Evaluation of the Anti-diabetic Properties of *Manniophyton fulvum*

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ABSTRACT

Aim*: To explore the anti-diabetic properties of the leaves of Manniophyton fulvum.*
Method: *Diabetes was induced in wistar albino rats by single intravenous injection of 120mg/kg body weight of alloxan monohydrate and different doses of 100, 200 and 400mg/kg bodyweight of the ethanol-extract of Manniophyton fulvum leaves were administered to the diabetic rats. The blood glucose level of the rats were determined at two(2) weeks intervals for six(6) weeks using a glucometer and the results obtained were compared by means of one way analysis of variance (ANOVA) to the normal rats, untreated diabetic rats and metformin-treated rats at $p \leq 0.05$.*
Results: *Results showed that the administration of the crude ethanol extract of M. fulvum leaves to alloxan-induced diabetic rats reduced the hyperglycaemic level from 13.70 ± 0.22mmol/l to 5.63 ± 0.13mmol/l (100mg/kg group), 14.85 ± 0.66mmol/l to 5.15 ± 0.13mmol/l (200mg/kg group) and 15.19 ± 0.15mmol/l to 3.87 ± 0.08 mmol/l (400mg/kg group) after 6 weeks of treatment while the administration of metformin (1.4mg/kg) reduced the hypoglycaemic level from 14.80 ± 0.17mmol/l to 4.24 ± 0.19 mmol/l.*
Conclusions*: The study suggests that the leaves of Manniophyton fulvum possess hypoglycaemic effect and supports the traditional use of M. fulvum for the treatment of diabetes indicating that the plant could be a good source of a potent anti-diabetic drug.*

KEYWORDS: *Antidiabetic; alloxan monohydrate; Manniophyton fulvum*

1. Introduction

Diabetes mellitus is a growing threat to public health in modern society (Xiang et al., 2007). It is a metabolic disorder of the pancreas in which blood sugar (glucose) levels are abnormally high (hyperglycaemia) because either the body does not produce enough insulin, the hormone produced by beta cells of the islet of Langerhans that controls the amount of sugar in the blood (Rother, 2007), or the insulin produced cannot be used by the body (Mayfield, 1998). While some non- insulin dependent diabetic mellitus patients can be managed by diet alone, others require hypoglycaemic therapy and/or insulin (Majekodunmi et al., 2011). Although insulin therapy affords an effective glycaemic control, drawbacks such as oral ineffectiveness, short shelf life, requirement of constant refrigeration, parenteral therapy with its attendant abscesses and fatal hypoglycaemia in the event of excess dosage limit its usage (Majekodunmi et al., 2011). On the other hand, pharmacotherapy with sulphonylureas, biguanides and thiazolidones is also associated with side effects (Swanston-Flatt, 1990). Therefore, there is an urgent need to

find safe and effective pharmacological interventions for diabetes mellitus (Monago et al., 2005). In recent years, the popularity of complementary medicine has increased considerably especially in areas where modern drugs are not readily available (Swanston-Flatt, 1990). Due to their relative safety and low costs, herbal medicinal plants are prescribed even when they are not standardized (Majekodunmi et al., 2008).

One of the known antidiabetic medicinal plants used in the south eastern part of Nigeria is *Manniophyton fulvum (M. fulvum)* and it remains one of the popular herbs amongst local traditional medicine practitioners in the region (Agbaire et al., 2013). It belongs to the family euphorbiacea(Ojieh et al., 2013). It is also geographically distributed widely in tropical Africa, from Sierra Leone to Sudan, and South-ward to Angola (Brown et al., 1913). In African traditional medicine the root, stem, bark and leaf are credited with analgesic properties, and are used to treat diarrhea, stomach ache, cough, bronchitis, oxidative stress and inflammation (Nia et al., 2005). According to unconfirmed folklore in the south-east of Nigeria, traditional herbalists have used the root extract of *M. fulvum* to treat erectile dysfunction. The red stem sap is credited with hemostatic properties, while the leaf sap is used against ear problems (Nia et al., 2005). In Congo (Brazzaville), it is considered a cicatrisant on wounds, and also good for treating dysentery and dysmenorrhea (Bouquet et al., 1969; Bouquet et al., 1974). The red stem-sap is used topically in Ivory Coast on herpes and other dermal infections (Burkill, 1994). Furthermore, a decoction of the young shoots, bark and stem, the husk of the nut and the sap are used as remedy for cough in Congo (Brazzaville), Ivory coast and Sierra Leone (Bouquet et al., 1969; Bouquet et al., 1974).

The leaf of *M. fulvum* is credited with antioxidant and antidiarrheal properties (Ezeigbo et al., 2010; Ojieh et al., 2013). The leaf extract of the plant was found less toxic than the root extract, being that the dosage required to cause death in 50% of the animals using the root extract is about 800 mg/kg whereas the leaf extract produced toxicity at a dose of 1050 mg/kg (Agbaire et al., 2013).

2. Materials and Methods

2.1 Chemicals

Alloxan monohydrate was obtained from Sigma Chemical Co. (St. Louis, M.O., USA) and a metformin tablet was obtained from the E-blend Pharmaceuticals (Port Harcourt, Rivers State, Nigeria.). All reagents were of analytical grade.

2.2 Collection of Plant Materials and extract preparation

Fresh leaves of *Manniophyton fulvum* were collected from Umuderim Village, Imo State, Nigeria. The leaves were identified and authenticated by the Hebarium unit of the Department of Plant Science and Biotechnology, University of Port Harcourt, Port Harcourt, Nigeria and a voucher specimen was deposited for reference purposes. The leaves of *M. fulvum* collected were sun-dried and grinded to fine-powder with an electric blender. The powdered leaves (600g) were subjected to soxhlet extraction using 1.5 litres of 95% ethanol at room temperature. The crude extract was oven-dried and powdered using an electric blender and then sieved.

2.3 Animals

Fifty-four (54) Wistar albino rats (150-200g) of both sexes were obtained and bred in the animal house of the Department of Biochemistry, Faculty of Chemical Sciences, University of Port Harcourt, Port Harcourt, Nigeria. After randomization into various groups, animals were acclimatized for a period of 7 days before the start of experiments. Animals described as fasting had been deprived of food for at least 16 hours but had been allowed free access to drinking water.

2.4 Induction of Diabetes

Diabetes was induced by single intravenous injection of 120mg/kg of alloxan monohydrate (dissolved just before use in 20ml of distilled water) to overnight fasted rats. Animals in which hyperglycaemia was confirmed 72 hrs after the administration of alloxan injection (blood glucose level range of 12-15 mmol/l) were used for experiments.

Table 1: Initial Concentration of Plasma glucose before treatment (72hrs after induction of diabetes)

Group	Treatment	Plasma Glucose Concentration Before Treatment (mmol/L)
I	Normal Control Rats (NCR)	6.55 ± 0.11^a
II	Diabetic Control Rats (DCR)	12.39 ± 0.12^a
	Diabetic Control Rats (DCR)on Reference drug (Metformin(1.4 mg/kg)	14.80 ± 0.17^a
IV	DCR on Ethanol extract (100mg/kg)	13.70 ± 0.22^a
V	DCR on Ethanol extract (200mg/kg)	14.85 ± 0.66^a
VI	DCR on Ethanol extract (400mg/kg)	15.19 ± 0.15^a

Values with common superscript(a) show significant difference when compared to the normal control rats at $p \leq 0.05$.

2.5 Hypoglycaemic activity

Wistar albino rats were randomly allocated into six groups (I - VI) of nine rats each. Group I consisted of non-treated rats (negative control). Group II consisted of diabetic control rats (alloxan induced diabetic rats), diabetic rats in group III received metformin (1.4mg/kg) as standard reference drug (Nwauche et al., 2014; Kelechi et al., 2014) while diabetic rats in groups IV, V and VI received 100, 200 and 400mg/kg body weight respectively of the crude ethanol

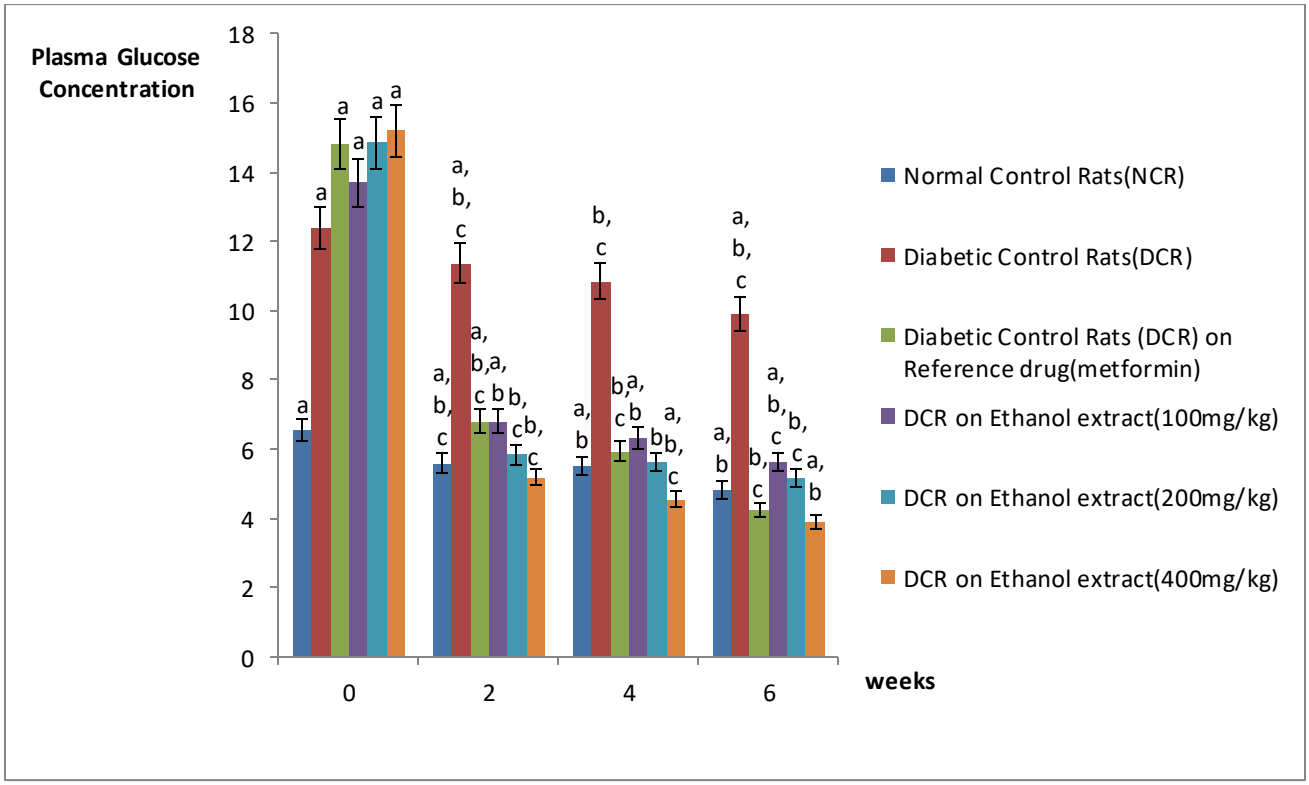
leaves extract orally. The LD_{50} carried out by Agbaire, et al., 2013 on *M. fulvum* leaves justified the selection of the treatment doses. Blood glucose levels were measured at 0 week (before receiving the extract) and at two weeks interval for 6 weeks using a glucometer (accu-check active, USA.)

2.6 Statistical analysis

The statistical analysis was carried out with Statistical Package for Social Sciences (SPSS) version 20. Data were expressed as mean ± SEM. Data were analysed using one way analysis of variance (ANOVA). Differences were considered to be significant when $P \leq 0.05$.

3. Results

Results of the effect of the crude ethanol leaves extract of *M. fulvum* on the blood glucose level in alloxan-induced diabetic rats are presented in **Figure 1**. It showed that normal control rats (group I) maintained a fairly stable level of glucose throughout the study period with the values 5.58 ± 0.12, 5.51 ± 0.27 and 4.82 ± 0.14 for 2, 4 and 6 weeks of the study respectively (Figure 1). There was however significant ($p \leq 0.05$) increase in the level of glucose concentration for the diabetic control rats (group II) with a hyperglycemic level of 12.39 ± 0.12, 11.36 ± 0.17, 10.85 ± 0.08 and 9.88 ± 0.16 for 0, 2, 4 and 6 weeks respectively. Diabetic animals in group III treated with standard drug (metformin 1.4mg/kg body weight) showed a significant ($p \leq 0.05$) decrease in plasma glucose levels on the 2nd, 4th and 6th week of treatment when compared with diabetic control group (group II) as shown in table 1. At the end of the 6 week administration of the ethanol extract of *M. fulvum* leaves to the diabetic rats, a significant decrease ($p \leq 0.05$) was observed in all the extract-treated groups when compared to the diabetic control group.



Error bars represent percentage

Superscript $A^{(a)}$ represents significant difference when group I (normal control rats) is compared with other groups at $p \leq 0.05$.
Superscript $B^{(b)}$ represents significant difference when group II (diabetic control rats) is compared with other groups at $p \leq 0.05$.
Superscript $C^{(c)}$ represents significant difference when group III (diabetic control rats on reference drug) is compared with other groups at $p \leq 0.05$.

Fig. 1: Effect of ethanol extract of the leaves of *Manniophyton fulvum* on Plasma Glucose levels of alloxan-induced Diabetic rats.

4. Discussion

Besides drugs classically used for the treatment of diabetes, several species of plants have been described as having hypoglycaemic activities (De Sousa et al., 2004; Colca, 2006). These herbal medicines have been recommended for the treatment of diabetes and are considered less toxic with fewer side effects than synthetic ones (De Sousa et al., 2004).

According to the preliminary phytochemical screening carried out by Ojieh et al., 2013, *Manniophyton fulvum* leaves contain alkaloids, flavonoids, phenols, saponins, tannins and terpenoids. The anti-diabetic effect observed with the administration of the extract could be attributed to the presence of flavonoids, phenols and saponins as they have been reported to possess anti-diabetic potentials (Ramesh et al., 2013; Gowri SS et al., 2000; Umar MS, 2015).

Flavonoids present in plants have been reported to exhibit hypoglycemic activity by regenerating damaged beta cells (Ramash et al., 2013), increasing insulin secretion (Cazarolli LH et al., 2009), significantly suppressing α – amylase and α – glucosidase (Sathya A and Siddhuraju P, 2012), showing insulin – mimetic role in glucose homeostasis (Cazarolli LH et al, 2013), inhibiting intestinal glucose uptake and renal reabsorption of glucose(Li JM et al, 2008) and diminishing carbohydrate reabsorption from intestine and reducing post prandial glucose levels (Ortiz-Andrade RR et al, 2008). Saponins and phenols have also been shown to inhibit glucose transport by inhibiting sodium glucose co-transporter-1 (S-GLUT-1) in the intestine (Hakkins F L et al, 2007; Tiwari A K et al, 2002).

The administration of ethanol extract of *M. fulvum* resulted in a significant ($P \leq 0.05$) reduction in the blood glucose level of the diabetic rats when compared with diabetic rats that received no treatment. There was a significant ($P \leq 0.05$) dose-dependent reduction in the blood glucose level after the extract administration. The maximium hypoglycaemic effect (3.87 ± 0.08) was achieved by the extract at a dose of 400mg/kg. There was a significant ($P \leq 0.05$) difference between the effects of administration of the extract and those of metformin (1.4mg/kg). The study indicates that the ethanol leaves extract of *M. fulvum* possess antidiabetic activities comparable with the standard drug, metformin. The study clearly supports the traditional use of the plant for the treatment of diabetes.

Conclusion

The results obtained from this study revealed that the ethanol extract of the leaves of *Manniophyton fulvum* possess anti-diabetic properties. The most effective dosage of the extract was observed at 400mg/kg body weight at the 6th week of study. This dosage, however, achieved more hypoglycaemic effect than the reference drug.

This suggests the possibility of its use in the management of diabetes mellitus thus indicating that *Manniophyton fulvum* could be a promising plant for the development of an anti-diabetic drug.

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Conflict of Interest statement

We declare that we have no conflict of interest.

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