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Lipid Profile of Albino Rats Fed with Ethanolic Extracts of Acacia Nilotica Fruit and Calotropis Procera Flower after Aspirin-Induced Toxicity

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Acacia nilotica; Calotropis procera; Hyperlipidaemia; Lipid Profile; Medicinal Plants

1. Abstract

Acacia nilotica and Calotropis procera parts are used for various purposes in traditional medicine. Because of their acclaimed medicinal uses, this study investigated the lipid profile of albino rats fed with ethanolic extracts of Acacia nilotica fruit and Calotropis procera flower after aspirin-induced toxicity. Twenty-one (21) male Wistar rats were used for this experiment. The animals were randomly distributed into seven groups with three animals in each group. They were administered various concentrations of ethanolic extracts of Acacia nilotica fruit and Calotropis procera flower after aspirin-induced toxicity. Total cholesterol (TC), Triglyceride (TG), High-density lipoprotein (HDL) and Low-density Lipoprotein (LDL) were determined using autochemistry analyzer; PKL PPC, China. The result showed that total cholesterol reduced non-significantly ($p>0.05$) in all the test groups, except in group 2 (administered aspirin only) where it increased non-significantly ($p>0.05$) when compared to the normal control. LDL increased non-significantly ($p>0.05$) in groups 2 and 5, but reduced non-significantly ($p>0.05$) in groups 3, 4, 6 and 7 when compared to the normal control. HDL reduced in all the test animals, while triglycerides increased in all the test animals when compared to the normal control. This study showed that ethanolic extracts of Acacia nilotica fruit and Calotropis procera flower may be useful in prevention or management of incidence of cardiovascular disease arising from alteration of lipid profile parameters.

2. Introduction

Plants, which have been the unique source of remedies for thousands of years, have been used in management of humans' as well as animals' diseases (El-Seedi et al., 2019). Currently, medicinal plants (MPs) are still the major source of primary health care in developing countries (Mulat et al., 2020). As per the World Health Organisation, around 80 % of world populace especially in developing countries rely on traditional medicines, particularly on MPs for their routine health problems (Fatima et al., 2018). However, only around 50 % of western drugs contain plants bioactive compounds or their analogues as their active ingredients. Medicinal plants have always been considered as healthy source of treatment due to their therapeutic effects and safety (Karunamoorthi et al., 2013). They find use in the treatment of diseases such as hyperlipidaemia.

Hyperlipidaemia refers to elevated levels of lipids and cholesterol in the blood. Lipid molecules are fatty acids, cholesterol, triglycerides and phospholipids that play key roles in metabolism of living organism (Nwanjo, 2005). Hyperlipidaemia plays an important role in the development of atherosclerosis, the main cause of death in the world (Elshourbagy et al., 2014). The formation of atherosclerotic plaque involves accumulation of LDL in intima, LDL oxidation, and uptake of oxidized LDL by macrophage scavenger receptors, influence of macrophages on foam cells, and stabilization of plaque. Inflammatory cytokines are involved in all

steps and make this process a chronic inflammatory disease (Xie et al., 2016). Different medicinal plants remedies were used to treat hyperlipidaemia, it decreased blood lipids by many mechanisms included inhibition of the expression of fatty acid synthase, decreasing free fatty acid release, inhibition of HMG-CoA reductase, increasing the faecal excretion of fat and cholesterol, inhibition of the activity of pancreatic lipase and inhibition of cholesterol absorption (Al-Snafi, 2016).

Acacia is a genus of shrubs and trees belonging to the subfamily Mimosoideae (Nadkarni, 2010), of the family Fabaceae or Leguminosae, first described by the Swedish botanist Carl Linnaeus (1773). They are pod-bearing, with sap and leaves typically bearing large amounts of tannins and condensed tannins that historically in many species found use as pharmaceuticals and preservatives. Different parts of A. nilotica are widely used in traditional medicine for the treatment of various ailments. In different countries of the West Africa, A. nilotica pods, bark, gum, root, flowers and leaves are very well solicited for the treatment of several diseases, such as gastrointestinal disorders (diarrhoea, dysentery, haemorrhoid, abdominal aches, toothaches, sore throat, etc), diabetes, asthma, hypertension, etc (Koube et al., 2016).

Acacia nilotica is very rich in secondary metabolites. It contains a variety of bioactive components, such as gallic acid, ellagic acid, isoquercetin, leucocyanidin, kaempferol-7-diglucoside, naringenin-7-O-β-D-(60- O-galloyl) glucopyranoside, rutin, apigenin-6,8-bisC-glucopyranoside, m-catechol and their derivatives, as well as galloylated derivatives of (+)-catechin and (+)-gallocatechin (Maldini et al., 2011). The seeds are very rich in phenolic constituents and also proteins. The fruits are reported to be very rich in saponins and tannins (Manzo et al., 2017). The leaves contain apigenin, 6-8-bis-D-glucoside, etc. The relative proportion of tannin in different parts of the plant is 50%, 7.6%, 13.5% for fruits, leaves and bark, respectively. The bark is rich in tannins (12-20%), terpenoids, saponins, etc. Its total extract is very rich in phenol. The root contains octaconsanol, betulin, etc.

Calotropis procera (C. procera) is a popular medicinal plant from the family Apocynaceae. It is a xerophytic perennial shrub (or small tree) with stems of 2 to 6 m tall and tap roots 3 to 4 m deep in the soil (Hassan et al., 2015). A thick milky sap or latex exudes out from the plant if its parts are cut or broken. C. procera grows on a variety of soils and it can tolerate different level of soil salinity, draught stress, intense light of arid and harsh environments. Hence, it is distributed in various tropical and subtropical countries (Hassan et al., 2015). C. procera showed a diverse array of biological activities such as antimicrobial, antidiarrheal, wound healing, anti-inflammatory, anticancer or cytotoxic, in vivo immunomodulatory, analgesic, anthelmintic, antioxidant, and in vivo antihyperglycemic (Ramos et al., 2020).

3. Materials and Methods

3.1. Collection of Plant Material

The fresh pre-matured leaves of Calotropis procera was collected from Angwan Roger Road, Wukari, Taraba State, Nigeria. It was dried at room temperature and then ground into powder form. The dried fruits of Acacia nilotica was purchased from old market Wukari, Taraba State, Nigeria. It was ground into powder form.

3.2. Animals Care and Management

A total of twenty-one (21) adult male Wistar rats (weighing between per b.w) was used for this experiment. The rats were purchased from the National Veterinary Research Institute, Vom, Plateau State. The rats were maintained under standard laboratory conditions and are allowed free access to standard diet and water ad libitum. They were allowed to acclimatize for 24 hours.

3.3. Preparation of the Extracts

70% ethanol solution was prepared and used to soak the two ground samples separately. The mixtures were then filtered after 48 hours and the filtrates collected separately. The filtrates were concentrated using a water bath set at 78oC in order to evaporate the ethanol. The concentrated extracts were diluted with normal saline at the rate of 100 mg per ml (Arowora et al., 2016).

3.4. Experimental Design and Grouping of Animals

21 Wistar rats were used for this experiment. The animals were grouped into seven (7) groups and received the extracts as follows:

Group 1 - Normal control.

Group 2 - Negative control: Aspirin: 500 mg/kg.

Group 3 - Positive control, Aspirin: 500 mg/kg and Omeprazole: 20 mg/kg.

Group 4 - Aspirin: 500 mg/kg and Acacia nilotica fruits extract 200 mg/kg.

Group 5 - Aspirin: 500 mg/kg and Acacia nilotica fruits extract 400 mg/kg.

Group 6 - Aspirin: 500 mg/kg and Calotropis procera flower extract 200 mg/kg.

Group 7 - Aspirin: 500mg/kg and Calotropis procera flower extract 400mg/kg.

Animals from group 2 to 7 were deprived of food for 24hours but allowed free access to water. After fasting the animals for 24 hours, toxicity was induced in the animals with 500 mg/kg body weight of aspirin via oral route. Water was removed from the cages 1 hour prior to the induction. Four hours after induction, feed and water were taken back to the cages. The animals were treated with omeprazole as the standard drug (group 3) while groups 4 and 5 received 200 mg/kg and 400 mg/kg of Acacia nilotica fruits extract respectively and Calotropis procera flower extract was given to groups 6 and 7 at low dose (200 mg/kg) and high dose (400 mg/kg) respectively. They were treated for 5 days and starved on the 6th

day. The animals were weighed and 3 animals from each group (1-7) were sacrificed and the blood collected directly from the heart for biochemical analysis.

3.5. Determination of Lipid Profile Level

Serum Concentrations / Levels of Lipid Profile: Total cholesterol (TC), Triglyceride (TG), High-density lipoprotein (HDL) and Low-density Lipoprotein (LDL) were determined using autochemistry analyzer; PKL PPC, China. The sera samples were brought to room temperature. The concentrations of the QC elected; the QC No in the QC lot also selected. The sample input was caped, saved and modified. The samples were loaded and the reagents status were checked. The OK button was clicked and all the samples were analyzed automatically where all the results were displayed on the screen.

3.6. Statistical Analysis

The results were expressed as Mean ± SEM (standard error of mean). All the analyses were conducted in triplicates in completely randomized design. The data were subjected to analysis of variance using Statistical Package for Social Science (SPSS) software. Means where significantly separated by the least significant difference (LSD) test and significance was accepted at $p<0.05$.

4. Results

The result of the lipid profile analysis is presented in the Table 1 below:

Total cholesterol reduced non-significantly ($p>0.05$) in all the test groups, except in group 2 (administered aspirin only) where it increased non-significantly ($p>0.05$) when compared to the normal control. LDL increased non-significantly ($p>0.05$) in groups 2 and 5, but reduced non-significantly ($p>0.05$) in groups 3, 4, 6 and 7 when compared to the normal control. HDL reduced in all the test animals, while triglycerides increased in all the test animals when compared to the normal control.

Table 1: Concentrations of lipid profile indices in aspirin-induced toxicity in albino rats administered ethanolic extracts of Acacia nilotica fruit and Calotropis procera flower (mmol/L)

Parameters	Group 1 (Normal control)	Group 2 (Aspirin: 500 mg/kg)	Group 3 (Aspirin: 500 mg/kg and omeprazole: 20 mg/kg bw)	Group 4 (Aspirin: 500 mg/kg and *Acacia nilotica* fruit extract: 200 mg/kg bw)	Group 5 (Aspirin: 500 mg/kg and *Acacia nilotica* fruit extract: 400 mg/kg bw)	Group 6 (Aspirin: 500 mg/kg and *Calotropis procera* flower extract: 200 mg/kg bw)	Group 7 (Aspirin: 500 mg/kg and *Calotropis procera* flower extract: 400 mg/kg bw)
Total cholesterol	$1.44 \pm 0.27^{a,b}$	1.62 ± 0.27^{b}	$1.16 \pm 0.02^{a,b}$	0.91 ± 0.08^{a}	$1.35 \pm 0.16^{a,b}$	$1.12 \pm 0.06^{a,b}$	0.97 ± 0.14^{a}
HDL	0.73 ± 0.10^{a}	$0.59 \pm 0.04^{a,b}$	0.46 ± 0.08^{b}	$0.36 \pm .03^{b}$	$0.49 \pm 0.12^{a,b}$	0.47 ± 0.08^{b}	0.36 ± 0.05^{b}
Triglycerides	0.28 ± 0.01^{a}	0.52 ± 0.12^{a}	0.34 ± 0.12^{a}	0.41 ± 0.11^{a}	0.55 ± 0.03^{a}	0.40 ± 0.07^{a}	0.37 ± 0.08^{a}
LDL	$0.57 \pm 0.16^{a,b}$	0.76 ± 0.25^{b}	$0.53 \pm 0.05^{a,b}$	0.35 ± 0.03^{a}	$0.59 \pm 0.06^{a,b}$	$0.44 \pm 0.05^{a,b}$	$0.43 \pm 0.06^{a,b}$

Result represent mean ± standard deviation of group serum result obtained (n=3).
Mean in the same row, having different letters of the alphabet are statistically significant ($p<0.05$).

5. Discussion

Aspirin has been used in different research studies and has been reported to induce toxicity at high dose in animals. The toxicity may cause alteration of some biochemical indices such as the lipid profile parameters. However, the use of herbal remedies for treatment of different disease conditions has been in vogue for a long time. The phytochemical constituents of plants are believed to be contributing to their medicinal effects. Some of these phytochemicals may include tannins, alkaloids, steroids, flavonoids, cardiac glycosides, saponins, and terpenoids (Elekwa *et al*., 2011). Hence in this study, the effects of ethanolic extracts of *Acacia nilotica* fruit and *Calotropis procera* flower on lipid profile in aspirin-induced toxic rats was investigated. Both plant parts are reported to be used by some indigenes of Taraba State in the management of ulcer. Since excess dose of aspirin is reported to induce ulcer (Cryer and Mahaffey, 2014), the effect of extracts of these plant parts on lipid profile will prove to the consumers and those who may be using them in nutrition their possible effects on lipid profile.

The result of this study (Table 1) showed that administration of aspirin (500 mg/kg) to the experimental animals caused an alteration of the lipid profile parameters. Although the alterations were not statistically significant ($p>0.05$), the results showed that consumption of excess dose of aspirin may increase total cholesterol, triglycerides, low density lipoprotein (LDL) levels and reduced high density lipoprotein (HDL) levels. The implication of these results is that without proper treatment, the animals may be at risk of suffering cardiovascular disease which may be caused by or related to increased cholesterol levels in the blood. It was reported in litera-

ture that elevated levels of cholesterol in the blood lead to atherosclerosis which may increase the risk of heart attack, stroke, and peripheral artery disease (Brunzell *et al*., 2008). Beside the elevated total cholesterol level, the elevated LDL level showed that there are more LDL available to transport cholesterol from the liver into the blood. This can result to hyper-cholesterolnaemia. The reduced HDL level confirmed that less cholesterol may be transported out of the blood of the test animals than in the normal control rats.

Treatment of the test animals with omeprazole, ethanolic extracts of *Acacia nilotica* fruit and *Calotropis procera* flower showed a reduction of the elevated total cholesterol level. This showed that omeprazole and the chemical constituents of the two plant parts may contain ingredient capable of reducing cholesterol levels. This implies that they may be used in prevention or management of coronary heart disease. These reductions in total cholesterol levels were below the level in normal control rats. A comparative analysis of the effects of the omeprazole, ethanolic extracts of *Acacia nilotica* fruit and *Calotropis procera* flower showed that *Acacia nilotica* fruit extract (200 mg/kg bw) showed better reduction effect on the cholesterol level followed by *Calotropis procera* flower extract (400 mg/kg bw). If cholesterol level is persistently high, there are several international guidelines on the treatment of hypercholesterolaemia (Mannu *et al*., 2012).

The effect of treatment with omeprazole, ethanolic extracts of *Acacia nilotica* fruit and *Calotropis procera* flower showed the same pattern of reduction of the LDL levels as in the cholesterol levels. It implies that these plant parts can cause reduction in the synthesis of LDL, thereby reducing the possibility of incidence of coronary heart disease or any other cardiovascular disease that may be caused by elevated LDL level. Treatment of the test animals with ethanolic extracts of the two plant parts did not reverse the reduced HDL caused by aspirin. HDL has been reported to exert a multiplicity of effects, from anti-inflammatory to anti-diabetic, antithrombotic, heart failure antagonism, and many others (Sirtori *et al*., 2019).

The result of this study therefore confirmed the fact that the two different plant part extracts may be used in treating or managing incidence of cardiovascular diseases arising from alteration of lipid profile parameters. It has been reported that reduction of severely elevated triglycerides is important to avert or reduce the risk of pancreatitis (Laufs *et al*., 2020), among other conditions.

6. Conclusion

Administration of aspirin (500 mg/kg) to the experimental animals caused an alteration of the lipid profile parameters, although the alterations were not statistically significant. Treatment of the test animals with omeprazole, ethanolic extracts of *Acacia nilotica* fruit and *Calotropis procera* flower showed a reduction of the elevated total cholesterol, triglycerides and low-density lipoprotein levels. This study showed that ethanolic extracts of *Acacia nilotica* fruit and *Calotropis procera* flower may be useful in prevention or management of incidence of cardiovascular disease arising from alteration of lipid profile parameters.

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