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Original

Concurrent Administration of the Methanol Leaf Extract of *Leptadenia Hastata* (Pers) Decne (*Apocynaceae*) With Metformin or Glibenclamide Influences Blood Glucose Handling in Normal Rats

Omobhude F. Aluefua[1], Aminu Chika[1], Aminu Ishaka[2], Kabiru Abubakar[3]

[1]Department of Pharmacology and Therapeutics, [2]Department of Medical Biochemistry, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.
[3]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

Abstract

Background: *Leptadenia hastata* (Pers) Decne (*Apocynaceae*) is a familiar medicinal plant utilized in northern Nigeria either singly or together with conventional drugs to treat diabetes mellitus.

Objective: This study investigated the influence of concurrent administration of the methanol leaf extract of *L. hastata* with Metformin or Glibenclamide on blood glucose handling in normal male Wister rats.

Methods: One hundred and twenty-eight normal rats were randomized into 16 groups of 8 rats each. The first group served as a vehicle-administered control. Three groups were treated with three increasing doses of the extract using a semilogarithmic scale (50mg/kg, 150mg/kg and 500mg/kg respectively). Another three groups were treated with Glibenclamide at doses of 0.3mg/kg, 1mg/kg and 3mg/kg respectively. Another three groups were treated with metformin at doses of 30mg/kg, 100mg/kg, and 300mg/kg respectively. Another three groups were treated with metformin (100mg/kg) co-administered with the extract at 50mg/kg, metformin 100mg/kg and the extract at 150mg/kg, metformin 100mg/kg and the extract at 500mg/kg respectively. The last three groups received Glibenclamide at 1mg/kg co-administered with the methanol extract at 50mg/kg, Glibenclamide at 1mg/kg with the extract at 150mg/kg, and Glibenclamide 1mg/kg with the extract at 500mg/kg respectively. Oral glucose tolerance test (OGTT) was conducted following 8 hours of fasting.

Results: The result revealed a significant reduction in the total area under the glucose tolerance curve when metformin was co-administered with the extract in normal rats.

Conclusion: The findings suggest that the methanol leaf extract of *L. hastata* when co-administered with metformin caused an enhancement of the effect of metformin following OGTT in normal rats.

Keywords: Blood glucose, extract, metformin, glibenclamide, glucose tolerance.

Corresponding author:
Omobhude Fidelis Aluefua,

Department of Pharmacology and Therapeutics,
College of Health Sciences,
Usmanu Danfodiyo University,
Sokoto, Nigeria.
Email: aluefuafo@gmail.com

Introduction

Plants with medicinal properties have been used since antiquity for the treatment and management of diabetic mellitus (DM) in traditional medicine systems of many cultures worldwide (1). Recently, the World Health Organization (WHO) encouraged the use of herbal medicinal plants for the management of DM and further promoted the expansion of the frontiers of scientific evaluation of hypoglycaemic properties of diverse plant species (2). Consequently, current statistics suggest that over 80% of the world population apply resources derived from traditional medicine for their primary health care (2). About one-third of outpatients attending the diabetic clinics in Nigeria use herbal products concurrently with conventional antidiabetic drugs (3). The concurrent use of herbal products with conventional antidiabetic drugs may have an influence on the antidiabetic effect of the drugs. (4–6) Metformin is the most frequently prescribed antidiabetic drug in Nigeria (7). *Leptadenia hastata* (pers) decne (*Apocynaceae*) is a common medicinal plant used in Northern Nigeria either singly or together with conventional drugs to treat diabetes mellitus (8,9). It is not known whether *L. hastata* will have an influence on the antidiabetic effect of metformin or glibenclamide when the two are used concurrently. This study aimed to evaluate the influence of concurrent administration of the methanol leaf extract of *L. hastata* with selected oral hypoglycaemic agents on blood glucose handling in normal rats.

Materials and Methods

Materials

Drugs and Reagents

Metformin and Glibenclamide were ordered from Chem Cruz, USA. Glucose (kermel) and methanol were ordered from Sigma Aldrich USA. All reagents were of analytical grade.

Animals

Male Wistar rats weighing 120-150g (8-10 weeks) were obtained from Institute for Advanced Medical Research and Training (IMRAT), University College hospital, Ibadan, Nigeria. The rats were maintained according to the minimum requirements of the international guidelines for the use of animals (10). During the 2 weeks of acclimatization, the animals were fed with a commercial diet (Vital Feed Nig. Ltd) and allowed access to water ad libitum (10). They were then fasted for 8 hours before the commencement of the experiment (10). Ethical clearance with clearance number PTAC/Lh/(Me)OT/35-21 was sought from UDUS ethical clearance committee before commencement of the in vivo study.

Methods

Collection of plant material

Fresh leaves of *L. hastata* were collected from the biological garden at the permanent site of Usmanu Danfodiyo University Sokoto. The plant sample was authenticated at the Herbarium of the Botany unit of the same Institution. A voucher number PCG/UDUS/ASCL/0002 was collected for the plant material specimen, which was deposited at the herbarium of the same unit.

Preparation of leaf extract

The leaves were air dried (to constant weight) at room temperature and pulverized into a fine powder. One hundred (100) grams of the sample was separately extracted with 95% methanol for 24 hr (8). The extract was filtered and concentrated to semisolid form under reduced pressure using rotary evaporator at 35oC. This was then finally dried in an aerated oven at 35oC. The percentage yield was calculated for the extract. The dried extract obtained was preserved at 4oC until needed for use.

Qualitative Phytochemical Screening

The extract was subjected to the various chemical tests for phytochemicals using standard procedures. (11–13).

Experimental Design of the In vivo Study

Determination of the antidiabetic drug which, when co-administered with the extract, is likely to have a better glucose handling in normal rat.

One hundred and twenty-eight normal rats were randomized (using Decision Analyst software) into 16 groups of 8 rats each. Due to practical reason, the experiment was conducted in 6 batches (of 3, 3, 3, 3, 2 and 2 groups respectively) separated one day apart. The first group served as a vehicle-administered control. Three groups were treated with three increasing doses of the extract using a semilogarithmic scale (50mg/kg, 150mg/kg and 500mg/kg respectively) (14). Another three groups were treated with Glibenclamide at doses of 0.3mg/kg, 1mg/kg and 3mg/kg respectively. Another three groups were treated with metformin at doses of 30mg/kg, 100mg/kg, and 300mg/kg respectively. Another three groups were treated with metformin (100mg/kg) co-administered with the extract at 50mg/kg, metformin 100mg/kg and the extract at 150mg/kg, metformin 100mg/kg and the extract at 500mg/kg respectively. The last three groups received Glibenclamide at 1mg/kg co-administered with the methanol extract at 50mg/kg, Glibenclamide at 1mg/kg plus the extract at 150mg/kg, Glibenclamide 1mg/kg combined with the extract at 500mg/kg respectively as shown in below (Table 1).

Table 1: Groups of Rats

Group 1:	Vehicle-administered control
Group 2:	*L. hastata* at 50mg/kg
Group 3:	*L. hastata* at 150mg/kg
Group 4:	*L. hastata* at 500mg/kg
Group 5:	Glibenclamide at 0.3mg/kg,
Group 6:	Glibenclamide at 1mg/kg
Group 7:	Glibenclamide at 3mg/kg
Group 8:	Metformin at 30mg/kg,
Group 9:	Metformin at 100mg/kg,
Group 10:	Metformin at 300mg/kg.
Group 11:	Metformin 100mg/kg plus extract at 50mg/kg,
Group 12:	Metformin 100mg/kg plus extract at 150mg/kg,
Group 13:	Metformin 100mg/kg plus extract at 500mg/kg
Group 14:	Glibenclamide 1mg/kg plus extract at 50mg/kg,
Group 15:	Glibenclamide 1mg/kg plus extract at 150mg/kg,
Group 16	Glibenclamide 1mg/kg plus extract at 500mg/kg

Oral glucose tolerance test was conducted as described below following 8 hours of fasting.

Oral glucose tolerance test

Glucose (2 g/kg) was given orally thirty minutes after the administration of the respective treatments. Blood samples were collected (by tail tipping) at 0 min (just before glucose loading), 30 min, 60 min, 90 min, and

120 min after glucose administration for glucose determination using standardized glucometer (Accu Check ®).

Statistical analysis

Data were analyzed using GraphPad Prism Version 7.0. The data were summarized as mean ± Standard Deviation. Analysis of Variance (ANOVA) with post hoc tests (Dunnet) was used to compare the difference between different groups. A p-value $p<0.05$ was considered to be statistically significant.

Results

Percentage yield of the methanol leaf extract of *L. hastata*

The percentage yield of the methanol leaf extract of *L. hastata* was 9.3%.

Phytochemical analysis of the methanol leaf extract of *L. hastata*

Phytochemical analysis of methanol leaf extract of *L. hastata* revealed the presence of cardiac glycosides, steroids/triterpenoids, phenol, flavonoids, carbohydrates, tannins, saponins, and alkaloids (Table 2).

Table 2. Phytochemical analysis of the methanol

S/N	Phytochemical constituents	Results
1.	Cardiac Glycosides	+
2.	Anthraquinones	ND
3.	Steroids/Triterpenoids	+
4.	Phenols	+
5.	Flavanoids	+
6.	Carbohydrates	+
7.	Tannins	+
8.	Saponins	+
9.	Alkaloids	+

+ = Present, ND = Not detected

Influence of co-administration of the methanol leaf extract of *L. hastata* and metformin on oral glucose tolerance curve in normal Rats following single dose treatment

The co-administration of the methanol leaf extract of *L. hastata* at 500mg/kg and metformin at 100mg/kg resulted in a significant (P 0.05) reduction in the total area under the oral glucose tolerance curve in normal Rats (Fig. 1.b). Compared with metformin at 100mg/kg, a significant reduction in the total area under the oral glucose tolerance curve was observed in the group of rats co-administered with metformin at 100 mg/kg and the *L. hastata* extract at 500 mg/kg concurrently (Fig.1.b).

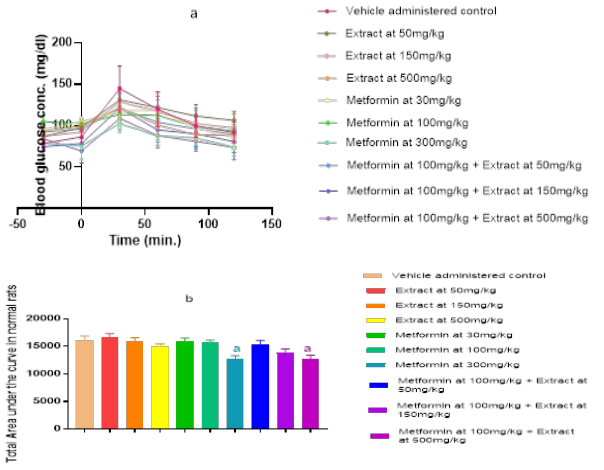

Figure 1: Effects of co-administration of the methanol leaf extract of *L. hastata* and metformin on (a) oral glucose tolerance test curve and (b) total area under the glucose tolerance curve in normal Rats. Data are presented as mean ± SEM; a signifies $P<0.05$ when compared with metformin at 100mg/kg, n= 8. One way ANOVA with Dunnett's multiple comparison post hoc tests was used to arrive at the P value.

Effects of co-administration of the methanol leaf extract of *L. hastata* and glibenclamide on oral glucose tolerance curve in normal rats following single dose treatment

Compared with the normal control group, no significant reduction in the total area under the oral glucose tolerance curve ($P>0.05$) was observed in the glibenclamide-treated group. There was also no significant difference between glibenclamide-treated group and the rats treated with the extract (at three doses) alone or in combination with glibenclamide (at 1 mg/kg; Fig. 2.b).

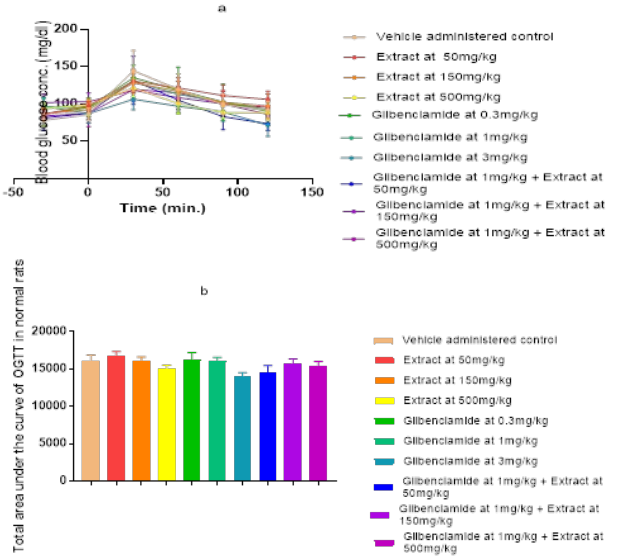

Figure 2: Influence of co-administration of the methanol leaf extract of *L. hastata* and Glibenclamide on (a) oral glucose tolerance test curve and (b) total area under the glucose tolerance curve in Normal Rats. Data are presented as mean ± SEM; No significant difference was observed when the treated groups were compared with Glibenclamide at 1mg/kg, n= 8. One-way ANOVA with Dunnett's multiple comparison post-hoc tests.

Discussion

Plant chemical constituents constitute an indispensable part of medicinal plants and are responsible for their numerous bioactivities. The preliminary phytochemical screening of the methanol leaf extract of *L. hastata* revealed the presence of cardiac glycosides, steroids/triterpenoids, phenols, flavonoids, carbohydrates, tannins, saponins, alkaloids. The presence of some of these phytoconstituents in the methanol leaf extract of *L. hastata* has been previously documented (15–17). Some of the phytochemicals found in the extract, namely cardiac glycosides, triterpenoids, flavonoids, tannins, saponins, alkaloids have been reported to possess antihyperglycaemic activity (18–21). This study revealed that the methanol leaf extract of *L. hastata* at all doses did not show any significant difference in the total area under the glucose tolerance curve between the extract-treated groups and the vehicle-treated controls in normal rats. However, compared with metformin-treated group (at 100 mg/kg), an improvement in glucose tolerance (evidenced by significant reduction in the total area under the oral glucose tolerance curve) was observed in normal rats when metformin at 100mg/kg was co-administered with the extract at 500mg/kg, indicating enhancement of effect of the drug. Therefore, the enhanced effect of metformin may be due to the presence of any of these phytochemicals either singly or in combination. On the other hand, no significant improvement in oral glucose tolerance was observed in normal rats when the extract at 500mg/kg was co-administered with glibenclamide at 1mg/kg, compared with the group of rats treated with glibenclamide (at 1 mg/kg) alone, suggesting no enhancement of the effect of Glibenclamide by concurrent use of the extract.

This study has also revealed that the methanol leaf extract of *L. hastata* may not cause acute hypoglycaemia in normal rats with maximum dose of 500mg/kg administered. Similar study has shown that the aqueous root extract of *L. hastata* administered at a dose of 400mg/kg did not show significant decrease in blood glucose level following daily administration for 2 weeks in normal rats. When administered daily at doses of 600 and 800mg/kg for 1 week, there was significant reduction from the zero-hour blood glucose level (22).

Conclusion

It could be concluded that the methanol leaf extract of *L. hastata* when co-administered with metformin caused a significant reduction in the total area under the glucose tolerance curve in normal rats. When administered singly, the methanol leaf extract of *L. hastata* may not cause acute hypoglycaemia in normal rats with maximum dose of 500mg/kg administered.

Conflict of interest

None

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