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Biochemical Changes in Wistar Rats on Oral Doses of Mistletoe (*Loranthus micranthus*)

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Abstract: Problem statement: To evaluate the effects of the water extract of the leaves of mistletoe (*Loranthus micranthus*) (traditionally used antidiabetic and antihypertensive) in male albino Wistar rats. **Approach:** The animals were divided into 4 groups (n = 6). Water extract of *L. micranthus* leaves was administered in graded doses of 0, 275, 551 and 827 mg kg^{-1} body weight (wt) of experimental animals for 21days. Blood samples were collected by cardiac puncture. The serum harvested was analyzed for some biochemical parameters, using assay kits. **Results:** There were no significant changes ($p>0.05$) in the levels of cholesterol, bilirubin, glucose, protein, urea, alkaline phosphatase and Aspartate Transaminase (AST). Alanine Transaminase (ALT) activities of the groups rats given 551 and 827 mg kg^{-1} body weight extracts (15.96 and 14.24 U L^{-1} respectively) showed significant decreases ($p<0.05$) when compared with the control (24.96 U L^{-1}) and the group fed 275 mg kg^{-1} body weight of extract (16.19U L^{-1}). Computed ALT/AST showed decreases in the test groups (0.77-0.78) when compared with the control (1.04). **Conclusion:** The results suggested no adverse biochemical changes being associated with the use of the extract or absence of hepatocellular damage at the investigated concentrations. Thus the use of the plant in the treatment and management of diabetes and hypertension should be encouraged.

Key words: Mistletoe, *Loranthus micranthus*, leaf extract, biochemical parameters

INTRODUCTION

The tropical rain forest of West Africa is endowed with enormous natural resources, mainly medicinal plants. Medicinal plants form the basis of medical treatment in many developing countries[1-4]. It is to this class that the African mistletoe (*Loranthus micranthus* Linn.) (family-Loranthaceae) belongs.

Mistletoe is a semi-parasitic evergreen plant found growing on a host of evergreen and deciduous trees all year round, around the branches of the tree. It is an obligate parasite, obtaining part of its food from the host plant. It depends on its host for minerals and water only, but photosynthesizes its carbohydrate by means of its green leathery, oblong leaves[5].

In Nigeria and some other parts of Africa, mistletoe has been used traditionally as antihypertensive and antidiabetic[6-9]. There have been reports on the phytochemical and antimicrobial properties of African mistletoe *Loranthus micranthus*[9]. However, information is scanty on the effects of the plant on biochemical parameters in experimental animals and on the possible risks associated with consumption of mistletoe extracts. The present study was designed to determine the biochemical changes associated with oral consumption of aqueous extracts of the leaves of the African mistletoe.

MATERIALS AND METHODS

Collection of plant materials and preparation of crude extract: Mistletoe plants obtained from host pear trees (*Persea americana*), were collected from 8 different locations of Ikot Essien Oku Village in Etinan Local Government Area of Akwa Ibom State, Nigeria. The plant was authenticated at the Department of Botany, University of Uyo, Akwa Ibom State of Nigeria. The leaves from the mistletoe plants were dried to constant weight in an oven (Stuart Scientific, UK) at 52°C for 24 h. They were ground into smooth powder (which passed through a 30-mesh sieve) and stored in air container at 4°C until when required.

One hundred grams (100 g) of the powdered material were brewed in 750 mL of boiled tap water and allowed to stand for at least 30 min. The decoction was then filtered (through a plug of cotton wool) and stored in a clean bottle to be administered to rats. Twenty mL aliquots of the decoction were evaporated

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to dryness using an electric heater. The residue left was then used to determine the concentrations of the mistletoe extract administered to different groups of the experimental animals.

Experimental animals and treatment: Twenty four male Wistar rats, weighing between 158.3 and 168.9 g were obtained from the animal house of the Department of Biochemistry, University of Calabar, Nigeria. The animals were divided into 4 groups of 6 rats each. All rats housed in well-ventilated cages (room temperature 24±1°C) were fed normal rat chow (Bendel Feed and Flour Mill Ltd., Benin City, Nigeria, without any restriction to food and drinking water during the experimental period. Rats in group 1 were the control that received distilled water. Animals in the test groups (II, III and IV) were orally fed once daily with 0.5, 1.0 and 1.5 mL of the extract respectively (prepared on each day of the experiment) corresponding to 275, 551 and 827 mg kg^{-1} body weights respectively. Administration of the extract was carried out daily for 21 days.

Preparation of serum: 24 h after the last administration of extract, the animals were anaesthetized under chloroform vapor and dissected. Blood for serum preparation was collected by cardiac puncture, using sterile syringes into sterile plain tubes without anticoagulant. Then serum was harvested for the clot by centrifugation at 3000 g for 5 min using a bench top centrifuge (MSE Minor, England) into sterile plain tubes. The serum was stored in the refrigerator for the analysis of biochemical parameters. All analyses on serum were completed within 24 h of sample collection.

Biochemical assays: Serum Alkaline Phosphate (ALP) Aspartate Transaminase (AST) and Alanine Transaminase (ALT) activities were determined using assay kits (Quimica Clinica Applicada [QCA], Amposta, Spain; Sigma Chemical, St. Louis, MO). Total cholesterol was assayed by enzymatic analysis[11] using a commercial kit from Randox Laboratories Ltd (Crumlin, North Ireland). Bilirubin and total protein were estimated using assay kits (Sigma Diagnostics, St Louis, MO). Urea was estimated by the diacetyl method of Wybenga *et al.*[12]. Glucose was assayed by the glucose oxidase-peroxidase method using a kit (Sigma Diagnostics, India; Sigma kit #315). The absorbances of all the tests were determined using a spectrophotometer (HAICH, DR 3000, Germany).

Statistical analysis: Results were expressed as means ± SD. The data were analyzed by exploring the Student's t-test and $p<0.05$ was regarded as significant.

RESULTS

The activities of the enzymes assayed as well as the concentrations of other biochemical parameters are shown in Table 1. The results indicated dose-related decreases in serum activities of ALT and AST in the test groups (14.26-16.19 and 18.61-21.53 U L^{-1} respectively) when compared with those of the control (24.96 and 23.96 U L^{-1} respectively). However, the groups fed 551mg kg^{-1} body weight and 827mg kg^{-1} body weight extracts had significantly lower ALT activities (15.96 and 14.24 U L^{-1} respectively) relative to those of the control ($p<0.05$). There were mild variations in the ALP activities (31.99-34.30 U L^{-1}) of the experimental rats as were the variations in the ALT/AST ratios of the experimental animals (0.77-1.04).

Table 2 shows the serum concentrations of bilirubin, total protein, total cholesterol, glucose and urea in the experimental animals. There were mild alterations in the serum concentrations of bilirubin (12.53-14.39 μmol L^{-1}), total protein (88.00-91.00 g L^{-1}) and total cholesterol (174.26-177.16 mg dL^{-1}).

Table 1: Effects of aqueous extracts of mistletoe on enzyme activities of rats*

Experimental Group	ALP Units L^{-1}	ALT Units L^{-1}	AST Units L^{-1}	ALT/AST
I 0 mg kg^{-1} body weight	31.99±2.47	24.96±6.11[a]	23.96±3.92	1.04±0.24
II 275 mg kg^{-1} body weight	34.30±5.30	16.19±7.52[a]	21.53±4.36	0.78±0.38
III 551 mg kg^{-1} body weight	32.85±2.11	15.96±2.98[b]	20.86±2.32	0.77±0.10
IV 827 mg kg^{-1} body weight	32.58±5.50	14.24±0.55[b]	18.61±2.00	0.77±0.07

*: Values are means ± SD (n = 6 rats per group). Values in some column with different superscripts represent means that are significantly different ($p<0.05$); ALP: Alkaline phosphatase; ALT: Alanine transaminase; AST: Aspartate transaminase

Table 2: Effects of aqueous extracts of mistletoe on serum chemistry of rats*

Experimental group	Total cholesterol mg dL^{-1}	Total bilirubin μmol L^{-1}	Glucose mmol L^{-1}	Protein g L^{-1}	Urea mmol L^{-1}
I 0 mg kg^{-1} body weight	177.16±3.04	13.83±1.90	4.91±0.50	88.00±2.20	6.63±0.48
II 275 mg kg^{-1} body weight	176.60±3.20	12.53±1.70	4.68±0.15	91.00±5.00	6.72±0.39
III 551 mg kg^{-1} body weight	175.92±3.01	13.28±2.30	4.44±0.19	89.60±5.20	7.09±0.59
IV 827 mg kg^{-1} body weight	174.26±3.16	14.39±2.40	4.35±0.47	86.90±4.30	7.21±0.84

*: Values are means ± SD (n = 6 rats per group)

There were very slight dose dependent decreases ($p>0.05$) in the serum glucose concentrations of the test rats (4.35-4.68 mmol L^{-1}) when compared with those of control rats (4.91 mmol L^{-1}). The urea concentrations of test rats (6.72-7.2 1mmol L^{-1}) were slightly higher ($p>0.05$) than those of the control rats (6.63 mmol L^{-1}).

DISCUSSION

The decreases in the serum AST activities and ALT/AST of the test groups when compared with the control are not significant ($p>0.05$). Serum ALT/AST has been used as an index to monitor liver pathology[14,15]. Ratios higher than unity are indicative of adverse pathological effects on the liver. From the studies, it has been shown that infusion of mistletoe leaves maintain the ALT/AST ratios at favorable levels. This might suggest a non-toxic effect or absence of hepatocellular damage at the investigated concentrations.

Serum ALP is a sensitive detector for intrahepatic and extrahepatic bile obstruction, the presence of infiltrative diseases of the liver and all bone diseases associated with osteoblastic activity (e.g., osteomalacia and rickets among others)[16,17]. From the results obtained, it is likely that the concentrations of mistletoe leaf extract used in this study did not adversely interfere with the calcification and/or metabolic activities involving the liver.

Bilirubin is formed by the breakdown of hemoglobin in the liver, spleen and bone marrow[17]. An increase in tissue or serum bilirubin concentrations results in jaundice. Jaundice occurs in toxic or infectious disease of the liver e.g., hepatitis or bile obstruction. Normal bilirubin concentration is 4-17 μmol L^{-1}. The sight variations (which are within normal limits) in bilirubin concentrations are indicative of non-adverse effects on haemoglobin metabolism. There were decreases in the serum glucose concentrations of the test rats (in a dose-dependent manner), when compared with those of control rats. Aqueous infusions of some medicinal plants have been reported to cause hypoglycemia in rats by increasing the level of insulin in the blood[15,18-21]. Reports by Osadebe *et al.*[10] indicated a significant reduction in blood glucose when crude methanolic extracts of mistletoe leaves were administered to rats. The observed differences in glucose-lowering effects could be attributed to the type of solvent used in preparing the extracts.

The total cholesterol levels of the test animals were lower than those of the control in a dose-dependent fashion. Obatomi *et al.*[8] reported significant reductions in total cholesterol when rats were dosed with 1.32 g kg^{-1} day^{-1} of aqueous extracts of the leaves of the African mistletoe plant, (in addition to reporting significant reductions in blood pressure of normotensive and hypertensive rats). The possible explanation for the observed effect on serum cholesterol levels may be based on the fact that herbs are generally rich in vitamins. Vitamins such as vitamins C and E are good antioxidants capable of preventing lipid peroxidation in both plasma and tissues[22]. This may explain why there were no adverse alterations in cholesterol levels of treated animals relative to the control.

CONCLUSION

From the foregoing, aqueous extracts of mistletoe leaves did not induce adverse alterations in biochemical parameters such as total cholesterol, protein, urea, total bilirubin, glucose, alkaline phosphatase and aspartate transaminase. Aqueous extract of mistletoe leaves may be a good candidate for alternative and /or complimentary medicine in the management of diabetes and hypertension. It may be useful in reducing the risk factor associated with arteriosclerosis, coronary artery and heart diseases. Thus use of infusions of the leaves in the management of hypertension and diabetes should be encouraged.

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