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Effect of ethanolic extract of *Daniella Oliveri* leaves on some cardiovascular indices in rats

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ABSTRACT - Effects of ethanolic extract of *Daniella oliveri* leaves (50, 100 and 200mg/Kg body weight) on the activities of alkaline phosphatase (ALP), acid phosphatase (ACP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in rat heart were investigated after fourteen days of oral administration. Effects of the extract on total cholesterol, HDL-cholesterol and triacylglycerol concentrations in the serum with the atherogenic index (total cholesterol concentration/HDL-cholesterol concentration ratio) were also evaluated. The extract had no significant effect ($P > 0.05$) on serum HDL- cholesterol and triacylglycerol concentrations when compared with controls. However, the extract significantly increased ($P < 0.05$) serum total cholesterol concentration at doses of 100 and 200 mg/kg body weight while it only significantly increased ($P < 0.05$) the atherogenic index at the dose of 200mg/kg body weight when compared with controls. The extract had no significant effect ($P > 0.05$) on heart AST activity while it significantly increased ($P < 0.05$) heart ALT activity at all doses administered when compared with controls. ALP activity was significantly reduced ($P < 0.05$) in the heart at doses of 50 and 100 mg/kg body weight of the extract when compared with controls. Moreover, heart ACP activity was significantly reduced ($P < 0.05$) at doses of 100 and 200mg/kg body weight of the extract whereas 50mg/kg body weight of the extract significantly increased ($P < 0.05$) it when compared with controls. The results of this study suggest that the administration of the ethanolic extract of *D. oliveri* leaves may predispose subjects to some cardiovascular problems.
KEYWORDS: Cardiovascular indices, Ethanolic extract, *Daniella oliveri*

INTRODUCTION

Daniella oliveri (Caesalpiniacea) is a plant found in the Amazon region and other parts of South America and Africa (1, 2). The tree may reach a height of 100 feet and trunk diameter of 4 feet (3). It produces liquid oleoresin which has been used as medicine by indigenous people for more than 400 years (4). The oleoresin is produced in the tree's trunk, stem, and leaves and it consists of large but varying amounts of volatile oils (primarily composed of sesquiterpene hydrocarbons usually including caryophyllene), non volatile resinous substances and small quantities of acids. The oleoresin is traditionally used as an anti-inflammatory agent and in the treatment of a variety of genito-urinary tract diseases and skin ailments (5, 6). Moreover, it is used as an anti-rheumatic, antiseptic, antibacterial, diuretic, and hypotensive agent, and also as an expectorant, laxative, purgative, vermifuge and vulnerary (7). The leaves are also used in folk medicine as an anti-diabetic agent. Modern scientific studies have authenticated some of these medicinal uses of oleoresin such as its effectiveness as an antibacterial, anti-inflammatory, and anti-oxidant agent (8, 9).

Coronary heart disease is now a serious threat all over the world due to increased number of deaths resulting from it (10). High blood cholesterol concentration is one of the important risk factors for cardiovascular disease (11, 12). In this study, we have sought to verify whether the ethanolic extract of *D. oliveri* leaves predisposes subjects to some cardiovascular problems by measuring some indicators of such.

MATERIALS AND METHODS

Animals.

Twenty male albino rats (*Rattus novergicus*) of the wistar strain weighing between 110 and 140 g were obtained from the small Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. Animals were housed 5 per cage in a standard environmental condition and were allowed free access to commercial pelleted rat chow (Bendel Feeds Ltd, Ewu, Nigeria).

Assay kits and chemicals

The assay kits for total cholesterol, HDL-cholesterol and triacyglycerol concentrations were obtained from Randox laboratories Ltd. (Co. Antrim, U.K) while all other reagents used were of analytical grade.

Preparation of plant extract

The leaves of *Daniella oliveri* were collected from trees within Bida town of Niger State, Nigeria. The leaves were air-dried and pulverized into fine powder. 200g of the fine powder was percolated in 500ml of absolute ethanol. The percolated mixture was filtered and evaporated at room temperature (13).

Extract administration

The animals were randomly divided into four groups (A, B, C and D) with five rats per group. Group A rats received appropriate volume of 0.25% Tween 80 solution orally while groups B, C and D rats were orally administered with 50, 100 and 200mg/kg body weight of the extract dissolved in 0.25% Tween 80 solution respectively for fourteen days.

Tissue preparation

At the end of the experimental period the rats were sacrificed and venous blood was collected into clean sample bottles containing no anticoagulant for the blood to clot. The clotted blood was centrifuged at 3000rpm for 5 minutes (14) and a Pasteur pipette was used to collect the supernatant (i.e. the serum) which was stored frozen until needed for analysis. The heart of each rat was also quickly isolated, cleaned of blood, weighed and suspended in ice-cold 0.25M solution (1:5w/v) in which it was homogenized. The homogenates were stored frozen overnight to ensure maximum release of enzymes (15).

Assay of biochemical parameters

Activities of alkaline phosphatase (ALP) and acid phosphatase (ACP) in the heart were determined by the method of Wright *et al* (16, 17) while the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the heart were assayed by the method of Reitman and Frankel (18). The total cholesterol concentration in the serum was assayed by the method of Frederickson *et al* (19) while the serum HDL-cholesterol concentration was determined using the method described by Albers *et al* (20). Serum triacylglycerol concentration was determined by the method of Jacobs and Demark (21). The atherogenic index was calculated by finding the ratio of the serum total cholesterol concentration to serum HDL-cholesterol concentration.

Statistical analysis

Data was analyzed using Duncan multiple range test following one-way analysis of variance (ANOVA) using SPSS 10.0 computer software package (SPSS Inc., Chicago, U.S.A). Differences at $P < 0.05$ were considered significant.

RESULTS

Serum lipid parameters

As seen in Table 1, the extract had no significant effect ($P > 0.05$) on serum HDL- cholesterol and triacylglycerol concentrations when compared with controls. However, the extract significantly increased ($P < 0.05$) serum total cholesterol concentration at doses of 100 and 200 mg/kg body weight while it only significantly increased ($P < 0.05$) the atherogenic index at the dose of 200mg/kg body weight when compared with controls (Table 1).

Activities of marker enzymes

The extract had no significant effect ($P > 0.05$) on heart AST activity while it significantly increased ($P < 0.05$) heart ALT activity at all doses administered when compared with controls (Table 2). ALP activity was significantly reduced ($P < 0.05$) in the heart at doses of 50 and 100 mg/kg body weight of the extract when compared with controls. Moreover, heart ACP activity was significantly reduced ($P < 0.05$) at doses of 100 and 200mg/kg body weight of the extract whereas the dose of 50mg/kg body weight of the extract significantly increased ($P < 0.05$) it when compared with controls (Table 2).

DISCUSSION

The increase in serum total cholesterol concentration and the atherogenic index at higher doses of the extract administered suggest that the extract may predispose patients to coronary heart disease. This is because increase in the two parameters is considered a strong indicator of cardiovascular disease risk (22, 23). Thus the use of the extract in treating diabetes in folk medicine may complicate the problem of hyperlipidemia commonly associated with diabetes (24). The increase in serum total cholesterol concentration observed may result from an up-regulation in the synthesis of β- hydroxyl- β -methyl glutaryl CoA reductase which catalyses the committed step in the biosynthesis of cholesterol *in vivo* (25). Some hypoglycemic plants have been reported to act by releasing insulin from the pancreatic beta-cells or by potentiating the action of insulin while others act by mimicking insulin (22, 26, 27). Due to the fact that insulin favours the formation of the active form of β-hydroxyl- β- methyl glutaryl CoA reductase thereby enhancing cholesterol biosynthesis, it may be that increase in serum total cholesterol concentration resulted from the release of insulin by the extract from the β - cells or that the extract contains some components that mimic the action of insulin like

Table 1: Effects of ethanolic extract of Daniella oliveri leaves on some serum lipid parameters in rats.

Experimental groups	Total cholesterol concentration (mmol/L)	HDL-cholesterol concentration (mmol/L)	Atherogenic index	Triacylglycerol concentration (mmol/L)
Control	1.80 ± 0.10[a]	0.84 ± 0.09 [a]	2.17 ± 0.07 [a]	0.36 ± 0.09 [a]
50mg/kg b.w. of extract	1.74 ± 0.26 [a]	0.90 ± 0.10 [a]	1.96 ± 0.09 [a]	0.38 ± 0.08 [a]
100mg/kg b.w. of extract	2.50 ± 0.32 [b]	1.08 ± 0.25 [a]	2.00 ± 0.14 [a]	0.42 ± 0.11 [a]
200mg/kg b.w. of extract	2.50 ± 0.14 [b]	0.84 ± 0.09 [a]	2.94 ± 0.05 [b]	0.44 ± 0.13 [a]

Values are mean ± SD of five replicates. Values with different letter superscripts in each column are significantly different (P < 0.05).

Table 2: Effects of ethanolic extract of Daniella oliveri leaves on some marker enzymes in rat heart.

Experimental groups	ALP activity (IU/L)	ACP activity (IU/L)	ALT activity (IU/L)	AST activity (IU/L)
Control	66.6 ± 8.50 [a]	47.0 ± 5.70 [a]	924.0 ± 8.74 [a]	1500.0 ± 224.17 [a]
50mg/kg b.w. of extract	46.2 ± 4.87 [b]	74.0 ± 9.62 [b]	1040.0± 13.24 [b]	1370.0 ± 212.25 [a]
100mg/kg b.w. extract	52.6 ± 7.86 [b,c]	36.0 ± 4.18 [c]	1016.0± 15.17 [c]	1390.0 ± 209.52 [a]
200mg/kg b.w. extract	58.2 ± 4.27 [a,c]	33.0 ± 5.70 [c]	973.0 ± 24.50 [d]	1604.0 ± 195.01 [a]

Values are mean ± SD of five replicates. Values with different letter superscripts in each column are significantly different (P < 0.05).

pinitol which has isolated from the alcoholic extract of *B. spectabilis* leaves (28). The increase may also result from decrease in the cellular uptake of cholesterol (25).

Alkaline phosphatase, acid phosphatase, alanine and aspartate aminotransferases in the heart are important marker enzymes which are used to assess the integrity of the cell membrane, cytosolic activity and cell death (29, 30). The reduction in heart ALP activity may not result from disruption of the cell membrane since there was no corresponding decrease in ALT and AST activities in the heart. The probable reason for the reduction in heart ALP activity is the inhibition of the existing enzyme molecules by components of the extract or reduction in the synthesis of the enzyme (29). The observed reduction in heart ALP activity may lead to less availability of phosphate groups required for oxidative phosphorylation to generate ATP molecules which in turn are used for the phosphorylation of some biomolecules like ethanolamine and choline needed for the synthesis of phosphatidyl ethanolamine and phosphatidyl choline (31). Inability to synthesize these two major membrane phospholipids may affect membrane fluidity thereby decreasing the permeability of the epithelial cells (31).

ACP is a lysosomal marker enzyme (32). The increased heart ACP activity at the least dose of the extract administered may be due to the response of the cellular systems to offset the stress imposed on the enzyme by exposure to the extract which may result from the inhibition of the enzyme activity *in situ* (29,33,34). This regulatory mechanism might have been overcome at higher doses (100 and 200mg/kg body weight), thus leading to the significant decrease in heart ACP activity at such doses. The same reasons may be advanced for the trend of results obtained for heart ALT activity. Heart ALT activity was significantly increased ($P < 0.05$) at all doses administered when compared with control but there was a dose-dependent decrease in activity from the lowest dose to the highest dose administered. This suggests that the strength of the cellular mechanism for offsetting the stress imposed on the enzyme by the extract is gradually being depleted. This implies that there may be a significant decrease in ALT activity at much higher doses than those administered in this study. Since AST activity was not also affected the results thus suggest that the activities of the aminotransferases were relatively spared than those of alkaline and acid phosphatases after administration of the extract.

The results of this study suggest that the administration of the ethanolic extract of *D. oliveri* leaves may predispose patients to some cardiovascular problems which may complicate the health problems of patients with cardiovascular diseases.

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