



Antidiabetic and Toxicological Evaluation of Aqueous Ethanol Leaf Extract of *Mitracarpus scaber* Zucc (Rubiaceae) in Rats.

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ABSTRACT

Mitracarpus scaber Zucc is used in ethnomedicine for diabetes, sickle cell anaemia, venereal diseases, skin diseases, headache, dyspepsia, toothaches and leprosy. This study was to evaluate the antidiabetic activity and toxicological profile of the aqueous ethanol extract to confirm and thus, justify its use in traditional medicine.

Doses of the extract ranging from 100 to 500 mg/kg were evaluated in normoglycaemic rats. The dose of 500 mg/kg was evaluated in Streptozotocin-induced diabetic rats. Glibenclamide 1.25 mg/kg was used as the reference standard. Acute toxicological evaluation was carried out in mice while 14-day assessment was done in rats.

Phytochemical screening revealed the presence of alkaloids, tannins, saponins and cardiac glycosides. The various doses of the extract produced significant ($p < 0.05$) reduction in blood glucose levels in normoglycaemic and streptozotocin-induced diabetic rats. These doses were more effective than glibenclamide in normoglycaemic rats. Oral doses as high as 5 g/kg did not cause death or toxicological symptoms in mice. No histological changes were seen in the slides of the major organs during acute toxicity test.

We conclude that *M. scaber* possesses antidiabetic properties in rats which lend credence to its use in ethnomedicine, but its overall safety profile needs to be further evaluated.

Keywords: *Mitracarpus scaber*, antidiabetic, toxicological profile

INTRODUCTION

Mitracarpus scaber Zucc belongs to the Rubiaceae family, popularly known as Madder family. It is a perennial annual herb 30cm tall or much smaller and possesses rough leaves. The flowers are white and located at both sides of the nodes within the divided stipules. The leaves are elliptic with whitish veins and have an entire margin with base. Stomata arrangement is anomocytic with numerous covering trichomes on both surfaces[1]. It is always found during the raining season and dies off during the dry season, leaving the seed to germinate during the next rainy season[2].

The leaf extracts are widely used in traditional medicine practices in West Africa for the treatment of diabetes, headaches, toothaches, amenorrhoea, dyspepsia, hepatic diseases, venereal diseases as well as leprosy. In Senegal, the plant is used for the treatment of sore throat and leprosy [3] and in Nigeria, the juice from the plant is applied topically

for the treatment of skin diseases such as ringworm, lice infestations, itching, rashes and other fungal diseases or applied to dressings for fresh cuts, wounds and ulcers[4]. It is claimed that the plant has both antibacterial and antifungal activities[5, 6].

The methanol extract and the isolated constituents of the aerial parts were reported to exhibit both antibacterial and antimycotic activities[7]. The antimicrobial activity was reduced when a 35% w/v of the extract was incorporated into a liquid soap[8]. The in-vitro antimicrobial activity of the leaf extract has been formulated as syrup¹. *M. scaber* decoction showed significant hepatoprotection against CCl₄-induced liver injury both *in-vivo* and *in-vitro*[9].

The enormous cost of modern medicines for the treatment of diabetes indicates that alternative strategies are required for better management. Traditional plant medicines including preparations from *M. scaber* are used throughout the world in the



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management of diabetes. Although, a number of research studies have been carried out on this plant, there is no scientific evidence on the antidiabetic activity of *M. scaber*. The present study was designed to investigate *M. scaber* for its antidiabetic activity as well as establish its toxicological profile.

MATERIALS AND METHODS

Preparation of plant extract

The leaves of *Mitracarpus scaber* Zucc (Rubiaceae) were collected in Ugbowo area of Benin City, Edo State, Nigeria. The plants were authenticated by the curator at the Forest Research Institute of Nigeria (FRIN), Ibadan where voucher specimens with the number FIH 107154 were deposited. The fresh leaves were air-dried for 72 h and powdered using an electric mill. The powder was extracted with 50% aqueous ethanol and concentrated *in vacuo* at 40 °C.

Animals

Swiss albino mice of both sexes (24. 46 ± 1. 73 g) and male Wistar rats (202. 00 ± 15. 79 g) were obtained from the Animal House, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City. All the animals were kept under standard environmental conditions and were handled according to international protocol for use of animals in experiments [10]. They were fed with standard pellets and tap water *ad libitum*. Ethical approval for the study was obtained from the College of Medicine, University of Benin Animal Ethics Committee (ADM/F. 22A/Vol. viii/349).

Phytochemical studies

Screening for secondary plant metabolites was carried out according to previously described methods [11-14]. These include chemical tests for tannins, alkaloids, cardiac, saponin, anthracene and cyanogenetic glycosides.

Toxicological evaluation

Swiss albino mice (5 animals per group) were orally administered the extract at doses of 1, 2, 3, 4 and 5 g/kg. The control group received only the vehicle (normal saline 5 ml/kg). Each group of mice was placed in the test cage for a 30 min habituation period before drug administration. The animals were observed for 10 min for the first 6 h and 10 min each day for the next two days. Lethality and gross toxicological features (convulsion, diarrhea, hyperactivity and pile-erection) were recorded for

each group [15]. The animals were further observed for fourteen days.

Thirty male Wistar rats were randomly distributed into three groups of ten rats each. The first (A) group served as control and received 5 ml/kg of normal saline (vehicle) while the second (B) and third (C) groups received oral doses of 250 and 500 mg/kg per day of the extract respectively for 14 consecutive days. The animals were observed for signs of toxicity (abnormal behaviours, writhing, convulsion, mood, motor activity and general body conditions) for 30 min each day. At the end of 14 days, the rats were sacrificed under chloroform anesthesia. The livers, kidneys, hearts and testes were removed and preserved in 10% formaldehyde solution. Each organ was sectioned (6 μ thick) embedded in paraffin wax and stained with hematoxylin and eosin [16].

Antidiabetic test

Diabetes was induced in rats fasted for 16 h by intraperitoneal injection of 55 mg/kg of freshly prepared Streptozotocin (STZ) in 0. 1M acetate buffer, pH 4. 5 after baseline blood glucose were estimated [17]. To overcome the hypoglycemia which occurs during the first 24 h following STZ administration, diabetic rats were orally given 5% glucose solution. After 5 days, rats with blood glucose levels of 250 mg/dl and above were considered to be diabetic and selected for the study. Oral doses of 100, 200, 300, 400 and 500 mg/kg of the extract and 1.25 mg/kg glibenclamide prepared in 1% aqueous Tween 80 were administered respectively to the groups of diabetic rats. Animals in the control group received only the vehicle (5 ml/kg) [18, 19]. The blood glucose levels were determined in the time intervals of 1, 4, 8 and 12 h for normoglycemic rats and at 0. 5, 1, 2, 4, 6, 8 and 12 h for STZ-induced diabetic rats. In all cases, blood was obtained from the tail tips of the rats.

Estimation of blood glucose level

Blood glucose concentration (mg/dl) was determined using a glucometer (Accu-Check, ROCHE, UK), the principle of which is based on the glucose oxidase method [20, 21].

The percentage glycaemic change at any time point was calculated using the formula:

$$\% \text{ Glycaemic change} = \frac{\text{FBG} - \text{GC}}{\text{FBG}} \times \frac{100}{1}$$

Where GC is the glucose concentration at different time points and FBG is the fasting blood glucose concentration representing baseline value.

Statistical analysis

Data are expressed as mean \pm SEM and "n" represents the number of rats used. The differences between the means were analyzed using one way analysis of variance (ANOVA). Values of $P < 0.05$ were taken to imply statistical significance between compared data.

RESULTS

Phytochemical screening

Phytochemical screening of the leaves of *M. scaber* for secondary plant metabolites revealed the presence of alkaloids, saponins, cardiac glycosides and tannins (Table 1).

Toxicological evaluation

The aqueous extract of *M. scaber* did not produce any mortality up to the oral dose level of 5 g/kg body weight in mice. There were no changes in behaviour, posture, nature and frequency of stooling, mood and motor activity. The animals did not convulse, exhibit writhing nor die.

Daily administration of the extract for 14 days did not produce gross toxicological symptoms nor deaths before the Wistar rats were sacrificed after 14 days treatment. General histopathological analysis of the liver (Fig. 1) in the *M. scaber* treated group showed a normal central vein and portal areas. The tubules in most parts of the kidney (Fig. 2) were normal and there was no damage to any heart cells (Fig. 3). There was no evidence of tissue necrosis in the testes (Fig. 4).

Hypoglycemic effects

Administration of doses of the aqueous ethanol extract of *M. scaber* to normoglycemic Wistar rats produced significant hypoglycemic effects after 1 h (Fig. 5). Percentage blood glucose reduction began at 1 h, maximized at the 8 h and reduced at the 12 h except at 300 mg/kg and 500 mg/kg. The percentage reductions were dose-dependent and were significant when compared with 1% aqueous Tween 80 ($p < 0.02$) and glibenclamide ($p < 0.05$). The highest percentage reduction of $46.89 \pm 0.42\%$ was recorded by 500 mg/kg at the 12 h.

Streptozotocin-induced diabetic rats showed significant increase in the levels of blood glucose when compared to normal rats. Oral administration of the 500 mg/kg of *M. scaber* extract to diabetic rats resulted in significant decrease in blood glucose levels ($p < 0.05$) when compared with distilled water (Fig. 6). On the other hand, glibenclamide 1.25 mg/kg produced the highest

percentage blood glucose reduction at 8 h ($38.25 \pm 2.22\%$).

DISCUSSION

Many plants accumulate biologically active complex organic chemicals in their tissues, hence the need for phytochemical evaluation of these medicinal plants. Phytochemical screening of the leaves of *M. scaber* for secondary plant metabolites revealed the presence of alkaloids, cardiac glycosides, saponin glycosides and tannins. *M. scaber* belong to the Gentianales Order, where all seven families are known to contain alkaloids, tannins and glycosides[13]. Studies on hypoglycaemic activities of plants have identified compounds like alkaloids[22], tannins[23], polypeptides [24] amongst others to be responsible for reported activity.

For the study of antidiabetic agents, streptozotocin-induced hyperglycemia in rats is considered to be a good preliminary screening model and is widely used[25]. Streptozotocin (N-[methyl nitro carbamoyl]-D-glucosamine) is a potent methylating agent for DNA and acts as nitric oxide donor in pancreatic β -cells and thus β -cells are more sensitive to damage by nitric oxide and free scavenging enzymes[26]. In this study, the aqueous ethanol extracts of the leaves of *M. scaber* produced significant hypoglycaemic effects after 4 h administration, confirming traditional indications. The hypoglycemic effects of variable doses of extracts had begun at 1 h and were maximum at between 4 h and 8 h in most groups. However, the responses decreased at 12 h in all doses except at 300 mg/kg and 500 mg/kg. Such a phenomenon of less hypoglycemic response at higher time is not uncommon with plants possessing hypoglycemic activities and may be due to reduced concentrations of active constituents in the system.

Percentage blood glucose reduction produced by 500 mg/kg of the extracts of *M. scaber* after oral administration in streptozotocin-induced diabetic rats produced a progressive increase after 0.5 h up to the 6 h, and was more pronounced at 4 h ($35.35 \pm 1.59\%$). On the other hand, glibenclamide 1.25 mg/kg produced the highest percentage blood glucose reduction at 8 h ($38.25 \pm 2.22\%$). These results of aqueous ethanol extracts of *M. scaber* indicated a potent action in short term study and reveal a defined role in reducing blood glucose level in diabetic patients.

Toxicological studies for all herbal medicines including the determination of their median lethal dose (LD 50) and other such parameters essential

for a proper dosage are desirable and necessary. If there is the suspected need for more detailed studies, such herbal medicines may be subjected to sub-acute tests. The general purpose of the sub-acute toxicity tests is to determine the organs that are likely to be susceptible to toxicity by the herbal medicines. Histopathological effects of the administration of 500 mg/kg per day of the aqueous ethanol extracts of *M. scaber* to rats showed no evidence of tissue necrosis on the liver, kidney, heart and testes. There were no marked adverse alterations or degeneration of tissues since these vital organs showed normal architectures suggesting no morphological disruptions as compared with the control group. It is an indication of the low toxicity of the extract [15], therefore *M. scaber* could be said to be relatively safe. Our results correlate with earlier findings [9] which demonstrated the Hepatoprotective effects of *M. scaber* in CCl₄-induced liver injury both *in-vivo* and *in-vitro*.

CONCLUSION

Herbal medicine can gain the confidence of orthodox health practitioners when there are scientifically established proofs of their claimed efficacies. On the basis of the results obtained from the pharmacological investigations, it could be said that *Mitracarpus scaber* possesses antidiabetic properties and can be considered safe on acute basis. The results from this study thus support the claimed traditional use of *M. scaber* in the management of diabetes.

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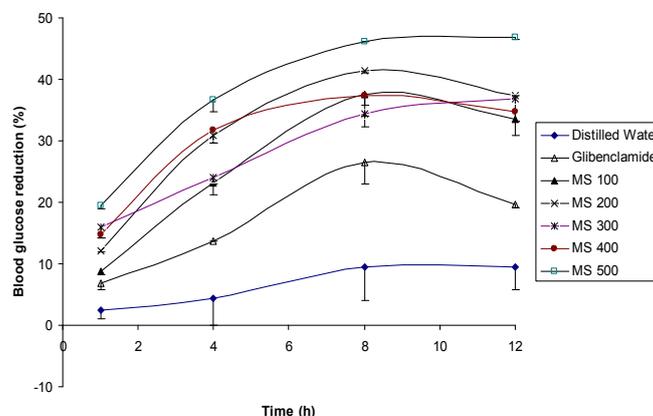
Table 1. Phytochemical constituents of *M. scaber* leaves

Classes of secondary metabolites	Inference
Alkaloids	+
Tannins	+
Anthracene derivatives	-
Saponin glycosides	+
Cardiac glycosides	+
Cyanogenetic glycosides	-

Key:

- = absent; + = present

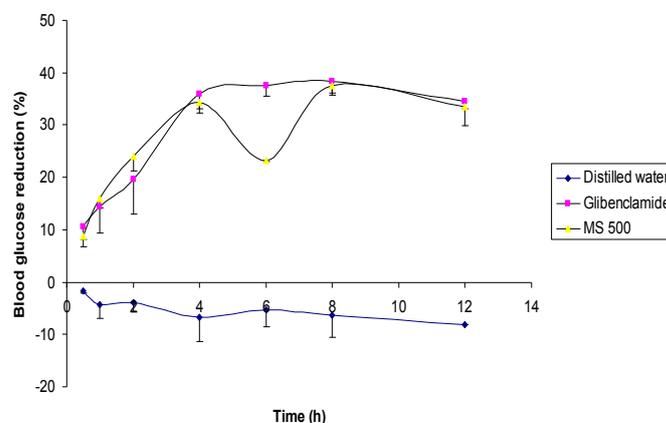
Fig. 5:
The hypoglycaemic effects of *M. scaber* extract on normoglycaemic rats



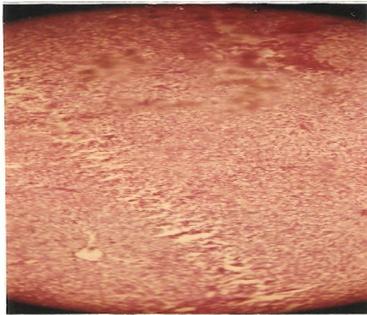
Blood glucose reduction by the various doses of *M. scaber* was significant all through the period of assay $p < 0.05$ compared to glibenclamide and $p < 0.02$ compared to distilled water. $n = 4$ per group.

Fig. 6

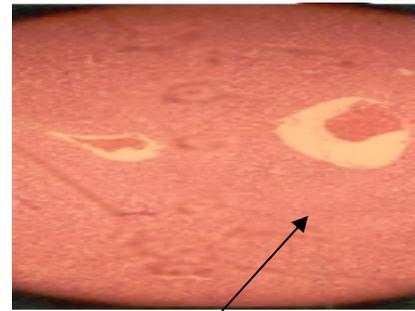
Hypoglycaemic effects of *M. scaber* on streptozotocin-induced diabetic rats



Blood glucose reduction by 500 mg/kg dose of *M. scaber* was significant all through the period of assay $p < 0.05$ compared to distilled water. $n = 4$ per group.



Saline control

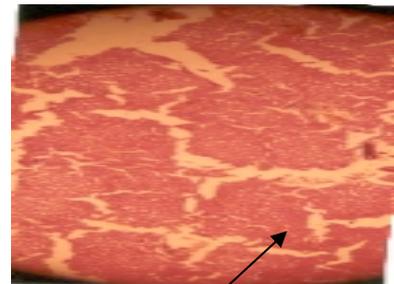


Normal central vein
M. scaber treated

Fig. 1: Photomicrographs of the liver of rats administered with 500 mg/kg extract of *M. scaber* and control for 14 days. There are no obvious differences in the histology of the livers (x 400)



Saline control

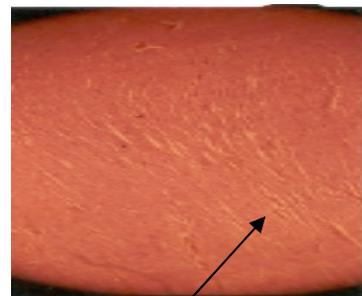


Normal tubule
M. scaber treated

Fig. 2: Photomicrographs of the kidney of rats administered with 500 mg/kg extract of *M. scaber* and control for 14 days. There are no obvious differences in the histology of the kidneys (x 400)



Saline control

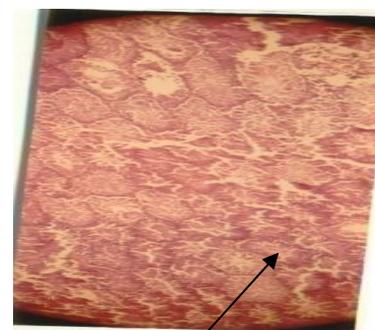


Normal cell
M. scaber treated

Fig. 3: Photomicrographs of the heart of rats administered with 500 mg/kg extract of *M. scaber* and control for 14 days. There are no obvious differences in the histology of the hearts (x 400)



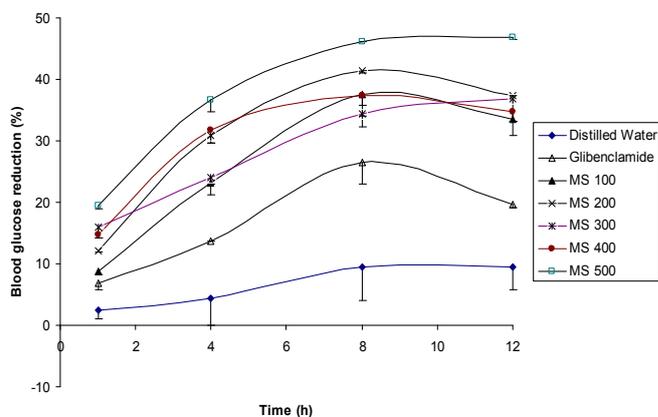
Saline control



Normal tissue
M. scaber treated

Fig. 4: Photomicrographs of the testes of rats administered with 500 mg/kg extract of *M. scaber* and control for 14 days. There are no obvious differences in the histology of the testes (x 400)

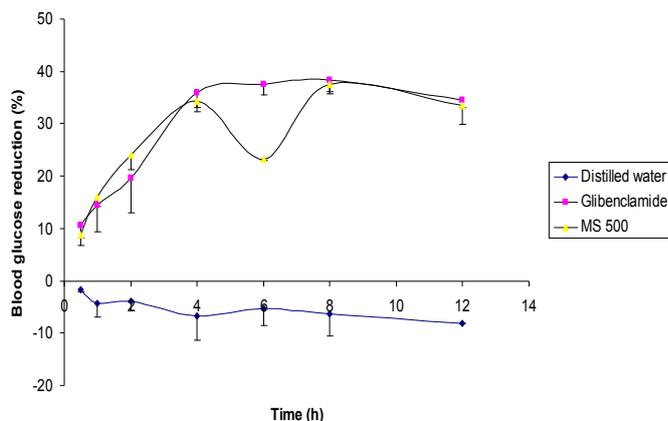
Fig. 5:
The hypoglycaemic effects of *M. scaber* extract on normoglycaemic rats



Blood glucose reduction by the various doses of *M. scaber* was significant all through the period of assay $p < 0.05$ compared to glibenclamide and $p < 0.02$ compared to distilled water. $n = 4$ per group.

Fig. 6

Hypoglycaemic effects of *M. scaber* on streptozotocin-induced diabetic rats



Blood glucose reduction by 500 mg/kg dose of *M. scaber* was significant all through the period of assay $p < 0.05$ compared to distilled water. $n = 4$ per group.

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