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Full Length Research Paper

Effect of *Peristrophe bicalyculata* on lipid profile of P- 407-induced hyperlipidemic Wistar rats

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The effect of the ethanolic extract of the leaves of *Peristrophe bicalyculata* on the lipid profile of hyperlipidemic rats was determined. Male Wistar rats were divided into two major groups; Group A, to determine the phytopreventive effect of the plant extract and group B, to determine its curative effect. Group A animals were administered the extract *ad libitum* for 26 days prior to induction of hyperlipidemia, while group B animals were administered the extract *ad libitum* also, for 2 days, beginning two hours after induction of hyperlipidemia. Atorvastatin was used as the standard drug. Rats were made hyperlipidemic by intraperitoneal injection of 1.0 g/kg poloxamer 407 (P-407) and blood samples collected 48 h after administration of P-407. On the 28th day, blood samples were collected to determine plasma lipid profile and atherogenic risk predictor indices. The levels of total cholesterol, LDL-cholesterol and triglycerides in animals treated with the extract in both groups were significantly lower ($p < 0.05$) than those in the hyperlipidemic control group; while HDL-cholesterol level significantly increased. In most cases, there were no significant differences in lipid levels of rats administered the extract, especially at a higher dose, compared to Atorvastatin. The Atherogenic risk predictor indices showed that rats in the hyperlipidemic control group and those treated to determine the curative effect of the extract were at risk of atherosclerosis. In conclusion, this study has demonstrated the phytopreventive, hypolipidemic and antiatherogenic effect of *P. bicalyculata.*

Key words: *Peristrophe bicalyculata,* poloxamer 407*,* hyperlipidemia, atherogenic.

INTRODUCTION

Coronary artery diseases (CAD) presents some of the major health problems across the globe today, with coronary heart disease, stroke and hypertension being the most common. Death due to coronary heart disease caused by atherosclerosis continues to be a cause of mortality in affluent nations of the world (Johnston et al., 2003; Johnston and Waxman, 2008).

It is well established that elevated blood lipid levels also known as hyperlipidemia constitute the primary risk factor for atherosclerosis. For example, elevated levels of cholesterol (hypercholesterolemia), low-density lipoprotein cholesterol (LDL-c) and triglycerides have been implicated. There is now overwhelming evidence that, dietary factor (Horn, 1997), nutritional habits and genetic origin influence the risk of CAD (Kourounakis et al., 2002).

Poloxamer 407 is a hydrophilic non-ionic surfactant belonging to the group known as poloxamers. It is a triblock copolymer consisting of a central hydrophobic block of polypropylene glycol flanked by two hydrophilic blocks of polyethylene glycol (PEG). The approximate length of the two PEG blocks is 101 repeat units while the approximate length of the propylene glycol block is 56 repeat units. This particular compound is also known by the BASF trade name Pluronic RF-127 (www.Wikipedia.com). Poloxamer 407 (Pluronic RF-127) has been used to induce hyperlipidemia in rats. P407 is a biocompatible, non-ionic surfactant, considered non-toxic and safe during chronic administration for long term studies (Megalli et al., 2005).

Peristrophe bicalyculata belongs to the Kingdom: Plantae, phylum: Magnoliophyta, class Magnoliopsida, order Lamiales, family Acanthaceae and to the genus *Peristrophe*. It is called 'tubanin dawaki' by Hausas in Northern Nigeria, meaning flour of the horse. In 'Serer' and 'Wolof' languages of Senegal, it is called 'buben' and 'môto' respectively (Burkill, 1985). In the Indore district of India, the local name is 'Chotiharjori' (Dwivedi, 2002). It is native to warm tropical regions of Africa, in the Sahel part of the region of Mauritania, Niger and northern Nigeria as well as in India, Burma and Thailand.

Although undocumented, the plant *P. bicalyculata* is used in South West Nigeria in the treatment of hypertension and other cardiovascular related diseases. However, very little research has been done to determine the efficacy of this plant in the treatment of CAD risk factors. Since elevated blood lipid levels are associated with CAD. It becomes pertinent to determine the efficacy of this plant in the treatment of hyperlipidemia. It is our belief that this investigation will take us another step forward in our quest to understand the mechanism of action of *P. bicalyculata* in prevention and treatment of arteriosclerosis and heart diseases. The broad objective of this study is to ascertain if the plant *P. bicalyculata* will reduce the blood lipoprotein level and subsequently reduce the risk of CAD.

The specific objectives include: To determine the level of total cholesterol, HDL-cholesterol, LDL-cholesterol and triacylglycerols in the blood of hyperlipidemic Wistar rats. To determine the atherogenic risk predicator indices (HDL-cholesterol/total cholesterol, LDL-cholesterol/ total cholesterol and log (triacylglycerols/HDL-cholesterol) in hyperlipidemic Wistar rats. To determine the phytopreventive and curative effect of the ethanolic extract of *P. bicalyculata* on hyperlipidemic Wistar rats.

METHODS

Animals

This study was conducted in accordance with the National Institute of Health standard for the care and use of experimental animals. Forty (40) clinically healthy male Wistar rats of approximately two months old, weighing between 130 – 200 g were purchased from the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The rats were housed five per cage, each cage representing a group, making a total of eight groups. They were fed rat pellet diet (PLS feeds, Zaria) throughout the experiment and allowed to acclimatize for 2 weeks before beginning the experiment. Doses were chosen after carrying out toxicity experiments as described by Lorke (1983) and the plant extract found to be non-toxic. Animals were grouped as follows: control group, hyperlipidemic control, phytopreventive and curative groups. The phytopreventive and curative groups were further subdivided into three groups.

Group 1 (control): Given distilled water only.

Group 2 (hyperlipidemic control): Intravenous administration of poloxamer 407 48 h prior to sample collection, that is, on the 26th day.

Group A (Phytopreventive group)

Animals in sub-groups 3, 4 and 5 that fall within this group were administered the drug and plant extract orally.

Group 3 (standard): Administered Atorvastatin at 70 mg/kg body weight for 26 days before induction of hyperlipidemia.

Group 4: Administered the plant extract at a dose of 250 mg/kg body weight for 26 days before induction of hyperlipidemia.

Group 5: Administered the plant extract at a dose of 500 mg/kg body weight for 26 days before induction of hyperlipidemia.

Group B (Curative group)

Animals in sub-groups 6, 7 and 8 that fall within this group were fed normally with rat pellet diet and on the 26th day, hyperlipidemia was induced. They were allowed for about two hours before oral administration of the drug and extract for two days.

Group 6 (standard): Given Atorvastatin at 70 mg/kg body weight.

Group 7: Given the plant extract at 250 mg/kg body weight.

Group 8: Given the plant extract at 500 mg/kg body weight.

In groups 3, 4 and 5, the treatment was administered for 26 days prior to the induction of hyperlipidemia (Phytopreventive), while in groups 6, 7 and 8, treatment began 2 h after the induction of hyperlipidemia (curative).

Preparation of plant material and preparation of ethanolic extract

The leaves of *P. bicalyculata* were obtained from Ibadan, Oyo state, Nigeria, in the month of July, 2008. They were identified and authenticated by the Botanist in the Herbarium of Ahmadu Bello University, Zaria, Kaduna State, Nigeria and then air-dried in the laboratory. The dried leaves were ground to powder and then sieved. 50 g of the powdered plant material was soaked in 150 ml of absolute ethanol (sigma Aldrich) for 24 h. The extract was filtered using Whatsman filter paper and concentrated to dryness by evaporation in a hot water bath at 40°C.

Preparation of standard drug

Atorvastatin (Pfizer Ireland pharmaceuticals, Ireland) was purchased in a tablet form at strength 20 mg. Tablets were crushed into powder, dissolved in distilled water and administered *ad libitum*.

Induction of hyperlipidemia

Hyperlipidemia was induced as described by Megalli *et al* (2005). Briefly, 1.0 g/kg dose of P407 (BASF Corporation; Mount Olive, NJ, USA) was introduced intraperitoneally. All syringes were placed on ice prior to P407 administration to maintain the polymer in a mobile viscous state during the injection, since P407 solutions at concentrations greater than about 23% w/w exhibit reverse thermal gelatin properties.

Sample collection

At the end of the experimental period, the rats were sacrificed by anesthesia using chloroform before sample collection. Blood was

collected after decapitation into EDTA bottles, centrifuged; to obtain the plasma which was used for lipid analysis.

Plasma lipid analysis

The plasma samples were analyzed for total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides according to the procedure described in the sigma cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides assay kits (Fortress diagnostics limited, Antrim).

Data analysis

Data obtained were expressed as mean ± standard error of mean (mean ± SEM). The significance of the results was evaluated using analysis of variance (ANOVA) and the means compared using Tukeys test. Values of $p < 0.05$ were regarded as statistically significant.

RESULTS

Phytopreventive effects of *Peristrophe bicalyculata*

Changes in lipoprotein levels

Changes in plasma lipoprotein levels in the Wistar rats are shown in Table 1. The total cholesterol level in rats within the control group was significantly ($p < 0.05$) lower (75.24 ± 1.63 mg/dl) than that of all the other groups. The level in hyperlipidemic control group (Group 2) was significantly ($p < 0.05$) higher (485.69 ± 1.16mg/dl) than all the other groups. Also, the total cholesterol level in rats administered Atorvastatin (405.04 ± 7.94 mg/dl) was significantly ($p < 0.05$) lower than those given the extract at 250 mg/kg (441.34 ± 3.33 mg/dl) and 500 mg/kg (423.97 ± 2.99 mg/dl), which was dose dependent.

The HDL-cholesterol level of rats given the standard drug (107.95 ± 5.08 mg/dl) and both doses of the extract increased significantly ($p < 0.05$) compared to the hyperlipidemic control group. The effect of the plant extract on the change in the HDL-cholesterol level was also dose dependent. On the other hand, levels of LDL-cholesterol and triglycerides of rats given atorvastatin and the extract was significantly ($p < 0.05$) lower than those in the control and hyperlipidemic group.

The values (mean ± S.E.M) of atherogenic risk indices of Wistar rats are shown in Table 2. The HDL-cholesterol/total cholesterol in rats administered the standard drug was not significantly ($p < 0.05$) different (0.27 ± 0.01) when compared to those given the extract at 500 mg/kg (0.27 ± 0.01). It was however significantly ($p < 0.05$) lower than that of the animals in the control group, whereas the value for hyperlipidemic control rats was significantly ($p < 0.05$) lower (0.20 ± 0.01) than that of the other groups.

The ratio of the LDL-cholesterol and HDL-cholesterol was significantly lower in the rats in the control group than in all the other groups. However, in the hyperlipidemic control group, the value was significantly ($p < 0.05$) higher (2.38 ± 0.13) than that of the other groups. The value in the animals administered Atorvastatin differed significantly ($p < 0.05$) from that of the animals given the extract at 250 mg/kg (2.12 ± 0.08) and at 500 mg/kg (1.72 ± 0.05).

The association of triglycerides and HDL-cholesterol in this simple ratio theoretically reflects the balance between risk and protective lipoprotein forces. There was no significant ($p < 0.05$) difference between the hyperlipidemic control group (1.18 ± 0.10) and those given the extract at 250 mg/kg (1.13 ± 0.01), however, it was significantly ($p < 0.05$) different from the animals given the standard (0.99 ± 0.03) and the extract at 500 mg/kg (0.98 ± 0.01). On the other hand, the difference was not significant in the animals given the extract at 500 mg/kg and those given Atorvastatin.

Curative effects of *Peristrophe bicalyculata*

Changes in lipoprotein levels

The values (mean ± S.E.M) of some plasma lipoproteins of Wistar rats are shown in Table 3. The total cholesterol level in rats within the control group was significantly ($p < 0.05$) lower (75.24 ± 1.63 mg/dl) than those given Atorvastatin (435.20 ± 2.64 mg/dl), the extract at 250 mg/kg (448.89 ± 3.32 mg/dl) and at 500 mg/kg (438.36 ± 3.90 mg/dl). The total cholesterol level in the hyperlipidemic control group increased by more than 6-folds when compared to the control group. The reduction in the level showed a dose dependent effect.

The HDL-cholesterol level was significantly ($p < 0.05$) higher in rats within the hyperlipidemic control group than those given the extract at 250 mg/kg and at 500 mg/kg. This could mean that acute administration of the extract after induction of hyperlipidemia does not increase HDL-cholesterol.

The LDL-cholesterol level in rats within the hyperlipidemic control group increased by about 9-folds when compared with the control. This increase was significantly higher than that of the animals administered the standard drug and both doses of the extract, which was dose dependent.

Also, the triglyceride level of hyperlipidemic control rats increased by about a 39-fold (1904.75 ± 89.93 mg/dl) when compared to control group. It was however not significant ($p < 0.05$) when control group is compared to those given atorvastatin and the extract.

The values (mean ± S.E.M) of atherogenic risk indices of Wistar rats are shown in Table 4. The HDL-cholesterol/total-cholesterol values in the control group was significantly ($p < 0.05$) higher (0.58 ± 0.04) than those given atorvastatin and the extract. The results obtained on giving the extracts and the standard were

found to be < 0.3, also, the values of LDL-cholesterol to HDL-cholesterol level, were < 2.3 except for the hyperlipidemic control group (2.38 ± 0.13). The values of log (triglycerides to HDL-cholesterol) was not significantly different between the hyperlipidemic control group, rats given atorvastatin and the extract, all indicating the rats may be at risk of atherosclerosis. This may be due to the short duration of administration.

DISCUSSION

The use of the plant *P. bicalyculata* in Nigeria in the treatment of CAD has not been documented. However, this study has demonstrated that the plant is capable of reducing blood lipids significantly and so may be effective in such treatments. In fact its action can be compared to the standard drug, atorvastatin, because TC and LDL were significantly reduced. It is important to state here that the effectiveness of atorvastatin in lowering cholesterol is dose-related, meaning that higher doses reduce cholesterol more, hence it is possible that if dosage is increased, the effect increases. Same can be said of the plant extract as most result were dose dependent. Atorvastatin is used for the treatment of elevated total cholesterol, LDL, triglycerides and to elevate HDL cholesterol. It prevents the production of cholesterol in the liver by blocking HMG-CoA reductase, however, the mechanism of action of our plant is still unknown, but it may be same as atorvastatin.

This study agrees with the work of Megalli et al. (2005) where P407 was shown to increase triglycerides, total cholesterol, LDL-cholesterol and HDL-cholesterol levels in plasma. However, the higher triglyceride levels compared to other lipids has been attributed to the inhibition of triglyceride degradation, due to a direct inhibitory effect on lipoprotein lipase bound to capillary endothelium. Lipoprotein lipase is vital in the metabolism of triglycerides and is involved in several pathological disorders, including atherosclerosis and obesity (Megalli et al., 2005, Wassan et al., 2003). Hence, it is possible that *P. bicalyculata* affects lipoprotein lipase activity since it was significantly effective in reducing triglyceride levels in this model.

Also, the significantly higher levels of LDL-cholesterol than HDL-cholesterol, has been demonstrated in studies by Johnston and Waxman (2008), that P407 treatment in mice induced a shift in the lipoprotein distribution from HDL-cholesterol to predominantly LDL-cholesterol, due to the presence of oxidized lipids believed to be oxidized LDL-Cholesterol. Thus, it is suggested that P407, by profoundly elevating plasma lipids, indirectly ensures that some of the lipids will undergo oxidation. It is well-known that lipoprotein oxidation plays a key role in atherosclerosis. LDL is oxidized in tissues, including the artery wall and serves to stimulate the release of oxidation products that activate an inflammatory response. In the present study, the hyperlipidemic control rats showed high plasma levels of total cholesterol, LDL-cholesterol and triglycerides, but oral administration of the extract significantly ($p < 0.05$) reduced their levels and also increased the level of HDL-cholesterol in a dose dependent manner (Table 1). Therefore, the results imply that *P. bicalyculata* extract has beneficial effects on plasma lipid profiles by reducing lipid levels.

HDL-cholesterol carries cholesterol and cholesterol esters from the peripheral tissues and cells to the liver, where cholesterol is metabolized into bile acids. This pathway plays a very important role in reducing cholesterol levels in the blood and peripheral tissues and in inhibiting atherosclerotic plaque formation in the aorta (Kim et al., 2008; Karmarkar, 2008). The increased levels of plasma HDL-cholesterol concentrations at a higher dose of 500 mg/kg body weight also suggest that *P. bicalyculata* may protect against cardiovascular diseases that result from hyperlipidemia. However, as a curative drug (group B), the triglycerides, total cholesterol and LDL-cholesterol levels decreased when compared to the hyperlipidemic control, thus it was not as effective as the phytopreventive group. This could be attributed to the duration of administration. It is important to note that the choice of duration was made to coincide with all groups and chosen because P407-induced hyperlipidemia is at maximum after 48 h of induction.

The Atherogenic indices predictor (AIP) reflects the delicate metabolic interactions within the whole lipoprotein complex. When using the results of well-standardized assays, AIP provides information about the atherogenicity of plasma and quantifies the response to therapeutic intervention (Ojiako and Nwanjo, 2005, Nwanjo, 2004). Although values of HDL-cholesterol /total cholesterol <0.3 are believed to be at risk of atherosclerosis, it can be seen that the value in the animals given the standard drug, Atorvastatin was not significantly different from that of the animals given the extract at 500mg/kg in the phytopreventive group (group A). This value increased significantly when compared to the hyperlipidemic control rats.

Also, the value 0.27 may not be significantly different when compared to the limit 0.3. The LDL-cholesterol to HDL-cholesterol ratio and log (triglyceride/HDL-cholesterol) also showed that the extract reduced the risk to atherosclerosis.

In summary, the present study has demonstrated that *P. bicalyculata* has hypolipidemic effects on poloxamer 407-induced hyperlipidemic rats, especially when the phytopreventive effects were studied. Utilizing the poloxamer P407 model, *P. bicalyculata* was shown to be effective in significantly lowering Triglycerides, total cholesterol, and LDL-cholesterol levels.

Although, we have just started work on this plant, these findings are of potential importance in the treatment and/or prevention of cardiovascular diseases. However, more work is needed to investigate the hypolipidemic

component(s) in *P. bicalyculata* andmechanism of action.

With the growing interest of the Western world in complementary and alternative medicines investigations such as these that scientifically examine traditional beliefs and experience are required and are ever increasingly forthcoming in the literature. Overall, the use of an effective herbal drug to supplement other drug treatments in controlling hyperlipidemia and enhancing cardiac functions could be potentially of clinical value if these models are translatable to human clinical studies and outcomes.

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