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HEPATOTOXICITY AND NEPHROTOXICITY EFFECT OF MURRAYA KOENIGII LEAVES EXTRACT IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT
Murraya Koenigii is a medicinal plant, which is claimed to have hypoglycemic potentials. The study was carried out to investigate the toxicity of long time usage of *Murraya Koenigii* in normal and alloxan-induced rats. In this study 30 rats were used which includes 5 normal rats, 5 diabetic untreated rats, 5 diabetic treated with aqueous extracts, 5 diabetic rats treated with ethanolic extract and 5 diabetic rats treated with standard drug. Aqueous and ethanolic extracts of this plant were prepared and blood glucose lowering effect and improvement of body weight gain in alloxan induced diabetic rats were measured and compared with that of standard drug. Rats were administered *Murraya Koenigii* extracts orally for 28 days, respectively. The animal were further sacrificed after the study and their serum collected to test for the effect of the extract on the liver and kidney. Although aqueous extract was more effective and no significant effect on the liver and kidney parameters when compared with non-induced untreated group. The data were compared statistically by using student's unpaired *t*-test. The present study clearly indicated that the ethanolic and aqueous extract of *Murraya Koenigii* has some hypoglycemic properties and maybe safe to use.

Key word: hypoglycemic, *Murraya Koenigii*, alloxan and diabetic rats.

INTRODUCTION
Diabetes Mellitus is a global disease found in all Nations of the World with high prevalence rate. Diabetes is a metabolic disorder characterized by the body inability to regulate blood glucose caused by relative or absolute deficiency in insulin producing cells in pancreas. The disease occurs when the insulin receptors are resistant to the functions of circulating insulin. (Ada, 2010) There are two main types of diabetes mellitus depending on its etiology and treatment. These are type 1 or insulin dependent diabetes mellitus which can only be controlled by insulin therapy and type 2 or non-insulin dependent diabetes mellitus which is the most common and starts in later life (over age 40) or obese individuals and can be improved with medication and exercise. (David, *et al.*, 2011)
To keep the normal level of glucose in the body, the kidney removes the extra sugar from the blood and excrete it through urine. When glucose level exceeds the renal threshold (160-180mg/L) glucosuria occurs with wastage of energy and increased excretion of water and sodium. Different techniques like drug therapy, dietary therapy, spices and natural product therapy has been employed to reduce the incidence rate of the disease, abolish the symptoms, minimize risk of hypoglycemia, minimize the long macro-vascular and micro-vascular complication which can result to death. Common Symptoms include weight loss, polyuria, polydipsia and polyphagia. (Cooke *et al.*, 2008).

Consequently, a number of plants indigenous to Nigeria have been studied, and found to have hypoglycemic effects. These effects were traced to phytochemicals like flavonoid called active principles that can be extracted from plants (Osadebe *et al.*, 2004). One of such anti-diabetic plants is *Murraya Koenigii*. Every part of the plant has shown to be of medicinal importance and has a wide

range of medicinal effects. Among the medicinal effects are; a vast documentation of the antidiabetic effects of *Murraya Koenigii*.

MATERIALS AND METHOD

Subjects and Grouping: 30 female albino rats of 7-10 weeks old weighing between 100-150g were used for the study. The rats were maintained in a well-ventilated cage at a controlled room temperature. They were allowed free access to food, water and allowed to acclimatize for 4weeks.

Extraction of Plant Sample: Exactly 100g each of powdered curry leaf was dissolved in 400ml of ethanol and 600ml of distilled water was refluxed for 4 hours at 78°c and 100°c respectively. The extract was filtered using muslin cloth. The filtrate was evaporated using a rotary evaporator and concentrated further using water bath. The extract was collected, weighed and stored in a sterile air tight container and kept in the refrigerator until required for use**.**

Preparation of Stock Solution: 1g each of ethanol, aqueous concentrated extract and metformin was dissolved in 10ml of distilled water and the prepared solution was stored in the refrigerator.

Experimental Design: Albino rats of 10-15 weeks old weighing between 180-250g were randomly grouped and used in the study after the LD_{50} was determined by Lorke (1983) method.

- Group A: Alloxan induced diabetes rats treated with 400mg/kg body weight of ethanol extract.
- Group B: Alloxan induced diabetes rats treated with 400mg/kg body weight of aqueous extract.
- Group c: Alloxan induced diabetes rats treated with 400mg/kg body weight of metformin.
- Group D: Alloxan induced diabetes rats with no treatment.
- Group E: Non induced rats.

Phytochemical analysis; the curry leaves was screened for phytochemical properties by Trease and Evans (1989); Sofowora (1993)

Diabetic Induction: Prior to diabetic induction, the blood glucose level of the animals were taken. The rats were fasted for 12-18 hours and a single dose of 400mg/kg body weight of alloxan was administered. After 3 days of induction, the animals with blood glucose level above 150mg/kg body weight were selected for the experiments.

Determination of blood glucose levels: All blood samples were collected from the tail artery of the rats after every seven day. Blood glucose levels were determined by the glucose-oxidase principle (Beach and Turner, 1958) using a digital glucometer (Accu-chek Advantage) and the value expressed in the unit of mg/dl.

Determination of change on body weight: This was determined after every seventh (7) day of treatment with *murraya koenigii* extract. The changes on body weight observed were recorded respectively.

Determination of biochemical parameters
This was carried out by method described by Thomas (1998). After the last day of treatment (28 days) all animals from each group were sacrificed on the following day and blood samples obtained through cardiac puncture for the determination of biochemical parameters such as determination of urea,

determination of creatinine level, alkaline phosphatase test, SGPT test, aspartate transferase test, serum total protein, serum albumin and electrolyte test; sodium, potassium, chlorine.

Statistical analysis

Data for haematological parameters of the animals from each group were expressed as mean±SEM. The data were statistically analysed using ANOVA with multiply comparisons versus control group. The values of $p<0.05$ were considered as significant (Duncan *et al.*, 1997)

RESULT

Hepatic assay

The activities of serum GPT, GOT and ALP are shown in the table below. The serum GOT activities show significant difference in the diabetic not treated (DNT) and diabetic extract treated (DTA and DTE) when compare with control group. The serum SGPT in DNT and DTE were significantly different when compare with control group. The alkaline phosphatase activities in DTA were significantly higher when compare to control group and DNT group but serum ALP activities in DTS group were significantly lower as compare to control group. The serum total protein were significantly lower in the DNT group but were not significantly different in the extract group (DTE and DTA) when compared with control group. The serum albumin concentration is higher in ethanol extract (DTE) but lower in aqueous extract (DTA) when compare with the control group. The albumin concentration in diabetic untreated were significantly lower when compared to the control and diabetic extract treated group (DTE and DTA).

Kidney function

The serum creatine level was significantly higher in the diabetic not treated rats and diabetic treated with extract group when compared with the control group. The serum urea level was significantly lower in diabetic not treated rats when compared with the rats treated with *murraya koenigii* extract. The serum urea concentration is significantly higher in control groups when compared with the other groups.

Table 1. Serum level of ALP, SGOT, SGPT, TP and Albumin

Sample		Albumin (g/L)	SGOT (µ/L)	TP (g/L)	ALP (µ/L)	SGPT(µ/L)
DNT	Mean	2.47	35.50	6.95	128.03	26.0
	Std. error of mean	±0.08a	±o.28c	±0.38a	±1.17d	±0.70a
NNT	Mean	3.68	31.50	7.12	107.53	26.50
	Std. error of mean	±0.11c	±0.86b	±0.29b	±0.94b	±0.95a
DTE	Mean	4.07	34.75	8.62	86.97	25.50
	Std. error of mean	±0.08d	±0.47c	±0.08b	±1.66a	±1.44a
DTS	Mean	4.30	32.25	7.32	1.134	34.25
	Std. error of mean	±0.14d	±0.47b	±0.06b	±0.1c	±0.25c

DTA	**Mean**	**3.40 ±0.10b**	**23.75 ±1.25a**	**7.22 ±0.04b**	**132.54 ±2.30f**	**30.50 ±1.19b**
	Std. error of mean					

Values followed by same superscript alphabet in a column, are not significantly different at p<0.05

Key: DNT (diabetes not treated), NNT (not induced not treated), DTE (diabetes treated with ethanolic extract), DTS (diabetes treated with standard), and DTA (diabetes treated with aqueous extract)

Electrolyte

There was no statistically significant difference in the serum chlorine concentration of diabetic not treated rat (DNT) and the diabetic treated with the extract group when compared with the control group. The concentration of sodium and potassium in the diabetic not treated group and the diabetic extract treated group were significantly lower when compared with the control and standard groups.

Table 1. Nephrotoxicity effect of aqueous and ethanolic extract of *murraya koenigii* on alloxan induced rats

Sample		**Chlorine (mol/L)**	**Sodium (mmol/L)**	**Potassium (mmol/L)**	**Creatinine (mg/dl)**	**Urea (mg/dl)**
DNT	**Mean**	**93.50 ±0.06a**	**1.34 ±o.25a**	**5.25 ±0.05b**	**1.32 ±0.04b**	**6.60 ±0.16a**
	Std. error of mean					
NNT	**Mean**	**92.20 ±1.93a**	**1.70 ±9.11c**	**6.50 ±0.07c**	**0.83 ±0.14a**	**9.47 ±0.06e**
	Std. error of mean					
DTE	**Mean**	**91.00 ±0.71a**	**1.33 ±1.29a**	**4.45 ±0.05a**	**1.30 ±0.07b**	**4.60 ±0.08a**
	Std. error of mean					
DTS	**Mean**	**113.04 ±0.41b**	**1.75 ±1.56c**	**10.65 ±0.12e**	**1.32 ±0.02b**	**8.45 ±0.08c**
	Std. error of mean					
DTA	**Mean**	**92.25 ±0.85a**	**1.48 ±1.50b**	**5.20 ±0.22b**	**1.30 ±0.13b**	**7.65 ±0.13b**
	Std. error of mean					

Values followed by same superscript alphabet in a column, are not significantly different at p<0.05

Key: DNT (diabetes not treated), NNT (not induced not treated), DTE (diabetes treated with ethanolic extract), DTS (diabetes treated with standard), and DTA (diabetes treated with aqueous extract)

DISCUSSION

In assessing the hepatic status of animals or hepato-protective or hepatotoxicity of plants in animal's model, determination of serum SGOT, SGPT, albumin, ALP and total protein play a major role. The alternation in the activities of these biomarkers seen in the DNT, DTA and DTE when compared to the control group indicate damages to the liver including the biliary system. The elevated level of the enzyme activities (serum SGPT) could be as a result of inflammation of liver cells, the SGPT leak into the blood stream leading to rise in serum level. The decrease in total protein in DNT could be as a result of continuing utilization of protein and lipid as a source of energy. Administration of *murraya koenigii* extract normalizes the total protein; this could be linked to the hypoglycemic potentials of the extract which potentiate the activities of insulin and thus makes carbohydrate available as source of energy.

The serum biochemical parameters related to kidney function analysed include urea, creatinine, chlorine, sodium and potassium. The kidney maintains the serum creatinine in a normal range. Creatinine has been found to be fairly reliable indicator of kidney function the elevated creatinine level in the diabetic rat and the rat treated with *murraya koenigii* is an indication of impaired kidney function resulting in the poor clearance of creatinine.

Maintenance of electrolyte balance depends primarily on excretion by kidney the decrease in serum K+ and Na+ in the diabetic extract treated group may be caused by reduce renal tubule function this effect could be attributed to the metabolic disorder and possibly the toxic effect of the extract, contrary to the finding of (Yankuzo *et al.,* 2011) reported significant restoration of serum electrolyte in diabetic rats treated with the *murraya koenigii.*

CONCLUSION

The observations of this study suggest that aqueous and ethanolic leaf extract of *Murraya koenigii* has no significant effect on the liver and kidney parameters and it's safe to use.

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