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Effect of Carmoisineorally Administered on Lipid Parameters of Albino Rats

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Abstract: ***In this study, the effect of carmoisine on lipid parameters of albino rats was evaluated. Thirty (30) rats weighing approximately 0.14kg were used. The rats were divided into five (5) groups of six (6) rats per group. Group 1, 2, 3, 4 and 5 were treated daily with 1.0ml of 0.0% (normal saline (0.00g/kg), 1.0% (0.07g/kg), 1.5% (0.11g/kg), 2.0% (0.14g/kg) and 2.5% (0.18g/kg) respectively. Lipid parameters such as TC, TG, HDL-C and LDL-C were evaluated. Data obtained were analysed using Graphpad prism 5.1 with statistical significance observed at P <0.05. The following results were obtained, Group 1 had 1.408± 0.002, 1.827±0.114, 1.143±0.159 and 0.0441±0.030 for TG, TC, HDL and LDL-c respectively. Group 2 had 1.440± 0.113, 2.233±0.819, 1.319±0.2626 and 1.248±0.733for TG, TC, HDL and LDL-C respectively. Group 3 had 1.464 ±0.173, 1.788 ± 0.280, 0.883±0.217 and 0.2319 ±0.176 for TG, TC, HDL and LDL-c respectively. Group 4 had 1.435± 0.025, 1.786±0.050, 0.934±0.122 and 0.1993±0.0951 for TG, TC, HDL and LDL-C respectively. Finally, group 5 had 1.429±0.135, 1.616±0.078, 0.554±0.163 and 0.4116±0.2169 for TG, TC, HDL and LDL-C respectively. The comparison between group 1 and group 2 and group 1 and group 3 showed that TG, TC, HDL and LDL-C were not significant except HDL-C and LDL-C was significant in group 1 vs group 3 (p<0.05). The comparison between group 1 and group 4 (table 4.3) showed that TG, HDL and LDL-C were significant (p<0.05) except TC. Finally, the comparison between group 1 and group 5 showed significant increase in TG, TC HDL-C and LDL-C at p<0.05). The result revealed that oral administration of carmoisine have effect on the lipid parameters of albino rats such as TG, TC, HDL, and LDL-C placing the system at the risk of developing cardiovascular diseases.***

Keywords: Carmoisine, cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol

1. Introduction

Lipid parameters such as total cholesterol (TC), Triglycerides (TG), High Density Lipoprotein (HDL-C) and low density lipoprotein (LDL-C) are important biochemical analytes that can serve as screening tool for abnormalities in lipids and may help in disease diagnosis such as cardiovascular diseases[1][2][3]. The term lipids refer to a category of biochemical substances that dissolves in organic solvent but are insoluble in water and chemically they contains primarily of non-polar carbon-hydrogen moiety which produces fatty acids and/or complex alcohols after hydrolysis[2][3]. It was also explained that some lipids also contain water-soluble moieties such as salic acids, phosphoryl, amino, sulfuryl and hydroxyl moieties which allows them to exist in aqueous interface of biological membrane[2][3]. Overall, lipids are divided into six categories depending on their chemical constituents namely cholesterol, fatty acids, acyl glycerol, shingolipids, prostaglandins and terpenes [2]. Lipids are important in serving as hormones, energy source and structural components in cell membranes [3][4]. For lipids to function effectively, they must be transported in the plasma binding to specific carrier proteins called lipoproteins [2][3]. Lipoproteins include chylomicrons, Very Low Density Lipoprotein (VLDL), Low Density Lipoprotein-cholesterol (LDL-C) and High Density Lipoprotein-cholesterol (HDL-C).Cholesterol is the most vital sterol in human system. It is found in almost all parts of the human body especially in endocrine glands [2]. Several substances such as drugs and chemical has been reported to affects lipid levels. As reported by [5], that oral administration of carmoisine affected the lipid metabolism with a resultant increase in cholesterol level. Triglyceride level aids in establishing the degree of risk for coronary artery disease in patients [3]. However, lipids and lipoproteins are intimately involved in the development of atherosclerosis, a pathogenic process that underlies the causes of the common cardiovascular disorder such as Myocardial infarction, cerebrovascular diseases and Peripheral vascular disease [2][3]. The aim of this work is to evaluate the effect of carmoisine food dye on lipid parameters such as TC, TG, HDL-C and LDL-C of albino rats orally administered.

Carmoisine is an azo dyes(benzene-like structure) derived from coal-tar, which imply that it is a compound where two hydrocarbon groups are linked by two nitrogen atoms [6][7][8].Azo dyes accounts for about 60 – 80% of all synthetic dyes used both commercially and domestically for cooking and production of cosmetics, rubber, leather, plastics and drugs respectively. Generally, food dyes are added to food materials not just to improve or develop color but also with the aim of improving food savor, taste, value, texture and conservation [8]. However, inasmuch as carmoisine have beneficial attributes, they can be harmful or toxic if misused, that is, when consumed excessively exceeding the acceptable daily intake of 0-4.0mg/kg body weight[9].The side effect ranges from organ damage, abdominal disturbances and chromosomal deletion [8][10]. It has been reported to be toxic and carcinogenic as well as haematological derangements, allergic reaction and attention deficit when consumed in excess [5][8][11][12].

2. Materials and Methods

2.1 Materials

Materials used include centrifuge, plain and lithium heparin bottles and spectrophotometer. Reagents used were Total cholesterol (TC), Triglyceride (TG), High Density Lipoprotein (HDL-C) reagent purchased from Randox Diagnostics, United Kingdom.

2.2 Animals

A total of thirty (30) rats of average weight of 0.14kg were used for this study. The rats were groupedinto five (5) namely; group 1, 2, 3, 4, and 5with each group containing a total of six (6) rats.The rats were purchased from the University of Portharcourt animal farm and transportedin a well ventilated plastic cage to Department of Medical Laboratory science animal house, University of Science and Technology, Portharcourt where they were acclimatized for10 days and fed with chickengrowers' mash and water *ad libitum* throughout.

2.3 Administration

The rats were treated orally using gavage tube with varying concentration of carmoisine. Group 1, 2, 3, 4 and 5 were treated daily with 1.0ml of 0.0% (normal saline) 0.0g/kg bodyweight), 1.0% (0.07g/kg bodyweight), 1.5% (0.11g/kg bodyweight), 2.0% (0.14g/kg bodyweight) and 2.5% (0.18g/kg bodyweight) of carmoisine respectively. The duration of treatment was for 4 weeks.

2.4 Collection ofsamples and analysis

About 5mls of fasting blood samples were collected after anesthetizing using cardiac puncture into lithium heparin bottle which was spun at 4000rpm for 5 minutes to obtain plasma for the analysis of total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL-C) while the low density lipoprotein (LDL-C) was calculated using Fieldewald, *et al*., (1972) equation.

2.5Statistical Analysis

The mean, standard deviation and inferential statistics using the student t-testwere used with the aid of Graphpad prism 5.1. Statistical significance were seen at $p<0.05$.

3. Results

The following results were obtained;Group 1 had 1.408± 0.002, 1.827±0.114, 1.143±0.159 and 0.0441±0.030 for TG, TC, HDL-C and LDL-C respectively. Group 2 had 1.440± 0.113, 2.233±0.819, 1.319±0.2626 and 1.248±0.733for TG, TC, HDL-C and LDL-C respectively. Group 3 had 1.464 ±0.173, 1.788 ± 0.280, 0.883±0.217 and 0.2319 ±0.176 for TG, TC, HDL and LDL-c respectively. Group 4 had 1.435± 0.025, 1.786±0.050, 0.934±0.122and 0.1993±0.0951 for TG, TC, HDL and LDL-C respectively. Finally, group 5 had 1.429±0.135, 1.616±0.078, 0.554±0.163 and 0.4116±0.2169 for TG, TC, HDL-C and LDL-C respectively. The comparison between group 1 and group 2 (table 3.1) and group 1 and group 3 (table 3.2) showed that TG, TC, HDL-C and LDL-C were not significant except HDL-C and LDL-C was significant ($p<0.05$) (table 3.2). The comparison between group 1 and group 4 (table 3.3) showed that TG, HDL-C and LDL-C were significant ($p<0.05$) except TC. Finally, the comparison between group 1 and group 5 (table 3.4) showed significant increase in TG, TC HDL-C and LDL-C at $p<0.05$).

Table 3.1 Comparison of group 1and group 2 ofcarmoisine treated rats

Parameter	TG (mmol/L)	TC (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
GROUP 1(0.00g/kg)	1.408± 0.002	1.827±0.114	1.143±0.159	0.0441±0.030
GROUP 2(0.07g/kg)	1.440± 0.113	2.233±0.819	1.319±0.2626	1.248±0.733
p value	0.5072	0.1754	0.7069	0.1662
t value	0.6878	1.458	0.392	1.493
REMARKS	NS	NS	NS	NS

Table 3.2: Comparison of group 1and group 3 of carmoisine treated rats

Parameter	TG(mmol/L)	TC (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
GROUP 1(0.00g/kg)	1.408 ± 0.002	1.827 ±0.114	1.143±0.159	0.0441±0.030
GROUP 3(0.11g/kg)	1.464 ±0.173	1.788 ± 0.280	0.883±0.217	0.2319±0.176
p value	0.272	0.764	0.039	0.027
t value	1.162	0.307	2.372	2.581
REMARKS	NS	NS	S*	S*

Table 3.3: Comparisonof group 1 and group 4 ofcarmoisine treated rats.

Parameter	TG (mmol/L)	TC (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
GROUP 1(0.00g/kg)	1.408± 0.002	1.827±0.114	1.143±0.159	0.0441±0.030
GROUP 4 (0.14g/kg)	1.435± 0.025	1.786±0.050	0.934± 0.122	0.1993±0.0951
p value	0.0246	0.5216	0.0288	0.0034
t value	2.643	0.644	2.552	3.812
REMARKS	S*	NS	S*	S**

Table 3.4: Comparison between group 1and group5 of carmoisine treated rats

Parameter	TG (mmol/L)	TC (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
GROUP1 (0.00g/kg)	1.408±0.002	1.827±0.114	1.143±0.159	0.0441±0.030
GROUP5(0.18g/kg)	1.429±0.135	1.616±0.078	0.554±0.163	0.4116±0.2169
p value	0.0039	0.01	<0.0001	0.021
t value	3.721	3.168	6.336	4.112
REMARKS	S*	S*	S***	S**

* Significant, ** moderate significant,*** highly significant; NS = Not Significant; S = Significant

4. Discussion

The result obtained when carmoisine treated rats of group1was compared with group 2, revealed that there were non-significant increase in TG, TC HDL and LDL-C levels.[13], reported a non-significant differences in TC and HDL-C levels among textile workers exposed to azo dyes such as carmiosine. The non–significant difference seen in TG, TC, HDL and LDL-C is contrary to the findings of [8][12], which reported a significant decrease in cholesterol and HDL-C and a significant increase in TG. The comparison of group 1 and group 3 revealed non-significant difference in TG and TC and a significant decrease and increase in HDL-C and LDL-C respectively. The significant decrease of HDL-C and increase of LDL-C is in line with the reports of [8][12] and [13] respectively. The increase in LDL-C is also in line with the reports of[5],when carmoisine (chocolate colour A and B) were administered to rats at varying concentrations. Though when the exposure of human to azo dyes among textile workers was studied, [13], reported an increase in LDL-C as well. However, contrary to this finding, [8], reported significant decrease in LDL-C levels when rats were treated with low and high doses of carmoisine. When group 1 was compared with group 4, there was a significant increase in TG and LDL-C and decrease in HDL-C. The increase in TG and LDL-C is in line with reports of[5][13]while the increase in TG and decrease in HDL-C is in line with the reports of [8], when carmoisine were administered to rats in low and high doses. Finally, when group 1 and group 5 were compared, there were significant increase in TG, TC and LDL-C and decrease in HDL-C. The increase in TC, TG and LDL-C is in accordance to the reports of [5] who reported an increase in lipid levels when carmoisine dye was administered in rats at varying concentrations. The significant decrease of HDL-C is also in line with the report of [8][12], who reported significant decrease in HDL-C levels when rats were treated with both low and high doses of carmoisine. However, the increase TC and LDL-C levels as seen in this report is contrary to the findings of [8][12], they reported a significant reduction in TC, HDL-C and LDL-C levels in rats treated with carmoisine dye. As reported by [8][10][12], that carmoisine as well as other azo dyes induces hepatic dysfunction resulting in increased liver enzymes in the plasma. [11], also reported that azo dyes when oxidized produces reactive oxygen species (ROS) and free radicals that induces organ damage by generating lesions. The significant increase in TC, TG, and LDL-C as well as the decrease seen in HDL-C in the respective groups could be as a result of the fact that damaged hepatocytes inability to produce enzymes such as lecithin cholesterol transferases and hepatic lipase necessary for the degradation of cholesterol and triglycerides respectively vis-à-vis affecting their transporter lipoprotein concentrations as well. The deficiencies of these hepatic enzymes usually results in hyperlipidaemia. The damage on the hepatocytes could be as a result of the reactive oxygen species produced during the intestinal biotransformation of carmoisine orally administered [14]. The increase in the levels of these lipid parameters (TG, TC and LDL-C) and decrease in HDL-C are usually associated with risks of developing cardiovascular disorder such as atherosclerosis, hypertension, coronary heart disease and so on [15]. Therefore, the consumption of carmoisine as food dye should be done with caution.

5. Conclusion

The result revealed that oral administration of carmoisine have effect on the lipid parameters of albino rats such as TG, TC, HDL-C, and LDL-C especially at doses slightly above the ADI of carmoisine placing the system with the risk of developing cardiovascular diseases.

6. Recommendation

As carmoisine is used to produce many edible food stuffs, domestic use should be carefully monitored, as excessive high doses may pose as risk for cardiovascular diseases. Further research should be done perhaps using other routes of administration at the recommended acceptable daily intake of 4mg/kg bodyweight in a longer duration to ascertain the effect of these dyes at this dose.

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