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Research Article

HYPOGLYCAEMIC INVESTIGATIONS OF MISTLETOE (*Loranthus micranthus*) LEAF EXTRACTS ON DIABETIC RATS

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ABSTRACT

This study investigated the hypoglycaemic activities of water extract of mistletoe leaf in the management of diabetes mellitus. Male Wistar rats were used for this study and they were housed to acclimatize in five different cages according to their groups. Each group contained four animals. Diabetes was induced in rats in all but groups 4 and 5 following the intravenous injection of alloxan monohydrate (90mg/kg) dissolved in normal saline through rat tail vein. Group 1 diabetic rats were treated with 600mg/kg body weight concentration of crude methanol extract of *L. micranthus* leaves orally. Group 2 diabetic rats were treated with 600mg/kg body weight concentration of crude water extract of *L. micranthus* leaves orally. Group 3 diabetic rats were treated with 250mg/kg body weight concentration of glibenclamide orally. Group 4 diabetic rats were not treated and served as positive control. Rats in Group 5, which were non-diabetic, received normal saline and served as negative control. The experiments were repeated using different Wistar rats for groups 1, 2, and 4 for the second, third, and fourth weeks. The results of this study showed that both the methanol and aqueous extracts of *L. micranthus* leaves significantly ($P<0.05$) reduced mean fasting blood sugar concentrations in normal rats. In diabetic rats, both extracts caused a significant ($P<0.05$) reduction in serum glucose levels.

Key Words: Hyperglycaemia, mistletoe, *Loranthus micranthus*, diabetes mellitus, histopathology, glucose, alloxan.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia with lipoprotein abnormalities (Scoppola *et al*., 2001). It is one of the main threats to the human health in the 21st century (Zimmet, 2000). Diabetes mellitus is of two types: Type 1 diabetes mellitus or insulin dependent diabetes mellitus (IDDM) and Type 2 diabetes mellitus or non-insulin dependent diabetes mellitus (NIDDM). This study focused on the use of traditional plant remedies in the management of blood glucose during diabetes mellitus using animal models. As we reported earlier, *Loranthus micranthus* leaf extracts indicated antilipidaemic properties (Eze-Steven and Njoku, 2010), so we investigated the hypoglycaemic properties of the aqueous and methanol extracts of African mistletoe (*Loranthus micranthus*). Traditional plant remedies have been in use for centuries in the treatment of diabetes, but only a few have been scientifically evaluated (Akhtar and Ali, 1984). In Nigeria, local herbal practitioners claim that extracts from mistletoe leaf is effective in the management of high blood glucose levels that is associated with diabetes mellitus (Osadebe *et al*., 2004). Mistletoe is a semi-parasitic woody perennial plant commonly found growing on oaks and other deciduous trees. Its semi-parasitic nature is because the plant synthesizes its own chlorophyll but depends on the host for its supply of water and minerals. (Duke, 1985). The plant relates to several different species of perennial, evergreen, parasitic shrubs from different genera including the American mistletoe (*Phoradendron leucarpum*), the European mistletoe (*Viscum album*), and the African mistletoe (*Loranthus micranthus*), which is the European equivalent of *Viscum album* and the American equivalent of *Phoradendron*. *Loranthus micranthus* belongs to the family of African bushy plants called Loranthaceae (Anderson and Phillipson, 1982 and Newall *et al*., 1996).

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MATERIALS AND METHODS

Animals

Male Wistar albino rats of about 8 to 11 weeks old and weight range of 100 to 200g were used for this study. The rats, obtained from the animal houses of both the Faculty of Biological Sciences and Veterinary Medicine of the University of Nigeria, Nsukka, Nigeria (UNN), were kept under standard conditions for 7 days with water and food *ad libitum* for acclimatization before the experiments commenced.

Animal Stock

Fifty-six male Wistar rats were used for this study. They were acclimatized and housed in separate cages according to their groups. Diabetes was induced in rats by the intravenous injection of alloxan monohydrate (90mg/kg) through the rat's tail vein. Diabetic condition in the rats was observed with an increase in the rat's blood glucose concentration and other clinical features including loss of weight, increased frequency of urination and ulceration in the peritoneum region.

Following the induction of diabetes, the rats were divided into five groups of four animals each (n = 4).

Group 1 was diabetic rats treated with 600mg/kg methanol extract (ME) of *Loranthus micranthus* orally.
Group 2 was diabetic rats treated with 600mg/kg of the aqueous (distilled water) extract orally and was called the AE.
Group 3 was diabetic rats treated with 250mg/kg of glibenclamide orally. Diabetic untreated rats named the positive control made up the
Group 4. And **Group 5** included non-diabetic rats given normal saline.

The experiments were repeated with different animals for groups 1, 2, and 3 for the second, third, and fourth weeks.

PLANT MATERIAL

The experiment was carried out in two parts with the same plant material. One part was carried out using methanol extract while the other was carried out using aqueous (distilled water) extract.

Preparation of the Methanol and Aqueous Extracts

The procedure for the preparation of the methanol extract was as we earlier reported (Eze-Steven and Njoku, 2010). The second part of the dried leaves were also pulverised into coarse form and used for the preparation of the aqueous extract.

Determination of the Concentration of Various Extracts

To determine this, known weights of both extracts were determined separately. The weight of dry crucible was also determined. Later, known weights of both extract were put into the dry crucible, respectively, and their weight determined before heating. The crucible with its content was heated and after the heating, the crucible was weighed with its heated content and the weight recorded. The concentrations of both extracts were then calculated from the various weights.

Acute Toxicity Test (LD_{50})

The median lethal doses (LD_{50}) of the methanol extract and aqueous extract were determined in mice using the oral route of administration (Lorke, 1983). Both extracts did not cause death in mice at dose concentrations above 2900mg/kg. Consequently, the methanol extract and aqueous extract of mistletoe (*Loranthus micranthus*) are considered non toxic (Lorke, 1983) at such concentration.

Determination of Plasma Glucose concentration

ONE-TOUCH blood glucose monitoring meter and test strips (LifeScan Inc. Johnson-Johnson Company, Multiplier California, USA) were used for this assay. The principle of the reaction is based on the glucose oxidase reaction. The blood glucose estimation involves the reaction between glucose in the blood and oxygen in the presence of glucose oxidase, which is immobilised in the test strip, to yield gluconic acid and hydrogen peroxide. The hydrogen peroxide subsequently oxidizes the dye in a reaction mediated by peroxidase to produce a blue-coloured product. The intensity of the colour produced is proportional to the glucose concentration in the blood sample. The colour intensity was read instantly from the ONE-TOUCH meter.

Statistical Analysis

The results of the experiment were presented as mean±SEM and were subjected to One Way Analysis of Variance (ANOVA). The differences between the means were tested using post Hoc L.SD at $P<0.05$ significance level.

RESULTS

The methanol and aqueous extracts significantly ($P<0.05$) reduced the fasting blood sugar in normal wistar rats. The reduction in fasting blood sugar caused by the methanol extract was higher within the first 6 h post-administration compared to that of the aqueous extract. The effect of the aqueous extract increased gradually within the first 2 h post-administration. Both extracts did not significantly ($P>0.05$) affect the fasting blood sugar in normal rats after 18 h post-administration.

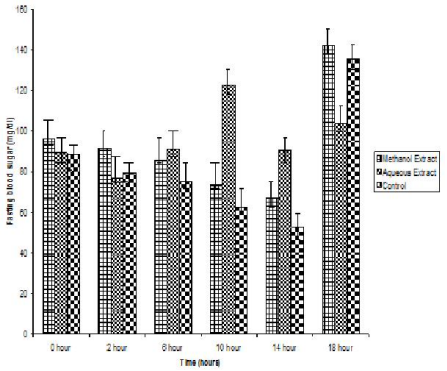

Fig. 1: Effect of extracts on fasting blood sugar concentration of normal rats

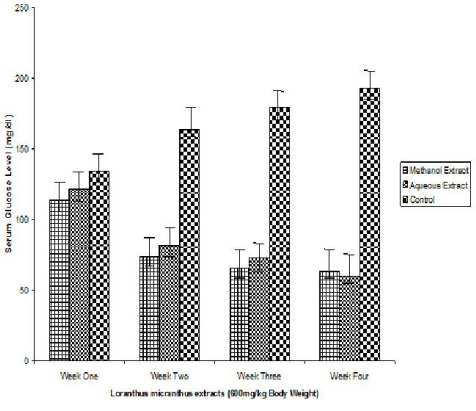

Fig. 2: Effect of extracts on blood glucose level of diabetic rats

The methanol and aqueous extracts significantly ($P<0.05$) reduced the blood glucose level of alloxan-induced diabetic rats within the four weeks of treatment. The extracts caused highest reduction in serum glucose level after the second week of treatment. The magnitude of effect caused by the methanol extract was significantly ($P<0.05$) higher than that of the aqueous extract.

DISCUSSION AND CONCLUSION

The results of this research work show a gradual reduction in the serum glucose concentrations in both fasting and non-

fasting states in the diabetic and non-diabetic rats. The crude extracts gradually reduced the serum fasting glucose concentrations in the non-diabetic rats. In the diabetic states, both extracts also indicated an antihyperglycaemic activity. This shows that the methanol extract and aqueous extract of *Loranthus micranthus* have antihyperglycaemic properties with the methanol extract having a greater effect than the aqueous extract. This result confirms that the methanol extract shows antilipidaemic properties as we reported earlier but might have some destructive effect on the hepatocytes of treated rats. This could be a result of destructive effect of the alcohol on the hepatocytes of treated rats. It further indicates that the methanol extract has a less destructive effect on the kidneys than it has on the liver. The liver is a vital organ involved in the detoxification and deamination reactions in the body. The kidneys are involved in the excretion of metabolites, mostly inactive, from the body. Thus, during the process of detoxification and deamination, some non-detoxified compounds in the methanol extract destroyed the hepatocytes of the organ of detoxification more than those of the kidney cell involved in excretion.

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