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

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Volume 13 Issue 3 was originally issued as K2

Global Journal of Medical Research: K
Interdisciplinary

Volume 13 Issue 3
Originally issued as K2

Global Journal of Medical Research: K Interdisciplinary

Volume 13 Issue 2 (Ver. 1.0)

Open Association of Research Society

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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*



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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),

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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.

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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

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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.

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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

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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.

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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

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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*



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

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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.

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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.

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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.

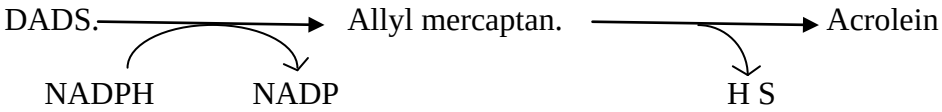
The observed in the present study clearly established that DPDS, a saturated, water soluble, well tolerated disulphide has a significant comparable hypolipidemic, hypocholesterolemic and antiatherosclerotic actions in atherogenic diet fed rats (ref. table. 1, 2 & fig 1-6).

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

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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.

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

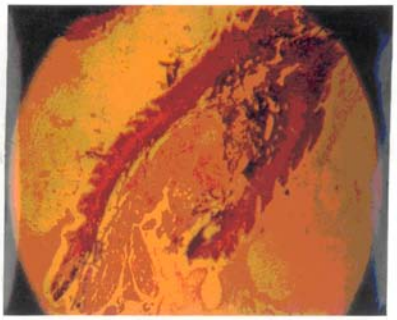


Fig 2 : Control Group (atherosclerotic) : Shows features of atherosclerosis/atheroma with fat deposition. (H & E, x 320)

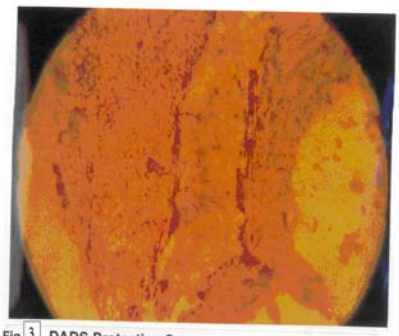


Fig 3 DADS Protective Group : Shows empty fat spaces with blood vessels and few cholesterol clefts and occasionally inflammatory cells. (H & E, x 320)

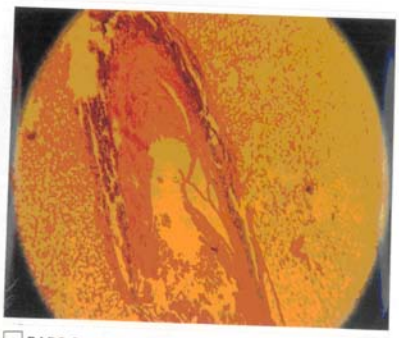


Fig 4 DADS Curative Group : Shows aorta with few cholesterol clefts and fat deposition (H & E , x 320)

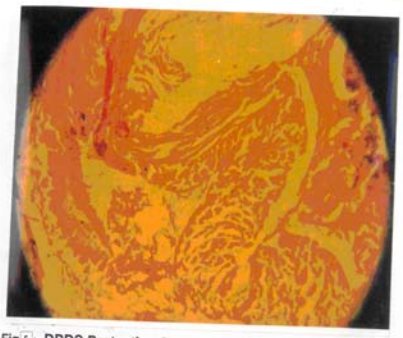


Fig 5 : DPDS Protective Group : Shows normal aorta with mild atherosclerotic changes (H & E, x 320)

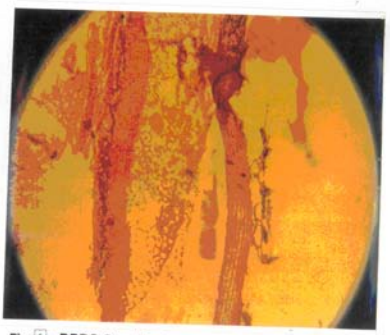


Fig 6 : DPDS Curative Group : Shows wall of the aorta with fatty infiltration (H & E, x 320)

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***

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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.

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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

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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



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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

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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.

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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

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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.

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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

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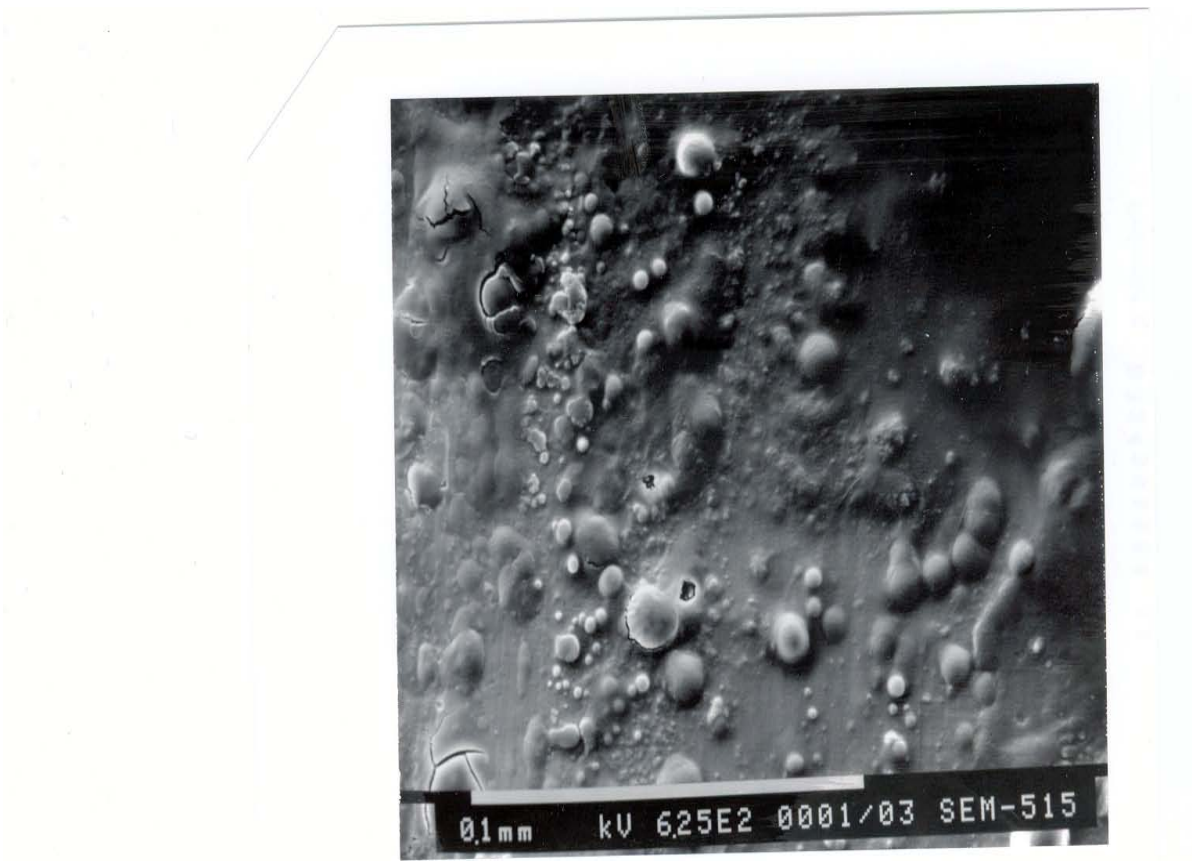
Table 3 : Quantitative Analysis of DNA in DOTAP:Cholesterol Liposome Complexes with DNA Using Ultra Violet Spectrophotometer

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

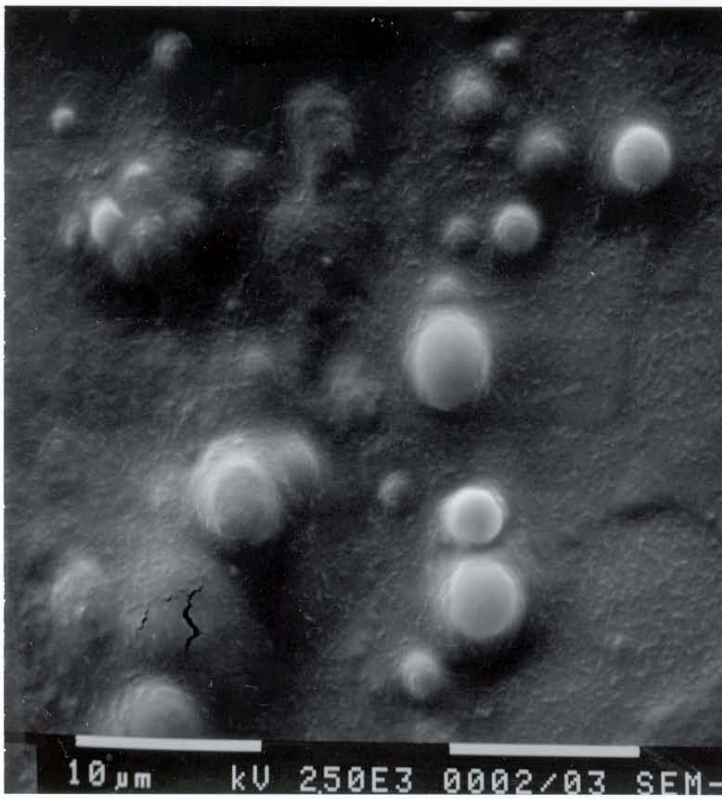
Samples	Initial Quantity (ug)	Mean Final Quantity in ug (n=3)	Percent Efficiency
Free DNA	1.50	1.15	76.5
Free DNA Treated with DNase	1.50	0.00	0
DC-Chol:DOPE Liposome Complexes with DNA	1.50	0.042	70.2
DC-Chol:DOPE Liposome Complexes with DNA and Treated with DNase	1.50	0.033	55.2

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



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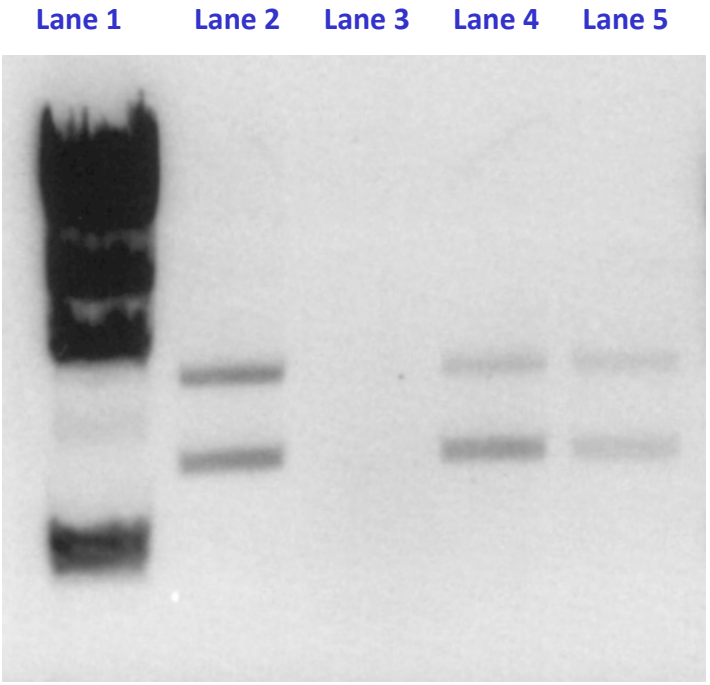
Figure 2 : Scanning Electron Microscope for DOTAP:Cholesterol Liposome Complexes with DNA at High Magnification



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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



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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

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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*



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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:

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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

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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.

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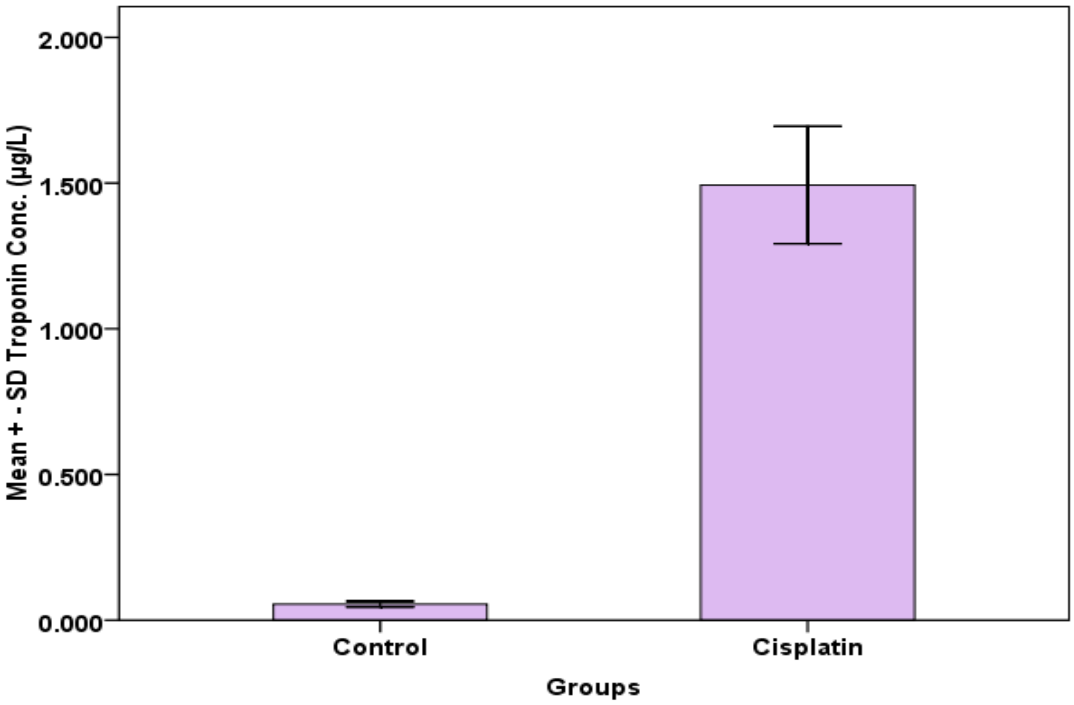


Figure 1 : The effect of cisplatin (10mg/kg, i.p. in 4 divided doses) on rats serum troponin concentration ($p < 0.001$)

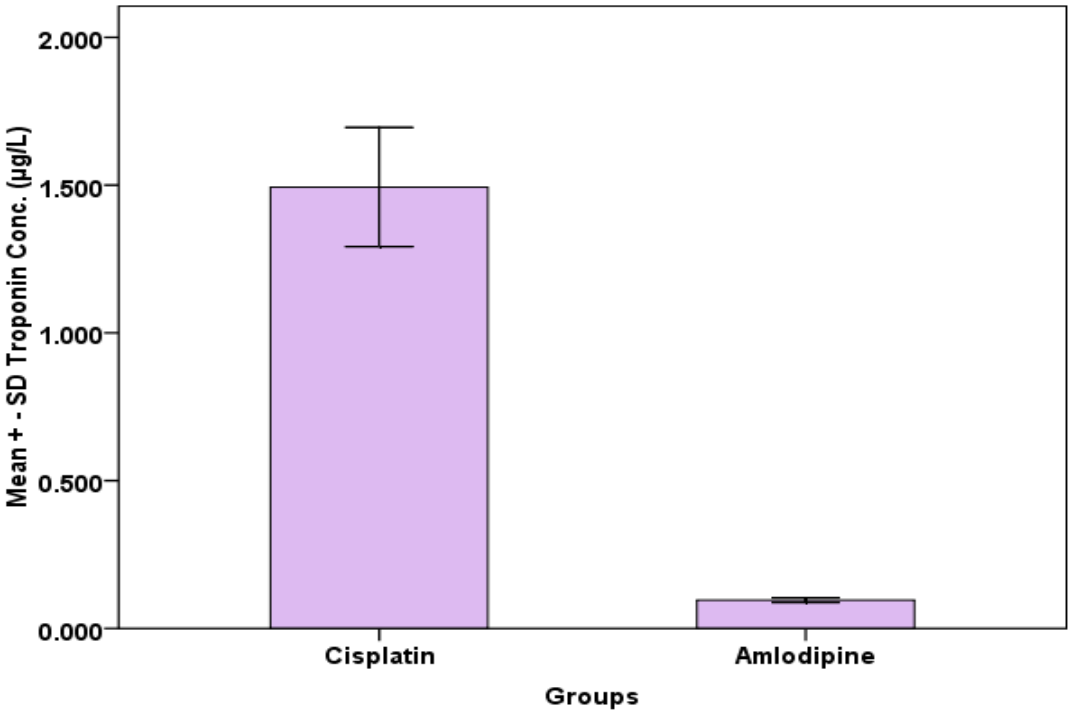


Figure 2 : The effect of amlodipine (5mg/kg, orally 2weeks before and during cisplatin administration) on rats serum troponin concentration ($p < 0.001$ versus cisplatin treated groups)

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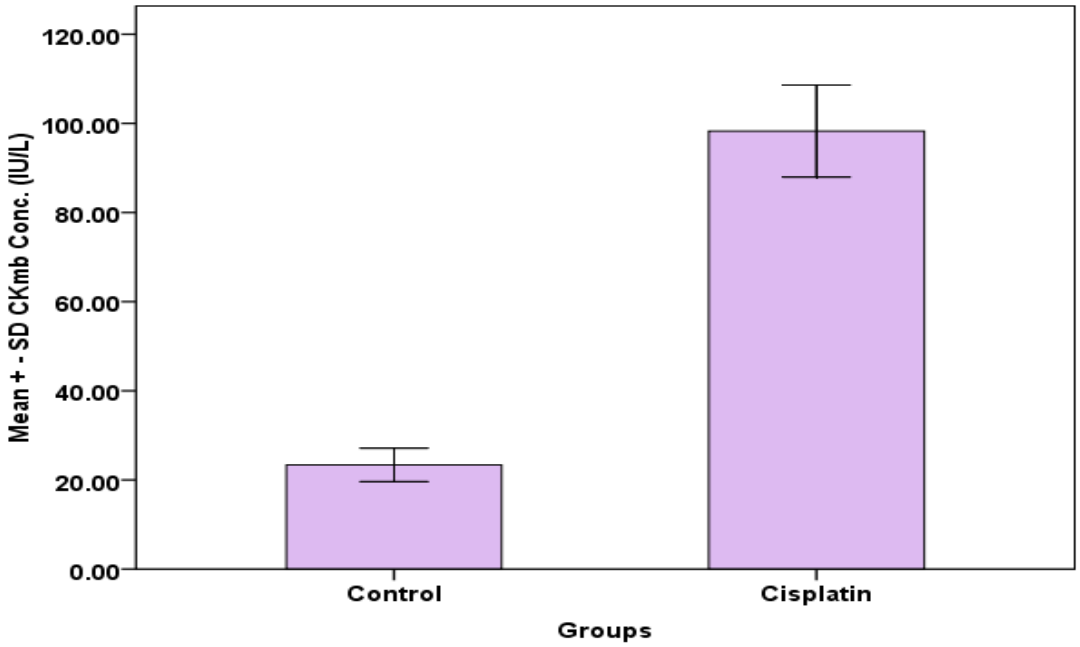


Figure 3 : The effect of cisplatin (10mg/kg, i.p. in 4 divided doses) on rats serum CK-MB concentration ($p < 0.001$ versus control groups)

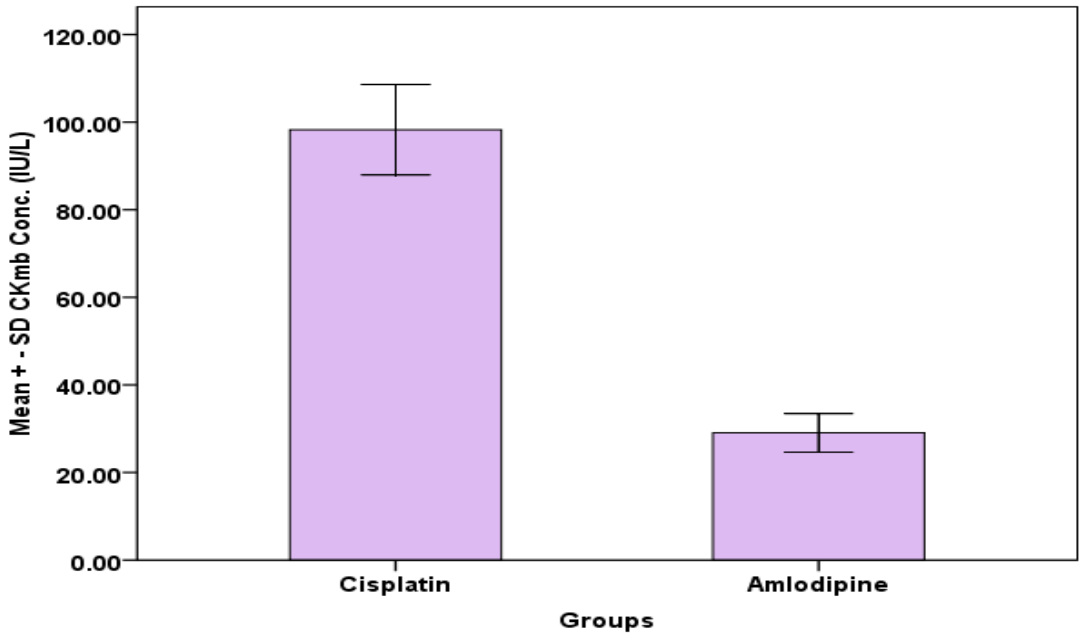


Figure 4 : The effect of amlodipine (5mg/kg, orally 2weeks before and during cisplatin administration), on rats serum CK-MB concentration ($p < 0.001$ versus cisplatin treated groups)

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Content

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References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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ISSN 09755888

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