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Effect of aqueous extract of *Loranthus micranthus* (mistletoe) on total protein, catalase and NOS activity in the kidney of albino rats induced with Pb acetate

Imade F¹, Imade M², Oyewole B³

1. Department of Botany, Ambrose Alli University, Ekpoma, Edo State, Nigeria
2. Biochemistry Department, Delta State University, Abraka.
3. Department of Botany, Olabisi Onabanjo University, Ago-Iwoye, Nigeria.

Corresponding Author: Imade F, Email: imadefrancis@gmail.com

The effect of aqueous extract of *Loranthus micranthus* on the kidney of albino rat induced with Lead acetate on catalase activity, nitric oxide scavenging (NOS) activity and total protein was investigated. 30 albino rats were used and they were divided into 6 groups. 200 mg/kg of lead acetate was injected intraperitoneally into the rats to intoxify and the extract was administered orally as a therapeutic agent. Group 1 (Negative control) received a normal rat feed, while group 2 to 5 were intoxicated and given 500 mg/kg, 100 mg/kg, 1500 mg/kg, 2000 mg/kg of extract respectively and group 6 serves as the positive control (without treatment). Duration of the experiment lasted for 3 weeks. Results indicate that at low concentration, extract significantly ameliorates total protein, nitric oxide radical scavenging activity (RSA), catalase activity ($p < 0.05$). 500 mg/kg of mistletoe extract significantly ameliorated the altered level of total protein, catalase activity and nitric oxide RSA status in group 2 when compared to group 6. The extract possessed antioxidant activity as seen from the ameliorating effect on oxidative stress in intoxicated rat. Therefore, it can be deduced that mistletoe extract has the potential of ameliorating the altered protein synthesis and antioxidant activity in a dose dependent manner.

Keyword: Catalase, protein, activity, kidney, albino rats.

1. Introduction

There is an increasing demand for medicinal plants and plant products as alternative to orthodox medicines, especially in developing countries (Murray,^[1] cited in Orji *et al.*,^[2]). The need for more potent, safe and affordable drugs has led to intensified research into herbal drugs, the result of which is the introduction of new herbal preparation for therapeutic uses^[3].

African mistletoe (*Loranthus micranthus*) belongs to the family Loranthaceae, and it is an obligate semi-parasitic plant (shrub) of *Kola acuminator*, *Baphia nitida*, *Citrus limon*, and *Pentaclethra macrophylla*, *K. nitida*, *Mangifera indica*, *Azadirachta indica*, *Jatropha curcas*, and *Persia* sp.^[4,5], 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]. It is commonly found in the tropical rain forest region of Nigeria and other parts of Africa, Europe and America^[2].

It has been reported in recent time that 50% of top pharmaceutical products sold are linked to natural product research (Falodun *et al.*, 2007). Mistletoe preparations are commonly used in complementary medicine as anticancer agents^[6], antidiabetic agents^[7], bacteriostatic agent and antihypertensive agent^[8]. The herb has been analyzed and observed to contain Lecithin, Viscotoxin, Polysaccharides and many Phytochemicals as active ingredient^[9].

In particular, Osadebe and Omeje, ^[10], Ameer *et al.* ^[11] reported the possibility of *Loranthus micranthus* acting as antihypertensive, which according to Egbuonu ^[12] is a class of drugs that should induce a reduction in blood pressure and prevent the complications of hypertension or high blood pressure with minimal induction of an adverse response. *L. micranthus* has been used in the treatment of atherosclerosis, AIDS, Skin Problems, such as acceleration of skin ageing ^[13, 14]. It has been reported to have hypoglycemic properties because it decreases the blood glucose level and has effects of controlling the loss of body weight which occurs during diabetes milletus ^[15, 16]. This plant has also been used in the effective treatment of epilepsy, infertility in men and women, menopausal syndrome and rheumatism ^[4].

Lead(Pb), a non- biodegradable heavy metals, continues to pose health hazards in man and animals in india and elsewhere in the world. It affects each and every organ and system in the body ^[17]. Being a cumulative poison, Pb is acumulated in various organs viz; liver, kidney, brain, bone and haemopoietic system ^[18]. Several metal chelators have been used to manage lead toxicity in the event of exposure, but none is suitable in reducing lead burden in chronic lead (Pb) exposure ^[19]. Moreover, these chelators have a toxic potential in themselves and often fail to remove Pb from all body tissues ^[20]. In th ancient indian system of medicine (Ajurveda), a number of plants and the herbs have been indicated for amelioration of metal poisoning ^[21].

Efforts are now being directed in obtaining drugs with different chemical features since many modern drugs originated from plants, the investigation of the chemical component of traditional medicinal plants could lead to development of new drugs. It is necessary to obtain more scientific information concerning the efficacy and safety of the remedies in use because many people already depend on herbal medicines such as mistletoe plant for the treatment of metabolic disease and cardiovascular disease. Health planners in developing countries also

requires such informational for evaluating the positive and effective use of traditional medicine In Nigeria and other parts of Africa, the plant is used in ethno medication against diabetes, hypertension, schizophrenia and also helps as an an immune booster ^[10, 22]. However, information is scanty on the effects of the plant on prophylactic effect on experimental animals.

The present study therefore was designed to investigate the prophylactic effect of aqueous extract of mistletoe (*Loranthus micranthus* Family Loranthaceae) on total protein, catalase activity and nitric oxide scavenging activity in kidney induced with Lead acetate.

2. Materials and Methods

2.1. Experimental animals

The experiment was conducted on thirty (30) albino wister rats obtained from the animal house of the department of medical physiology, Delta State University, Abraka, Delta state, Nigeria. They were housed in wooden cages under controlled conditions with proper bedding and fed wit growers mash obtained from Top feeds, Sapele, Delta state and were given water ad libitum. They were acclimatized for three (3) weeks before the starting of the experiment.

2.2. Source of plant material

Fresh mistletoe plant were collected from a natural habitat around Delta State University, Abraka. They were identified, confirmed and authenticated at the Department of Botany, Delta State University, Abraka.

2.3. Extract preparation

Fresh leaves of mistletoe from host plant (kolanut) were collected, the leaves were first washed free of sand and debris, wash water blotted off and then it was dried and pulverised separately into coarse powder. the resulting powder was then used for extraction.

50 g of the grinded sample was weighed and boiled with 50 ml of water for 30 minutes. It was then sieved and the filtrate made up to 50 ml with

warm water to have a final concentration of 100% w/v.

100% w/v or 1 g/ml= 1000mg/ml boiled extract stock.

2.4. Administration of lead acetate and aqueous mistletoe extract

Lead acetate group rats were given lead acetate diluted with normal saline. Lead acetate was injected at a dose of 200 mg/ kg body weight (intraperitoneal injection) and the mistletoe aqueous extract was given to groups orally induced with lead acetate except group 6, which served as a positive control, at the end of this period , the rats were fasted overnight and sacrificed under chloroform anaesthesia

2.4.1. Experimental protocol

The rats were divided into six groups of five rats per group treated as follows:

Group 1: (Negative control): These rats were given their normal diet without induction of toxicity with lead acetate nor given mistletoe extract.

Group 2: These rats were intoxicated with 200 mg/kg bwt lead acetate and thereafter given their normal diet and aqueous mistletoe extract (500 mg/ml).

Group 3: These rats were intoxicated with 200 mg/kg bwt lead acetate and thereafter given their normal diet and aqueous mistletoe extract (1000 mg/ml).

Group 4: These rats were intoxicated with 200 mg/kg bwt lead acetate and thereafter given their normal diet and aqueous mistletoe extract (1500 mg/ml).

Group 5: These rats were intoxicated with 200 mg/kg bwt lead acetate and thereafter given their normal diet and aqueous mistletoe extract (2000 mg/ml).

Group 6: (Positive control): These rats were intoxicated with 200 mg/kg bwt lead acetate and thereafter given their normal diet. No treatment with aqueous mistletoe extract.

2.4.2. Collection and weighing of organs

The rats were sacrificed under chloroform anaesthesia. They were quickly dissected and their kidney, heart and blood vessels were removed and weighed using the meter P163 weighing balance. A Half gram of wet kidney organ was homogenized in 4.5 ml of normal saline. Supernatant was obtained by centrifugation at 1000 rpm for 15 minutes. The supernatants were then stored in a freezer until required for the various biochemical investigations.

2.4.3. Chemicals

Lead acetate, sulphanilamide, naphthyethylenediamine and dihydrochloride, sodium nitroprusside and glacial acetate were obtained from the BDH chemical Co., England. Every other chemical used for the animal experiments was purchased locally from associated laboratories in Obiaruku, Delta State, Nigeria and was of analytical grade.

2.5. Total protein estimation

Total protein estimation was determined using the Biuret method. The principle involves Cupric ions in an alkaline medium, interact with protein peptide bonds resulting in the formation of a coloured complex.

Procedure used include as shown:

	Reagent Blank	Standard	Sample
Distilled water	0.02 ml	-	-
Standard	-	0.02 ml	-
Sample	-	-	0.02 ml
Reagent 1	1.0 ml	1.0 ml	1.0 ml

1. Test tubes were arranged in a test tube rack, labelled reagent blank, standard and sample;
2. To each test tube was added 1.0 ml of reagent 1;

3. To test tube labelled reagent blank was added 0.02 ml of dist. Water. To test tube labelled standard; 0.02 ml of standard was added. To test tube labelled sample; 0.02 ml of sample was added.

4. The test tubes were mixed gently and incubated for 30 minutes at 25 °C.

5. The absorbance were read at a wave length of 546 nm against reagent blank.

2.6. Determination of catalase activity

2.6.1. Preparation of reagents

Dichromate/ acetic acid solution (5% $K_2Cr_2O_7$ IN 0.1 M glacial acetic acid). 5 g of dichromate (BDH Chemical CO., England) was dissolved in distilled water and made up to 100 ml. Thereafter, 570 μ l of acetic acid was added and thoroughly mixed (this will bring the acetic acid concentration to 0.1 M).

0.2 m H_2O_2 (Hydrogen peroxide): 6.8 of 30% (w/w) aqueous H_2O_2 (M. Wt 34g/mol) was mixed with distilled water and the solution made up to 300ml

10 Mm Phosphate Buffer (pH 7.0): 3.581g of $Na_2HPO_4 \cdot H_2O$ and 1.19 g of $NaH_2PO_4 \cdot 2H_2O$ was dissolved in distilled water. The pH was adjusted to 7.0 using NaOH and HCL as required. The solution was made up to 1 litre

2.6.2. Methods

This experiment was carried out using the method described by Sinha ^[23] with some modifications. 1ml of sample was mixed with 1ml distilled H_2O give 1 in 2 dilution of the sample. The assay mixture contained 2 ml of H_2O_2 solution (0.2 M) and 2.5 ml of phosphate buffer in a 10 ml flat bottom flask. 0.5 ml of properly diluted enzyme preparation was rapidly mixed with the reaction mixture by a gentle swirling motion. The reaction was run at room temperature. A 1ml portion of the reaction mixture was withdrawn and blown into 1ml dichromate/acetic acid reagent at 30 and 60 seconds. A blank was prepared in a similar way in the absence of sample and absorbance

read at 610 nm also at 30 and 60 seconds, the percentage hydrogen inhibition and catalase activity were calculated as described below:

Calculation

$$\text{Decrease in absorbance per minute} = \frac{A_{60} - A_{30}}{0.5\text{min}}$$

Where A_{30} = Absorbance after 30 seconds, A_{60} = absorbance after 60 seconds

$$\% \text{ inhibition of } H_2O_2 = \frac{\text{Decrease in absorbance for sample}}{\text{Decrease in absorbance of blank}} \times 100$$

1 unit of CAT activity was given as the amount of CAT necessary to cause 50% inhibition of the oxidation of hydrogen peroxide during 1 minute. Hence, unity of Activity = % inhibition / 50

2.7. Nitric oxide scavenging activity assay

The scavenging effect on nitric oxide (NO*) radical was measured according to the method of Marocci *et al.* ^[24]

2.7.1. Preparation of reagents

25 mM Sodium nitroprusside: This was prepared by dissolving 372.5 mg of sodium nitroprusside, Mwt. 298.0 (Hopkins and Williams) in distilled water and made up to 50 ml.

Griess reagent: This was prepared by dissolving 1 g of sulphanilamide in 50 ml of distilled water containing 0.1 g of naphthylenediamine dihydrochloride and 5.0 ml of H_3PO_4 and finally made up to 100 ml with distilled water. This brings the final concentration to sulphanilamide, naphthylenediamine and H_3PO_4 to 1%, 0.1% and 5% respectively.

Procedure: 0.2 ml of organ homogenate (diluted 1:1) was added in the test tubes to 1ml of sodium nitroprusside solution (25 mM) and the tubes incubated at 37 °C for 2 hours. An aliquot (0.5 ml) of the incubated solution was removed and diluted with 0.3 ml of Griess reagent (1%

sulphanilamide in 5% H₃PO₄) and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed was immediately read at 570 nm against distilled water as blank. Also absorbance of reagent blank was read as control.

Result were expressed as percentage radical scavenging activity (RSA):

$$\% \text{ RSA} = \frac{(1 - \Delta \text{Abs of sample})}{\Delta \text{abs of reagent blank}} \times 100$$

2.8. Statistical analysis

The values were expressed as Mean \pm SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) using SPSS(Statistical package for social science)

3. Results

3.1. Catalase Activity

Values in the table indicates that there was an increase in the catalase activity when administered 500 mg/kg mistletoe extract which was significantly different from the positive control.

3.2. Nitric Oxide Scavenging activity

The table also shows that there was a significant decrease in group 6 relative to other groups. Also, there was no significant difference amongst group 1, 2 and 3; 4 and 5

3.3. Total Protein

The table above shows a significant increase in group 2 when compared to other groups and also a significant difference between group 1 and 6.

Table 1: The effect of mistletoe extract on catalase, radical scavenging assay, and total protein in the kidney of lead acetate induced albino rat.

Groups	Catalase activity (unit/mg protein)	% Nitric oxide radical scavenging activity (%RSA)	Total protein (mg/ml)
1	1.0 \pm 0.416e	186 \pm 3.6a	93.0 \pm 2.6c
2	3.52 \pm 92b	184 \pm 2.6a	159.4 \pm 16.4a
3	1.92 \pm 0.0005c	182.4 \pm 3.5a	106.2 \pm 8.0b
4	1.28 \pm 0.176	176 \pm 4.6b	106.2 \pm 8.0b
5	7.04 \pm 2.92a	162 \pm 4.2c	93.0 \pm 8.0c
6	1.67 \pm 0.176d	147 \pm 8.4d	73.0 \pm 1.8d

Mean with similar letters are not significant at p>0.05

4. Discussion

Lead is a persistent and a common environmental contaminant. Like other commonly found persistent toxic metals e.g. mercury, lead damages cellular material and alters cellular genetics, Kidneys are particularly susceptible to the effect of toxic agents that can cause renal damage and even renal failure. Moreover, many studies show a strong association between lead exposure and renal effects. In our present work, administration of lead caused lead accumulation and toxicity. This has been confirmed by a significant elevation of lead level in the kidney of rat. The observed toxicity induced by lead acetate

in rats was similar to those previously reported by Patra *et al.* [24] and Shalan *et al.* (2005 [25]) cited in Gamil *et al.* 2009 [26].

Catalase is an enzymatic antioxidant that is widely distributed in all animal tissues and the highest activity was recorded when treated with plant extract. Catalase detoxifies hydrogen peroxide [27] and protects the tissue from highly reactive hydroxyl radicals. Thus the reduction in the levels of these enzymes as a result of the Lead acetate poison that was injected into the animals may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide.

Results obtained for catalase activities (antioxidant enzyme) shows that the generation of ROS induce the biological system to secrete of catalase which will mop up the free radical and as it increases catalase activity drops which is in agreement with earlier report by [28]. The amount of antioxidants present under normal physiological conditions is just adequate to quench the free radicals that are generated at a normal physiological rate. Any further increase in free radicals can cause an imbalance between free radicals and antioxidant leading to oxidative stress [29]. This is where the role of antioxidant becomes relevant. The recovery of the antioxidant enzyme in the extract, treated group, justifies the antioxidant potentials of the African mistletoe, making it useful in combating oxidative stress and it was found to be effective at a concentration of 500 mg/ml this is the one of the most significant finding in this study.

Also, the hepatotoxic and haematotoxic effects of lead poisoning earlier documented has been established [30]. The detoxification effect of *L. miranthus* (anti-nephrototoxic effect) was observed as shown by a statistical significant increase in catalase activities, there was an initial increase in catalase activity in group 6 when Lead acetate is administered alone. Nwokocha *et al.* (2012) [30] reported that lead toxicity increased catalase activities which is in agreement with this study.

The level of protein was significantly decreased in lead acetate treated group when compared to control group (Table 1). the decrease in total protein is as a result of the generation of ROS, which mediate damage to structures including; lipids and membranes, protein and nucleic acids, this damage is often referred to as oxidative stress [31] (Poli *et al.*, 2004). when lipid peroxidation increases, DNA is damaged and when DNA is damage translation and transcription cannot take place thus, protein synthesis is impeded. Administration of the extract of *L. miranthus* (African mistletoe) significantly increased the level of total protein at a concentration of 500 mg/ml but at higher concentration total protein

decreased showing that at higher concentration mistletoe becomes toxic.

NO is a free radical produced in the mammalian cells and is involved in regulation of various physiological processes. However, excess production of NO during oxidative stress could be deleterious. In the present study lead acetate reduces the scavenging activity of the biological system as can be seen in group 6 and mistletoe was found to increase nitric oxide scavenging activity in a dose dependent manner.

The administration of extract of *Loranthus micranthus* (African mistletoe) at a dose dependent manner with lead acetate represents its ability to maintain the normal functional status of kidney.

4.1. Conclusion and Recommendation

In conclusion, there is a heightened concern of the general toxic substances which affects our environment and human health, and the need for ways of ameliorating the adverse effect on the physiological system. The result of the study reveals that mistletoe has considerable antioxidant potentials as at a dose dependent manner as seen from the percentage nitric oxide radical scavenging activity and catalase activity of the animals, justifying its therapeutic use in herbal medicine. In addition, the plant has proven to have anti-nephrotoxicity potentials and could be useful in managing the complications arising from kidney diseases such as, impaired renal function, protein metabolism and antioxidant status.

In this study *L. miranthus* (African mistletoe) has been established to have anti-nephrotoxic and antidote effect. Therefore, the ingestion of *L. miranthus* should be encouraged, especially in individuals who may be exposed to increased risk of lead poisoning.

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