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Glycaemic Evaluation of *Murraya koenigii* in Alloxan-induced Diabetic Rabbits

T. Akande[1], S. T. Balogun[2], H. Abdullahi[3], T. O. Ogundeko[4*] and M. S. Ramyil[5]

[1]Department of Chemical Pathology, College of Medicine and Health Sciences, Bingham University, Jos, Nigeria.
[2]Department of Pharmacology and Therapeutics, College of Medical Sciences, University of Maiduguri, Nigeria.
[3]Department of Medical Laboratory Sciences, Faculty of Basic Medical Sciences, Ambrose Ali University, Ekpoma, Nigeria.
[4]Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, Bingham University, Jos, Nigeria.
[5]Department of Medical Microbiology, College of Medicine and Health Sciences, Bingham University, Jos, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors TA designed the study, authors STB and HA wrote the protocol. Authors STB and HA handled, managed the animals and collected all data. Authors TA, STB and HA did the literature review. Authors TOO and MSR fine-tuned the final design and protocol while all authors read and approved the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Study was aimed to evaluate the antidiabetic activity of *Murraya koenigii,* a traditional medicinal plant (Curry leaf) in normoglycemic and alloxan-induced diabetes rabbits.
Methods: Antidiabetic activity of aqueous extract of *M. koenigii* in 100, 200 300 mg/k doses was determined by estimating blood glucose before and at 1, 2, 4, 8, 24, and 72 hours post treatment intervals in treated rabbits.

**Corresponding author: E-mail: tim_ogundeko@yahoo.com;*

Results: Aqueous extract of *Murraya koenigii* showed a dose dependent antidiabetic activity with maximum effect established at 300 mg/kg. The extract also exhibited a significant ($p<0.05$) dose-dependent hypoglycemic effect on normal and alloxan-induced diabetic rabbits.
Conclusion: *Murraya koenigii* causes a reduction in blood glucose. This hypoglycemic property supports-its usein folkloric medicine as an antidiabetic agent and thus suggests a place for it in nutritional therapy in the management of diabetes mellitus and thus as an oral hypoglycaemic agent.

Keywords: Murraya koenigii; hypoglycemic effect; alloxan-induced diabetes.

1. INTRODUCTION

Diabetes mellitus is one of the leading causes of death, illness, and economic loss in the United Statesand developing countries [1]. There is a global trend towards the increase of the incidence and prevalence of diabetes mellitus in African populations [2]. Indeed, Africa is experiencing one of the most rapid demographic and epidemiological transitions in the world [3].

Most antidiabetic drugs are hypoglycemic or anti-hyperglycemic. However, most of these drugs are adipogenic [4]. Most desirable situation would be the development of new types of antidiabetic drugs that are either hypoglycemic or anti-hyperglycemic without the side effects and of promoting weight gain (adiposity). Herbal medicines known to be useful in diabetes treatment may be able to lead to compounds with such a combination of ideal therapeutic properties [5,6]. Animal experimentation has a long history in the field of diabetes research. To induce diabetes in animals, toxic chemicals such as streptozotocin and alloxan have been used appropriately in experimental models which are essential tools for understanding the pathogenesis, complications, and genetic or environmental influences that increase the risks of type2 diabetes and testing of various therapeutic agents [7,8].

Patients with diabetes mellitus are likely to develop complication such as retinopathy, nephropathy and neuropathy as a result of oxidative stress and overwhelming free radicals. Alloxan is a β-cytotoxin, induces Diabetes mellitus by damaging the insulin secretion β -cells of the pancreas, resulting in decreased endogenous insulin release [9]. Alloxan-administered rabbits become hyperglycemic in a short period of time, followed by hepatic glucose overproduction. Reactive oxygen species (ROS) are important mediators of β -cell death during the development of diabetes mellitus [9,10]. High glucose has been postulated to generate ROS and nitrogen species in numerous cell types. Generation of superoxide by high glucose is well described and arises principally via the mitochondrial electron transport chain [11]. Another source of glucose-induced oxidative stress is via the polyol pathway where glucose is reduced to sorbitol by aldose reductase in a process that consumes nicotinamide adenine dinucleotide phosphate (NADPH): This will impair the NADPH-dependent generation of glutathione, and essential cellular antioxidant [12,13]. Others studies showed that alloxan further induced a marked decrease in serum insulin level at test interval in the fasted-treated control rabbits with maximum hyperinsulinemia observed at 4hours post-treatment of the medicinal plant [14].

A fairly narrow normal range of serum glycaemia is regulated by the release of insulin (β -cells) and glucagon (α-cells) from pancreatic islets [15]. In general, insulin is the hormone responsible for glucose disposal, glucagon for glucose availability. Hyperglycemia promotes insulin release, which in turn increases formation of glycogen stores, facilitated glucose uptake in muscle and adipose tissues and supposes hepatic glucose output in part via paracrine suppression of glucagon release. The reduced glucagon secretion limits hepatic glucose output through suppression of glycogenolysis and gluconeogenesis. The opposite series of events occurs during the fasting state, in which the effects of glucagon predominate and insulin secretion is minimal [16]. Losses of glycemic control due to abnormalities of insulin action, including deficiency and insulin resistance [17] may cause hyperglycemia leading to Diabetes mellitus [18]. This study was planned to lend scientific support to the traditional use of *Murraya koenigii* as a hypoglycemic agent by way of evaluating the antidiabetic activity of aqueous extract of *Murraya koenigii* (AMK) in normoglycemic and alloxan- induced diabetic rabbits.

1.1 Ethical Issues

Due processes were adhered to in purchase, handling of animals, and research methodology.

2. MATERIALS AND METHODS

Study was designed for three days. Student T- and Standard Mean Error (SME) were used to analyze results. Effects obtained from various concentrations (100, 200 and 300 mg/kg b.w) were compared with that of the reference drug (Metformin – 500 mg/kg b,w) in order to draw conclusions.

2.1 Extraction Procedure

Fresh leaves of the plant *Murraya koenigii* were purchased from the local herbal dealer at Jos, Plateau State Nigeria. The plant materials were authenticated by Botany Department of University of Jos, Nigeria. The leaves were oven-dried at 45ºC for 72 hours and grinded into powder with mortar and pestle. 20 g of the powder was dissolved in 200 ml distilled water giving a stock concentration of 1 x 10^{-1}g/ml (100 mg/ml), after which the filtrate was evaporated to dryness in hot air oven at 72ºC for 24hours. Dried extract obtained was stored in sealed container and stored in a refrigerator before use.

2.2 Animals Preparation

A total of 24 adult, healthy male rabbits (*Oryctolagus coniculus*) weighing between 1.20-1.50 kg, were purchased from the National Veterinary Research institute Vom, Jos, Nigeria. The animals were acclimatized under standard conditions of temperature, humidity and light, kept under observation for a week in animal house and were provided with a free access to a balanced rabbit's diet. They were fed according to a strict schedule and subjected to overnight fasting before day of commencement of experiment. The rabbits (average b.w = 1.35 kg) were divided into two major groups (control and experimental) with further division into four sub-groups. Each of the sub-group was divided into another group of three with two animals in each case according to the schedule of drug administration viz:

- Group I (control – normal untreated) - 6 animals.
- Group II (Alloxan treated) – 6 animals.
- Group III (crude extract *Murraya koenigii)* - 6 animals.
- Group IV (Metformin) - 6 animals.

2.3 Administration of Drugs

Drugs were administered to the animals in the various groups and sub-groups after which mean values were tabulated for analysis. At experimental day (01) three groups of fasted-normal and three groups of fasted-treated rabbits were administered 100, 200 and 300 mg/kgb.w of (1 ml, 2 ml and 3 ml) aqueous extract of *Murraya koenigii* respectively orally, while fasted-reference control rabbits were given Metformin (500-mg/kg, b.w) according to Aritajat et al. [19]. Fasted-untreated (control) rabbits were administered with Lactose (500-mg/kg b.w) in gelatin capsule for three consecutive days (1-3) orally also.

One hour later, the treated-control and reference-control rabbits were injected with Alloxan Monohydrate intravenously with an equal volume of vehicles to all other rabbits.

Diabetes mellitus (DM) was induced by a single intravenous (iv) injection of Alloxan monohydrate (150 mg/kg b.w.), dissolved in 0.1 m sodium citrate buffer ($_P$H4.5). The control group received similar volume of the vehicle (citrate buffer, 1 ml/kg).

2.4 Drugs and Chemicals

Alloxan Monohydrate, Sodium Carbonate - Sigma Chemical Co. USA. Glucose Estimation kit -Randox-Diagnostic UK. Metformin (reference diabetic drug) – Hovid Perak Malaysia.

2.5 Laboratory Analysis

Blood samples from all the rabbits under test were collected before and after 1, 2, 4, 8, 24, 48, and 72 intervals following Alloxan injection. Plasma harvested from blood samples was used for the estimation of glucose by the glucose oxidase method.

3. RESULTS

Drugs were administered to the animals in the various groups and sub-groups after which SME values were presented in tables for analysis.

Table 1 illustrates the effect of Alloxan on blood glucose levels in rabbits. The single dose of alloxan (150 mg/kg) induced significantly (P<0.05) high blood glucose level at 2-72 hours intervals in the fasted-treated control rabbits and maximum hyperglycemic effect was observed at 8hours post-treatment Tables 2, 3 and 4 illustrate gradual increased antidiabetic activity of extract (aq) of *Murraya Koenigii* (100, 200 and 300 mg/kg b.w) respectively on alloxan –induced (150 mg/kg) diabetic rabbits.

4. DISCUSSION

Aqueous extract of *Murraya koenigii* (AMK) exhibited a dose dependent hypoglycemic effect in the normoglycemic fasted normal rabbits. The 300 mg/kg, b.w dose caused the most potent effect. A significant hypoglycemic effect (P<0.05) in comparison to the treated-control rabbits was observed at 24 and 48hours test points in agreement with [20]. Other test doses of AMK showed similar but less potent hypoglycemic effect in descending order (200-100 mg/kg). This finding is in consistent to [21,22]. The single dose of Alloxan (150 mg/kg,) induced significantly (P<0.05) high blood glucose level at 2-72 hours intervals in the fasted-treated control rabbits and maximum hyperglycemic effect was observed at 8 hours post-treatment 100 and 200 mg/kg b.w. Similar effects in alloxan-induced diabetic rabbits was described by [20,21].

Table 1. Effect of Alloxan on blood glucose levels in rabbits

Blood glucose level (mmo1/L)			
Time internal (Hours)	**Untreated control (SME)**	**Alloxan treated diabetic (SME)**	**P-value**
0	5.69±0.23	5.80±0.20	>0.05
1	5.57±0.25	6.70±0.24	<0.05
2	5.62±0.29	10.30±0.35	<0.05
4	5.65±0.28	11.83±0.35	<0.05
8	5.46±0.23	12.33±0.40	<0.05
24	5.59±0.23	12.20±0.44	<0.05
48	5.66±0.29	11.83±0.51	<0.05
72	5.73±0.18	11.92±0.49	<0.05

Table 2. Effect of extract of *Murraya koenigii* (100 mg/kg) on Alloxan–induced (150 mg/kg) diabetic rabbits

Blood glucose level (mmol/L)			
Time interval (Hours)	**Alloxan treated diabetic (SME)**	**Alloxan + *Murraya koenigii* treated (SME)**	**P-Value**
0	5.80±0.20	1.88±0.20	> 0.05
1	6.70±0.24	1.92±0.23	<0.05
2	10.30±0.35	1.96±0.24	<0.05
4	11.83±0.35	1.96±0.26	<0.05
8	12.33±0.40	1.98±0.26	<0.05
24	12.20±0.44	1.97±0.24	<0.05
48	11.83±0.51	1.93±0.27	<0.05
72	11.92±0.49	1.94±0.24	<0.05

Table 3. Effect of extract of *Murraya koenigii* (200 mg/kg) on Alloxan-induced (150 mg/kg) diabetic rabbits

Blood glucose level (mmol/L)			
Time interval (Hours)	**Alloxan treated diabetic (SME)**	**Alloxan + *Murraya koenigii* treated (SME)**	**P-value**
0	5.80±0.20	3.75±0.20	>0.05
1	6.70±0.24	3.84±0.21	<0.05
2	10.30±0.35	3.95±0.23	<0.05
4	11.83±0.35	3.97±0.25	<0.05
8	12.33±0.40	3.95±0.21	<0.05
24	12.20±0.44	3.93±0.22	<0.05
48	11.83±0.51	3.87±0.25	<0.05
72	11.92±0.49	3.89±0.24	<0.05

Table 4. Effect of extract of *Murraya koenigii* (300 mg/kg) on Alloxan–induced (150 mg/kg) diabetic rabbits

Blood glucose level (mmol/L)			
Time interval (Hours)	**Alloxan treated diabetic (SME)**	**Alloxan + *Murraya koenigii* treated (SME)**	**P-value**
0	5.80±0.20	5.68±0.22	>0.05
1	6.70±0.24	5.78±0.23	<0.05
2	10.30±0.35	5.92±0.25	<0.05
4	11.83±0.35	5.98±0.26	<0.05
8	12.33±0.40	5.93±0.23	<0.05
24	12.20±0.44	5.90±0.24	<0.05
48	1.83±0.51	5.80±0.27	<0.05
72	11.92±0.49	5.83±0.26	<0.05

Table 5. Effect of Metformin (500 mg/kg) on Alloxan-induced (150 mg/kg) diabetic rabbits

Blood glucose level (MMol/L)			
Time interval (Hours)	**Alloxan treated diabetic (SME)**	**Alloxan + Metformin (SME)**	**P-value**
0	5.80±0.20	5.73±0.26	>0.05
2	10.30±0.35	7.56±0.29	<0.05
4	11.83±0.35	8.12±0.30	<0.05
8	12.33±0.40	9.91±0.26	<0.05
24	12.20±0.44	11.96±0.30	<0.05
48	11.83±0.51	11.84±0.29	<0.05
72	11.92±0.49	11.90±0.26	<0.05

The alloxan-induced hyperglycemic effect in the fasted-treated rabbits was attenuated successfully by AMK in a dose-dependent manner. 300 mg/kg, b.w dose ameliorated the effect significantly (P<0.05), followed by 200 and 100 mg/kg, b.w doses. Treated rabbits also exhibited antagonistic activity (P<0.05 at 1-72hours test points) by 300 mg/kg dose. This also agrees with the reports on the activity of various indigenous medicinal plants by [23,24].

Metformin (500-mg/kg, b.w) a reference agent successfully antagonized alloxan-induced hyperglycemia at (1-8hourstest points) as expected [25,26].

Data indicated that Alloxan - induced a significant hyperglycemic effect (P<0.05) in fasted treated control rabbits. AMK (300 mg/kg, b.w) antagonized completely alloxan-induced effects on serum levels of glucose in fasted-treated rabbits.

Aqueous extract of *Murraya koenigii* exhibited a dose dependent hypoglycemic effect in the normoglycemic fasted normal rabbits. Antagonizing activity of extract of *Murraya koenigii* (aq) compares with that of Metformin. The foregoing suggests thatthe orally administered *Murraya koenigii* (aq) lower blood glucose by way of acting on the sulfonylurea receptor which may have been done by reducing insulin resistance from an increase uptake and utilization of glucose in skeletal muscle i.e. by uplifting regulation of body tissues receptors. The closely related hypoglycaemic activity with Metformin could in other words describe the mechanism of action of extract of *Murraya koenigii* (aq) as increasing the release of insulin possibly by encouraging release of Ca^{2+} into the islet β-cells and exhibited Evaluation agreed with the potential use of *Murraya koenigii* as a traditional antidiabetic tool.

5. CONCLUSION

Study confirms that aqueous extract of *Murraya koenigii* (curry leaf) of has a modest anti-diabetic effecton the alloxan-induced diabetic rabbits. This hypoglycemic property supports its use in folkloric medicine as an antidiabetic agent and thus suggests a place for it in nutritional therapy in the management of diabetes mellitus and thus as an oral hypoglycaemic agent.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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