninet , and then email the paper directly to dean@globaljournals.org.

Offline Submission: Author can send the typed form of paper by Post. However, online submission should be preferred.

Preferred Author Guidelines

MANUSCRIPT STYLE INSTRUCTION (Must be strictly followed)

Page Size: 8.27" X 11'"

- Left Margin: 0.65
- Right Margin: 0.65
- Top Margin: 0.75
- Bottom Margin: 0.75
- Font type of all text should be Swis 721 Lt BT.
- Paper Title should be of Font Size 24 with one Column section.
- Author Name in Font Size of 11 with one column as of Title.
- Abstract Font size of 9 Bold, "Abstract" word in Italic Bold.
- Main Text: Font size 10 with justified two columns section
- Two Column with Equal Column with of 3.38 and Gaping of .2
- First Character must be three lines Drop capped.
- Paragraph before Spacing of 1 pt and After of 0 pt.
- Line Spacing of 1 pt
- Large Images must be in One Column
- Numbering of First Main Headings (Heading 1) must be in Roman Letters, Capital Letter, and Font Size of 10.
- Numbering of Second Main Headings (Heading 2) must be in Alphabets, Italic, and Font Size of 10.

You can use your own standard format also.
Author Guidelines:

1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

1. GENERAL

Before submitting your research paper, one is advised to go through the details as mentioned in following heads. It will be beneficial, while peer reviewer justify your paper for publication.

Scope

The Global Journals Inc. (US) welcome the submission of original paper, review paper, survey article relevant to the all the streams of Philosophy and knowledge. The Global Journals Inc. (US) is parental platform for Global Journal of Computer Science and Technology, Researches in Engineering, Medical Research, Science Frontier Research, Human Social Science, Management, and Business organization. The choice of specific field can be done otherwise as following in Abstracting and Indexing Page on this Website. As the all Global

Journals Inc. (US) are being abstracted and indexed (in process) by most of the reputed organizations. Topics of only narrow interest will not be accepted unless they have wider potential or consequences.

2. ETHICAL GUIDELINES

Authors should follow the ethical guidelines as mentioned below for publication of research paper and research activities.

Papers are accepted on strict understanding that the material in whole or in part has not been, nor is being, considered for publication elsewhere. If the paper once accepted by Global Journals Inc. (US) and Editorial Board, will become the copyright of the Global Journals Inc. (US).

Authorship: The authors and coauthors should have active contribution to conception design, analysis and interpretation of findings. They should critically review the contents and drafting of the paper. All should approve the final version of the paper before submission

The Global Journals Inc. (US) follows the definition of authorship set up by the Global Academy of Research and Development. According to the Global Academy of R&D authorship, criteria must be based on:

1) Substantial contributions to conception and acquisition of data, analysis and interpretation of the findings.

2) Drafting the paper and revising it critically regarding important academic content.

3) Final approval of the version of the paper to be published.

All authors should have been credited according to their appropriate contribution in research activity and preparing paper. Contributors who do not match the criteria as authors may be mentioned under Acknowledgement.

Acknowledgements: Contributors to the research other than authors credited should be mentioned under acknowledgement. The specifications of the source of funding for the research if appropriate can be included. Suppliers of resources may be mentioned along with address.

Appeal of Decision: The Editorial Board's decision on publication of the paper is final and cannot be appealed elsewhere.

Permissions: It is the author's responsibility to have prior permission if all or parts of earlier published illustrations are used in this paper.

Please mention proper reference and appropriate acknowledgements wherever expected.

If all or parts of previously published illustrations are used, permission must be taken from the copyright holder concerned. It is the author's responsibility to take these in writing.

Approval for reproduction/modification of any information (including figures and tables) published elsewhere must be obtained by the authors/copyright holders before submission of the manuscript. Contributors (Authors) are responsible for any copyright fee involved.

3. SUBMISSION OF MANUSCRIPTS

Manuscripts should be uploaded via this online submission page. The online submission is most efficient method for submission of papers, as it enables rapid distribution of manuscripts and consequently speeds up the review procedure. It also enables authors to know the status of their own manuscripts by emailing us. Complete instructions for submitting a paper is available below.

Manuscript submission is a systematic procedure and little preparation is required beyond having all parts of your manuscript in a given format and a computer with an Internet connection and a Web browser. Full help and instructions are provided on-screen. As an author, you will be prompted for login and manuscript details as Field of Paper and then to upload your manuscript file(s) according to the instructions.

To avoid postal delays, all transaction is preferred by e-mail. A finished manuscript submission is confirmed by e-mail immediately and your paper enters the editorial process with no postal delays. When a conclusion is made about the publication of your paper by our Editorial Board, revisions can be submitted online with the same procedure, with an occasion to view and respond to all comments.

Complete support for both authors and co-author is provided.

4. MANUSCRIPT'S CATEGORY

Based on potential and nature, the manuscript can be categorized under the following heads:

Original research paper: Such papers are reports of high-level significant original research work.

Review papers: These are concise, significant but helpful and decisive topics for young researchers.

Research articles: These are handled with small investigation and applications

Research letters: The letters are small and concise comments on previously published matters.

5.STRUCTURE AND FORMAT OF MANUSCRIPT

The recommended size of original research paper is less than seven thousand words, review papers fewer than seven thousands words also.Preparation of research paper or how to write research paper, are major hurdle, while writing manuscript. The research articles and research letters should be fewer than three thousand words, the structure original research paper; sometime review paper should be as follows:

Papers: These are reports of significant research (typically less than 7000 words equivalent, including tables, figures, references), and comprise:

(a)Title should be relevant and commensurate with the theme of the paper.

(b) A brief Summary, "Abstract" (less than 150 words) containing the major results and conclusions.

(c) Up to ten keywords, that precisely identifies the paper's subject, purpose, and focus.

(d) An Introduction, giving necessary background excluding subheadings; objectives must be clearly declared.

(e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition; sources of information must be given and numerical methods must be specified by reference, unless non-standard.

(f) Results should be presented concisely, by well-designed tables and/or figures; the same data may not be used in both; suitable statistical data should be given. All data must be obtained with attention to numerical detail in the planning stage. As reproduced design has been recognized to be important to experiments for a considerable time, the Editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned un-refereed;

(g) Discussion should cover the implications and consequences, not just recapitulating the results; conclusions should be summarizing.

(h) Brief Acknowledgements.

(i) References in the proper form.

Authors should very cautiously consider the preparation of papers to ensure that they communicate efficiently. Papers are much more likely to be accepted, if they are cautiously designed and laid out, contain few or no errors, are summarizing, and be conventional to the approach and instructions. They will in addition, be published with much less delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and to make suggestions to improve briefness.

It is vital, that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

Format

Language: The language of publication is UK English. Authors, for whom English is a second language, must have their manuscript efficiently edited by an English-speaking person before submission to make sure that, the English is of high excellence. It is preferable, that manuscripts should be professionally edited.

Standard Usage, Abbreviations, and Units: Spelling and hyphenation should be conventional to The Concise Oxford English Dictionary. Statistics and measurements should at all times be given in figures, e.g. 16 min, except for when the number begins a sentence. When the number does not refer to a unit of measurement it should be spelt in full unless, it is 160 or greater.

Abbreviations supposed to be used carefully. The abbreviated name or expression is supposed to be cited in full at first usage, followed by the conventional abbreviation in parentheses.

Metric SI units are supposed to generally be used excluding where they conflict with current practice or are confusing. For illustration, 1.4 l rather than 1.4 × 10-3 m3, or 4 mm somewhat than 4 × 10-3 m. Chemical formula and solutions must identify the form used, e.g. anhydrous or hydrated, and the concentration must be in clearly defined units. Common species names should be followed by underlines at the first mention. For following use the generic name should be constricted to a single letter, if it is clear.

Structure

All manuscripts submitted to Global Journals Inc. (US), ought to include:

Title: The title page must carry an instructive title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) wherever the work was carried out. The full postal address in addition with the e-mail address of related author must be given. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining and indexing.

Abstract, used in Original Papers and Reviews:

Optimizing Abstract for Search Engines

Many researchers searching for information online will use search engines such as Google, Yahoo or similar. By optimizing your paper for search engines, you will amplify the chance of someone finding it. This in turn will make it more likely to be viewed and/or cited in a further work. Global Journals Inc. (US) have compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Key Words

A major linchpin in research work for the writing research paper is the keyword search, which one will employ to find both library and Internet resources.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy and planning a list of possible keywords and phrases to try.

Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

Choice of key words is first tool of tips to write research paper. Research paper writing is an art.A few tips for deciding as strategically as possible about keyword search:

- One should start brainstorming lists of possible keywords before even begin searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in research paper?" Then consider synonyms for the important words.
- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

Acknowledgements: Please make these as concise as possible.

References

References follow the Harvard scheme of referencing. References in the text should cite the authors' names followed by the time of their publication, unless there are three or more authors when simply the first author's name is quoted followed by et al. unpublished work has to only be cited where necessary, and only in the text. Copies of references in press in other journals have to be supplied with submitted typescripts. It is necessary that all citations and references be carefully checked before submission, as mistakes or omissions will cause delays.

References to information on the World Wide Web can be given, but only if the information is available without charge to readers on an official site. Wikipedia and Similar websites are not allowed where anyone can change the information. Authors will be asked to make available electronic copies of the cited information for inclusion on the Global Journals Inc. (US) homepage at the judgment of the Editorial Board.

The Editorial Board and Global Journals Inc. (US) recommend that, citation of online-published papers and other material should be done via a DOI (digital object identifier). If an author cites anything, which does not have a DOI, they run the risk of the cited material not being noticeable.

The Editorial Board and Global Journals Inc. (US) recommend the use of a tool such as Reference Manager for reference management and formatting.

Tables, Figures and Figure Legends

Tables: Tables should be few in number, cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g. Table 4, a self-explanatory caption and be on a separate sheet. Vertical lines should not be used.

Figures: Figures are supposed to be submitted as separate files. Always take in a citation in the text for each figure using Arabic numbers, e.g. Fig. 4. Artwork must be submitted online in electronic form by e-mailing them.

Preparation of Electronic Figures for Publication

Even though low quality images are sufficient for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit (or e-mail) EPS (line art) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings) in relation to the imitation size. Please give the data for figures in black and white or submit a Color Work Agreement Form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution (at final image size) ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs) : >350 dpi; figures containing both halftone and line images: >650 dpi.

Color Charges: It is the rule of the Global Journals Inc. (US) for authors to pay the full cost for the reproduction of their color artwork. Hence, please note that, if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a color work agreement form before your paper can be published.

Figure Legends: Self-explanatory legends of all figures should be incorporated separately under the heading 'Legends to Figures'. In the full-text online edition of the journal, figure legends may possibly be truncated in abbreviated links to the full screen version. Therefore, the first 100 characters of any legend should notify the reader, about the key aspects of the figure.

6. AFTER ACCEPTANCE

Upon approval of a paper for publication, the manuscript will be forwarded to the dean, who is responsible for the publication of the Global Journals Inc. (US).

6.1 Proof Corrections

The corresponding author will receive an e-mail alert containing a link to a website or will be attached. A working e-mail address must therefore be provided for the related author.

Acrobat Reader will be required in order to read this file. This software can be downloaded

(Free of charge) from the following website:

www.adobe.com/products/acrobat/readstep2.html. This will facilitate the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof.

Proofs must be returned to the dean at dean@globaljournals.org within three days of receipt.

As changes to proofs are costly, we inquire that you only correct typesetting errors. All illustrations are retained by the publisher. Please note that the authors are responsible for all statements made in their work, including changes made by the copy editor.

6.2 Early View of Global Journals Inc. (US) (Publication Prior to Print)

The Global Journals Inc. (US) are enclosed by our publishing's Early View service. Early View articles are complete full-text articles sent in advance of their publication. Early View articles are absolute and final. They have been completely reviewed, revised and edited for publication, and the authors' final corrections have been incorporated. Because they are in final form, no changes can be made after sending them. The nature of Early View articles means that they do not yet have volume, issue or page numbers, so Early View articles cannot be cited in the conventional way.

6.3 Author Services

Online production tracking is available for your article through Author Services. Author Services enables authors to track their article - once it has been accepted - through the production process to publication online and in print. Authors can check the status of their articles online and choose to receive automated e-mails at key stages of production. The authors will receive an e-mail with a unique link that enables them to register and have their article automatically added to the system. Please ensure that a complete e-mail address is provided when submitting the manuscript.

6.4 Author Material Archive Policy

Please note that if not specifically requested, publisher will dispose off hardcopy & electronic information submitted, after the two months of publication. If you require the return of any information submitted, please inform the Editorial Board or dean as soon as possible.

6.5 Offprint and Extra Copies

A PDF offprint of the online-published article will be provided free of charge to the related author, and may be distributed according to the Publisher's terms and conditions. Additional paper offprint may be ordered by emailing us at: editor@globaljournals.org .

Before start writing a good quality Computer Science Research Paper, let us first understand what is Computer Science Research Paper? So, Computer Science Research Paper is the paper which is written by professionals or scientists who are associated to Computer Science and Information Technology, or doing research study in these areas. If you are novel to this field then you can consult about this field from your supervisor or guide.

TECHNIQUES FOR WRITING A GOOD QUALITY RESEARCH PAPER:

1. Choosing the topic: In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

2. Evaluators are human: First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

3. Think Like Evaluators: If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

4. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

5. Ask your Guides: If you are having any difficulty in your research, then do not hesitate to share your difficulty to your guide (if you have any). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work then ask the supervisor to help you with the alternative. He might also provide you the list of essential readings.

6. Use of computer is recommended: As you are doing research in the field of Computer Science, then this point is quite obvious.

7. Use right software: Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

8. Use the Internet for help: An excellent start for your paper can be by using the Google. It is an excellent search engine, where you can have your doubts resolved. You may also read some answers for the frequent question how to write my research paper or find model research paper. From the internet library you can download books. If you have all required books make important reading selecting and analyzing the specified information. Then put together research paper sketch out.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

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

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

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

Informal Guidelines of Research Paper Writing

Key points to remember:

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

Final Points:

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

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

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

General style:

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

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

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

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

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

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

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

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

Title Page:

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

Abstract:

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

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

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

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

Approach:

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

Introduction:

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

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

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

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

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)

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals Inc. (US).

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

INDEX

Global Journal of Medical Research

Visit us on the Web at www.GlobalJournals.org | www.MedicalResearchJournal.org
or email us at helpdesk@globaljournals.org

ISSN 9755896