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Effect of sub-acute administration of *Phoradendron leucarpum* ethanolic extract on hepatocytes of Wistar Rats

Tugbobo OS *, Idowu KS, Awonegan AP, Apata DA and Olorunoje HA

Department of Science Technology, Federal Polytechnic, Ado-Ekiti.

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Abstract

The rapid incidence of liver disorders is rampant in our society today and there is this very need to salvage the sudden and untimely death resulting from liver disorders. In this study, aqueous extract of *Phoradendron leucarpum* leaf was administered orally at doses of 50, 100 and 200 mg/kg body weight to wistar rats. Twenty (20) rats were divided into four groups of five in each group of which one group served as the control. The other groups were treated with the ethanolic leaf extract 50, 100, and 200 mg/kg of *Phoradendron leucarpum* (mistletoe). The extract was administered orally and the administration of the extract lasted for 21-days. Effect of the extract on liver function indices was assessed. Treatment of the rats with the ethanolic extract of *Phoradendron leucarpum* leaf showed significant ($p<0.05$) increase in liver biomarker enzymes activities in group IV animals fed with the extract only when compared to the control. There was no considerable increase in enzyme activities in group II and III animals compared with the control group. Hence, *Phoradendron leucarpum* leaf extract could possibly offer protective effects on liver cell membranes at considerably low concentration.

Keywords: *Phoradendron leucarpum*; Biomarker-enzymes; Liver; Hepatocytes; Sub-acute

1. Introduction

Myriads of indigenous plants have been available for use in our environment since time immemorial for curing various ailments without the actual knowledge of their toxic potential. One of such plants used by indigenous people is *Phoradendron leucarpum* otherwise known as mistletoe. The growing interest in herbal medicine demands information on the toxicity risk assessment on different plant preparations used in the management of diseases. *Phoradendron leucarpum* is a family of hemi-parasitic evergreen plants of order Santalales with white berries that usually grow in the crown of apple trees, oaks and other forest trees [1]. The plant is mostly used locally to treat various diseases such as colic, nasal ulcer, asthma, hypertension, diabetes amongst others.

Besides, herbal medicine has generated considerable lot of interest globally for its contribution to the overall health care delivery [2]. A fact from research report revealed that estimated 80% of the population in developing nations depends mostly on herbal medicine and natural products [3]. Disease management and treatment of illness as well as maintenance of health using herbal medicine is an ancient health care practice known to humanity and practiced by all cultures in the history of civilization [4]. Hence, it is in the light of this that the present investigation was set out to provide research information on safety or toxicity risk potential of *Phoradendron leucarpum* leaf extract on liver hepatocytes in wistar rats.

* Corresponding author: Tugbobo OS
Department of Science Technology, Federal Polytechnic, Ado-Ekiti.

2. Material and method

2.1 Plant Materials

Samples of *Phoradendron leucarpum* leaves were obtained from a private farm in Ado-Ekiti, Ekiti-State, Nigeria. The leaves were air dried in the laboratory, pulverized and stored in an air-tight container.

2.2 Reagent and Chemicals

All reagents and chemicals were of analytical grade.

2.3 Extract Preparation

Phorandendron leucarpum leaves were air dried for 45 days at room temperature in the laboratory. The air- dried samples were ground to fine powder using blender. 300g of the powder sample was soaked in 1500ml ethanol for 72hrs and was later filtered using a clean white cloth and freeze dried to obtain the dried extract.

2.4 Experimental Design

Twenty (20) male wistar rats weighing between 130-150g, purchased from central animal house, Department of Science Technology, Federal Polytechnic, Ado-Ekiti were used for the study. The animals were kept under standard condition (12hr light/dark cycle), fed with commercial rat chow (Vital Feeds Nig., Ltd.) *ad libitum* and liberally supplied with water. The animals were divided into four groups designated I, II, III and IV for the experiment in which group II, III and IV were orally administered 50, 100 and 200 mg/kg body weight of *Phorandendron leucarpum* ethanolic leaf extract respectively. Group I animals received distilled water only throughout the duration of the experiment and served as the control. The experiment was carried out for period of 21 days and lethal concentration (LC_{50}) was determined with five different concentrations (50, 100, 200, 400, 1000 mg/kg) of the extract. Mortality rate was detected at two concentrations (800 and 1000 mg/kg). The animals were sacrificed and liver was quickly excised and homogenized where the liver homogenate was centrifuged and the supernatant collected was used for the assay.

2.5 Biochemical Assay

The liver was excised and homogenized in ice cold sucrose solution in a ratio 1: 5 w/v. The homogenate was centrifuged at 3500 rpm for 15 min and the supernatant was separated and stored at -20 0C which was used for the biochemical assay. ALT activity was conducted by adding 0.2 ml of the sample homogenate to 1 ml L-ALT buffered substrate. The mixture was incubated at 37 0C for 30 min. 1 ml 2,4-dinitrophenylhydrazine was added to the mixture and the solution was allowed to stand for 20 min while 0.4 M NaOH was also added and allowed to stand for 5 min. The absorbance was read at 505 nm and the enzyme activity was determined from calibration curve of L-ALT while similar procedure was observed in other enzymes assay. The biochemical parameters were determined colorimetrically by employing the use of Randox kits where activities of aspartate amino transferase (AST) and alanine amino transferase (ALT) described by [5], alkaline phosphatase (ALP) described by [6] were assessed. Besides, the concentration of total protein (T.P), albumin (Alb), bilirubin (B), urea (U), uric acid (U.A) and creatine kinase (CK) in the rat liver were determined as described by [7].

2.6 Statistical Analysis

All values are expressed as mean ± SD and statistical evaluation was done using one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). The significant level was set at ($p<0.05$).

3. Results

Table 1 Effect of *Phoradendron leucarpum* ethanolic leaf extract on liver function indices

Parameters		Group I	Group II	Group III	Group IV
ALT	(U/I)	18.10± 1.02^{a}	19.01±1.20^{a}	19.62±1.03^{a}	25.42±1.02^{b}
AST	(U/I)	22.17±0.12^{a}	23.10±0.4^{a}	24.92±0.73^{a}	27.20±0.98^{bc}
ALP	(mg/dl)	56.24 ± 0.53^{a}	58.06±0.82^{a}	57.03±0.54^{a}	61.26±0.30^{b}
BIL	(U/I)	19.84± 0.57^{a}	20.72±0.52^{a}	20.18±0.39^{a}	26.03± 0.66^{b}

ALB	(g/l)	11.17±0.21[a]	10.23±0.17[a]	12.16±0.10[a]	12.19±0.13[a]
CK	(U/I)	27.84± 0.67[a]	25.72±0.62[a]	26.18±0.43[b]	29.73± 0.85[b]
Urea	(U/I)	10.17±0.70a	9.23±0.05[a]	9.16±0.02[a]	8.96±0.15[a]
Uric. A	(U/I)	4.17±0.09[a]	4.23±0.06[a]	3.16±0.05[a]	3.09±0.02[a]
TP	(mg prot/ml)	1.17±0.03[a]	1.23±0.02[a]	2.16±0.02[a]	2.09±0.04[a]

Values (mean ± SE) with different superscript are significant (P<0.05)

4. Discussion

The effect of ethanolic leaf extracts of *Phoradendron leucarpum* on liver function indices of wistar rats shown in table 1 indicates insignificant increase in activity of liver ALP, ALT, AST, CK, uric acid, albumin, total protein and urea in rats treated with 50 mg/kg and 100 mg/kg of leaf *Phoradendron leucarpum* leaf extract compared to control. However, significant (P<0.05) increase in activity of the enzymes was observed in group IV animals administered 200 mg/kg ethanolic leaf extract of *Phoradendron leucarpum* when compared to control. The result of this study revealed a concentration dependent increase in activity of liver enzymes with remarkable increase at 200 mg/kg *Phoradendron leucarpum* ethanolic extract fed to animals in group IV which was significant (P<0.05) compared to values obtained for animals in group I, II and III. Similar result has been reported for a study on administration of ethanolic extract of *Pilostigma thonningii* on creatinine concentration [8]. Specific marker enzymes for the plasma and endoplasmic reticulum are frequently used to access the integrity of plasma membrane. Their alteration in the tissue and serum would likely indicate damage to the external boundary of the cell [9]. The significant (P<0.05) increase in the marker enzymes ALT, AST and ALP activities observed at 200 mg/kg extract concentration compared to control may be due to particles interference or de novo synthesis in the liver cells in response to contaminant. The insignificant difference in the liver biomarker enzymes activities observed in this study suggests that administration of *Phoradendron leucarpum* leaf extract may not contribute to liver damage at lower concentration.

5. Conclusion

In conclusion, this study re-emphasizes the needful consumption of *Phorandendron leucarpum* leaves for its medicinal values and its essential protection on liver function indices.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there is no conflict interest on this research.

Statement of ethical approval

The research was ethically approved by the centre for research innovation and development (CRID), Federal Polytechnic, Ado-Ekiti, Nigeria.

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