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Exacerbation risk of Shortened and Prolonged Administration of Acetylsalicylic Acid on the Lipid Profile of Wistar Rats

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Abstract: **Aspirin is a non-steroidal anti-inflammatory drug (NSAID) that is used in relieving pain and reducing fever. In this study, the short-term and prolonged effects of aspirin on the lipid profile were investigated using wistar rats. Eighteen (18) male wistar rats weighing between 135g -217g were divided into 2 main batches (Short-term effects and prolonged effects), each of which has 3 groups containing 3 animals each. Animals in the control group were fed daily with normal growers feed and water *ad libitum.* Animals in groups 2 and 3 were administered daily with aspirin at doses 2.5mg/kg and 5.0mg/kg respectively. They were also given water and food *ad libitum.* After the first 6 weeks, animals in batch 1 were sacrificed and the blood samples collected were taken for lipid profile analysis. Six weeks later (after 12 weeks), animals in batch 2 were also sacrificed and the blood samples collected were also taken for lipid profile analysis. After the first, a significant decrease in the triglyceride level and a corresponding increase in the HDH level were observed for groups 2 and 3 when compared with the control group. A significant decrease in the LDL level was also observed in group 3 when compared to the control group, however, there was little or no changes in the LDL level of group 2 when compared with the control group. After 12 weeks, animals in groups 2 and 3 maintained a significant increase in the HDL level and a corresponding decrease in the triglyceride level. This present study gives scientific support on the medicinal value of aspirin in treating hypertriglyceridemia.**

Keywords: **Aspirin, lipid profile, acetylsalicylic acid, cholesterol.**

1. INTRODUCTION

Aspirin (acetylsalicylic acid) is a non-steroidal anti-inflammatory drug (NSAID) used in various pathological conditions for its anti-inflammatory, antipyretic, and analgesic benefits (Fuster and Sweeny, 2014).The use of salicylic acid in medicine stretches back to antiquity. Early medicines containing salicylic acid were derived from willow bark and other salicylate-rich plants. These formulations were recognized for their antipyretic, analgesic and anti-inflammatory properties, but were also found to have gastrointestinal side effects. The modern form, aspirin or acetylsalicylic acid, is the acetylated version of the natural product and was developed with the aim of improving the tolerability of the drug. More recently, research into the mechanism of action of aspirin led to the discovery that it inhibited the production of prostaglandins. This has resulted in a multitude of new applications for aspirin encompassing conditions such as cardiovascular disease, pre-eclampsia, and cancer prevention. The increasing numbers of people being exposed to aspirin has also led to the awareness of the significant potential harm arising from the prolonged intake of aspirin.

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According to Beckman *et al.*, (2002), Lipids, represented by phospholipids, cholesterol, triglycerides (TG) and fatty acids, are considered essential to the human body, both by making up the basic structure of cell membranes (phospholipids), and by acting as a precursor to steroid hormones, bile acids and vitamin D, as well as being a constituent of cell membranes, acting on the fluidity of the latter and in the activation of the enzymes located there (cholesterol). As for TG, these are formed from three fatty acids bound to a glycerol molecule and constitute one of the most important forms of energy storage in the body, and are deposited in the adipose and muscle tissue. In relation to lipoproteins, it is emphasized that these allow the solubilization and transport of lipids, usually hydrophobic substances, in aqueous plasma. It is important to note that there are four major classes of lipoproteins separated into two groups: those that are TG-rich, larger and less dense, represented by chylomicrons, of intestinal origin, and very low density lipoproteins, of hepatic origin, and those rich in cholesterol, forming low density (LDL) and high density (HDL) particles.

In many situations, the concentrations of these lipids and/or lipoproteins are not in normal amounts in the human body, in what is known in the scientific literature as dyslipidemia. Studying the lipid profile (total cholesterol biochemical determinations – TC, HDL-c, TG and LDL-c) after fasting for 12 to 14 hours, has been an activity of great value, considering that the research already carried out, and correlation between the morphology of the arteries obtained from autopsies and cardiovascular risk factors, has allowed it to be demonstrated that dyslipidemia is a factor of great importance for the development of atherosclerosis in later life (Baigent, 2009).

A lipid profile is a blood test that measures the amount of cholesterol and fats called triglycerides in the blood. It is a combination of tests conducted together to check for any risks of coronary heart disease, or as a preventive measure to check any risks depending on factors like eating habits, diet, stress, exercise and life-style related. Lipids are the fats and fatty substances that are stored in your blood and tissues and are used by the body as a source of energy. While lipids help keep the body functioning normally, lipid disorders, like high cholesterol, might lead to life-threatening conditions like heart attacks, strokes, or coronary artery disease. The results from this test might be used to prevent, monitor, or diagnose various medical conditions. It may also be performed to evaluate the success of various treatments, or the effectiveness of drug therapies or lipid-lowering lifestyle changes (American Heart Association, 2003).

2. MATERIALS AND REAGENTS

2.1 Drug and Equipment.

Aspirin 300mg was purchased from Dooka Pharmacy Ltd, opposite University of Port Harcourt Teaching Hospital Main gate, Alakahia, Port Harcourt, Rivers State. All other reagents were of analytical grade.

2.2 Animals

Eighteen (18) adult male wistar rats weighing between 135g-217g were purchased from the Animal House of the department of Biochemistry, University of Port Harcourt, Port Harcourt, Nigeria. The animals were divided into two main batches, each of which comprises of three groups of three animals of similar weights each. The animals were acclimatized for a period of ten days. During this period, they were fed with water and Grower's feed *ad libitum.*

2.3 Experimental Design

The rats were weighed using a weighing balance and divided into 2 different batches (Short-term and prolonged effects). Each batch had 3 groups of rats and each group had a total of 3 rats. The rats were grouped based on their weight as follows:

Group	Description
Group 1 (Control)	Treated with grower's feed and water
Group 2 (Aspirin 2.5mg/ml)	Treated with grower's feed, water and 2.5mg/ml aspirin
Group 3 (Aspirin 5.0mg/ml)	Treated with grower's feed, water and 5.0mg/ml aspirin.

N.B: This grouping applies to both batches 1 & 2 as animals in batch A were used to analyze the short-term effects of the drug after the first-six (6) weeks of administration while animals in batch B were used to analyze the prolonged effects of the drug after 12 weeks.

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2.4 Lipid Profile Analysis

Determination of Plasma Triglyceride Concentration

Plasma triglyceride (TG) concentration in the rats was assayed enzymatically with commercial test kits (Randox Laboratories, Crumlin, England).

Triglyceride is determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen-peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

$$\text{Triglycerides} + H_2O \longrightarrow \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol Kinase}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone} + \text{Phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4- Aminophenazone} + \text{4-chlorophenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + HCL + 4H_2O$$

Procedure

Three test tubes were set up labelled T_1 (blank), T_2 (Randox triglyceride standard) and T_3 (test sample). T_1 contained 0.01ml distilled water, T_2 contained standard triglyceride solution while T_3 contained 0.01ml plasma sample. To each tube was added 1.0ml of Randox triglyceride reagent. The contents were thoroughly mixed, placed in a water bath at 25°C for 10min, after which the absorbance (A) was read at 546nm, against the blank in a spectrophotometer.

Determination of Plasma Total Cholesterol Concentration

Plasma total cholesterol (TC) was assayed enzymatically with commercial test kits (Randox Laboraties, Crumlin, England).

Principle

The cholesterol released to enzymatic hydrolysis is oxidized with the concomitant release of hydrogen peroxide, whose breakdown leads to the conversion of 4-aminoantipyridine to quinoneimine (the indicator) whose concentration can be determined spectrophotometrically at 500nm.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol-esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol-esterase}} \text{Cholesterol-3-one} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

Procedure

Three test tubes were set up labelled T_1 (blank), T_2 (standard) and T_3 (test sample). T_1 contained 0.01ml distilled water, T_2 contained 0.01ml Randox standard cholesterol solution while T_3 contained 0.01ml plasma sample. To each tube was added 1.0ml of Randox cholesterol reagent. The contents were thoroughly mixed, placed in water bath at 25°C for 10min, after which their absorbance (A) were read at 546nm, against the blank, in spectrophotometer.

Determination of Plasma HDL-Cholesterol Concentration

Plasma HDL-cholesterol (HDL-C) was assayed enzymatically with commercial test kits (Randox Laboratories, Crumlin, England).

Principle

In the presence of magnesium ions, low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid. After centrifugation, the cholesterol concentration of the high densiy lipoprotein (HDL) fraction, which remains in the supertant, can be determined.

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Procedure

Three test tubes were set up labelled T_1 (blank), T_2 (Randox standard cholesterol) and T_3 (test sample). T_1 contained 0.10ml of the supernatant from the blank tube above; T_2 contained 0.10ml of supernatant from Randox standard cholesterol solution tube while T_3 contained 0.10ml supernatant from plasma sample tube. To each tube was added 1.0ml of Randox cholesterol reagent. The contents were thoroughly mixed, placed in water bath at 25°C for 10min, after which their absorbance (A) were read at 546nm, against the blank, in a spectrophotometer.

Determination of Plasma LDL – Cholesterol Concentration

Plasma LDL- Cholesterol (LDL - C) was calculated using Friedewald equation (Friedewald *et al*., 1972) as follows:

i. [LDL cholesterol] (mmol/L) = [Total cholesterol] – [HDL cholesterol] – [estimated VLDL cholesterol] (mmol/L)

ii. [estimated VLDL cholesterol] (mmol/L) = $\frac{[\text{Triglyceride}]}{5}$

Where 5 is Friedewald's value

Determination of Atherogenic Indices

The atherogenic indices of the rats were calculated as earlier reported by Nimmy *et al*., 2012 using the following formula.

i. Atherogenic Index of Plasma (AI) = $\frac{[\text{Triglyceride}]}{[\text{HDL cholesterol}]}$

2.5 Statistical analysis

All data for lipid profile analysis were analyzed for statistical differences and in rat treatment groups by means of one-way ANOVA and post hoc LSD, on SPSS 20. In all, $p<0.05$ was considered significant. Data are presented as mean ±S.D (standard deviation).

3. RESULTS

The table below shows the effects of aspirin on the lipid profile of male Wistar rats after 6 weeks of administration.

Group	TC (mg/dL)	TG(mg/dL)	HDL(mg/dL)	LDL (mg/dL)	AI
Group 1	1.37±0.15	0.70±0.10^b	0.20±0.10	0.83±1.53	3.50±0.00
Group 2	1.50±0.10^a	0.40±0.10^a	0.23±0.58	1.80±0.10^a	1.73±0.00
Group 3	1.32±0.10^a	0.43±0.58^a	0.29±0.00	0.83±0.58^a	1.48±0.00

Each value is a mean of three replicates expressed as mean ± S.D. Values in the same column with common superscript letters (a,b,…) are significantly different at $p<0.05$ when compared with one another.

The table below shows the effects of aspirin on the lipid profile of male Wistar rats after 12 weeks of administration.

Group	TC (mg/dL)	TG(mg/dL)	HDL(mg/dL)	LDL(mg/dL)	AI
Group 1	3.50±0.1	1.07±0.5^a	0.53±0.58	0.27±0.15	2.01±0.00
Group 2	3.60±0.10^a	1.02±0.58^a	0.57±0.58	0.27±0.15	1.80±0.00
Group 3	4.10±1.73^a	1.05±0.58	0.60±0.58^a	0.34±0.15^a	1.75±0.00

Each value is a mean of three replicates expressed as mean ± S.D. Values in the same column with common superscript letters (a,b,…) are significantly different at $p<0.05$ when compared with one another.

4. DISCUSSION

The study was undertaken to investigate the effect of aspirin on the lipid profile of male wistar rats. There was no adverse effect or death recorded in the groups of rats that received different doses of aspirin and this may be attributed to the low concentration of aspirin administered.

It was observed that after the first six weeks, the cholesterol level for group 2 increased (1.50±0.10) when compared to that of the control (1.37±0.15). That of group 3 however reduced when compared to the value of the control (1.32±0.10). Ahmad *et al.,* (2015) reported that aspirin is known to reduce total cholesterol levels. After twelve weeks, the level of cholesterol increased in groups 2 and 3 (3.60±0.10 and 4.10±1.73) when compared to the control which was 3.50±0.10. From this it could be deduced that prolonged administration of aspirin led to a notable increase in the cholesterol level of the rats. The high cholesterol absorbed is transported to the liver through chylomicrons remnants which suppresses the synthesis of LDL receptors raising the LDL-concentration by decreasing the uptake of VLDL remnants, resulting in conversion of VLDL to LDL which delayed the clearance of circulating LDL (Fungwe, 1992).

From the results, the triglyceride level for groups 2 and 3 (0.40±0.10and 0.43±0.10) reduced when compared to that of group 1(0.70±0.10) after the first six weeks. The triglyceride level of group 2 and group 3 was also observed to be increasing after the next six weeks when compared to the control which was 1.07±0.58. The results obtained is in line with the work of Coutinho *et al.,* (1999) who opined that aspirin reduces hypertriglyceridemia by lowering triglyceride levels in rats. This thus indicates the antihypertriglyceridemic property of aspirin which may be employed in the management of hypertriglyceridemia, induced atherogenesis, ischemic heart disease, obesity and cholesterol deposition in the body.

For the High Density Lipoprotein level (HDL), a slight increase was observed in group 2 (0.23±0.58) and group 3 (0.29±0.00) when compared to the control after six weeks. A similar pattern of increase was observed after the next six weeks in group 2 (0.57±0.58) and group 3 (0.60±0.58) when compared to the control which was 0.53±0.58. From this, it was deduced that aspirin increases the level of HDL at higher concentrations.

High density lipoprotein is the main cholesterol carrier from the body cells to the liver, including those from the arterial walls. In the liver, cholesterol is transformed into bile acids and then excreted through the intestine (Hayes *et al*., 1991). This could be the reason why the level of HDL is high in blood of rats different concentration of aspirin than the normal control rats (group I). This is desirable because HDL is usually termed the "good cholesterol". Aspirin at all concentrations in this study increased the plasma HDL-cholesterol levels of treated rats. In contrast to HDL the HDL-C is strongly bound by receptors at endothelial cells that have a high affinity for LDL. This suggests that HDL may protect against atherosclerosis by interfering with LDL binding to the endothelial cells which would reduce the cholesterol uptake by arterial cells.

After the first six weeks, the Low Density Lipoprotein Level (LDL) was observed to reduce in group 2 (0.80±0.10) when compared to the control (0.83±1.53). The LDL level for group 3 remained constant with the control. After the next six weeks, the LDL level for group 2 remained the same with the control (0.27±0.15) while that of group 3 increased to 0.34±0.15.

A research reported elevated LDL in wistar rats due to the suppression of LDL receptor activity (Grundy and Denke, 1990) or newly secreted lipoproteins enriched with cholesteryl ester at the expense of triglycerides (Johnson *et al*., 1983). The liver regulates total body and plasma cholesterol level by decreasing biliary cholesterol synthesis and absorption efficiency whereby excess cholesterol is converted to bile acid and eventually are excreted through faeces.

A significant decrease in the rat atherogenic indices was observed in groups 2 and 3 when compared to group 1 for 6 and 12 weeks indicating a decreased risk of cardiovascular diseases since high atherogenic index has been positively correlated with cardiovascular risk (Igwe *et al*., 2007). Atherosclerotic index indicates the deposition of lipids or plaque in heart, coronary arteries, aorta, liver and kidney.

5. CONCLUSION

In this study, the effect of short-term and prolonged administration of aspirin on the lipid profile were investigated using wistar rats. The finding in this study suggests that aspirin possess anti-hyperlipidemic potentials. The atherogenic index also was decreased in a dose dependent manner. This study has indicated the usefulness of aspirin as a hypolipidemic agent as indicated by its hypolipidemic potential, and this justifies the use of the aspirin as medicine at least for the treatment of those diseases caused by high cholesterol content in the blood.

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