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

Global Journal

of Medical Research: K

Interdisciplinary

Practices and Nutritional Liposome-DNA Complexes

Highlights

Disulphide in Experimental Properties of Amlodipine

Discovering Thoughts, Inventing Future

VOLUME 13 ISSUE 2 VERSION 1.0

Global Journal of Medical Research: K
Interdisciplinary

Global Journal of Medical Research: K
Interdisciplinary

Volume 13 Issue 2 (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 Inc., Headquarters Corporate Office, Cambridge Office Center, II Canal Park, Floor No. 5th, ***Cambridge (Massachusetts)***, Pin: MA 02141 United States
USA Toll Free: +001-888-839-7392
USA Toll Free Fax: +001-888-839-7392

Offset Typesetting

Open Association of Research Society, Marsh Road, Rainham, Essex, London RM13 8EU
United Kingdom.

Packaging & Continental Dispatching

Global Journals, 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: *investers@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)

Editorial Board Members (HON.)

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

President Editor (HON.)

Chief Author (HON.)

Dr. R.K. Dixit
M.Sc., Ph.D., FICCT
Chief Author, India
Email: authorind@computerresearch.org

Dean & Editor-in-Chief (HON.)

Contents of the Volume

Global Journal of Medical research
Interdsciplinary
Volume 13 Issue 2 Version 1.0 Year 2013
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN : 0975-5888

Feeding Practices and Nutritional Parameters of Children Aged 6-14 Years from Cameroon

By Djeukeu A.W., Kana-Sop. M. M., Gouado I., Nolla N. P., Mananga M. J., Amvam Z. P. H. § & Ekoe T.
University of Douala

Abstract - Malnutrition in all the forms is highly prevalent in Cameroon. The aim of this study was to evaluate some nutritional parameters of children aged of 6 to 14 years in Douala. The study evaluated nutritional status of 265 children of 6 to 9 years (63.9%) and 10-14 years (36.1%) using anthropometric measures and albuminemia of 99 children, determined by the colorimetric method. Foods habits and practices were assessed using questionnaires. Statistical analyses were performed by Graph Pad prism version 5. Stunting, wasting and overweight were observed at 18.0 %, 5.1 % and 16 % respectively. Stunting was frequent in families of more than 5 persons and in those with illiterate mothers. There was a significant difference ($p<0.001$) between the average albuminemia of stunted children (38.1 ± 7.7g/l) and that of non stunted children (48.7± 11.1 g/l). The daily energy intake of the boys ranged between 89.5% and 100.6% of their energy requirement, and that of girls ranging between 100.9% and 114.1%. The foods of those children were diversified but minerals intake were low. Nutritionals problems observed may be due to poor knowledge of food practices and poor food habits.

Keywords : *malnutrition, children 6-14 years, nutritional-status, albuminemia, food-intake, cameroon.*

GJMR-K Classification : *NLMC Code: WB 410, WS 115*

Strictly as per the compliance and regulations of:

Feeding Practices and Nutritional Parameters of Children Aged 6-14 Years from Cameroon

Djeukeu A.W. [α], Kana-Sop. M. M. [σ], Gouado I. [ρ], Nolla N. P. [ω], Mananga M. J. [¥], Amvam Z. P. H. [§] & Ekoe T. [χ]

Abstract - Malnutrition in all the forms is highly prevalent in Cameroon. The aim of this study was to evaluate some nutritional parameters of children aged of 6 to 14 years in Douala. The study evaluated nutritional status of 265 children of 6 to 9 years (63.9%) and 10-14 years (36.1%) using anthropometric measures and albuminemia of 99 children, determined by the colorimetric method. Foods habits and practices were assessed using questionnaires. Statistical analyses were performed by Graph Pad prism version 5. Stunting, wasting and overweight were observed at 18.0 %, 5.1 % and 16 % respectively. Stunting was frequent in families of more than 5 persons and in those with illiterate mothers. There was a significant difference ($p<0.001$) between the average albuminemia of stunted children (38.1 ± 7.7g/l) and that of non stunted children (48.7± 11.1 g/l). The daily energy intake of the boys ranged between 89.5% and 100.6% of their energy requirement, and that of girls ranging between 100.9% and 114.1%. The foods of those children were diversified but minerals intake were low. Nutritionals problems observed may be due to poor knowledge of food practices and poor food habits.

Keywords : malnutrition, children 6-14 years, nutritional-status, albuminemia, food-intake, cameroon.

I. Introduction

Nutrition plays a key role in health and development of an individual. Good nutrition protects the infants, the children and the mother, strengthens the immune system and reduces the risk of non communicable diseases related to foods. It also enhances the productivity of the population and can help to get out gradually from the vicious circle of poverty and hunger (UNICEF, 2011). The nutritional needs of an individual require the consumption of balanced diet. However, not everyone has access to optimal feeding. Inappropriate food habits linked to poor nutrients intakes are unable to cover nutrients needs of the body, leading to malnutrition (Baneko, 2008).

Malnutrition is then a result of less or excess of one or more nutrients (FAO, 2003). In all its forms it has serious health consequences and now, there is a double burden of malnutrition especially in developing countries. According to FAO (2006), more than 3.5 billion people are suffering in the world, for malnutrition and hunger. Each year, almost 9 million deaths of children fewer than 5 years, estimated from 33 to 56% are attributable to malnutrition (UNICEF, 2011). Malnutrition if not control at the youngest age will lead to chronic diseases at adulthood. Schoolchildren are also one of groups severely affected by malnutrition, after infants and young children. Long term poor eating habits affect lifestyle and cause related chronic diseases including obesity, diabetes, cardiovascular diseases and some cancers (Kobayassi et al., 2010). In recent years, obesity has become prevalent not only among adults but also in children in Japan (Kouda et al., 2004). Those who are obese in childhood tend to remain obese as adults (Freedman et al., 2005; Guo et al., 2000; Fried et al., 2005; Whilhok et al., 2005). When children are overweight, they are more likely to develop metabolic syndrome later in life (Vanhaha et al., 1998). Furthermore, the longer individuals rae overweight, the greater their risk of cardiovascular diseases (Baker et al., 2005). Various factors contribute to obesity, including physical inactivity, an irregular and unbalanced diet, and over-eating (Sugiura et al., 2007). Dietary habits are formed during childhood (Mikkala et al., 2005). To prevent adult obesity, it is desirable that individuals acquire appropriate dietary habits in childhood. Habitual dietary intake among children should be assessed to evaluate childhood dietary problems, enabling the correction of any bad dietary habits malnutrition consequences comprising essentially, the impairment of cognitive, and learning capacities resulting in the quickly and early drop out from school (Alaimo et al., 2001; Sanokho, 2005, Victoria et al., 2008).

In Cameroon, despite the quantity and diversity of food resources, populations are not exempt to nutritionals problems (PAM, 2007). According to statistics from the Department of Public Health, the prevalence of stunting among children under five rose from 23% in 1991 to 32% in 2004. Malnutrition is implicated in more than 50% of infant mortality. Deficiencies in vitamin A and iron affect respectively 38% and 58% of children under 5 years (EDSCIII, 2004). Beyond the age of 5 years there is very little information on child malnutrition in Cameroon. Available data concerns preschool children nutritional status and feeding (Kana Sop et al., 2008, Kana Sop et al., 2011),

Author α σ ρ ω : University of Douala, Faculty of Science, PO Box 24157 Douala, Cameroon.
Author ¥ : University of Yaoundé I, Faculty of Science.
Author § : University of Ngaoundéré.
Author χ : University of Yaoundé I, Faculty of Medicine and Biomedical Sciences.

very little work was carried on school age children (Ponka et al., 2006). This work was thus initiated to assess the nutritional status of the population of children in Makèpè Missokè in order to contribute to the optimization of food and nutrition security in this locality.

II. Materials and Methods

a) Study Area

The study took place in Douala. Douala is the most popular city of Cameroon (with 14.4% of the Cameroonian's population, which is 2,510,283 inhabitants) (CRTV news, 2010)). Makèpè Missokè is one of the poorest sub-quarter or locality of Douala according to AFEF (2011).

b) Subjects Recruitment

All the children (6-14 years of age, n = 255) were registered at the Bilingual Confidence Primary School of Makèpè Missokè and enrolled in anthropometrics measurements. Ninety nine of them were randomly selected for serum albumin determination. A sub-sample of 25 children (8 females and 17 males), Representing about 10% of the sample size, were involved in 3 day Weighed food records. The study was approved by the Cameroon National Ethics committee. The study aim and methods were explained orally, and written informations were provided to all parents/guardians (i.e., the person whose prepared the child's were enrolled). Then, the parents/guardians provided their written informed consent.

c) Weight Measurements

Using Salter scale (1 - 120 Kg Cap, AMSUA at 0, 01 Kg), subjects were made to stand on the platform without touching anything. Shoes were removed. Readings were taken to the nearest 0.2Kg. Weighing was done when the stomach was virtually empty.

d) Height Measurements

The children were made to stand without shoes on the platform of the vertical toise. The head erect comfortably was held in the same vertical plane as the external auditory meatus. The head piece was then lowered gently, crushing the hair and making contact with the top of the head. Readings were taken to the nearest 0.5 cm.

e) Biochemical Analyses

Blood samples were collected only on children whose parents gave their consent and signed the inform consent form. Thus, blood samples of 99 children were taken on an empty stomach in the morning between 8 and 10 h. Approximately 2 ml of venous blood were collected from each child. The blood collected was introduced in dry tubes and sent to the biochemistry laboratory at the University of Douala. Centrifugation of the blood was performed using a Sigma 2-6 E centrifuge type at the speed of 3600 x g for 20 min. This technique allowed us to separate serum from whole blood. The serum (supernatant) was extracted using a micropipette "eppendorf" (1000 mL) and introduced into cryotubes (1.2 mL). The serum obtained was then used to determine the albumin content.

f) Food Intakes

Three days weighed food records were conducted during a week. Foods intakes of children was quantified by using household weighing measuring tools, such as standard measuring cups and spoons, ruler for Measuring dimensions. We help children parents and guardians to fill the form detailing each menu according to breakfast, lunch, dinner, or snack in each investigation day. We determined the child's daily intake of food items by weighing their meal before an after each meal. We then calculated the nutrient intake for each child using the nutrients composition of dishes consumed in Douala of Kana Sop et al., 2008 and other item present in Nutrisurvey software. We defined food as not only a single food item (e.g., banana) but also as a mixed dish (e.g., banana stew). We calculated the composition of nutrients in the food per 100 g. For even the coating data sheets, we had the help of investigators trained for the occasion.

g) Anthropometric Analyses

Data from anthropometric measurements were analyzed using WHO (2007) standard references. Nutritional state indicators used were Body Mass Indices (BMI) for age, weight for age, height for age Z-scores. By the use of the indicator's above Z-score results, percentages of stunted, wasted, overweight and normal children were calculated. The overall prevalence rates of malnutrition were obtained by setting the threshold of normality to -2 z-scores below the baseline average for indicators P/A and T/A and 2 z-scores above the average reference indicator for BMI/A. Serum albumin concentrations of the children were compared according to their nutritional status. Food composition tables published by Kana Sop et al. (2008) and Nutrisurvey 2007 software were used to calculate and estimate the energy and micronutrients intakes of dishes consumed by any of the enrolled subjects. The results then were compared with the requirement or daily recommended values. We used two methods to develop the list of food types. First, we used the method reported by Block et al., 1998 and modified by Kobayashi el al (2010) and ranked all of the reported food types according to the contribution analysis. We were especially interested in the total energy, protein and calcium, magnesium, phosphor, zinc, copper and iron. The percentages were calculated by dividing the nutrient contents of each food type by the total nutrient amounts. All of the food types that contributed at least 0.15% to the total energy and nutrients were combined. In addition, we excluded food types eaten by fewer than 15 subjects.

III. Statistical Analyses

Mean and standard deviation of the height and weight measurements and serum albumin concentration were determined using Graph pad prism version 5. Significance was considered with $p \leq 0.05$.

IV. Results and Discussions

In this study, male sex was the most represented with 136 boys against 119 girls. Sixty seven percent (67%) of children live in families with at least five individuals. In addition, nearly 78.6% of mothers of investigated children had primary level of education against only 21.4% for secondary school level and beyond (table 1). Table 2 shows mean weights and heights of the children ranged from 19.2 ± 3.1 to 36.5 ± 8.0 kg and 108.7 ± 5.3 to 143.6 ± 7.8 cm respectively. Evolution of height and weight of these children shows that there growth faltering in many cases (table 2).

Prevalences of various nutritional disorders encountered in the study population were 18.0 % for stunting, 5.1 % for underweight and 1.6 % for overweight (table 3). These prevalences were much lower than those of children under 5 years in Cameroon according to the ESDCIII (2004). These results showed a decrease of nutritional disorders with age in Cameroon. No significant difference ($p > 0.05$) between age of children suffering from stunting (9.5 ± 1.8 years), low weight (10.2 ± 2.4 years) and overweight (9.0 ± 1.4 years) were observed. From the children suffering from nutritionals disorders, height and weight were lower than that of normal children. On the number of children with wasting and overweight, girls were most affected with respectively 53.84 and 75% of cases (Table 4). The content of serum albumin of children who are underweight was the lowest (31.2 ± 2.9 g / l) and below standard (35-55 g/l) recommended by the assay method (table 4). Serum albumin levels of all children except of those suffering of wasting were in the normal range (table 4). Boys took between 89.5% and 100.6% of their daily energy requirement and girls between 100.9% and 114.1%. Mean daily protein intake of the Children was above 50% for all the children. However their daily calcium, magnesium, potassium, zinc, and iron intakes were below the daily requirement values. Copper intakes of 10-12 years children were above requirement for all the two sexes. The food type intake frequencies were classified into many levels linking consumption mode. For example, we used seven (i.e., everyday, 5-6 times per week, 3-4 times per week, 1-2 times per week, 2-3 times per month, 1 time per month, or never); eight ("2-3 times per day" was added to seven categories); nine ("4-5 times per day" was added to eight categories) and eleven ("8-10 times per day", "6-7 times per day" were added to nine categories) according to general intake frequency of each food type. The estimation of portion size was classified into six categories referring to the photographs in full-scale size; that is, one-third, one-half, the same amount, 1.5 times, twice, and other.

Table 1 : Characteristics of the study population

Parameters	Effective	Frequency (%)
Sex		
Male	136	53.3
Female	119	46.7
Age (years)		
6-10	163	63.9
10-14 years	92	3.1
Size of household		
< 5 persons	84	33
≥ 5 persons	168	67
Mothers instruction level	252	
Primary	198	78.6
Secondary and beyong	54	21.4
M= Male; F= female, SD= Standard Deviation		

Table 2 : Mean weights, heights and Body Mass Indices (BMI) of the 6-14 years old children

Age (years)	Sex	Number	Mean weight (± SD) (Kg)	Mean height (± SD) (cm)	BMI (± SD) (Kg/m²)
6	M	11	19.2 ± 3.1	110.2 ± 6.3	15.7 ± 1.9
	F	9	18.3 ± 2.6	108.7 ± 5.3	15.2 ± 2.6
7	M	14	22.5 ± 3.0	118.4± 5.5	16.5 ± 1.2
	F	18	21.3 ± 3.5	116.5 ± 5.3	15.3 ± 1.8
8	M	20	25.4 ± 4.5	121.7 ±10.6	16.8 ± 2.7
	F	21	25.8 ± 3.2	123.9 ± 4.5	16.9 ± 1.3
9	M	39	26.6 ± 3.2	126.3 ± 6.9	17.0 ± 1.9
	F	31	27.4 ± 5.2	128.6 ± 7.2	16.2 ± 2.2

10	M F	24 14	29.2 ± 4.3 27.4 ± 2.5	130.8 ± 4.8 129.7 ± 4.3	16.9 ± 0.9 16.0 ± 0.7
11	M F	24 19	32.0 ± 5.1 33.0 ± 7.4	134.0 ± 4.7 134.1 ±11.8	17.8 ± 1.3 18.3 ± 2.1
12	M F	3 6	36.3 ± 5.0 36.5 ± 8.0	138.6 ± 10.9 143.6±7.8	19.2 ± 1.4 17.8 ± 1.7
13	M F	1 1	30.0 ± 0.0 36.0 ± 0.0	131.0± 0.0 143.0 ± 0.0	17.5 ± 0.0 17.6 ± 0.0
Total		255			

Figures are means ± standard deviation (SD); SD=Standard Deviation F= Famale; M= Male; BMI= Body Mass Indices.

Table 3 : Percentage of children classified as normal, wasted, stunted, underweight and overweight

Indicators	Normal <-2 SD, >+2SD	Wasting ≤- 2SD	Stunting ≤- 2SD	Underweight ≤- 2SD	overweight ≥ + 2SD	Total
Weight for age	94.5 (241)	51 (13)	-		0.4 (1)	100 (255)
Height for age	81.6 (208)	-	18 (46)		0.4 (1)	100 (255)
BMI for age	96.47 (246)	-	-	1.96 (5)	1.6 (4)	100 (255)

The numbers in brackets represent the number of subjects. SD= Standard Deviation

Table 4 : Characteristics of children according to their nutritional status

Parameters	Group				
	Normal (n=192)	Stunting (n=46)	Wasting (n=13)	Overweight (n=4)	P
Individuals factors					
Mean age (years)	8.8 ± 1.5 ^{a}	9.5 ± 1.8 ^{a}	10.2 ± 2.4 ^{ab}	9.0 ± 1.4 ^{a}	*
Mean weight (Kg)	28.0 ± 9.3^{ab}	24.7 ± 5.9^{a}	21.4 ± 5.6^{a}	34.0 ± 7.8 b	**
Mean height (cm)	127.9 ± 9.3 ^{b}	118.5 ± 10.8 ^{ab}	117.0 ± 12.4 ^{ab}	129.0 ± 7.7 ^{b}	*
Sex distribution					
Female % Male %	45.4 54.6	43.47 5.53	53.84 46.16	75 25	NR
Serum albumin levels (g/l)	48.7 ± 11.1^{b}	38.1 ± 7.7^{a}	31.2 ± 2.9 ^{a}	46.2 ± 2.1^{ab}	***

n= number of subjects.
* = Significant at P <0.05
** = Significant at P <0.01,
*** =Significant at P <0.001, NR = not relevant.
Figures in the same line carrying the same superscript letters are not significantly different at least at p≤0.05.

Table 5 : Estimated daily energy and nutrient intakes of 6 - 14 years old children compared with FAO/WHO (1990) daily needs

Sex	Nutrients/Age	Age (yrs)	Energy (kcal)	Protein (g)	Ca (mg)	Mg (mg)	P (mg)	Zn (mg)	Cu (mg)	Fe (mg)
Female	Mean daily intake	6-9	1916.6±241.4	38.6±11.3	275.7±45.9	103.3±9.7	320.6±25.4	3.7±0.9	1.16±0.3	3.74±0.7
	FAO/WHO daily needs		1900	34	800	200	3800	9	0.75	8
	% of coverage		100.9	113.3	34.5	51.6	8.4	41.1	154.6	37.4
	Average daily intake	10-12	2172.9±308.1	40.2±84	194.9±18.4	108.0±13.7	274.2±29.2	4.7±1.1	2.5±0.6	4.1±0.9
	FAO/WHO daily needs		1905	49	1200	280	4500	12	0,77	10
	% of coverage		114.1	82.0	16.2	38,.5	6.0	39.2	324.6	51.2
Male	Average daily intake	6-9	1700.9±129.3	31.2±10.5	355.3±54.2	106.3±19.3	324.7±47.5	2.9±0.6	0.4±0.03	3.8±0.09
	FAO/WHO daily needs		1900	34	800	200	3800	9	0,75	8
	% of coverage		89.5	91.7	44.4	53.2	8.5	32.2	53.3	38
	Average daily intake	10-12	2133±419.6	42.2±10.5	196±33.4	100.3±22.1	316.6±50.4	4.6±1.3	1.9±0.02	3.7±0.04
	FAO/WHO daily needs		2120	48	1200	280	4500	12	0,73	10
	% of coverage		100.6	87.9	16.3	35.8	7.0	38.3	260.2	46.2

Macronutrients and micronutrients intake levels were estimated from Kana Sop *et al.* (2008)

It was observed that children diet was very monotonous. Stunting and falter growth observed may be due to poor knowledge on optimal feeding. Intakes of nutrients estimated by weighed foods dairy record (WDR) showed very insufficient coverage of daily needs apart for energy. We used weighed foods dairy record (WDR) for easy estimation of nutrients intakes.

WDR is quantified either by weighing or determining volumes using a household measuring tool, such as standard measuring cups and spoons, and a ruler for measuring dimensions. Usually, general WDR performers weigh the raw ingredients (Buzzard, 1998), but we were interested by the weight of eaten portions of the meals. We used a digital cooking scale as an index of the size of the dish. Energy, protein, was calculated directly. To obtain the necessary open-ended data from children, we conducted our WDR personally. Even if energy needs were meeting globally, the recipes were imbalanced in tem of macronutrients contribution to energy intakes. The fact that in this study, male group was the most represented with 136 boys against 119 girls may be linked to ignorance. Girls are always in high number than boys in our society. However, girls drop out from school earlier to help household activities. In poor families, when funds lack, parent preferred to send boys at school (ESDCIII, 2004). Sixty seven percent (67%) of children live in families with at least five individuals and this condition was linked to poor growth. The number of children and the family size was inversely correlated malnutrition indicators. According to Emel et al., (2005) household size has a very big influence on young children nutritional state. There is therefore competition on the household's financial resources which could affect the nutritional status of children living in poorest families were also most malnourished. According to Madginzira et al., (1995), the educational level of mothers is very important especially when living conditions are difficult. Poverty and malnutrition form a vicious cycle. Poverty prevents individuals to access good nutrients sources. For example, meat, fishes and animal foods sources are very rich in bioavailable minerals, but it is very inaccessible to poor population. They are forced to consume mainly vegetal foods that contain many antinutritional substances that inhibit micronutrients bioavailability and sometimes micronutrients digestibility. Illiteracy is another underlying factor of poor feeding. Individuals in these cases are limited in knowledge and cannot master optimal feeding.

It is well known that those stunted children would have poor school result as they may be usually ill. Malnourished children tend to start school later, progress less rapidly, have lower attainments, and perform less well on cognitive achievement tests, even into adulthood. These indirect effects of malnutrition on productivity are substantially more than the direct effects of height on schooling and hiring. Malnourished children may receive less education than their well-nourished peers for a number of reasons. Caregivers may invest less in their education or schools may use physical size as a rough indicator of school readiness, and thus bar children of short stature from entering school at the appropriate age. Malnourished children are also sick more often and so absent more often, and learn less well when they are in school. Studies showed that delayed entry to school leads to lower expected lifetime earnings because of fewer years in the workforce (Behrman et al., 2004). In addition to its impact on adult productivity through less schooling, severe malnutrition also affects learning capacity or cognitive development directly, with consequent impact on schooling productivity and labor productivity. Birth weight and breast-feeding both correlate with cognitive development; malnourished children perform poorly on cognitive tests, have poorer psychomotor development and fine motor skills, have lower activity levels, interact less frequently with their environments and fail to acquire skills at normal rates (Grantham-McGregor et al., 1999).

Stunting prevalences were still high in these group affecting mostly boys. Similar results were found by Wamani et al. (2007) in Congo, in children less than 5 years. In fact, malnutrition that start during preschool age is usually not corrected among affected children that are exposed to the same food habits. However, the stunting rate is lower than those found in preschool infant (ESDC, 2004). As indicated earlier, growth deficits in the first 2 to 3 years of life are only partially regained during childhood and adolescence, particularly when children remain in poor environments. Stature at age 3 is strongly correlated with attained body size in adulthood in several countries (Martorell et al., 1994; Simondon et al., 1998).

Actions need to be taken because affected children may have poor scores in school and are more exposed to diseases. The synergy between malnutrition and infectious diseases is well established (Schrimshaw et al., 1968). In a widely quoted study, Pelletier et al. (1995) estimated that 56% of child deaths can be attributed to the potentiating effect of malnutrition (including low birth weight), with most of those deaths linked to mild or moderate malnutrition, rather than severe malnutrition. Although severely malnourished children are more likely to die, they are far fewer in number. Children with mild, moderate or severe malnutrition would be, respectively, 2.5, 4.6 and 8.4 times more likely to die than children whose weights are within the normal range for their ages. Not only a significant proportion of child can deaths from common infectious diseases be attributed to malnutrition (measles, 44.8 %; malaria, 7.3 %; diarrhea, 60.7 %; and pneumonia, 52.3 %) but malnutrition also increases the likelihood of having an attack of malaria, diarrhea or pneumonia (but not measles) (Caulfield et al., 2004). There is also increasing evidence that fetal malnutrition predisposes to the metabolic syndrome later in life (Barker, 1998). This result suggests that boys recover less of their growth retardation with age.

The contents of serum albumin of children who were underweight were low (31.2 ± 2.9 g / l) and below standard (35-55 g / l) (table 4). Serum albumin levels of

all children except those affected by wasting were in the normal range. However, the children suffering from nutritionals disorders have their heights and weights lower than those of normal children. This decrease was also reported by Diouf et al., 2000; Simpore et al., 2009 in children suffering from severe malnutrition, and Yapi et al., (2010) among children under 5 years suffering from moderate or minor malnutrition.

Coverage of energy by boys ranging between 89.5% and 100.6% of their daily energy requirement where lower than those of girls, ranging between 100.9% and 114.1%. This may be explained by the fact that girls eat more frequently than boys. No matter the age group considered, girls energy intake was above WHO (1985) and FAO (1990) standard. This observation could be due to the high number of snack found in their diaries. Most of their proteins intakes were from plant foods but there are some good nutritionally combinations they make in the area that can help in improving the quality of their protein intake. For example, plant foods like beans were being prepared with animal foods like dried fish. Vegetables and cereals were usually blended together in their meals and this combination gives protein of very high quality. Calcium, magnesium, potassium, zinc and iron intakes were low in the diet of these children. The problem with micronutrients like calcium, iron, zinc, magnesium and copper from plant sources is poor bioavailability because of phytates and fibres contains of plants (Kana Sop et al., 2008 Kana Sop et al., 2012). Another explanation of the low intake of minerals was poor consumption of vegetables and fruits, poor consumption of animal foods and practices in preparations process in the area (these include, reheating of vegetables in meals several times before consumption, fruits and vegetables of exposure to some degree of sun-drying before eating).

This study highlights types and causes of nutritional problems in the area of Makèpè Missokè. Stunting, wasting and overweight were the physical forms of malnutrition identified in that area. Besides macronutrients, there were poor intakes of micronutrients due to inappropriate feeding linked to poor knowledge of available foods and poverty. The solution therefore remains the intensification of nutrition education, dietary diversification and fortification, optimal processing, post harvest improvement in storage and handling techniques.

Acknowledgements

The authors thank responsible of Bilingual Confidence School, parents and children who participated to the study.

References Références Referencias

1. Alaimo K, Olson CM, Frongillo EA (2001). Development food insufficiency and American school-aged children's cognitive, academic, and psychosocial. Pediatrics, 108:44-53.
2. Baker J, Olsen L, Sorensen T (2007). Children body-mass index and the risk of coronary heart disease in adulthood. N Engl J Med., 357:2329-2337.
3. Baneko Y (2008). Potentiel énergétique de trois plats consommés dans quelques restaurants classe moyenne de Douala: Water fufu et sauce éru, Plantain mur et sauce ndolè, Patate et morelle sautée. Mémoire de Maitrise en Biochimie, Université de Douala.
4. Barker D (1998). Mothers, Babies and Health in Later Life. 2nd ed. London: Churchill Livingstone Pp 1-7.
5. Behrman JR, Alderman H, Hoddinott J (1995). Hunger and malnutrition. In: Lomborg B (ed). Global Crisis, Global Solutions. Cambridge, Bull WHO, 73 (4):443–8.
6. Block G, Hartman AM, Dresser CM, Carroll MD, Gannon J, Gardner L (1986). A data-based approach to diet questionnaire design and testing. Am J Epidemiol, 124:453-469.
7. Buzzard M (1998). 24-hour dietary recall and food record methods. Nutritional epidemiology Oxford: Oxford University Press. Willett W, 2, 50-73.
8. Caulfield LE, de Onis M, Blossner M (2004). Undernutrition as an underlying cause of child deaths associated with diarrhea, pneumonia, malaria, and measles. Am J Clin Nutr., 80:193–8.
9. CRTV News of 15/04/2010. Statistics on population in 2010 Cameroon.
10. Diouf S, Diallo A, Camara B (2000). La malnutrition protéino-calorique chez les enfants de moins de 5 ans en zone rurale sénégalaise (Khombole). Med Afr N., 47 (5): 225-228.
11. EDSCIII. (2004). Institut national de la statistique. Site web http: www.measuredhs.com/pubs/pdf/FR 163/ FR163-CM04.pdf. (Assessed 12/05/2012).
12. Emel G, Inci Y, Tiraje C, Guneycan, Semra A, Ahmet A, Sima G, Serdar C (2005). Prévalence of anémia and risk factors among school children in Istanbul. J. Trop pediatr, 51: 346-350.
13. FAO (2003). Ciblage et amélioration de la nutrition. Moyen d'évaluer le statut nutritionnel. Rome, Italie, 107p.
14. FAO (2006). The state of food insecurity in the World. Eradicating world hunger taking stock ten years after the World Food Summit Rome, Italia, pp 30-34.
15. Field A, Cook N, Gillman M (2005). Weight status in childhood as a predictor of becoming overweight or hypertensive in early adulthood. Obes Res., 13:163-169.
16. Freedman D, Khan L, Serdula M, Dietz W, Srinivasan R, Berenson G (2005). The relation of childhood BMI to adult adiposity: the Bogalusa Heart Study. Pediatrics, 115:22-27.

17. Grantham-McGregor S, Fernald L, Sethuraman K (1999). Effects of health and nutrition on cognitive and behavioural development in children in the first three years of life. Low birthweight, breastfeeding and protein-energy malnutrition. Food Nutr Bull., 20:53–75.
18. Guo SS, Huang C, Maynard LM, Demerath E, Towne B, Chumlea WC, Siervogel RM (2000). Body mass index during childhood, adolescence and young adulthood in relation to adult overweight and adiposity: the Fels Longitudinal Study. Int J Obes Relat Metab Disord., 24:1628-1635.
19. Kana Sop MM, Gouado I, Teugwa M, Miro S, Fotso M, Tetanye E (2008). Mineral content in some Cameroonian household foods eaten in Douala. African Journal of Biotechnology, 7 (17), pp. 3085-309.
20. Kana Sop MM., Kikafunda JK, Meli FC, Gouado I, Zollo PHA, Oberleas D and E Tetanye (2011). Young children feeding and zinc levels of complementary foods in western cameroon AJFFAND, 4 (11) 4953-4967.
21. Kana Sop MM, Gouado I, Mananga M., Djeukeu WA, Amvam Zollo PH, Oberleas D (2012). Tetanye Ekoe. Trace elements in foods of children from Cameroon: A focus on zinc and phytates content. J Trace Elem Med Biol., 26 (2012) 201– 204.
22. Kobayashi T, Tanaka S, Toji T, Shinohara H, Kamimura M, Okamoto N, Imai S, Fukui M, Date I (2010). Development of a food frequency questionnaire to estimate habitual dietary intake in Japanese children. Nutrition Journal, 9:17.76-81.
23. Kouda K, Nakamura H, Tokunaga R, Takeuchi H (2004). Trends in levels of cholesterol in Japanese children from 1993 through 2001. J Epidemiol, 14:78-82.
24. Madzingira N (1995). Malnutrition in children under five in Zimbabwe: effect of socioeconomic factors and disease. Soc. Biol., 42 (3-4): 239-246.
25. Martorell R, Khan KL, Schroeder DG (1994). Reversibility of stunting: epidemiological findings in children from developing countries. Eur J Clin Nutr., 52 (S1): S43–53.
26. Mikkilä V, Räsänen L, Raitakari OT, Pietinen P, Viikari J (2005). Consistent dietary patterns identified from childhood to adulthood: the cardiovascular risk in Young Finns Study. Br J Nutr, 93:923-931.
27. PAM (2007). Analyse globale de la sécurité alimentaire et de la vulnérabilité, République au Cameroun, P.9.
28. Pelletier DL, Frongillo EA Jr, Schroeder DG, Habicht JP (1968). The effects of malnutrition on child mortality in developing countries. World Health Organization, 1-9.
29. Ponka R, Fokou E, Fotso M, Mbiapo-Tchouanguep F, Leke R, Souopgui J & Achu- Bih M (2006). Composition of dishes consumed in Cameroon International Journal of Food Science and Technology, 41: 361–365.
30. Sanokho MY (2005). Enquête nutritionnelle dans les quatre groupes scolaires de la commune urbaine de ke-macina. Thèse de doctorat en médecine (Pharmacie et d'Odontostomatologie), Université de Bamako Fac médecine. 99 p.
31. Schrimshaw NS, Taylor CE, Gordon JE (2004). Interaction of Nutrition and Infection. Monogr Ser 57. Cambridge University Press, Geneva ; pp. 363–420.
32. Simondon K, Simondon F, Simon A (1998). Preschool stunting, age at menarche and adolescent height: a longitudinal study in rural Senegal. Eur J Clin Nutr., 52: 412–8.
33. Simpore J, Kabore F, Zongo F (2009). Nutrition rehabilitation of undernourished children utilizing Spiruline and Misola. Nutr J, 5:345-350.
34. Sugiura R, Sakamoto M, Murata M (2007). Risk of life-style related diseases in young children. Jpn J Nutri Diete., 65:67-73.
35. UNICEF (2011). La malnutrition responsable de la moitié des décès. www.unicef.fr/la-malnutrition-responsable-de-la-moitie-des-deces-d'enfants (Accessed -05/03/2011).
36. Vanhala M, Vanhala P, Kumpusalo E, Halonen P, Takala J (1998). Relation between obesity from childhood to adulthood and the metabolic syndrome: population based study. BMJ, 3:317-319.
37. Victoria CG (2008). Maternal and child undernutrition: consequences for adult health and human capital. The Lancet, 371:340–357.
38. Wamami MM (2007). École de Santé Publique, Université de Lubumbashi. Facteurs prédictifs de la malnutrition chez les enfants âgés de moins de cinq ans à Lubumbashi (RDC). 1-5.
39. Whitlock EP, Williams SB, Gold R, Smith PR, Shipman SA (2005). Screening and interventions for childhood overweight: a summary of evidence for the US Preventive Services Task Force. Pediatrics, 116:e125-e144.
40. Yapi H, Yapo A, Yeo D, Ahiboh H, Nguessan J, Attoungré M, Monnet D, Djaman A (2010). Effet des malnutritions mineures et modérées sur les protéines immunitaires inflammatoires et nutritionnelles chez l'enfant en cote d'ivoire. Mali médical 2010 tome XXV n°2, 12-43.

This page is intentionally left blank

Global Journal of Medical research
Interdsciplinary
Volume 13 Issue 2 Version 1.0 Year 2013
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN : 0975-5888

Comparative Study of Diallyl-Disulphide and Dipropyl-Disulphide in Experimental Atherosclerosis

By Govindaswamy. K.S., Basavaraj B. Devaranavadagi, Kashinath. R .T. & Nagendra. S.

Shimoga Institute of Medical Sciences, Shimoga

Abstract - Diallyl disulphide, the principle organosulphur compound of garlic oil, is known to possess many clinical beneficial effects, but its overuse or abuse has been reported to cause certain harmful side effects due to its possible metabolite acrolein. It was thought that the disulphide nature of diallyl disulphide is responsible for its hypolipidemic effect and the unsaturation may be for its toxic effects. Recently few synthetic disulphides are successfully employed in experimentally induced hyperlipidemia. The present study was under taken to compare the hypolipidemic as well as toxic effects of saturated disulphide, Dipropyl disulphide with Diallyl disulphide. The atherogenic diet fed male albino rats were given orally 100mg/kg body weight of disulphide (DADS or DPDS) for 60 days, later the rats were sacrificed and the plasma lipid profile, glycoproteins, calcium and transaminases were estimated. The aortic homogenates were employed for the estimation of thiobarbituric acid reactive substances and total sulphhydryl group. The results indicate a significant hypolipidemic effect with dipropyl disulphide with a comparative lower toxic side effect. It is concluded that DPDS is much safer and equally good hypolipidemic agent in experimentally induced hyperlipidemia in albino rats.

Keywords : *diallyl disulphide, dipropyl disulphide, atherosclerosis, lipid profile, acrolein.*

GJMR-K Classification : *FOR Code: 250302p*

Strictly as per the compliance and regulations of:

Comparative Study of Diallyl-Disulphide and Dipropyl-Disulphide in Experimental Atherosclerosis

Govindaswamy. K.S.[α], Basavaraj B. Devaranavadagi[σ], Kashinath. R .T.[ρ] & Nagendra. S.[ω]

Abstract - Diallyl disulphide, the principle organosulphur compound of garlic oil, is known to possess many clinical beneficial effects, but its overuse or abuse has been reported to cause certain harmful side effects due to its possible metabolite acrolein. It was thought that the disulphide nature of diallyl disulphide is responsible for its hypolipidemic effect and the unsaturation may be for its toxic effects. Recently few synthetic disulphides are successfully employed in experimentally induced hyperlipidemia. The present study was under taken to compare the hypolipidemic as well as toxic effects of saturated disulphide, Dipropyl disulphide with Diallyl disulphide. The atherogenic diet fed male albino rats were given orally 100mg/kg body weight of disulphide (DADS or DPDS) for 60 days, later the rats were sacrificed and the plasma lipid profile, glycoproteins, calcium and transaminases were estimated. The aortic homogenates were employed for the estimation of thiobarbituric acid reactive substances and total sulphhydryl group. The results indicate a significant hypolipidemic effect with dipropyl disulphide with a comparative lower toxic side effect. It is concluded that DPDS is much safer and equally good hypolipidemic agent in experimentally induced hyperlipidemia in albino rats.

Keywords : diallyl disulphide, dipropyl disulphide, atherosclerosis, lipid profile, acrolein.

I. Introduction

Garlic and its extracts are known to have proved hypolipidemic as well as anti atherosclerotic effects[1]. The principle organo sulphur compound, Diallyl disulphide (DADS) is thought to be responsible for the hypolipidemic and hypocholesterolemic effects of garlic[2]. However few recent studies have shown that Garlic and DADS May induce certain biochemical toxic effects like increased in blood urea levels, increased plasma transaminases levels[3] as well as increased TBARS production[4]. It was presumed that the disulphide nature of DADS is responsible for its hypolipidemic and hypocholesterolemic effects where as the unsaturation or allyl groups present in DADS may be responsible for its toxic effects. Further a few synthetic disphulphide have been employed with moderate success in regulating hyperlipidemia[1].

The present study was under taken to compare the hypolipidemic as well as toxic effects of saturated aliphatic low molecular weight disulphide Dipropyldisulphide (DPDS) with Diallyl dispulphide (DADS).

II. Materials & Methods

All the chemicals employed in the present study were of Analer (AR) Grade DADS & DPDS were procured from sigma Aldrich Company, USA.

a) Atherogenic Diet

The atherogenic diet to feed & to induce atherosclerosis in male albino rats was prepared by mixing whole milk powder, dalda (vegetable ghee) and pure cholesterol in the ratio of 1:0.5:0.1 with an extra vit D_2 supplement of 4 mg/100 g.

b) Experimental Animals

Male albino rats of 6 to 8 weeks old weighing 150 g - 200 g were selected randomly for the present study from the animal house of Dr. B.R. Ambedkar Medical College Bangalore, upon approval of the committee of ethics in animal experimentation (132/1999/CPSEA). These rats were kept on stock laboratory diet (Amruth rat feed Nava maharatara Chakan oil Ltd. Pune.) and tap water adlibitum.

i. *Group-1 (Normal group)*

Consisting 6 male albino rats fed stock laboratory diet and given orally 30 ml of normal saline per kg body weight daily for 60 days.

ii. *Group-2 (Control group)*

Consisting 6 male albino rats fed atherogenic diet adlibitum for 60 days and given normal saline 30 ml per kg body weight daily.

Author α : PhD. Former Research Scholar, Biochemistry Department, Ambedkar Medical College, Bangalore, Presently Associate Professor, Department of Biochemistry, Shimoga Institute of Medical Sciences, Shimoga. E-mail : ksgovindaswamy@rediffmail.com
Author σ : Former PG in Biochemistry, J N Medical College, Belguam, Presently Associate Professor, Department of Biochemistry, B.M. Patil Medical College, Bijapur.
Author ρ : Ph.D. Formerly. Prof. & Head, Biochemistry, Ambedkar Medical College, Bangalore, Presently Professor and Head, Department of Biochemistry, Subbaiah Institute of Medical Sciences & Research Centre, Shimoga. E-mail : drkashinath_1945@ yahoo.co.in
Author ω : MBBS, MD. Former Staff, Medicine Department, K M C, Manipal, Presently Medical Director, Subbaiah Institute of Medical Sciences & Research Centre, Shimoga.
E-mail : smcshimoga@yahoo.co.in

iii. *Group-3 (DADS Protective group)*

Consisting 6 male albino rats maintained on atherogenic diet adlibitum for 60 days and given 100 mg of DADS as 30 ml warm aqueous solution/kg body weight for 60 days using gastric tube.

iv. *Group-4 (DADS Curative group)*

Consisting 6 male albino rats maintained on atherogenic diet adlibitum for 60 days and later given 100 mg of DADS as 30 ml warm aqueous solution/kg body weight daily for next 60 days using gastric tube. During DADS feeding, the rats were maintained on stock laboratory diet, water was provided adlibitum to all these rats always.

v. *Group-5 (DPDS Protective group)*

Consisting 6 male albino rats maintained on atherogenic diet adlibitum and were given 100 gm DPDS as 30 ml warm aqueous solution/kg body weight for 60 days using gastric tube.

vi. *Group-6 (DPDS Curative group)*

Consisting 6 male albino rats maintained on atherogenic diet adlibitum for 60 days and later given 100 mg of DPDS as 30 ml warm aqueous solution/kg body weight daily using gastric tube for next 60 days. During DPDS feeding, the rats were maintained on stock laboratory diet and tap water, water was provided adlibitum to all these rats always. The rats of the group 1.2,3 & 5 were sacrificed by decapitation on the 61st day and the rats of group-4&6 were sacrificed by decapitation on the 121st day. Blood samples were collected using heparin as anti coagulant. The blood samples were centrifuged at 3600rpm for 5 minutes, the separated plasma were employed for estimation of total lipids (TL)[5], triacyl glycerol (TAG)[6], total cholesterol (TC)[7] phospholipids (PL)[8] HDL cholesterol[9], free fatty acid (FFA)[10], esterified fatty acid (EFA)[10], calcium [11], glycoprotein[12], fibrinogen[12], lipoperotein lipase[13], aspartate amino transferase (AST)[14], and alanine amino transferase (ALT)[14] . Aorta was procured and put into a pre weighed dry watch glass.

A portion of aorta was immediately fixed in buffered formalin and was employed for histopathological study.

A second portion of aorta was homogenized with chloroform methanol (1:1v/v) mixture and the extracts were used for estimation of lipid parameters. (TL, TAG, TC & PL).

A third portion of aorta was homogenized with 5% cold TCA and the extracts were used for the estimation of thiobarbituric acid reactive substances (TBARS)[15].

A fourth portion of aorta was homogenized with phosphate buffer (p^H 7.4) and the extracts were used for the estimation of total protein[16] (TP) and total sulphydryl groups[17] (SH).

III. Statistical Analysis

Data obtained were analyzed comparing the results of groups using students 't' test. Probability values less than 0.02 were considered as significant.

IV. Results

Results obtained in the present study are given in table 1 & 2 as well as in figure 1-6.The plasma levels of TL, TAG, TC, PL, HDL- cholesterol, EFA, FFA, calcium, glycoprotein, fibrinogen, LPL, AST & ALT in normal group (group 1), control group (group 2), DADS protective group (group 3), DADS curative group (group 4), DPDS protective group (group 5) and DPDS curative group (group 6) are given in table 1. As seen from the table there is a significant rise in plasma lipid levels in control group as compared to normal group whereas a significant decrease is observed in DADS Protective group, DADS curative group, DPDS protective group and in DPDS curative group as compared to control group suggesting that both DADS and DPDS have a significant lipid lowering effect in atherogenic diet fed rats.

Table-2 narrates aortic levels of TL, TAG, TC, PL, TBARS, SH groups and TP (Total protein) in normal, control, DADS protective, DADS curative, DPDS protective and DPDS curative group of rats.

It is seen from the table-2 there is a significant rise in aortic levels of TL, TAG, TC, PL and TP in control group as compared to normal group suggesting feeding atherogenic diet leads to accumulation of lipids and proteins in aorta. These values are significantly reduced in DADS protective, DADS curative DPDS protective and DPDS curative group establishing that feeding DADS and DPDS decreases the accumulation of lipids in aorta.

The aortic TBARS levels decreased and total SH group – increased in DADS protective, DADS curative, DPDS protective & DPDS curative group as compared to control group as seen from Table 2.

Figures 1-6 shows the histopathological findings of aortic cross section (H &E stain) of normal, control, DADS protective, DADS curative, DPDS protective and DPDS curative group of rats. It is evident from table 1 all the lipid parameters except HDL cholesterol are increased in control group as compared to normal group. These parameters were significantly reduced in DADS protective, DADS curative, DPDS protective and DPDS curative group of rats compared to control group establishing both DADS and DPDS has hypolipidemic effects. Further a raise in Glycoprotein and Fibrinogen levels seen in control group as compared to normal group. Whereas feeding DADS & DPDS significantly reduces these values in protective as well as curative group as compared to control groups. The plasma AST and ALT levels are elevated in control group as compared to normal group showing a possibility of tissue damage.

The histological aortic cross section of group 1-6 rats are given in figures 2-6. It is evident from the figures that there is an accumulation of lipids in aortic walls in control group (ref fig-2) as compared to normal group (ref fig-1). Further there is a significant decrease in this accumulation in both protective (ref fig 3 & 5) as well as curative groups (ref fig 4 & 6)

V. Discussion

The optimum dosage of DADS (100 mg/kg body weight) or DPDS (100 mg/kg body weight) employed in the present study clearly establishes the hypolipidemic, hypocholesterolemic and anti-atherosclerotic effects of these disulphides. A significant reduction is observed in both plasma and aortic lipids in DADS protective group (group 3), DADS curative group (group 4), DPDS protective group (group 5) and in DPDS curative group (group 6) as compared to antherogeinc diet fed control group (group 2) as evident from the tables 1 & 2. Further it is established by the histological studies of the aortic sections of these group of rats (fig 3-6) that both these disulphides have significant antiatherosclerotic effects in atherogenic diet fed rats (ref fig 2) . It has been repeatedly established by the earlier workers[18] that garlic has hypolipidemic, hypocholesterolemic and anti atherosclerotic effects[19] and the possible constituent of garlic bringing up this effect is DADS, as it is known that DADS is the principle organo sulphur compound of garlic oil[20].

Both DADS and DPDS are disulphides and similar to any other disulphide may undergo degradation to their respective thiols utilizing NADPH [21]. This leads to the depletion of cellular available NADPH levels and affects the synthesis of fatty acid, fats and cholesterol as their synthesis requires NADPH[22] hence resulting in a decrease in the plasma and aortic tissue lipid parameters including cholesterol as observed in DADS or DPDS treated atherogenic diet fed rats (group 3, 4, 5 & 6) as compared to control atherogenic diet fed rats (group 2).

HMG CoA reductase is the key enzyme of cholesterol biosynthetic and it is known that DADS has significant inhibition action against this enzyme [19, 23]. Through such an inhibition DADS can effect lowering of plasma as well as aortic cholesterol levels as evident from the result given in table 1 & 2. DPDS being a disulphide may induce inhibition of HMG CoA reductase similar to DADS, hence causing a significant lowering of cholesterol levels in plasma & in aorta (refer table).

It is known that disulphide can undergo sulphydrly exchange reaction with tissue proteins and thiol enzymes as depicted below-

$$R - S - S - R + ENZ - SH \longrightarrow R - S - S - ENZ + R - SH$$

DADS and DPDS are disulphides and may possibly undergo similar sulphydrly exchange reactions with the tissue proteins as well as thiol enzymes. Such a possible sulphdrly exchange reaction with Fatty acid synthase, HMG CoA reductase, glycerol phosphate dehydrogenase, squalene synthase and squalen oxidase leading to a conformational change in these enzymes resulting in a possible inhibition of these enzymes thereby causing in a significant reduction in fat, fatty acid and in cholesterol synthesis[21].

The atheromatus plaques in blood vessels are produced by an over accumulation of certain proteins and calcium as well as cholesterol[24]. The disulphide, DADS and DPDS significantly lowers the plasma levels of calcium, glycoproteins and fibrinogen in DADS as well as DPDS treated groups (group 3 & 4, group 5 & 6) as compared to control group (group 2). Suggesting that these disulphides promote a decrease in the plasma levels of calcium, glycoprotein's and fibrinogen thereby reduces their accumulation in the intima of blood vessels resulting in showing down of atheromatous plaque formation. This is evidenced by the histological aortic cross section of these rats (ref. fig 1-6) It is clear from these histological findings that both DADS and DPDS not only slow down the atheromatus plaque formation in treated groups (group 3, 4, 5 & 6) but also favors regression on the atherosclerotic plaques (fig 3 & 4).

Lipoprotein lipase, also known as clearing factor, helps in the clearing of triacylglyerols from plasma. The activity of this enzymes is significantly higher both group 3 & group 4 as compared to group 2 suggesting that both DADS & DPDS improves clearing of plasma triacylglycerols hence favours reduction in plasma / aortic tracylglycerols which is evident from the results given in table 1 & 2 . The disulphide DADS and DPDS might have undergone a sulphydryl exchange reaction with the lipoprotein lipase probably activating the enzyme or increasing the lifespan of the enzyme resulting in a significant reduction in plasma/ aortic triacylglycerol levels.

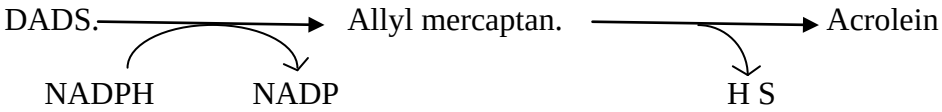
This observed reduction in plasma and tissue triacylglycerol levels may be in part due to a possible sulphydryl exchange reaction of these disulphides with glycerol phosphate dehydrogenate thus resulting in a partial inhibition of the enzyme leading to a decreased glycerol phosphate formation hence a decreased triacylglycerol production.

The observed in the present study clearly established that DPDS, a saturated, water soluble, well tolerated disulphide has a significant comparable hypolipidemic, hypocholesterolemic and antiatherosclerotic actions in atherogenic diet fed rats (ref. table. 1, 2 & fig 1-6).

Recently it has been shown by many workers[25] that feeding garlic extracts or garlic oil to experimental

animals do induce certain biochemical abnormalities like increases in blood urea levels increases in serum Bilirubin levels, elevation is serum transaminases[3] etc. Feeding 100mg/kg body weight garlic oil go an overnight fasted rat proved fatal and the cause of death was acute pulmonary edema[3].These findings of garlic oil attributed to its organosulphur compound, DADS.

The disulphide DADS may undergo catabolism in tissues to give rise to allyl mercaptan which might have converted to acrolein by an unknown mechanism.



The toxicity of DADS been evidenced by a significant rise on aortic TBARS levels (ref. table 2) whereas the increases in aortic TBARS levels is comparatively lower in DPDS treated atherogenic diet fed rats (ref. table 2).

Hence it is concluded by the results of the present experiments that DPDS is much safer, well tolerated and better hypolipidemic compound as compared to garlic principle, DADS.

References Références Referencias

1. Syed M.B. Inamdar M.N. Asad M. "Effect of conventional anti hypertension drugs on hypolipidmic action of garlic in rats". Indian journal of experimental Biology 2009 Mar; 47(3); 176-81.
2. Stephenwarshafsky, MD; Russel S. Kamer, MD; "Effect of garlic on total serum cholesterol" Ann Intern Med, 1993; 119; 599-605.
3. Joseph P.K. Rao K.R., Sundaresh C.S. "Toxic effects of garlic extract and garlic oil in rats" Indian journal of Exp physiology 1989 Nov 27 (11); 977-9.
4. G. Dilip Reddy, A Gopala Reddy, G Krishana Rao, C. Haritha, Jyothi "Interaction study on garlic and Atorvastatin with Reference to nephrotoxicity in dyslipidemic rats". Toxicol Int. 2010 Jul-Dec 17(2); 90-93.
5. Choudhary. K: in Biochemical techniques Edn-1 New Delhi, Jaypee Bros. 1989; 112-114.
6. Alan HG. Maurice B, Lipids and lipoproteins. In, varely H practical clinical Biochemistry 5th ed. London Heineman professional publishing Ltd., 1980; 625.
7. Richard J. Henry, Donald C, Connan, James. W. Winkelman: in Clinical Chemistry-Principles and Practice, 2nd Edn. Newyork Harper Row Publishers, 1438-1441.
8. Nath RL. In practical Biochemistry in clinical medicine 2nd Edn. Calcutta, India, Academic publishers, 1990; 120-122.
9. Alan H. Gowenlock, janet R, Mc Murray and Donald. M, Mc Lauchlan in Varley's practical clinical chemistry 6th Edn. London 1988; 461-462.
10. Nath R.L in Practical Biochemistry in clinical medicine 2nd Edn. Calcutta India, Academic publishers, 1990; 125-128.
11. Lorentz, K, Determination of serum calcium with 2-cresopthalein complexone. Norbert. W. Teitz in Fundamentals of clinical chemistry. Philadelphida. W.B. Sanders. 1986; 1350.
12. Harold Varley, Alan H. Gowen lock, Maurice Bell practical clinical Biochemistry, 5th Edn. London, Heineman professional publishing Ltd, 1980; 557-591.
13. Korn ED. Lipoprotein Lipase. In Methods in Enzymol. 5thedn. New York, Academic Press, 1962; 543-545.
14. Mass DW, Henderson AR, Kachmar JF. Enzymes. In Text book of Clinical Chemistry, by Tietz, Philadelphia. WB Sanders, 1986; 669-677.
15. Nadigar HA, Marcus S.R. Chandrakala, MV, Kulkarni DD, Malony1 dialdehyde levels in different organs of rats subjected to acute alcohol toxicity. Ind. J. clin. Biochem 986; 1: 133.
16. Peter TJ, Biamonte GT, Doumas BT. Determination of toal protein by Binret Method. In fundamentals of clinical chemistry of Narbert W. Teitz, Philadephia, W.B. Sanders. 1986; 583-584.
17. Colowick and Kaplan: Enzymes in lipid metabolism in methods of Enzymology (Academic Press, New York) 1957; 623-624.
18. Sanjay K. Banerjee, Subir K Maulik "Effect of garlic on Cardiovascular disorders" Nutrition Journal 2002; 2891-1-4.
19. Yu-Yan Yeh, Lijuan Liu "cholesterol lowering effects of garlic extracts and Orgarnosulphur compounds; Human and Animal studies" The journal of nutrition 2001; 131(3) 9895-9935.
20. Lawson L D. (1998). Garlic: a review of its medicinal effects and indicated active compounds. Phytomedicines of Europe". Chemistry and Biological activity. 691, pp. 176-209.
21. Black.S. Reduction of sulfoxide and disulfides. Methods in Enzymology. Vol-5, Academic Press, New York 1962; 992.
22. Sheela CG, Augu. KT. Antiperoxide effects of S-allyl Cysteine sulphoxide isolated from allium sativum linn and gugulipid in cholesterol diet fed rats. Ind. J. Exp. Biol. 1995; 33: 337-341.
23. Stevinson C, Pittler MH and Ernst E. (2000). Garlic for treating Hypercholesterolemia. Ann. Intern Med. 133, pp. 420-429.

24. Donald voet & Judith G. Voet "Text Book of Biochemistry 1990; 64; 308-309.
25. Sodimu O, Joseph. PK. Augusti KT. Certain biochemical effects of garlic oil in rats maintained on high fat, high cholesterol diet. Experientia 1984; 40: 78-80.

Fig 1 : Normal Group : Shows normal blood vessel/aorta (H & E, x 320)

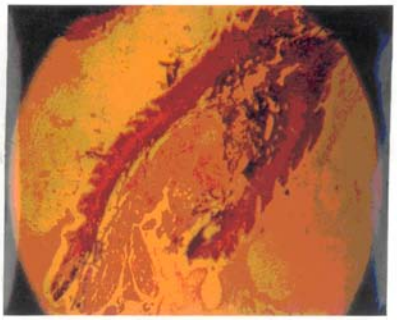


Fig 2 : Control Group (atherosclerotic) : Shows features of atherosclerosis/atheroma with fat deposition. (H & E, x 320)

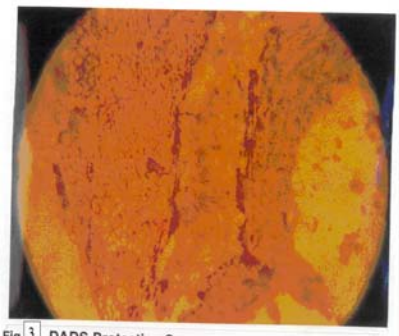


Fig 3 DADS Protective Group : Shows empty fat spaces with blood vessels and few cholesterol clefts and occasionally inflammatory cells. (H & E, x 320)

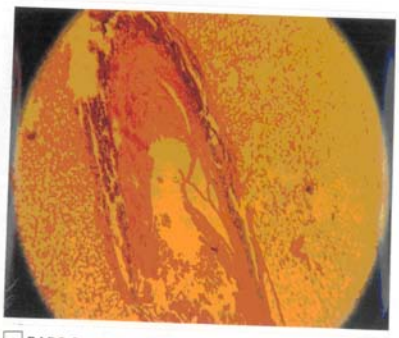


Fig 4 DADS Curative Group : Shows aorta with few cholesterol clefts and fat deposition (H & E , x 320)

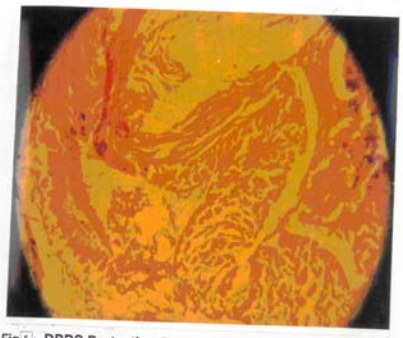


Fig 5 : DPDS Protective Group : Shows normal aorta with mild atherosclerotic changes (H & E, x 320)

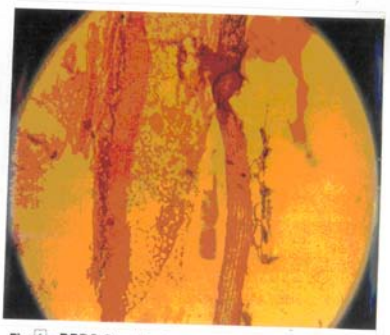


Fig 6 : DPDS Curative Group : Shows wall of the aorta with fatty infiltration (H & E, x 320)

Table 1 : Table showing the plasma levels of TL, TAG, TC, PL, HDL – Cholesterol, FFA, EFA, LPL, Calcium, Glycoprotein Fibrinogen, AST & ALT in normal rats (group-1), in atherogenic diet fed rats (groups-2), in rats fed atherogenic diet and given diallyl disulphide daily (DADS protective group-3), in atherosclerotic rats fed diallyl disulphide daily (DADS curative group-4), in rats fed atherogenic diet and given dipropyl disulphide daily (DPDS protective group-5) and in atherosclerotic rats fed dipropyl disulphide daily(DPDS curative group-6).

Analyte	Group 1 (Normal)	Group 2 (Control)	Group 3 (DADS Protective)	Group 4 (DADS Curative)	Groups5 (DPDS Protective)	Groups6 (DPDS Curative)
Total lipids (mg %)	303.5±17.9	610.0±79.9**	354.1 ±13.6***	374.9 ± 8.9***	321.3±17.8***	342.2±18.5***
Triacylglyceol (mg%)	102.3±0.55	206.9±3.4***	118.3 ± 0.81***	122.0 ± 1.12**	112.7±0.98***	119.5±0.41***
Total Cholesterol (mg%)	136±2.55	296.5±3.3***	140.4 ± 2.54***	143.2 ± 1.65***	137.1±2.54***	139.9±1.7***
Phospholipids (mg%)	17.4±1.35	41.0±5.5**	18.4±0.68***	24.40.±26***	17.8±0.36***	20.4±0.26***

HDL cholesterol (mg%)	6.5±1.43	33.63±0.9	56.3±0.8**	51.6±0.59***	59.1±0.47***	55.2±0.2**
Free fatty acids (Meq/L)	0.312±0.02	0.836±0.02**	0.48±0.024***	0.496±0.027**	0.488±0.013***	0.504±0.024***
Esterifed fattyacids (mmol/hr)	440.6±13.5	646.3±13.7***	438.3±2.7*	449.9±4.12***	435.6±2.6***	446.2±9.15***
Lipoprotein lipase (mmol/ml/hr)	17.1±0.17	7.9±0.1***	18.2 ± 0.9***	14.8 ± 0.2***	17.6±0.4***	16.3±0.7***
Calcium (mg%)	9.8±064	18.3±1.62***	10.2 ± 0.59**	11.9 ± 0.64***	9.5±1.0***	10.2±2.66***
Glycoprotein (g/L)	1.28±0.1	4.4±0.26***	1.37 ± 0.1**	1.69 ± 0.01**	1.21±0.05***	1.48±0.03***
Fibrinogen (g/L)	3.06±0.058	9.1±0.8***	3.8 ± 0.80***	4.2 ± 0.1***	3.4±0.1***	4±0.9**
AST (U/ml)	15.3±0.35	22.8±0.62***	26.5±0.61***	30.7±0.15***	31.7±0.1***	34.3±0.17***
ALT (U/ml)	12.4+0.45	18.2±0.17***	24.5±0.22***	26.6±0.81**	25.1±0.26***	27.1±0.1**

Note:

1. Values are expressed as mean ±SD.
2. No. of animals in each group is 6.
3. Group 2 is compared to Group 1, Group 3 to 6 are compared to group 2, Significance levels: *P < 0.02 **P < 0.01 *** P <0.001.

Table 2 : Table showing the plasma levels of TL, TAG, TC, PL, HDL – Cholesterol, FFA, EFA, LPL, Calcium, Glycoprotein Fibrinogen, AST & ALT in normal rats (group-1), in atherogenic diet fed rats (groups-2), in rats fed atherogenic diet and given diallyl disulphide daily(DADS protective group-3), in atherosclerotic rats fed diallyl disulphide daily (DADS curative group-4), in rats fed atherogenic diet and given dipropyl disulphide daily (DPDS protective group-5) and in atherosclerotic rats fed dipropyl disulphide daily(DPDS curative group-6).

Analyte	Group 1 (Normal)	Group 2 (Control)	Group 3(DADS Protective)	Group 4 (DADS Curative)	Group 5 (DPDS Protective)	Group 6 (DPDS Curative)
Total lipids (mg/g)	53.5 ±1.78	104.0 ± 4.47***	55.3 ± 3.5***	57.0 ± 4.7**	54.1±2.7***	56.5±2.7***
Triacylglycerol (mg/g)	38.6 ± 0.15	78.0 ± 0.30***	41.6 ± 0.55***	43.3 ± 0.80***	39.6±0.73***	42.8±0.21***
Total cholesterol (mg/g)	17.3 ± 0.50	40.5 ± 0.65***	20.6 ± 0.80***	24.6 ± 0.3***	18.6±0.3**	22.±0.55***
Phospholipids (mg/g)	19.3±0.25	42.±0.2**	22.4±0.25*	24±0.76***	19.9±0.3***	22.8±0.6**
Total proteins (mg/g)	49.4±0.55	109.9±0.78*	51.7±0.36**	53.1±0.55***	49.9±0.4***	52.5±0.86***
TBARS (μmolMDA/g)	4±0.26	10.4±0.26***	11.7±0.25**	12.5±0.26***	6±0.27***	6.8±0.28**
SH group (μmol/g)	63±0.51	30.7±0.2***	40.1±0.57***	42.±0.2***	56.2±0.45**	54.6±0.25**

Note:

1. Values are expressed as mean ±SD.
2. No. of animals in each group is 6.
3. Group 2 is compared to Group 1, Group 3 to 6 are compared to group 2.
4. Significance levels: *P < 0.02; **P < 0.01;***, P <0.001.

Global Journal of Medical research
Interdsciplinary
Volume 13 Issue 2 Version 1.0 Year 2013
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN : 0975-5888

Mediated Liposome for Gene Delivery to Mice Brain Part I. Design and Characterization of Liposome-DNA Complexes

By Dr. Evone S. Ghaly, J. Wang & E.S. Ghaly
University of Puerto Rico

Abstract - The purpose of this research is to develop a novel liposome-mediated system for delivery of expression plasmid into specific regions in the rat brain. Complexes of plasmid DNA and different liposome were prepared in phase 1 of the study. The composition, method of preparation were varied and the physico-chemical characterization of the different systems were investigated two different methods of preparation were used, in the first method the liposome were prepared simultaneously with the DNA entrapped into the liposome and in the second method, the liposome were prepared first and then complexes with the DNA were performed. The liposome formulations were composed of DOTAP: Cholesterol; and DC-Chol: DOPE and different lipid helpers. The particle size of liposomes prepared with DNA entrapped into the liposome was larger than those prepared with liposome-DNA complexation. All liposome formulations were spherical, uniform in size and have smooth surface. In vitro DNase digestion experiments demonstrated that liposome protects 60-80% plasmid DNA from DNase digestion. The plasmid: DNA lmediated liposome can be widely prepared, have less risk than the use of viral vectors, can protect DNA from DNase digestion, none toxic and therefore can be used repeatedly in vivo.

Keywords : mediated liposomes; gene delivery; targeting; gene expression; plasmid-DNA expression; targeting to mice brain.

GJMR-K Classification : FOR Code: 270299p

Strictly as per the compliance and regulations of:

Mediated Liposome for Gene Delivery to Mice Brain Part I. Design and Characterization of Liposome-DNA Complexes

Dr. Evone S. Ghaly [α], J. Wang [σ] & E.S. Ghaly [ρ]

Abstract - The purpose of this research is to develop a novel liposome-mediated system for delivery of expression plasmid into specific regions in the rat brain. Complexes of plasmid DNA and different liposome were prepared in phase 1 of the study. The composition, method of preparation were varied and the physico-chemical characterization of the different systems were investigated two different methods of preparation were used, in the first method the liposome were prepared simultaneously with the DNA entrapped into the liposome and in the second method, the liposome were prepared first and then complexes with the DNA were performed. The liposome formulations were composed of DOTAP: Cholesterol; and DC-Chol: DOPE and different lipid helpers. The particle size of liposomes prepared with DNA entrapped into the liposome was larger than those prepared with liposome-DNA complexation. All liposome formulations were spherical, uniform in size and have smooth surface. In vitro DNase digestion experiments demonstrated that liposome protects 60-80% plasmid DNA from DNase digestion. The plasmid: DNA lmediated liposome can be widely prepared, have less risk than the use of viral vectors, can protect DNA from DNase digestion, none toxic and therefore can be used repeatedly in vivo.

Keywords : mediated liposomes; gene delivery; targeting; gene expression; plasmid-DNA expression; targeting to mice brain.

I. Introduction

Liposomes are self-closed spherical particles where one or several lipid membranes encapsulate (s) part of the solvent in which they freely float into their interio (1-5). Liposome (6) is distinguished by large multilamellar vescicles (MLV) and unilamellar vesicles which can be small (SUV), large (LUV) or giant unilamellar vesicles (GUV).

The major purpose of gene therapy is to deliver genetic material into target cells to rproduce specific therapeutic proteins needed to correct or or to modulate disease. However, developing appropriate biotherapeutics, such as plasmid-based gene expression vectors delivered successfully to the target cell is one of the major practical problem in gene therapy today (7). Approaches available for introduction of DNA into cells include viral transduction or plasmid transfection. These systems are effective for the expression of a variety of trangenes in brain issues. However, several technical problems are associated with issues such as immunogenicity, scale up, random integration and cellular tropism, which may limit them as therapeutic agents (8).

Many efforts have been devoted to the development of non-viral delivery due to the disadvantages of viruses used for gene delivery (9-12). Cationic liposomes have several attractive features as as vectors for gene transfer: They are non-immunogenic and non-toxic; cationic liposome as DNA carriers can transfect postmitotic, non-dividing cells including neurons; cationic liposome can deliver multiple genes of any type (linear or super coiled) nucleic acid and finally, cationic liposome are relatively simple to prepare and can be administered to the body by different several routes.

Gene therapy is potentially powerful method for treatment of neurological diseases for which classical pharmacotherapy is unavailable or not easily applicable (13-14). Transfection within the brain has distinct advantages over other administration sites. The post-mitotic stage of nature neurons may prolong transgene expression. Moreover, the liquid volume in which in which the delivery vectors needs to be distributed and the metabolism of the plasmid can be limited because of the lack of major clearance mechanisms, such as those in the liver or kidney. In addition the cerebrospinal fluid has limited nuclease activity as compared to plasma and thus provides for longer half-life of the administered DNA in the nervous system (15).

The hypothesis of this investigation is that mediated liposome-DNA complex may protect the DNA from degeneration by DNase; use of cationic mediated liposome for targeting plasmid DNA is more safe, non-toxic compared to the use of viral vector. Also, the intra-hipocampus infusion of liposome-DNA complex may leads to DNA expression in specific brain region. The overall goal of this research is to develop a novel liposome mediated system for delivery of expression plasmids into specific brain regions in the rat. The specific objectives of this study are to prepare different

Author α : Professor of Pharmaceutics, School of Pharmacy, Medical Sciences Campus, University of Puerto Rico, G.P.O. Box 365067, San Juan, Puerto Rico-00936-5067, (787) 758-2525 ext. 5417.
E-mail : evone.ghaly@upr.edu
Author σ ρ : School of Pharmacy, Medical Sciences Campus, University of Puerto Rico, G.P.O. Box 365067, San Juan, Puerto Rico-00936-5067.

liposome formulations; use B-galactosidase reporter plasmid construct; determine complexation between cationic liposome formulations and the plasmid-DNA; and examine the efficiency of the mediated liposome to protect DNA plasmid from DNase digestion.

II. Experimental and Methods

a) Materials

DOTAP, N-[1{2,3-deoxypropyl]-N, N, N trimethyl ammonium., lot No. 181TAP-65, Avanti Polar Lipids, AL, USA.; DOPE, dioleoyl phosphatidylethanolamine, lot No. 181PE-228, Avanti Polar Lipids, AL, USA.; DC-Chol., 3B-(N,N,N,-dimethylaminoethane}-carbimol}cholesterol, lot No. 017H8476, Sigma, MO, USA.; Cholesterol, lot No. 96H8476, Sigma, MO, USA; Protamine sulfate, lot No. 02K7400, Sigma, MO, USA; pSV-B-Galactosidase control vector, lot No. 12706113, Promoga Corporation, Madison, USA; XL-10-Gold^R , ultracomponent cellls, Stratagene Services, USA; Turbo DNase, Ambion Inc., USA; Wizard^R PlusMaxipreps DNA Purification System, lot No. 241531, Promega Corporation, Madison, USA. All other ingredients are of chemical grade.

b) Methods

i. *Plasmid Preparation*

a. Transformation of XL-10-Gold^R ultracompetent cells with plasmid pSV-pSV-B-Galactosidase. Plasmid psV-B-Galactosidase contains SV40 early promoter and enhancer drive transcription of the lacZ gene, which encodes the B-Galactosidase enzyme. The plasmid was propagated in XL-10-Gold^R ultracompetent cells (Stratagene) following the manufacturer's instruction.

b. Production and purification of plasmid pSV-Galactosidase. The pSV-B-Galactosidase was purified by Wizard^R PlusMaxipreps DNA purification system following the manufacturer's instruction.

ii. *Design of Liposome and Preparation of Liposome-pSV-B-Galactosidase Complex*

a. *Preparation of liposomes containing DOTAP and cholesterol with DNA entraped in the liposome*

A mixture of DOTAP and cholesterol at 1:1 molar ratio (8.38 mg DOTAP and 4.64 mg of cholesterol was dissolved in 12 ml chloroform. The organic solvent was removed using rotary evaporator at 40^{0}C and vaccum for 2 hours. The thin layer of lipid film formed on the wall of the flask was hydrated using 1 ml of 5% dextrose solution containing 1 mg of DNA and 0.6 mg of protamine sulfate. The mixture of the hydrated thin film of the lipids and DNA was agitated by vortexing for 30 seconds and then incubated at 37^{0}C for 30 seconds. This process was repeated 8 times (n=8). The liposome suspension was sonicated for 20 seconds and vortexed for 30 seconds.

b. *Preparation of liposome containing DOTAP and cholesterol*

The cationic DOTAP was mixed with cholesterol at equimolar concentration (8.38 mg DOTAP with 4.64 mg cholesterol). The mixture of lipid was dissolved in HPLC grade chloroform using 1 litre round bottom flask. The organic solvent was evaporated using rotary evaporator at 30^{0}C for 30 minutes and then dried under vacuum for 15 minutes. The dried thin film was hydrated in 5% dextrose solution to give a final concentration of 20 mM DOTAP and 20 mM cholesterol (20 mM DOTAP-Cholesterol). The hydrated lipid film was agitated in a water bath at temperature of 50^{0}C for 45 minutes and then at 35^{0}C for 10 minutes. The mixture was covered and kept overnight at room temperature. After 24 hours, the mixture was sonicated for 10 minutes at 50^{0}C. DOTAP-cholesterol liposome (75 ul) was mixed with protamine sulfate (30 ug) and the mixture was kept at room temperature for 10 minutes before use. 75 ul of plasmid DNA (0.6 ug/ul) was slowly added while stirring and the mixture was incubated at room temperature for 10 minutes before use. The final concentration of DNA was 0.3 ug/ul.

c. *Preparation of liposomes containing DC-Chol and DOPE*

DC-chol-DOPE cataionic liposomes were prepared by mixing DC-Chol and DOPE at 1.5:1 molar ratio (6.37 mg DC-Chol and 6.13 mg DOPE). The mixture of the two lipids was dissolved in HPLC grade chloroform. The organic solvent was evaporated in a rotary evaporator at temperature of 55^{0}C for 60 minutes, then dried under vacuum for 30 minutes. The film was hydrated in 5% dextrose solution to give a final concentration of DC-Chol:DOPE liposome (1.25 mg/ml). The hydrated film was agitated in a water bath at 55^{0}C for for 45 minutes and at 35^{0}C for an additional 10 minutes and the mixture was kept overnight at room temperature. After 24 hours, the mixture was sonicated for 5 minutes at 50^{0}C, transferred to a tube and heated at 50^{0}C for 10 minutes. Then the mixture, was extruded through a Millipore filters in a descending order of 1 um, 0.45 um, 0.2 um, 0.1 um using syringe. Portion of the liposome that did not pass through 0.1 um filter was heated again at 50^{0}C for 5 minutes before passing through a new 0.1 um filter. The filtered fractions were stored under argon gas at 4^{0}C. 75 ul of Plasmid DNA (0.6 ug/ul) was added slowly while stirring to equal amount of DC-Cho:DOPE liposome and the mixture is incubated at room temperature for 10 minutes before use. The final concentration of DNA is 0.3 ug/ul.

iii. *Characterization of Liposome and Plasmid DNA Complexes*

a. *Particle size distribution*

The particle size was determined by Malvern particle size diffraction analyzer using a scale constant of 300 nm, the laser beam passed through the liposome dispersion and the light scattered was measured in 19 to 30 seconds. A blank of distilled water was used.

b. *Morphology of liposome and plasmid DNA complex using scanning electron microscope (SEM)*

The liposomes were coated with conducted film into pin mount and tighten. The image appeared on the screen after clicking on beam and high voltage /xKV buttoms. The brightness and the magnification were adjusted and the image was saved.

iv. *DNase digestion study*

Four reactions were performed:

Components	Reaction 1	Reaction 2	Reaction 3	Reaction 4
pSV-B-Gal	N/A	N/A	1.5 ug	N.A
pSV-B-Gal lipos.complex	N/A	5 ul	N/A	5 ul
DNase reaction buffer	100 ul	100 ul	100 ul	100 ul
DNase	3 ul	3 ul	N/A	N/A

All mixtures were incubated for 2.5 hours at 37^0C and 100 um of 1:1 phenol:chloroform mixture was added, mixed gently and centrifuged at 14,000 rpm for 4 minutes at room temperature. The supernatant was transferred to tube and mixed with 100 ul chloroform and centrifuged at 4,000 rpm for 4 minutes at room temperature. The supernatant was removed and the pellets were washed with 70% alcohol and dried. The DNA was dissolved in 50 ul buffer. Aliquots of plasmid DNA were analyzed using agarose gel electrophoresis and UV spectrophotometer.

III. Results and Discussion

In phase 1 study, the plasmid DNA was successfully encapsulated into different liposome formulations and or formed DNA:liposome complexes. The liposome formulations prepared were containing DOTAP and cholesterol with entrapped DNA; DOTAP and cholesterol and DC-Chol:DOPE liposomes complexes with DNA. All liposomes were cationic, DOTAP and DC-Chol are two cationic lipids and they provided a positive charge for the liposome. They are considered to be as a lipids helper. Cholesterol was also used as an alternative lipid helper that resulted in more stable complexes than those containing DOPE.

Two manufacturing methods were also used to prepare the liposomes. In the first method, plasmid DNA solution was used to hydrate the lipid film and the lipids formed the bilayers membrane while the DNA was encapsulated into the liposomes. In the second method, after preparation of the liposome, a complex is formed between the DNA and the liposome. The first method gave better entrapment efficiency of DNA and better protection of the DNA.

The particle size of the different mediated liposome-DNA systems are shown in Table 1. The particle sizes for all formulations were larger than expected (50 nm – 200 nm). Liposome prepared with DOTAP-Cholesterol have the largest particle size while liposome prepared with DC-Chol:DOPE were of the smallest particle size.

Figures 1 and 2 show the surface morphology of liposome prepared with DOTAP:Cholesterol and entrapped DNA. The liposomes appear to be spherical and of smooth surface.

Figure 3 shows the electrophoresis spectra of free liposome; DNA s and DNA encapsulated liposome after exposure to DNase enzyme. Adding DNase to free DNA resulted in complete degradation of DNA while DNA encapsulated liposome was not affected by DNase, indicating that the liposome was able to protect the DNA. Tables 2 to 4 show that the recovery of efficiency of the DNA from DNA entrapped liposome was between 67% to 83% using UV spectrophotometer. The lost quantity of DNA was due to the difficulty of avoiding loss during the extraction and the precipitation processes. The particle size was large when DNA was entrapped into the liposome. This may be due to that the hydration of lipid with DNA solution resulted in formation of very heterogeneous population with possible large size. On the other hand when liposome was first made and then complexed with DNA, it is assumed DNA and cationic liposome aggregate because of electrostatic attractive forces and formation of small stoichiometric complexes.

IV. Conclusions

Mediated liposomes with entrapped DNA lipid or liposome complexes with DNA plasmid were successfully prepared. Liposome prepared with DC-Chol: DOPE complexes with DNA were the smallest in size while liposome prepared with DOTAP:Cholesterol and entraped DNA plasmid into liposome gave the highest efficiency entrapment and best protection of DNA against DNase digestion. The composition of the liposome and the method of preparation have an effect

on the physico-chemical properties of the liposome. All mediated liposome formulations showed spherical particles and smooth surface. The mediated liposomes were able to protect DNA against DNase digestion and degradation.

References Références Referencias

1. Golbey WT., Mikos AG. Recent progenies in gene delivery using 24 non-viral transfer complexes. J. Controlled Release 2001; 72:115-125.
2. Allen TM., Hansen C., Martin F., Redeman C., Yan-Young A. Liposomes containing synthetic lipid derivatives of polyethylene glycol show prolonged circulation half life in vivo. Biochem. Biophys. Acta, 1991; 1066:29-36.
3. Princen F., Lechanteur C., Lopez M., Gielen J., Bours V., Merville MP. Similar efficiency of DNA-liposome complexes and retrovirus-producing cells for HSVtk suicide gene therapy of peritoneal carcinomatosis. J. Drug Target 2000; 8:79-89.
4. Templeton NS., Lasic DD., Frederic PM., Strey HH., Roberts DD.; and Pavlaki GN. Improved DNA; liposome complexes for increased systemic delivery and gene expression. Nature Biotechnology. 1997; 15: 647-652.
5. Yang Y., Numes FA., Berenesi K., Furth E E., GoE NezoE IE., Wilson, I.M.. Cellular immunity to viral antigens limits el detected adinoviruses for gene therapy. PNAS. 1994: 91:44-07-4411.
6. May S., Haries D., Beh-Shaul A. The phase behavior of cationic liposome-DNA lipid-DNA complexes related. Biophys. J. 2000; 78:1681-169.
7. Templeton NS. Cationic liposome mediated gene delivery in vivo. Biosci. Reps. 2002; 22:283-295.
8. Templeton NS., Lasic D.D. New directions in liposome gene delivery. Mol. Biotechnol. 1999; 11:175-180.
9. Aton EW., Stern M., Early R., Jaffe, A., Shadwick S.L., Philips J.; Davis J., Smith SN., Browning J., Davies MG., Hodson, ME., Durham SR,, Li D, Jeffery PK., Scallan M., Balfour R., Eastman SJ., Cheng SH., Smith EA.E., Meeker D., Geddes D.M. Cationic lipid mediated CFTR gene transfer to the lungs and nose of patients with cystic fibrosis. A double blind placebo-controlled trial. Lancet 1999; 353, 947-954.
10. Zaumer W.. Brumer S., Buschle M., Ogris M., Wagner E. Differential behavior of lipid based and polycation based gene transfersystem in transferring primary human fibroblasts: a potential role of polylysine in nuclear transport. Biochem. Biophys. Acta. 1999; 1428, 57-67.
11. Bragonzi A, Boletta A., Biffi, A., Muggia A., Sesale G., Cheng SH., Bordignon B.M. Gene delivery to the lungs. Gene Ther. 1999; 6:1995-2004.
12. Felgmer PL., Gadek YR,; Holm M., Roman R., Chan HW., Wenz M., Norhp JP, Ringold GM., Damielson M. Lipofectin: a highly efficient, lipid-mediated DNA-transfection procedure. Proc. Natl. Acad. Sci., USA. 1987; 84: 7413-7417.
13. Fahr A., Seelig J. Liposomal formulations of cyclosperin. A biophysical approach to pharmacokinetics and pharmacodynamics. Crit. Rev. Ther. Drug Carier Syst. 2001; 18:141-172.
14. Halfez IM., Cullis PR. Roles of lipid polymorphism in intracellular delivery. Adv. Drug Delv. Rev. 2001: 47:139-148.
15. Trachant I., Thompson B, Nicolazzi C., Mignit M.. Scherman, D. Physico-chemical optimization of plasmid delivery by cationic lipids. J. Gene Med. 2004: 6: 524-535).

Table 1 : Mean Particle Size of the Different Mediated Liposome-DNA Systems

Formulations	Mean Particle Size ib nm (n=3)
DOTAP:Cholesterol with DNA Entrapped in the Liposome	512.5
DOTAP:Cholesterol Liposome Complexes with DNA	723.7
DC-Chol:DOPE Liposome Complexes with DNA	309.8

Table 2 : Quantitative Analysis of DNA in DOTAP:Cholesterol with Entraped DNA to the Liposome Using Ultra Violet Spectrophotometer

Samples	Initial Quantity (ug)	Mean Final Quantity in ug (n=3)	Percent Efficiency
Free DNA	1.50	1.15	83.33
Free DNA Treated with DNase	1.50	0.04	2.89
DNA Entraped in Liposome	1.50	1.06	70.7
DNA Entraped in Liposome treated with DNase	1.50	0.91	60.9

Table 3 : Quantitative Analysis of DNA in DOTAP:Cholesterol Liposome Complexes with DNA Using Ultra Violet Spectrophotometer

Samples	Initial Quantity (ug)	Mean Final Quantity in ug (n=3)	Percent Efficiency
Free DNA	1.50	1.18	78.5
Free DNA Treated with DNase	1.50	0.03	2.0
DOTAP:Cholesterol Liposome Complexes with DNA	1.50	0.83	66.1
DOTAP:Cholesterol Liposome Complexes with DNA and Treated with DNase	1.50	0.78	52.3

Table 4 : Quantitative Analysis of DNA in DC-Cholesterol:DOPE liposome Complexes with DNA Using Ultra Violet Spectrophotometer

Samples	Initial Quantity (ug)	Mean Final Quantity in ug (n=3)	Percent Efficiency
Free DNA	1.50	1.15	76.5
Free DNA Treated with DNase	1.50	0.00	0
DC-Chol:DOPE Liposome Complexes with DNA	1.50	0.042	70.2
DC-Chol:DOPE Liposome Complexes with DNA and Treated with DNase	1.50	0.033	55.2

Figure 1 : Scanning Electron Microscope for DOTAP:Cholesterol Liposome Complexes with DNA at low Magnification

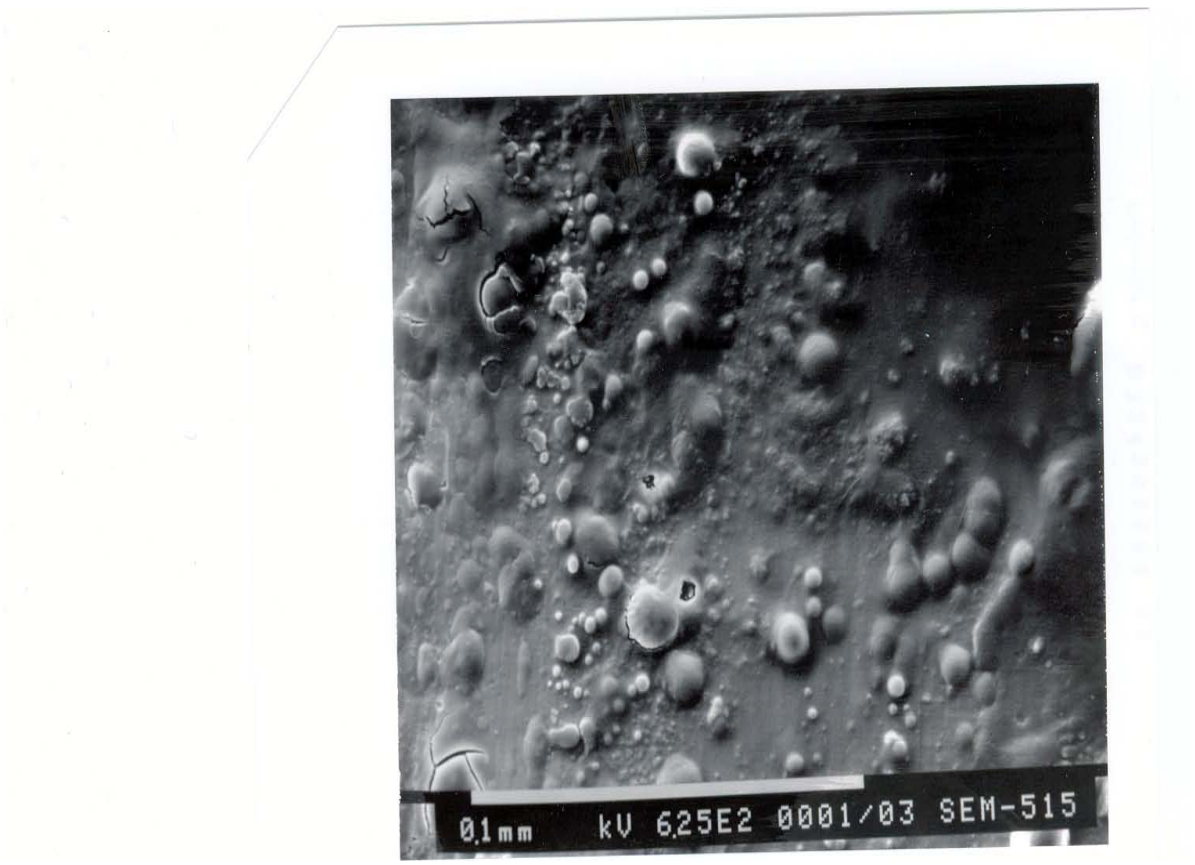


Figure 2 : Scanning Electron Microscope for DOTAP:Cholesterol Liposome Complexes with DNA at High Magnification

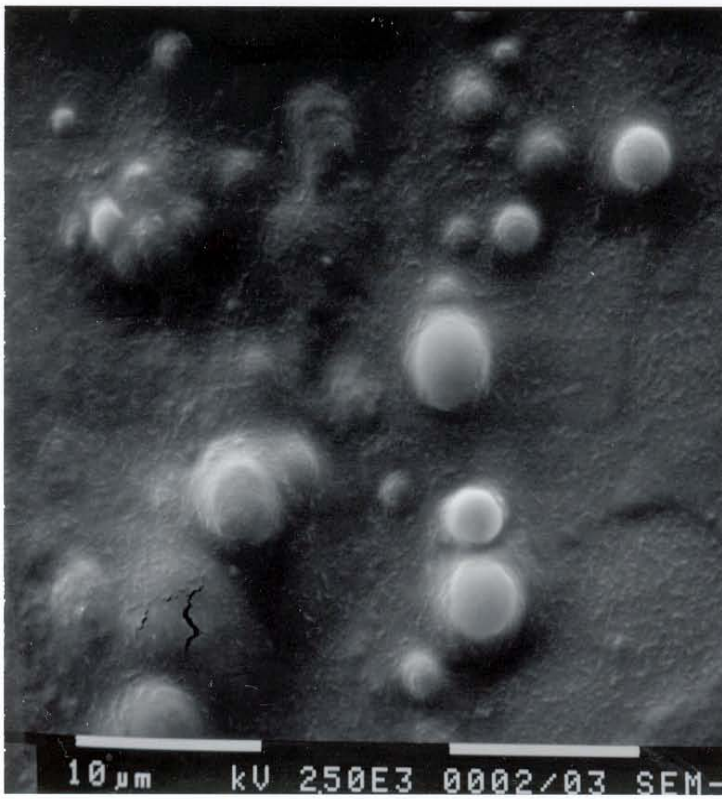
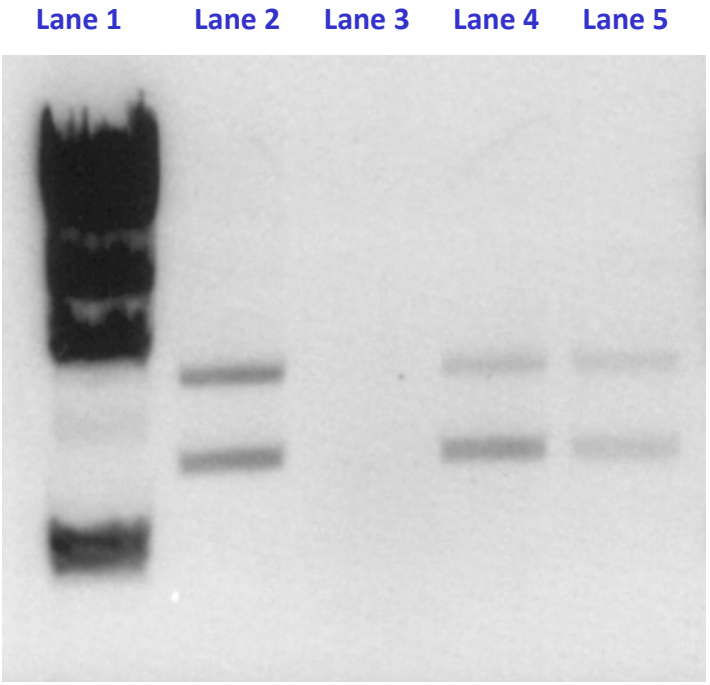


Figure 3 : Agarose Gel Electrophoresis Analysis

Lane 1 : Molecular Weight Marker
Lane 2 : Free DNA
Lane 3 : DNA Treated With DNase
Lane 4 : Liposome:DNA Complexes
Lane 5 : Liposome:DNA Complexews Treated with DNase



This page is intentionally left blank

Global Journal of Medical research
Interdsciplinary
Volume 13 Issue 2 Version 1.0 Year 2013
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN : 0975-5888

Evaluation of the Protective Properties of Amlodipine, on Cisplatin Induced Cardiotoxicity in Male Rats

By Dr. Ammar Abdulkareem Hadi & Prof. Dr. Sabah N. Al-Thamir
Babylon University

Abstract - This study was aimed to evaluate the cardiotoxicity of cisplatin in rats and to investigate the cardioprotective effect of amlodipine on cisplatin treated rats by using cardiac biomarkers troponin, CK-MB. Thirty five healthy male swiss albino rats were used in this study. The study design was divided into two patterns:

Pilot study design: The animals were randomly divided into two groups, In the first treated group, all rats received cisplatin in a single dose, while in the second treated group, rats received cisplatin in four divided doses every 2 days.

Keywords : *cisplatin, amlodipine, cardiotoxicity, troponin, CK-MB.*

GJMR-K Classification : *FOR Code: 111506*

Strictly as per the compliance and regulations of:

Evaluation of the Protective Properties of Amlodipine, on Cisplatin Induced Cardiotoxicity in Male Rats

Dr. Ammar Abdulkareem Hadi [α] & Prof. Dr. Sabah N. Al-Thamir [σ]

Abstract - This study was aimed to evaluate the cardiotoxicity of cisplatin in rats and to investigate the cardioprotective effect of amlodipine on cisplatin treated rats by using cardiac biomarkers troponin, CK-MB. Thirty five healthy male swiss albino rats were used in this study. The study design was divided into two patterns:

Pilot study design: The animals were randomly divided into two groups, In the first treated group, all rats received cisplatin in a single dose, while in the second treated group, rats received cisplatin in four divided doses every 2 days.

Active study design: The animals were randomly divided into 3 groups (7 rats/ group) and treated as follows:- a- Normal saline (N.S) treated group. b- Cisplatin treated group. c- Amlodipine treated group with cisplatin. Blood samples were collected and used to determine the biomarkers serum troponin, CK-MB. The result from pilot study shows that in cisplatin treated groups (single dose) serum troponin, CK-MB are not significantly change with control group. While, when we give cisplatin in multiple doses, there is a significantly increase in serum troponin, CK-MB. Amlodipine show a potent cardioprorective effect against cisplatin cardiotoxicity.

Keywords : cisplatin, amlodipine, cardiotoxicity, troponin, CK-MB.

I. Introduction

Cardiotoxicity is the most feared adverse effect of anticancer therapy, due to the fact that life expectancy obtained as a result of the anticancer treatment, may be reduced by the death rate determined by cardiac problems arising as a consequence of the treatment[1] cisplatin is an antineoplastic drug widely used for the treatment of several human malignancies (as standard component of treatment regimens) including bladder cancer[2] cervical cancer[3], non-small cell lung cancer[4] ovarian cancer[5] squamous cell carcinoma of the head and neck[6] testicular cancer[7]. Nephrotoxicity of cisplatin was the main complication of cisplatin[8]. Earlier studies reported cardiotoxicity with cisplatin treatment[9]. Cisplatin cardiotoxicity can present in a number of ways. However, the most serious complication of the toxicity includes electrocardiographic changes, arrythmias, myocarditis, cardiomyopathy and congestive heart failure[10][11]. Several investigators hypothesized that the oxidative stress mechanism of cisplatin induced toxicity is related to:

a. Many studies found that rats treated with cisplatin show significant elevation in plasma, heart, kidney and liver thiobarbituric acid reactive substances (TBARS) while the activities of antioxidant enzymes (SOD and CAT) and the levels of glutathione (GSH) were decreased. [12]
b. Many report show that treatment of rats with cisplatin results in a significant increase in NO production in the cardiac tissues [10].

II. Subject and Methods

a) Animals

This study was carried out at animal house in college of medicine Babylon University in May 2012. A total of 35 adult male Albino Swiss rats aged 16 - 24 weeks with body weight of (170 – 255g) were used. The animals were obtained from Animal Resource Centre, College of Veterinary Medicine/ Baghdad University. The animals were apparently healthy and they were housed in individual cages at temperature controlled environment (25±5◦C) with an ambient humidity. Lights were maintained on a 12-h light/dark cycle. The rats received standard chow diet with water (ad libitum). Rats used in the study were maintained and handled in accordance with the Guide for the Care and Use of Laboratory Animals USA (1996).

The study design was divided into two patterns:

i. *Pilot study design*

After 4 weeks acclimatization period, the animals were randomly divided into 2 groups each of (7 rats/group) and treated as follows:

In the first treated group, all rats received cisplatin (10 mg/kg, i.p.) in a single dose, while in the second treated group, all rats received cisplatin (10mg/kg, i.p.) in four divided doses every 2 days.

ii. *Active study design*

After 4 weeks acclimatization period, the animals were randomly divided into 6 groups (7 rats/ group) and treated as follows:

Author α σ : Faculty of Pharmacy, Babylon University.

a. *Normal saline (N.S) treated group*

All rats of this group received normal saline (1ml/kg, orally) by oral gavages once daily for 14 days.

b. *Cisplatin treated group*

All rats of this group received cisplatin (10mg/kg, i.p.) in 4 divided doses.

c. *Amlodipine treated group (5mg/kg) plus cisplatin*

All rats of this group were given amlodipine (5mg/kg, orally) by oral gavages once daily for 14 days before and during cisplatin (10mg/kg, i.p.) injection regimen.

b) Sample collection and preparation

After 24hr from the last injection of any treatment, the rats were anesthetized with phenobarbital (50mg/kg) subcutaneously. Blood samples (3ml-5ml) were obtained from each rat by an intra cardiac puncture[18]. Each blood sample was placed in a plain tube and left for 15 - 20 minutes at room temperature for promote blood coagulation. Serum was obtained after centrifugation at 3000 rpm for 10 minutes and preserved at -20 °C until the determination of serum troponin I, CK-MB.

c) Statistical analysis of data

Statistical analyses were performed using SPSS version 18[19] computer program. Independent sample t test was used to compare means between two groups. Data are expressed as means ± standard deviation (M±SD). The ($p<0.05$) level of probability was chosen as a criterion for the lowest level of significance.

III. Results

a) The effect of cisplatin (10mg/kg, i.p. single dose) on rats serum troponin concentration

The administration of cisplatin (10mg/kg, i.p. in single dose) showed no significant increase in serum troponin concentration of treated rats (0.063μg/l ± 0.005) when compared with that of the control group (0.05μg/l ± 0.005).

b) The effect of cisplatin (10mg/kg, i.p. in 4 divided doses) on rats serum troponin concentration

The administration of cisplatin (10mg/kg, i.p. in 4 divided doses) significantly ($p<0.001$) increased serum troponin concentration of treated rats (1.49μg/l ± 0.1) when compared with that of the control group (0.05μg/l ± 0.005), figure 1.

c) The effect of amlodipine (5mg/kg, orally) on cisplatin treated (10mg/kg, i.p in 4 divided doses) rats serum troponin concentration

The administration of amlodipine in a dose (5mg/kg, orally) once daily for 2 weeks before and during cisplatin (10mg/kg, i.p.) administration, significantly ($p<0.001$) reduced serum troponin concentration of treated rats (0.09μg/l ± 0.04) when compared with cisplatin treated groups (1.49μg/l ± 0.1) figure 2.

d) The effect of cisplatin (10mg/kg, i.p. single dose) on rats serum CK-MB concentration

The administration of cisplatin (10mg/kg, i.p., single dose) showed no significant increase in serum CK-MB concentration of treated rats (26.48 IU/l ± 1.13) when compared with the control group (23.36 ± 1.89 IU/l).

e) The effect of cisplatin (10mg/kg, i.p. in 4 divided doses) on rats serum CK-MB concentration

The administration of cisplatin (10mg/kg, i.p. in 4 divided doses) significantly ($p<0.001$) increased serum CK-MB concentration of treated rats (98.26l U/l ± 5.15) when compared with the control group (23.36 ± 1.89IU/l), figure 3.

f) The effect of amlodipine (5mg/kg orally) on cisplatin treated (10mg/kg, i.p in4 divided doses) rats serum CK-MB concentration

The administration of amlodipine in a dose (5mg/kg, orally) once daily for 2weeks before and during cisplatin (10mg/kg, i.p.) administration, significantly ($p<0.001$) reduced serum CK-MB concentration of cisplatin treated rats (29.06IU/l ± 2.2) when compared with cisplatin treated groups (98.26IU/l ± 5.15) figure 4.

IV. Discussion

Renal toxicity of cisplatin was insured by many authors such as[20][21]. The main mechanism behind this selective organ toxicity is the generation of free radicals such as (¬ O2.-, HO., NO) which in turn damaged the renal tissues. However, cisplatin cardiotoxicity was rarely indicated. In our pilot study, we follow cisplatin induced toxicity as it was introduced by[22]. Our results showed a high mortality rate due to renal toxicity rather than cardio-toxicity as indicated by the normal levels of cardio-selective markers (CK-MB and Troponin) unlike the results of[22]. From the results presented in this study, we can confirm the resistibility of cardiac tissues to the free radical generating property of cisplatin when administered in a high dose/ single shoot. This cardiac resistibility was not insured when the drug cisplatin administered in a low dose but with more frequency and duration. These results are consistent with studies presented by[22][23][10], although they used a different protocol in the dose, frequency of administration and the duration (10 mg /kg, 7 mg /kg, 7 mg /kg i.p, all in single dose) respectively.

It seems obviously, that the oxidative stress plays an important role in the mediation of cardiotoxicity and this in return would influence the levels of serum cardiac markers. This fact had been proven by many

worker such as [22], [23], [10]. The proposed mechanism of induced cardiotoxicity of cisplatin could be explained as in the following: During the physiological process, the mitochondrial respiratory chain continuously generates ROS. Approximately 2% of the electrons which flow along the respiratory chain escape from the chain and partially reduces molecular oxygen, originating superoxide anion (O2−•). Superoxide anion, the precursor of most of the reactive oxygen species generated in mitochondria as for example hydroxyl radicals HO[24][25]. An efficient mitochondrial antioxidant defense system maintains the balance between ROS generation and detoxification. Cisplatin unbalances the oxidant–antioxidant ratio by (i) Augmenting ROS generation, mainly hydroxyl radical and (ii) Inhibition of the antioxidant defense system which are SOD, CAT and GSH[26])[27]. These radicals can evoke extensive tissue damage, reacting with membrane lipids, proteins and nucleic acids. This will lead sequencelly to an increase in leakage of cardiac enzymes such as CK-MB and troponin I, which were released from damaged myocytes and considered as sensitive indicators of cardiac injury[24][28]. Also, when cisplatin generates reactive oxygen species, it triggers the opening of the mitochondrial permeability transition pore that permits the release of cytochrome c from mitochondria to cytosol and hence it will activate the mitochondrial dependent pathway leading to apoptosis[29][30]. Additionally, once in a cell, cisplatin is equated into a highly reactive form, which can rapidly react with the thiol-containing molecules namely glutathione. Depletion of glutathione and related antioxidants by cisplatin shifts the cellular redox status, leading to the accumulation of endogenous reactive oxygen species within the cells[31]. The decline in GSH level in cisplatin-treated rat resulted in an enhanced lipid peroxidation which is supported by an increment in MDA could be another pathway for the cardiac cells damage[32].

The results of this study confirm the protective activity of the calcium channel blocker ; amlodipine (5mg/kg, orally) as indicated by the levels of studied serum cardiac biomarkers In fact, the protective effect of amlodipine can be explained according to its antioxidant property which was previously provoked by[33][34]. Amlodipine antioxidant activity could be related to its endogenous property as a dihydropyridine compound (physicochemical properties) which has a reductant nature or hydrogen donor properties, respectively – The ability of donating protons and electrons to the lipid peroxide molecules, thereby blocking the peroxidation process[33]. Also, the antioxidant activity of amlodipine was attributed to both of its high lipophilicity and a chemical structure that facilitates proton-donating and resonance-stabilization mechanisms that turn off the free radical reaction[34].

V. Conclusion

1. Low doses of cisplatin with more frequency and duration can induce cardiotoxicity rather than high dose/single shoot.
2. Oxidative stress has a role in cisplatin induced cardiotoxicity.
3. The protective effect of amlodipine is evident by the significant reduction in serum troponin, CK-MB.

References Références Referencias

1. Rădulescu, M. L., Duncea, C.L., Rădulescu, D., Militaru, V., and Pârv, A. (2011); Cardiotoxicity of antineoplasic agents; onset, risk factors and clinical manifestation, Journal HVM Bioflux. 3(2): 150-157.
2. Coppin, C.M., Gospodarowicz, M.K., James, K., Tannock, I.F., Zee, B., Carson, J., Peter, J. and Sullivan,L. D. (1996); Improved local control of invasive bladder cancer by concurrent cisplatin and preoperative or definitive radiation. J. Clinical Oncology. 14(11): 2901-2907.
3. Rose, P.G., Bundy, B.N., Watkins, E.B., Thigpen, J.T., Deppe, G., Maiman, M.A., Clarke-Pearson, D.L. and Insalaco, S. (1999); Concurrent cisplatin-based radiotherapy and chemotherapy for locally advanced cervical cancer.The new. Engl. J. Med. 340(15): 1144-1153.
4. Gatzemeier, U., Von Pawel, J., Gottfried,M., Ten Velde, G., Mattson, K., DeMarinis, F., Harper, P., Salvati, F., Robinet, G., Lucenti, A., Bogarerts, J. and Gallant, G. (2000); Phase III comparative study of high-dose cisplatin versus a combination of paclitaxel and cisplatin in patients with advanced non-smallcell lung cancer. J. Clinic. Oncol. 18(19): 3390 - 3399.
5. Hoskins, P., Eisenhauer, E., Vergote, I., Dubuc-Lissoir, J., Fisher, B., Grimshaw, R., Oza, A., Plante, M., Stuart, G. and Vermorken, J. (2000); Phase II feasibility study of sequential couplets of Cisplatin/Topotecan followed by paciltaxel/cisplatin as primary treatment of advanced epithelial ovarian cancer: American Society of Clinical Oncology 18(24): 4038-4044.
6. Planting, A.S.T., Catimel, G., de Mulder, P.H.M., de Graeff, A., Hoppener, F., Verweij, J. Oster, W. and Vermorken, J.B. (1999); Randomized study of a short course of weekly cisplatin with or without amifostine in advanced head and neck cancer. Ann. Oncol.10 (6): 693–700.
7. Loehrer, P. J., Gonin, R., Nichols, C. R., Weathers, T. and Einhorn, L. H. (1998); Vinblastine plus ifosphamide plus cisplatin as initial salvage therapy in recurrent germ cell tumor. J. Clin. Oncol.: 16(7): 2500-2504.

8. Van-Hoff, D. D., Schilsky, R. and Reichert, C. M. (1979); Toxic effects of cis-dichlorodiammine platinum in man. Cancer Treat. Rep. 63: 1527–1531.
9. Florea, A.,and , Büsselberg, D.(2011); Cisplatin as an Anti-Tumor Drug: Cellular Mechanisms of Activity, Drug Resistance and Induced Side Effects. Cancers: 3(1) 1351-1371.
10. Al-Majed, A.A., Sayed-Ahmed, M.M., Al-Yahya, A.A., Aleisa, M.A., Al-Rejaie, S.S., and Al- Shabanah, O.A. (2006); Propionyl-L-carnitine prevents the progression of cisplatin induced cardiomyopathy in a carnitine-depleted rat model. Pharmacol. Res. 53: 278–286.
11. Shanmugasundaram, S., Bharathithasan, R., and Elangovan, S. (2002);5-fluorouracil induced cardiotoxicity. Indian Heart J.54:86–97.
12. Yousef, M.I., Saad, A.A., and El-Shennawy, L.K. (2009); Protective effect of grape seed proanthocyanidin extract against oxidative stress induced by cisplatin in rats. Food and Chemical Toxicology. 47: 1176–1183.
13. Weijl, N. I., Hopman, G. D., Wipkink-Bakker, A., Lentjes, E. G., Berger, H. M., Cleton, F. J., and Osanto, S. (1998); Cisplatin combination chemotherapy induces a fall in plasma antioxidants of cancer patients. Ann. Oncol. 9: 1331-1337.
14. Silva, C. R., Greggi Antunes, L. M. and Bianchi, M. L. (2001); Antioxidant action of bixin against cisplatin-induced chromosome aberrations and lipid peroxidation in rats. Pharmacol. Res. 43: 561-566.
15. Linds, D. S., Kontaridis, M. I., Edwards, P. D., Josephs, M. D., Moldawer, L. L. and Copeland, E. M. (1997); Nitric oxide contributes to adriamycin's antitumor effect. J. Surg. Res.: 69: 283-287.
16. Ischiropoulos, H., Zhu, L. and Beckman, J. S. (1992); Peroxynitrite formation from macrophage-derived nitric oxide. Arch. Biochem. Biophys.298: 446-451.
17. Radi, R., Beckman, J. S., Bush, K. M. and Freeman, B. A. (1991); Peroxynitrite oxidation of sulfhydryls. The cytosolic potential of superoxide and nitric oxide. J. Biol. Chem. 266: 4244-4250.
18. Hong, M. E., Hong, J. C., Stepkowski, S. and Kahan, B. D. (2005); Correlation between cyclosporine-induced nephrotoxicity in reduced nephron mass and expression of kidney injury molecule-1 and aquaporin-2 gene. Transplant. Proc. 37(10): 4254 -4258.
19. GrraphPad, ISI software, Philadelphia, PA, USA, 1993.
20. Van-Hoff, D. D., Schilsky, R. and Reichert, C. M. (1979); Toxic effects of cis-dichlorodiammine platinum in man. Cancer Treat. Rep. 63: 1527–1531.
21. Sastry, J. and Kellie, S. J. (2005); Severe neurotoxicity, ototoxicity and nephrotoxicity following high-dose cisplatin and amifostine. Pediatr. Hematol. Oncol. 22: 441-445.
22. ElAwady, E.E., Moustafa,Y.M., Abo-Elmatty,D.,M., and Radwan A.(2011); Cisplatin-induced cardiotoxicity: Mechanisms and cardioprotective strategies. Eur. J. Pharma. 650: 335–341.
23. Wang, J., He, D., Zhang, Q., Han, Y., Jin, S.,and Qi, F.(2009); Resveratrol Protects Against Cisplatin-Induced Cardiotoxicity by Alleviating Oxidative Damage: Cancer biotherapy and radio pharmaceuticals. 6:675-681.
24. Conklin, K.A., and Nicolson, G.L. (2008); Molecular replacement in cancer therapy: reversing cancer metabolic and mitochondrial dysfunction, fatigue and the adverse effects of cancer therapy. Curr. Cancer Ther. Rev. 4: 66–76.
25. Fariss, M.W., Chan, C.B, Patel, M., Van Houten, B., and Orrenius, S. (2005); Role of mitochondria in toxic oxidative stress, Mol. Interv. 5 (2): 94–111.
26. Santos, N.A. , Bezerra, C.S. , Martins, N.M. , Curti, C. , Bianchi, M.L. , and Santos, A.C.(2008); Hydroxyl radical scavenger ameliorates cisplatin-induced nephrotoxicity by preventing oxidative stress, redox state unbalance, impairment of energetic metabolism and apoptosis in rat kidney mitochondria, Cancer hemother. Pharmacol. 61 (1):145-155.
27. Santos, N.A., Bezerra, C.S., Martins, N.M., Curti, C., Bianchi, M.L., and Santos, A.C (2007); Cisplatin-induced nephrotoxicity is associated with oxidative stress, redox state unbalance, impairment of energetic metabolism and apoptosis in ratkidney mitochondria, Arch. Toxicol. 81 (7): 495–504.
28. Satoh, M., Kashihara, N., Fujimoto, S., Horike, H., Tokura, T., Namikoshi, T., Sasaki, T., and Makino, H.(2003); A novel free radical scavenger, edarabone, protects againstcisplatin-induced acute renal damage in vitro and in vivo. J. Pharmacol. Exp. Ther.305: 1183–1190.
29. Kim, J.S., He, L., and Lemasters, J.J. (2003); Mitochondrial permeability transition: a common pathway to necrosis and apoptosis. Biochem. Biophys. Res. Commun. 304: 463–470.
30. Kroemer, G., and Reed, J.C., (2000). Mitochondrial control of cell death. Nat. Med. 6: 513–519.
31. Arany, I., and Safirstein, R.L. (2003); Cisplatin nephrotoxicity. Semin. Nephrol. 23: 460–464.
32. Karthikeyan, K., Bai, B.R., and Devaraj, S.N. (2007); Cardioprotective effect of grape seed proanthocyanidins on isoproterenol-induced myocardial injury in rats. Int. J. Cardiol. 115(3): 326–333.
33. Begum,S., and Akhter, N.(2007); Cardioprotective effect of amlodipine in oxidative stress induced by experimental myocardial infarction in rats. Bangladesh J Pharmacol. 2: 55-60.

34. Mason, R.P., Walter, M., F., Trumbore, M., W., Olmstead Jr, E., G., Mason P.,E.(1999); Emerging approaches in preventing cardiovascular disease. J. M. and C. Cardiology, 31(1): 275-281.

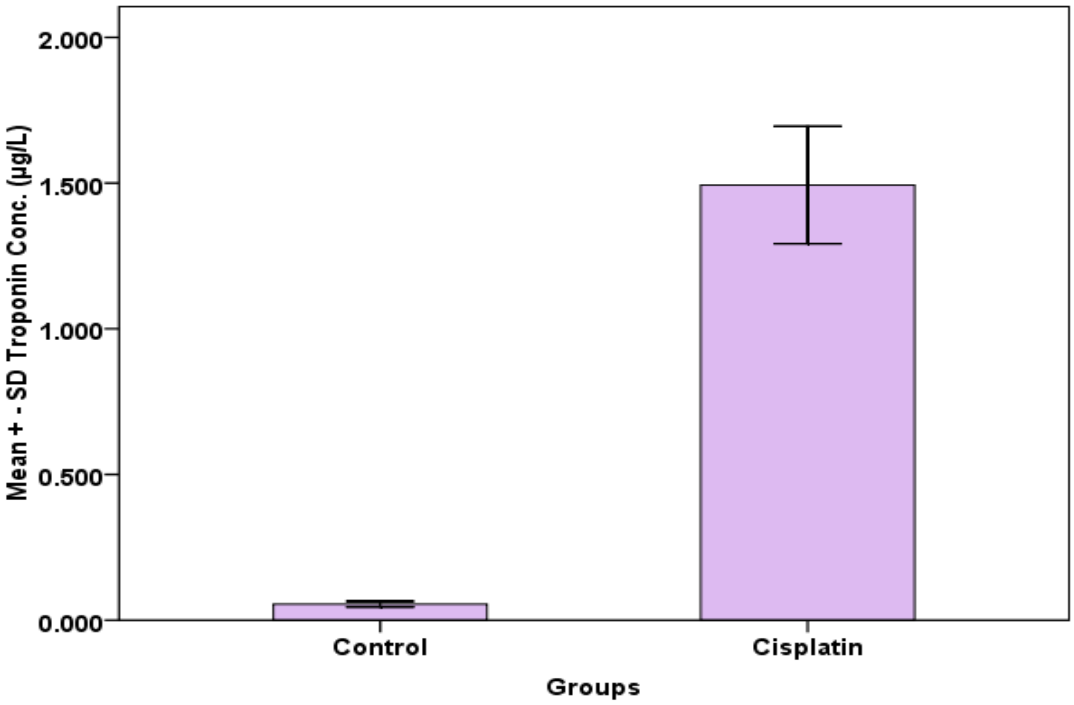


Figure 1 : The effect of cisplatin (10mg/kg, i.p. in 4 divided doses) on rats serum troponin concentration ($p < 0.001$)

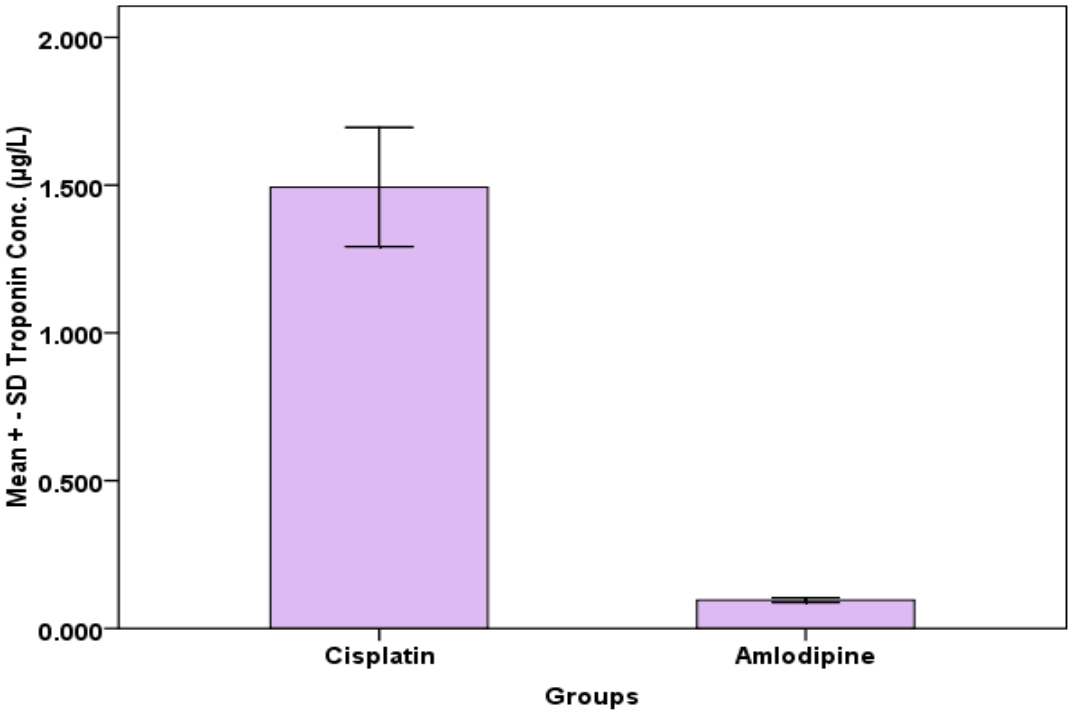


Figure 2 : The effect of amlodipine (5mg/kg, orally 2weeks before and during cisplatin administration) on rats serum troponin concentration ($p < 0.001$ versus cisplatin treated groups)

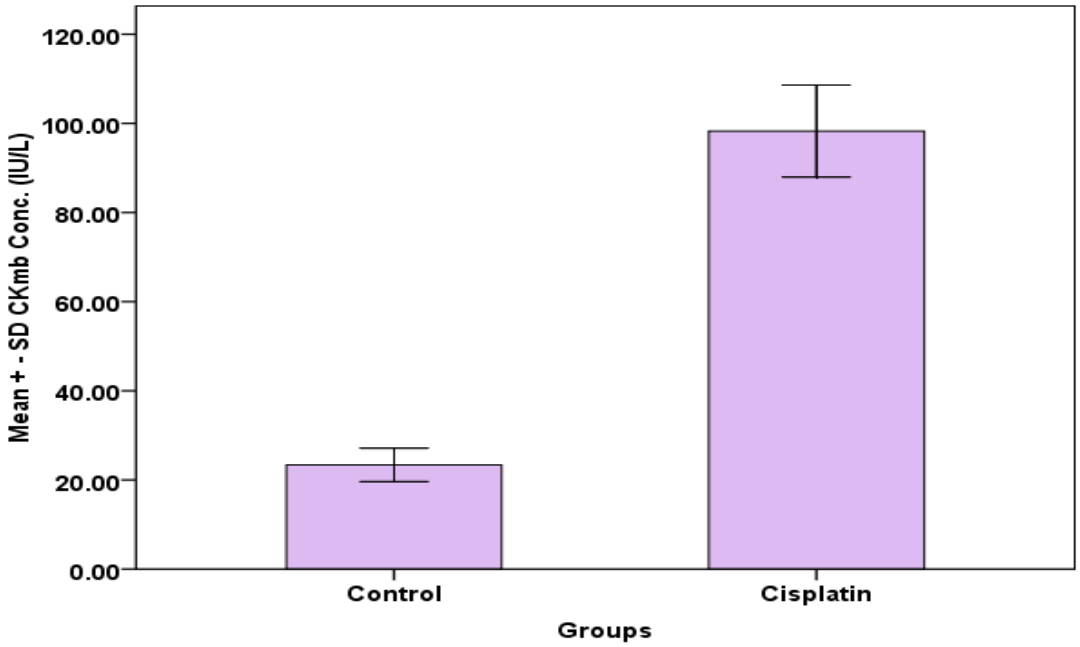


Figure 3 : The effect of cisplatin (10mg/kg, i.p. in 4 divided doses) on rats serum CK-MB concentration ($p < 0.001$ versus control groups)

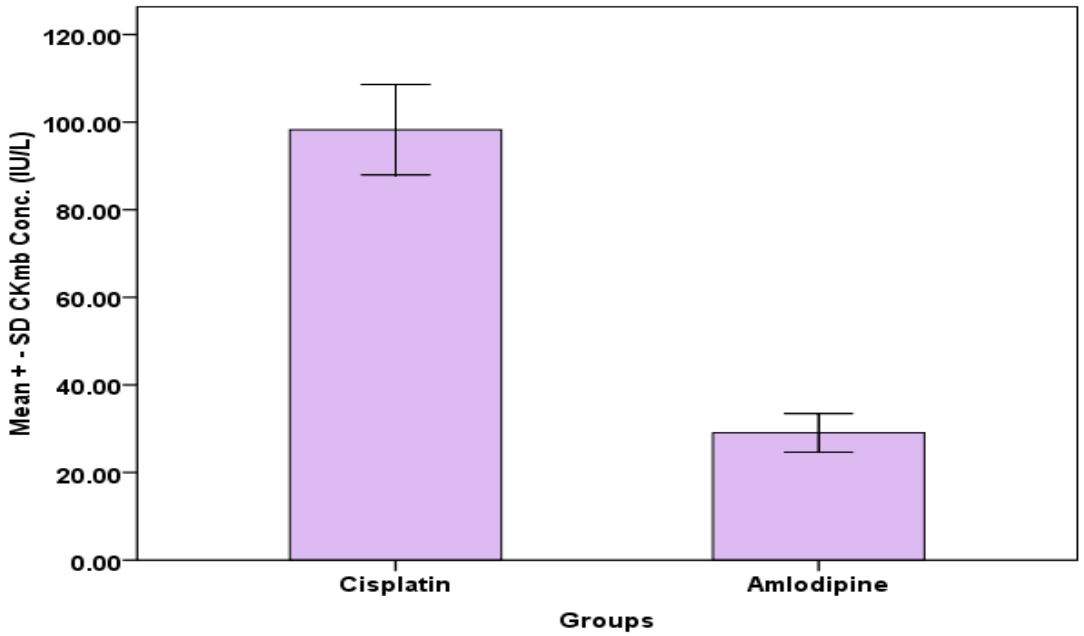


Figure 4 : The effect of amlodipine (5mg/kg, orally 2weeks before and during cisplatin administration), on rats serum CK-MB concentration ($p < 0.001$ versus cisplatin treated groups)

Global Journals Inc. (US) Guidelines Handbook 2013

www.GlobalJournals.org

FELLOW OF ASSOCIATION OF RESEARCH SOCIETY IN MEDICAL (FARSM)

- 'FARSM' title will be awarded to the person after approval of Editor-in-Chief and Editorial Board. The title 'FARSM" can be added to name in the following manner. eg. Dr. John E. Hall, Ph.D., FARSM or William Walldroff Ph. D., M.S., FARSM
- Being FARSM is a respectful honor. It authenticates your research activities. After becoming FARSM, you can use 'FARSM' title as you use your degree in suffix of your name. This will definitely will enhance and add up your name. You can use it on your Career Counseling Materials/CV/Resume/Visiting Card/Name Plate etc.
- 60% Discount will be provided to FARSM members for publishing research papers in Global Journals Inc., if our Editorial Board and Peer Reviewers accept the paper. For the life time, if you are author/co-author of any paper bill sent to you will automatically be discounted one by 60%
- FARSM will be given a renowned, secure, free professional email address with 100 GB of space eg.johnhall@globaljournals.org. You will be facilitated with Webmail, SpamAssassin, Email Forwarders, Auto-Responders, Email Delivery Route tracing, etc.
- FARSM member is eligible to become paid peer reviewer at Global Journals Inc. to earn up to 15% of realized author charges taken from author of respective paper. After reviewing 5 or more papers you can request to transfer the amount to your bank account or to your PayPal account.
- Eg. If we had taken 420 USD from author, we can send 63 USD to your account.
- FARSM member can apply for free approval, grading and certification of some of their Educational and Institutional Degrees from Global Journals Inc. (US) and Open Association of Research,Society U.S.A.
- After you are FARSM. You can send us scanned copy of all of your documents. We will verify, grade and certify them within a month. It will be based on your academic records, quality of research papers published by you, and 50 more criteria. This is beneficial for your job interviews as recruiting organization need not just rely on you for authenticity and your unknown qualities, you would have authentic ranks of all of your documents. Our scale is unique worldwide.
- FARSM member can proceed to get benefits of free research podcasting in Global Research Radio with their research documents, slides and online movies.
- After your publication anywhere in the world, you can upload you research paper with your recorded voice or you can use our professional RJs to record your paper their voice. We can also stream your conference videos and display your slides online.
- FARSM will be eligible for free application of Standardization of their Researches by Open Scientific Standards. Standardization is next step and level after publishing in a journal. A team

of research and professional will work with you to take your research to its next level, which is worldwide open standardization.

- FARSM is eligible to earn from their researches: While publishing his paper with Global Journals Inc. (US), FARSM can decide whether he/she would like to publish his/her research in closed manner. When readers will buy that individual research paper for reading, 80% of its earning by Global Journals Inc. (US) will be transferred to FARSM member's bank account after certain threshold balance. There is no time limit for collection. FARSM member can decide its price and we can help in decision.

MEMBER OF ASSOCIATION OF RESEARCH SOCIETY IN MEDICAL (MARSM)

- 'MARSM' title will be awarded to the person after approval of Editor-in-Chief and Editorial Board. The title 'MARSM" can be added to name in the following manner. eg. Dr. John E. Hall, Ph.D., MARSM or William Walldroff Ph. D., M.S., MARSM
- Being MARSM is a respectful honor. It authenticates your research activities. After becoming MARSM, you can use 'MARSM' title as you use your degree in suffix of your name. This will definitely will enhance and add up your name. You can use it on your Career Counseling Materials/CV/Resume/Visiting Card/Name Plate etc.
- 40% Discount will be provided to MARSM members for publishing research papers in Global Journals Inc., if our Editorial Board and Peer Reviewers accept the paper. For the life time, if you are author/co-author of any paper bill sent to you will automatically be discounted one by 60%
- MARSM will be given a renowned, secure, free professional email address with 30 GB of space eg.johnhall@globaljournals.org. You will be facilitated with Webmail, SpamAssassin, Email Forwarders, Auto-Responders, Email Delivery Route tracing, etc.
- MARSM member is eligible to become paid peer reviewer at Global Journals Inc. to earn up to 10% of realized author charges taken from author of respective paper. After reviewing 5 or more papers you can request to transfer the amount to your bank account or to your PayPal account.
- MARSM member can apply for free approval, grading and certification of some of their Educational and Institutional Degrees from Global Journals Inc. (US) and Open Association of Research,Society U.S.A.
- MARSM is eligible to earn from their researches: While publishing his paper with Global Journals Inc. (US), MARSM can decide whether he/she would like to publish his/her research in closed manner. When readers will buy that individual research paper for reading, 40% of its earning by Global Journals Inc. (US) will be transferred to MARSM member's bank account after certain threshold balance. There is no time limit for collection. MARSM member can decide its price and we can help in decision.

AUXILIARY MEMBERSHIPS

ANNUAL MEMBER

- Annual Member will be authorized to receive e-Journal GJMR for one year (subscription for one year).
- The member will be allotted free 1 GB Web-space along with subDomain to contribute and participate in our activities.
- A professional email address will be allotted free 500 MB email space.

PAPER PUBLICATION

- The members can publish paper once. The paper will be sent to two-peer reviewer. The paper will be published after the acceptance of peer reviewers and Editorial Board.

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 convenient, 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.

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 .

You must strictly follow above Author Guidelines before submitting your paper or else we will not at all be responsible for any corrections in future in any of the way.

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.

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

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

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

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

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

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

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

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 09755888