ScientificScholar®
Knowledge is power
Publisher of Scientific Journals

https://ijpp.com

Indian Journal of Physiology and Pharmacology

Original Article

Influence of concurrent administration of the methanol leaf extract of *Leptadenia hastata* (Pers) decne (*Apocynaceae*) with metformin on blood glucose in diabetic rats

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

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

***Corresponding author:**
Omobhude Fidelis Aluefua, Department of Pharmacology and Therapeutics, Usmanu Danfodiyo University, Sokoto, Nigeria.

aluefuafo@gmail.com

Received : 21 July 2022
Accepted : 01 December 2022
Published : 29 December 2022

DOI
10.25259/IJPP_333_2022

Quick Response Code:

ABSTRACT

Objectives: *Leptadenia hastata* (Pers) Decne (*Apocynaceae*) is a common medicinal plant used in northern Nigeria either singly or together with conventional drugs to treat diabetes. This study investigated the influence of concurrent administration of the methanol leaf extract of *L. hastata* with metformin in streptozotocin/nicotinamide-induced diabetic rats.

Materials and Methods: Possible synergistic activity between the extract and metformin was assessed using 3 models of synergy analysis (Loewe additivity, Bliss independent and highest single agent [HSA] models). Eleven groups of Wistar rats (eight animals per group) consisting of ten groups of diabetic rats and one normal control group were used in this study. Six groups were administered with either the extract or metformin at three different doses each (50, 150 and 500 mg/kg for the extract and 30, 100 and 300 mg/kg for metformin), while another three groups were co-administered with the extract and metformin at three different ratios each (50 mg/kg: 30 mg/kg, 150 mg/kg: 100 mg/kg and 500 mg/kg: 300 mg/kg). An oral glucose tolerance test (OGTT) was conducted at baseline and on day 14.

Result: The results revealed that the extract-metformin combination brought about a synergistic reduction in the total area under the OGTT curve (based on Loewe and HSA models) as well as a synergistic reduction in blood glucose (based on Loewe, Bliss and HSA models).

Conclusion: The methanol leaf extract of *L. hastata* produced a synergistic antidiabetic activity in streptozotocin/nicotinamide-induced diabetic rats when combined with metformin.

Keywords: Diabetes, Blood glucose, *Leptadenia hastate*, Metformin, Synergy

INTRODUCTION

The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterised by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs.[1] The worldwide prevalence of diabetes mellitus and impaired glucose tolerance in adults has been on the rise in recent years. The rate at which the prevalence of diabetes mellitus changes in many countries

and regions has been enhanced by rapid urbanisation and the increasing tendencies for a sedentary lifestyle. According to the world health organisation, about 108 million people were estimated to be diabetic in 1980. This estimate rose to 422 million individuals in 2014.[2] There has been increasing interest in drugs derived from higher plants especially those with medicinal value in recent times.[3] Awodele and Osuolale, reported that 48.7% of patients occasionally or always use herbal medication alongside their antidiabetic medications. Herbal medications have been discovered to cause interactions with conventional medications; these interactions can either be beneficial or harmful depending on the agent implicated. Some herbal medications were found to have either synergistic effects or antagonistic effects while others had no evidence of efficacy.[4]

About one-third of outpatients attending diabetic clinics in Nigeria use herbal products concurrently with conventional antidiabetic drugs.[4] Concurrent use of herbal products with conventional antidiabetic drugs, which is common in Nigeria, may influence the antidiabetic effect of the drugs.[5-7] Metformin is the most frequently prescribed antidiabetic drug in Nigeria.[8] *Leptadenia hastata* is a common medicinal plant used in Northern Nigeria either singly or together with conventional drugs to treat diabetes mellitus.[9,10] It is not known whether *L. hastata* will have an influence on the antidiabetic effect of metformin when the two are used concurrently. The study aimed to evaluate the influence of concurrent administration of the methanol leaf extract of *L. hastata* (pers) decne (*Apocynaceae*) with metformin on blood glucose in streptozotocin/nicotinamide-induced diabetic rats.

MATERIALS AND METHODS

Materials

Drugs and reagents

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

Animals

Ethical clearance with clearance number PTAC/Lh/(Me) OT/35-21 was gotten from Usmanu Danfodiyo University Sokoto (UDUS) ethical clearance committee on Jan 18, 2021 before the commencement of the study. Male Wistar rats weighing 120–150 g (4–6 weeks) were obtained from IMRAT, UCH, Ibadan, Nigeria. The rats were maintained according to the minimum requirements of the International guidelines for the use of animals.[11] During the 2 weeks of acclimatisation, the animals were fed a commercial diet (Vital Feed Nig. Ltd) and allowed access to water *ad libitum*.[11] They fasted for 8 h before the commencement of the experiment.

Methods

Collection of plant material

Fresh leaves of *L. hastata* were collected from the Biological garden on the main Campus of Usmanu Danfodiyo University, Sokoto (UDUS). 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 allowed to air dry (to constant weight) at room temperature and pulverised into a fine powder. One hundred grams of the sample was separately extracted with 500 mL of 95% methanol for 24 h.[9] The extract was filtered and concentrated to semisolid form under reduced pressure using a rotary evaporator at 35°C. This was then finally dried in an aerated oven at 35°C. The percentage yield was calculated for the extract. The dried extract obtained was preserved at 4°C until needed for use.

Phytochemical analysis

Qualitative phytochemical screening

The extract was subjected to various chemical tests using standard procedures.[12-14]

Gas chromatography-mass spectroscopy (GCMS) analysis of plant extract

GCMS analysis was performed using Agilent Intuvo 9000 GC system coupled with detector system 5977B MSD with split/splitless injector. A DB - 5 MS (5% phenyldimethylsiloxane) fused silica capillary column 30 m, 320 μm i. d., 0.25 μm of film thickness was used with Helium gas (99.999% purity) as carrier gas at flow rates of 1.2 mL/min. Inlet temperature was set at 300°C, MS Source at 230°C and MS Quad at 150°C. The oven temperature was programmed as follows: 50°C for 5 min, increased to 300°C at 10°C min^{-1} and hold for 20 min. The analysis was run for a total of 50 min. Data were acquired by GCMSD/Enhanced Mass Hunter Software and processed GCMSD Data analysis software incorporated with the 2017 Version of NIST Library. 1 μL of the sample extract was injected in split less mode into the GC system using Agilent Automated Liquid Sampler G4513A.

Experimental design of the *in vivo* study

Induction of diabetes

Out of 88 male Wistar rats, eight were randomly selected to serve as normal control. Diabetes mellitus was induced in the

remaining animals using streptozotocin reconstituted in 0.1M citrate buffer (pH 4.5) and nicotinamide 230 mg/kg dissolved in normal saline. Using an insulin syringe, streptozotocin (65 mg/kg) was administered intraperitoneally 15 min after the intraperitoneal injection of nicotinamide.[15,16] Fasting blood glucose was determined after 72 h using a standardised glucometer (AccuChek® Active). Rats with blood glucose levels of 198–252 mg/dL (moderate diabetes) were selected for the subsequent study.[17]

Determination of the effect of co-administration of the extract and metformin (at selected ratios) on blood glucose of streptozotocin/nicotinamide-induced diabetic rats

Eighty-eight male Wistar rats (150–180 g each) comprising 80 diabetic rats and eight normal rats were employed. The 80 diabetic rats were randomised using decision analyst into ten groups of eight rats each. Two groups served as vehicle-administered normal and diabetic control, respectively, and received distilled water. Six groups were administered with either the extract or the metformin at three different doses each (50, 150 and 500 mg/kg for the extract and 30, 100 and 300 mg/kg for metformin), while the remaining three groups were co-administered with the extract and metformin at three different ratios each (50 mg/kg: 30 mg/kg, 150 mg/kg: 100 mg/kg and 500 mg/kg: 300 mg/kg). After an 8-h fast, an oral glucose tolerance test (OGTT) was conducted (as described below) at baseline and on day 14, a day before the sacrifice of the animals at the end of the experiment. The duration of this stage of the study was 15 days.

OGTT

Glucose (2 g/kg) was given orally 30 min 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 a standardised glucometer (Accu Check®). Gentle digital pressure was applied to the tail tip for 30–45 s with a clean gauze pad to stop any bleeding before the animals were placed back in their cages.

Statistical analysis

Data were analysed using GraphPad Prism Version 7.0. The data were summarised as mean ± Standard Deviation. Analysis of Variance (ANOVA) with *post hoc* tests (Tukey Kramer) was used to compare the difference between different groups. $P < 0.05$ was considered to be significant. Synergy was analysed employing Combenefit software (Version 2.02), using Loewe additivity, Bliss independent and highest single agent (HSA) models. The sum of synergy and antagonism was calculated by the use of Combenefit software and a single value was given. Value >0 was synergism, if <0, antagonism. 0 indicated additivity.

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% [Table 1].

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

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

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

GCMS analysis of the methanol leaf extract of *L. hastata* revealed the presence of active principles with their retention time, molecular formula, molecular weight and peak area (%) as shown in [Table 3 and Figure 1].

Induction of diabetes

Following intraperitoneal administration of streptozotocin and nicotinamide, the majority (75.2%) of the rats induced exhibited an elevation in the level of their fasting blood glucose (compared with normal controls), within the acceptable range of 198–252 mg/dl.

Table 1: Weight and percentage yield of methanol leaf extract of *L. hastata*.

Plant	Weight of extract (g)	% yield
L. hastata	186	9.3

L. hastata: Leptadenia hastata

Table 2: Phytochemical analysis of the methanol leaf extract of *Leptadenia hastata.*

S. No.	Phytochemical constituents	Results
1.	Cardiac Glycosides	+
2.	Anthraquinones	ND
3.	Steroids/Triterpenoids	+
4.	Phenols	+
5.	Flavonoids	+
6.	Carbohydrates	+
7.	Tannins	+
8.	Saponins	+
9.	Alkaloids	+

+: Present, ND: Not detected

Table 3: Gas chromatography-mass spectroscopy analysis of the methanol leaf extract of *Leptadenia hastata.*

S. No.	Retention time (min)	Name of the compound	Molecular formula	Molecular weight	Peak Area %
1.	5.911	Docosanoic acid	$C_{22}H_{44}O_2$	340.6	0.24
2.	7.891	Phosphoric acid	H_3PO_4	97.994	0.19
3.	8.023	2 (1H)-Pyrimidinone	$C_4H_4N_2O$	96.09	0.36
4.	8.635	2-butenedioic acid	$C_4H_4O_4$	174.15	0.24
5.	13.745	Silane	SiH^4	32.12	0.28
6.	20.205	Naphthalene	$C_{10}H_4$	128.17	3.32
7.	20.663	2-Dodecene	$C_{12}H_{24}$	168.32	1.31
8.	22.476	Cyclohexane, octyl-	$C_{14}H_{26}$	196.37	3.00
9.	28 0.336	2-Tetradecene, (E)-	$C_{14}H_{28}$	196.37	7.61
10.	28.622	Tridecane	$C_{13}H_{28}$	184.37	1.93
11.	35.185	Cetene	$C_{16}H_{33}$	225.43	14.93
12.	35.431	Hexadecane	$C_{16}H_{34}$	226.41	2.74
13.	37.634	Pentacos-1-ene	$C_{25}H_{50}$	350.7	10.57
14.	41.353	1-Octadecene	$C_{18}H_{36}$	252.49	14.85
15.	43.700	Thiazole	C_3H_3NS	85.13	3.45
16.	43.917	Benzenepropanoic acid	$C_9H_{12}0_2$	152.19	9.28
17.	46.973	1-Hexacosene	$C_{26}H_{52}$	364.7	5.40

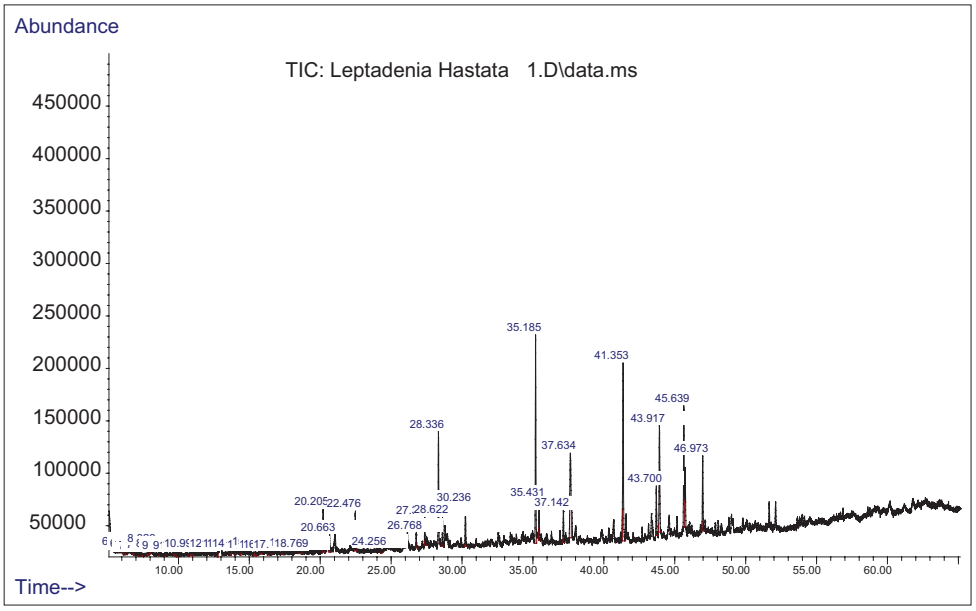

Figure 1: Mass spectra of identified compound from methanol leaf extract of *L. hastata.*

Effect of co-administration of the methanol leaf extract of *L. hastata* and metformin on OGTT curve in streptozotocin/nicotinamide-induced diabetic rats at baseline

At baseline, all the diabetic groups exhibited a significant increase in the total area under the OGTT curve ($P < 0.05$), compared with the vehicle-treated normal control group. Administration of the extract or metformin (at three increasing doses each), either singly or in combination, brought about a significant reduction in the total area under the OGTT curve ($P < 0.05$) when compared with the vehicle-treated diabetic rats. Other inter-group comparisons were not statistically significant [$P > 0.05$; Figure 2].

Effect of co-administration of the methanol leaf extract of *L. hastata* and metformin on OGTT curve in streptozotocin/nicotinamide-induced diabetic rats at day 14 of treatment

Administration of the extract or metformin (at three increasing doses each) either singly or in combination, brought about a significant reduction in the total area under the OGTT curve ($P < 0.05$) compared with the vehicle-treated diabetic rats [Figure 3]. Other inter-group comparisons were not statistically significant [$P > 0.05$; Figure 3].

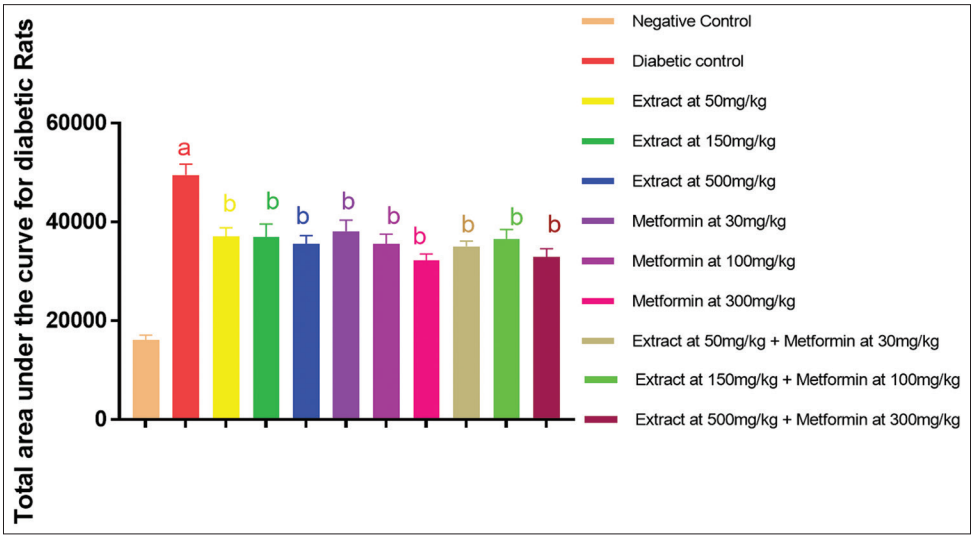

Figure 2: Effects of co-administration of the methanol leaf extract of *Leptadenia hastata* and metformin on the total area under the glucose tolerance curve in streptozotocin/nicotinamide-induced diabetic rats at baseline. Data are presented as mean ± SEM; [b]signifies $P < 0.05$ when compared with [a]the vehicle-treated diabetic group, n= 8. One-way ANOVA with Tukey Kramer multiple comparison *post hoc* tests were used to arrive at the *P* value.

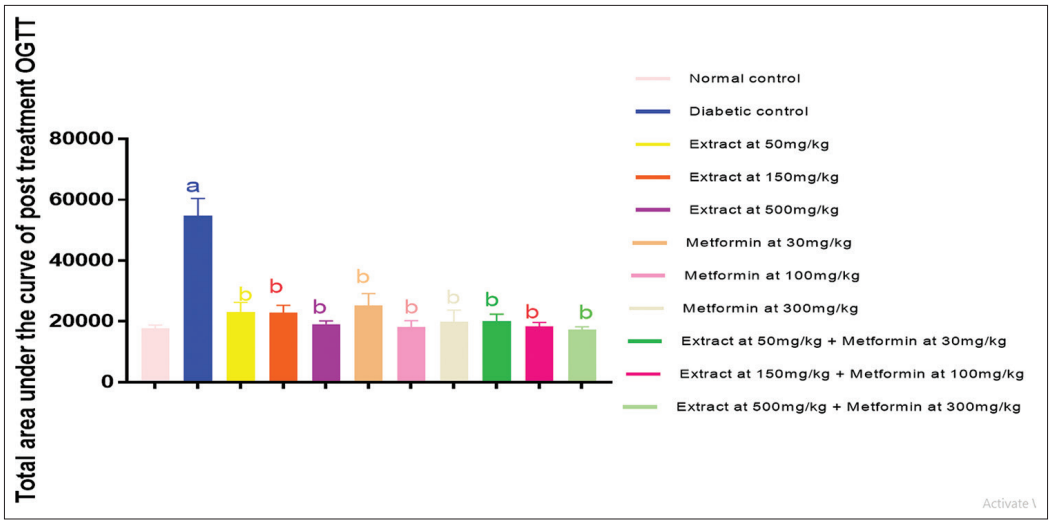

Figure 3: Effects of co-administration of the methanol leaf extract of *Leptadenia hastata* and metformin on the total area under the glucose tolerance curve in streptozotocin/nicotinamide-induced diabetic rats on day 14 of treatment. Data are presented as mean ± SEM; [b]Signifies $P < 0.05$ when compared with [a]the vehicle-treated diabetic group, $n = 8$. One-way ANOVA with Tukey Kramer multiple comparison *post hoc* tests were used to arrive at the *P* value. OGTT: Oral Glucose Tolerance Test.

Dose-response curves of the change in total area under the glucose tolerance test curve following 14 days of treatment with either the methanol leaf extract of *L. hastata* or metformin in streptozotocin/nicotinamide-induced diabetic rats

The dose-response curves of the methanol leaf extract of *L. hastata* and metformin showed median effective doses (ED_{50}s) of 8.078 mg/kg and 29.56 mg/kg, respectively[Figure 4].

HSA synergy and antagonism metrics of the change in total area under the glucose tolerance test curve following 14 days of co-administration of the methanol leaf extract of *L. hastata* and metformin in streptozotocin/nicotinamide -induced diabetic rats

With the HSA model, the value of the sum of synergy and antagonism of the change in total area under the glucose tolerance test curve for increasing doses of methanol leaf

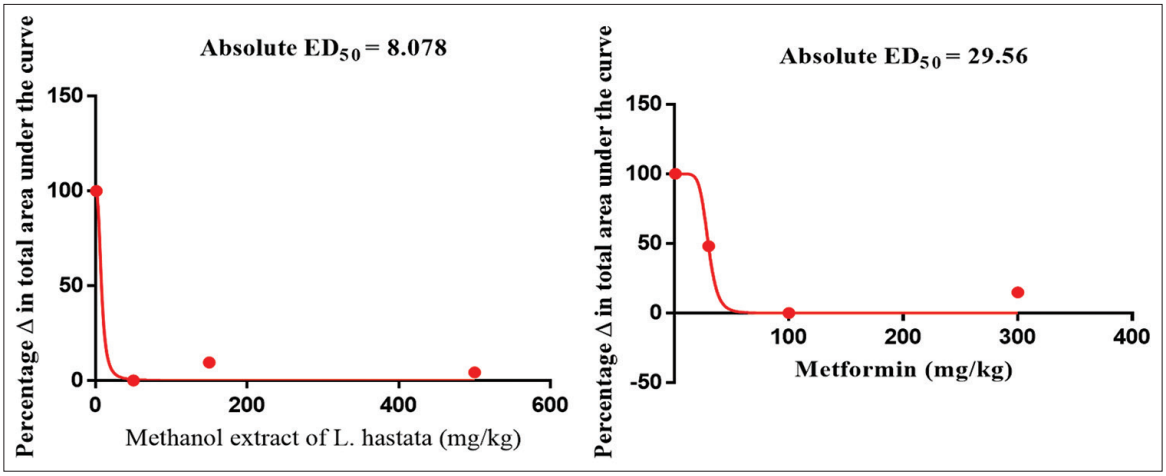

Figure 4: Dose-response curves of the change in total area under the glucose tolerance test curve following 14 days of treatment with either the methanol leaf extract of *Leptadenia hastata* or Metformin in streptozotocin/nicotinamide-induced diabetic rats. ED_{50} = Effective $dose_{50}$.

extract of L. hastata co-administered with metformin to streptozotocin/nicotinamide-induced diabetic rats for 14 days was 1.189958. The maximum synergy and antagonism values were 9.366548 and −3.31782, respectively [Figure 5].

Loewe's synergy and antagonism metrics of the change in total area under the glucose tolerance test curve following 14 days of co-administration of the methanol leaf extract of *L. hastata* and metformin in streptozotocin/nicotinamide-induced diabetic rats

With the Loewe's model, the value of the sum of synergy and antagonism of the change in total area under the glucose tolerance test curve for increasing doses of methanol leaf extract of *L. hastata* co-administered with metformin to streptozotocin/nicotinamide-induced diabetic rats for 14 days was 0.659006. The maximum synergy and antagonism values were 3.3329 and −3.31782, respectively [Figure 6].

Bliss synergy and antagonism metrics of the change in total area under the glucose tolerance test curve following 14 days of co-administration of the methanol leaf extract of *L. hastata* and metformin in streptozotocin/nicotinamide-induced diabetic rats

With the bliss model, the value of the sum of synergy and antagonism of the change in total area under the glucose tolerance test curve for increasing doses of the methanol leaf extract of L. hastata co-administered with metformin to streptozotocin/nicotinamide-induced diabetic rats for 14 days was −0.54078. The maximum synergy and antagonism values were 2.505578 and −3.31782, respectively [Figure 7].

Loewe synergy and antagonism metrics of the mean fasting blood glucose following 14 days of co-administration of the methanol leaf extract of *L. hastata* and metformin in streptozotocin/nicotinamide-induced diabetic rats

With Loewe's model, the value of the sum of synergy and antagonism of the mean fasting blood glucose for increasing doses of the methanol leaf extract of L. hastata co-administered with metformin to streptozotocin/nicotinamide-induced diabetic rats for 14 days was 3.309851. The maximum synergy and antagonism values were 12.49756 and −12.4975, respectively [Figure 8].

HSA synergy and antagonism metrics of the mean fasting blood glucose following 14 days of co-administration of the methanol leaf extract of *L. hastata* and metformin in streptozotocin/nicotinamide-induced diabetic rats

With the HSA model, the value of the sum of synergy and antagonism of the mean fasting blood glucose for increasing doses of the methanol leaf extract of *L. hastata* co-administered with metformin to streptozotocin/nicotinamide-induced diabetic rats for 14 days was 3.660699. The maximum synergy and antagonism values were 12.49763 and −12.4975, respectively [Figure 9].

Bliss synergy and antagonism metrics of the mean fasting blood glucose following 14 days of co-administration of the methanol leaf extract of *L. hastata* and metformin in streptozotocin/nicotinamide-induced diabetic rats

With the Bliss model, the value of the sum of synergy and antagonism of the mean fasting blood glucose for

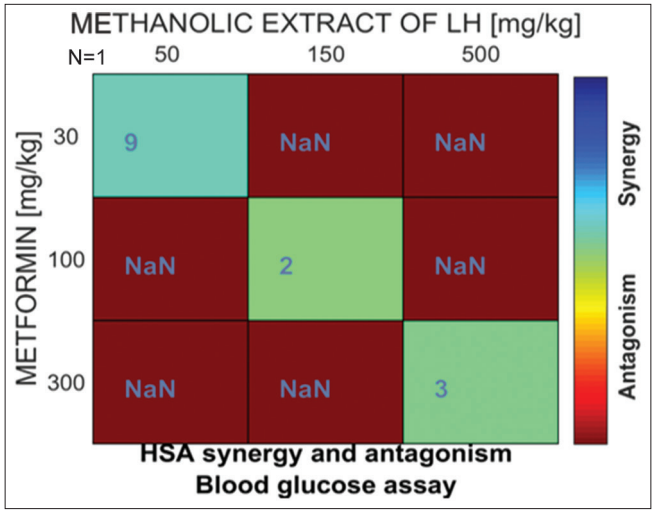

Figure 5: HSA synergy and antagonism Matrix of the change in total area under the glucose tolerance test curve following 14 days of concurrent treatment with the methanol leaf extract of *Leptadenia hastata* (LH) and metformin in streptozotocin/nicotinamide-induced diabetic rats. Value of the sum of synergy and antagonism (as shown in the square brackets) > or < 0 indicated synergism or antagonism, respectively. NaN: No data inserted.

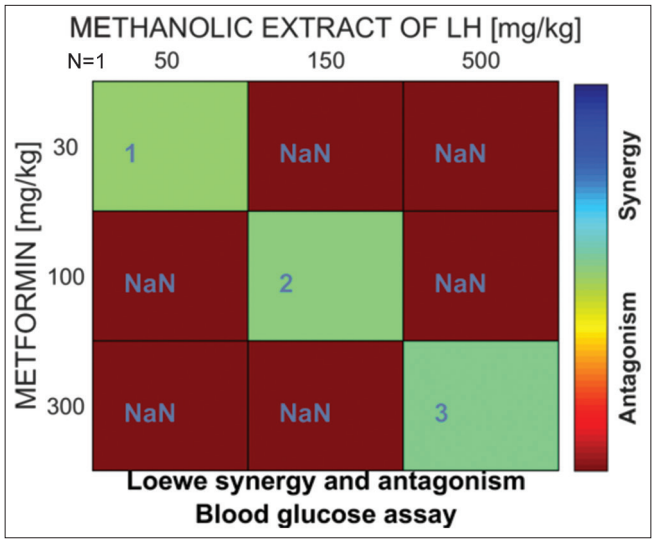

Figure 6: Loewe's synergy and antagonism Matrix of the change in total area under the glucose tolerance test curve following 14 days of concurrent treatment with the methanol leaf extract of *Leptadenia hastata* (LH) and metformin in streptozotocin/nicotinamide-induced diabetic rats. Value of the sum of synergy and antagonism (as shown in the square brackets) > or < 0 indicated synergism or antagonism, respectively. NaN: No data inserted.

increasing doses of the methanol leaf extract of *L. hastata* co-administered with metformin to streptozotocin/nicotinamide-induced Diabetic rats for 14 days was 0.06061. The maximum synergy and antagonism values were 12.49763 and −12.4975, respectively [Figure 10].

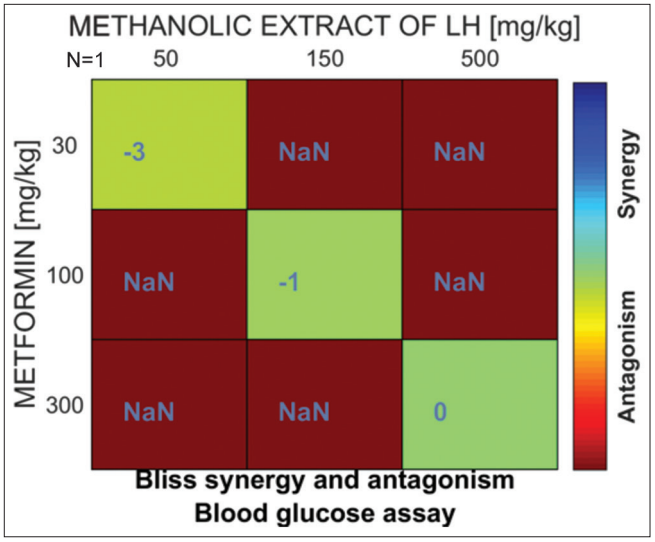

Figure 7: Bliss synergy and antagonism metrics of the change in total area under the glucose tolerance test curve following 14 days of concurrent treatment with the methanol leaf extract of *Leptadenia hastata* (LH) and metformin in streptozotocin/nicotinamide-induced Diabetic Rats. Value of the sum of synergy and antagonism (as shown in the square brackets) > or < 0 indicated synergism or antagonism, respectively. NaN: No data inserted.

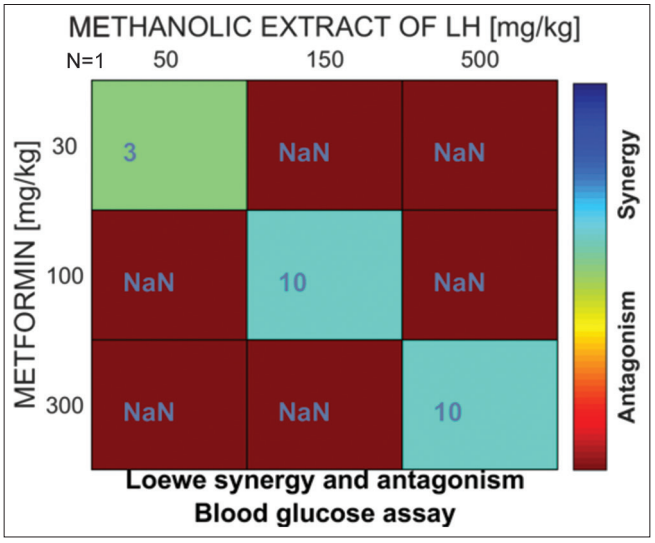

Figure 8: Loewe's synergy and antagonism metrics of the mean fasting blood glucose following 14 days of concurrent treatment with the methanol leaf extract of *Leptadenia hastata* (LH) and metformin in streptozotocin/nicotinamide-induced Diabetic Rats. Value of the sum of synergy and antagonism (as shown in the square brackets) > or < 0 indicated synergism or antagonism, respectively. NaN: No data inserted.

DISCUSSION

Plant chemical constituents constitute an indispensable part of medicinal plants and are responsible for their numerous bioactivities. The preliminary phytochemical screening of the

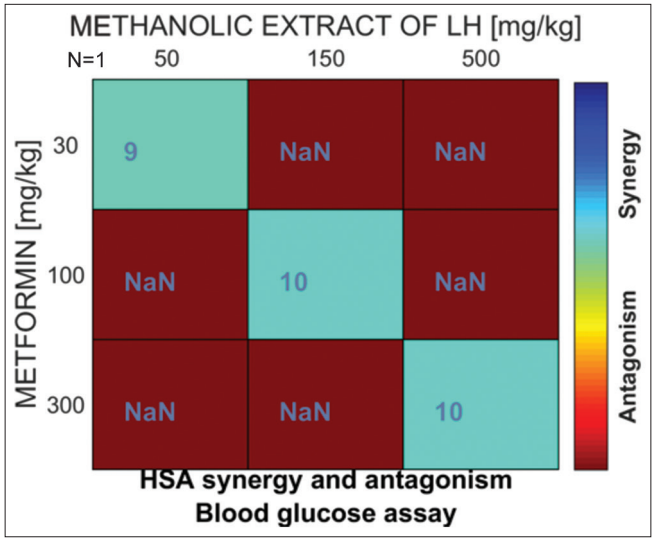

Figure 9: HSA synergy and antagonism metrics of the mean fasting blood glucose following 14 days of concurrent treatment with the methanol leaf extract of *Leptadenia hastata* (LH) and metformin in streptozotocin/nicotinamide-induced diabetic rats. Value of the sum of synergy and antagonism (as shown in the square brackets) > or < 0 indicated synergism or antagonism, respectively. NaN: No data inserted.

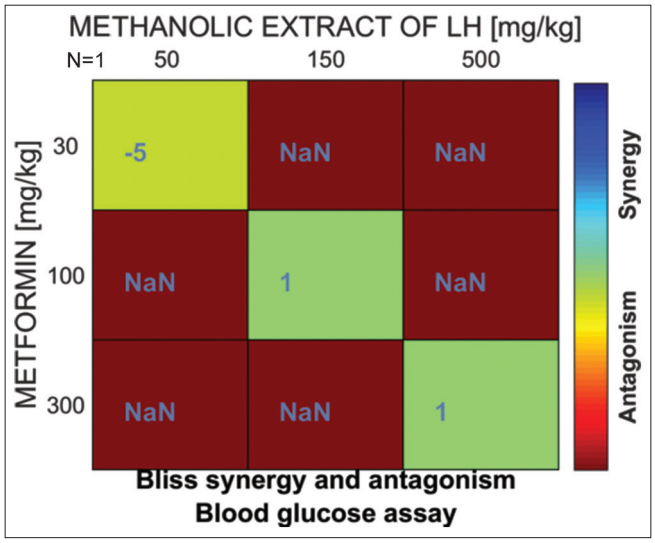

Figure 10: Bliss synergy and antagonism metrics of the mean fasting blood glucose following 14 days of concurrent treatment with the methanol leaf extract of *Leptadenia hastata* (LH) and metformin in streptozotocin/nicotinamide-induced diabetic rats. Value of the sum of synergy and antagonism (as shown in the square brackets) > or < 0 indicated synergism or antagonism, respectively. NaN: No data inserted.

methanol leaf extract of *L. hastata* revealed the presence of cardiac glycosides, steroids/triterpenoids, phenols, flavonoids, carbohydrates, tannins, saponins and alkaloids. The presence of some of these phytoconstituents in the methanol leaf extract of *L. hastata* has been previously documented.[18-20]

In this study, type 2 diabetes mellitus was induced in Wistar rats using streptozotocin in combination with nicotinamide. Streptozotocin induces diabetes mellitus in experimental animals by the process of methylation, generation of free radicals and production of nitric oxide which brings about the destruction of pancreatic islet cells.[21] It is preferable to alloxan in the induction of diabetes mellitus because of its greater selectivity towards β-cells, reduced mortality rates as well as more prolonged or irreversible induction.[22] Nicotinamide has been used together with streptozotocin to induce diabetes mellitus within 3–7 days after a single intraperitoneal injection, especially in the Wistar rat model. Several studies have shown that nicotinamide can enhance the energy status in ischaemic tissues, and exhibits antioxidant properties as well as slow apoptosis.[23-25]

Streptozotocin/nicotinamide-induced diabetic murine model is one of the commonest models of type 2 diabetes mellitus.[22] The antihyperglycaemic effect of the plant as observed in this study agrees with the findings of several studies using different leaf extracts of the plant in both alloxan- and streptozotocin-induced murine models.[9,19,26,27] Since streptozotocin-induced diabetes mellitus is associated with resistance to insulin action, the mechanism of *L. hastata* methanol leaf extract may be through the improvement of insulin sensitivity.[22] Some of the phytochemicals found in the extract, namely, cardiac glycosides, triterpenoids, flavonoids, tannins, saponins and alkaloids have been reported to possess antidiabetic activity.[28-31] Therefore, the antidiabetic activity of the extract may be due to the presence of any of these phytochemicals either singly or in combination.

The mass spectra of identified compounds from *L. Hastata* are presented in [Table 3 and Figure 1]. Some of the identified phytocompounds with possible antidiabetic potentials include Docosanoic acid,[32] 2(1H)-Pyrimidinone,[33] 2-Tetradecene,[34] Hexadecane,[35] 1-Octadecene,[36] Thiazole,[37] Benzenepropanoic acid,[38] and 1-Hexacosene.[32]

At baseline, single-dose treatment with either the extract or metformin (at three escalating doses each), alone or in combination resulted in a significant reduction in the total area under the OGTT curve compared to the vehicle-treated diabetic rats. This suggests that the methanol leaf extract of *L. hastata* possesses some antihyperglycaemic properties in diabetic rats even after a single dose treatment. Metformin is relevant in this study as a standard drug for type 2 diabetic models because it is the recommended first-line agent in the management of type 2 diabetes mellitus with proven clinical efficacy and lower incidence of adverse effect.[39] Furthermore, it is the most frequently prescribed oral antidiabetic agent globally. Metformin is ineffective in pancreatectomised animals and type 1 diabetes.[39] The dose-response curves of the methanol leaf extract of *L. hastata* and metformin showed median effective doses (ED_{50}s)

of 8.078 mg/kg and 29.56 mg/kg, respectively. Doses that give around 50% of the maximum possible drug effect (the effective dose 50 [ED_{50}]) often prove to be sufficient. The ED_{50} helps clinicians decide the initial drug dose in patients as it is an essential clinical starting point for practitioners when prescribing medications, as adjustments are made to balance efficacy and toxicity.[40,41]

We believe that this study is the first of its kind to find the synergistic activity in reducing the level of fasting blood glucose (using all the three models of synergism assessment) of *L. hastata* methanol leaf extract when co-administered with metformin, as evidenced by the observed values of the sum of synergy and antagonism being greater than 0 as well as a synergistic activity between the extract and metformin in improving OGTT (using Loewe and HSA models, but not Bliss model).

The bliss-independent model assumes independence of the mechanism of action of the individual drugs in the combination. It also does not take into consideration the dose-response curve of the effect of the combination. It is only accurate if the mechanism of action is independent which has not been established in this study. The findings from the Loewe model are standard and accurate and it takes into consideration the dose-response curve of the combination.[42]

We also believe that this is the first study to evaluate the potential synergistic activity between herbal extracts and antidiabetic agents, using the internationally accepted models: Loewe additivity and Bliss independent models. However, various studies have reported synergistic activity between metformin and extracts of different plants using other models such as the HSA model.[43-45] Synergism was assessed with three increasing doses of the extract-metformin combination. Experiments in which any drug is tested at less than three dose levels are therefore not likely to be sufficient to demonstrate synergy.[42]

The antihyperglycaemic effects observed by *L. hastata* extracts could be attributed to one or more possible mechanisms which include the activation of β-cells and subsequent release of insulin and stimulation of the insulin receptors. It may also be due to the facilitation of blood glucose transport to the peripheral tissue.[9,26] Other possible mechanisms include the inhibition of the activity of alpha-glucosidase, inhibition of glycogenolysis or stimulation of glycogenesis in the liver and skeletal muscle of diabetic rats.[19] However, the exact mechanism of the antihyperglycaemic activity of the extract administered singly as well as the mechanism of the observed synergistic antihyperglycaemic activity between the extract and metformin has not been investigated in this study.

One of the limitations of this study is that the possibility of pharmacokinetic interaction between the extract and metformin has not been evaluated by assaying the concentration of metformin in the blood.

CONCLUSION

It could be concluded that the methanol leaf extract of *L. hastata* when coadministered with metformin act synergistically in producing antidiabetic activity in streptozotocin/nicotinamide-induced diabetic rats.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. World Health Organization. Definition, Diagnisis, and Classification of Diabetes Mellitus and its Complications. Report of a WHO Consultation. Part 1, Diagnosis and Classification of Diabetes Mellitus. Vol. 1. Geneva: World Health Organization; 2016. p. 20-33.
2. Cho NH, Shaw JE, Karuranga S, Huang Y, Rocha JD, Ohlrogge AW, *et al*. IDF diabetes atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract 2018;138:271-81.
3. Ijomone OR, Ekpe EL. Antidiabetic potentials of common herbal plants and plant products: A glance. Int J Herb Med 2016;4:90-7.
4. Awodele O, Osuolale J. Medication adherence in Type 2 diabetes patients. Afr Health Sci 2015;15:513-22.
5. Hassanpour-Fard M, Hosseini M, Vazifeshenas-Darmiyan K, Hassanzadeh-Taheri M, Doostabadi M, Moodi H. Co-administration effects of aqueous extract of turnip leaf and metformin in diabetic rats. J Tradit Complement Med 2017;8:178-83.
6. Bugudare US, Divakar K, Krishnaveni BR, Ashfaq M. Influence of hydroalcoholic extract of *Cinnamomum cassia* on anti-diabetic effect of glibenclamide, metformin alone and their combination. Pharmacologyonline 2011;2:798-807.
7. Ohadoma S, Michael H. Effects of co-administration of methanol leaf extract of Catharanthus roseus on the hypoglycemic activity of metformin and glibenclamide in rats. Asian Pac J Trop Med 2011;4:475-7.
8. Eraga SO, Arhewoh MI, Oruh EP, Iwuagwu MA, Eraga S, De A. A comparative evaluation of the pharmaceutical quality of different brands of metformin hydrochloride tablets available in Abuja, Nigeria. West Afr J Pharm 2017;28:61-71.
9. Bello A, Aliero AA, Saidu Y, Muhammad S, Musa U, Katsina PM. Hypoglycaemic and hypolipidaemic effects of *Leptadenia hastata* (Pers.) decne in alloxan-induced diabetec rats. Niger J Basic Appl Sci 2011;19:187-92.
10. Aliero AA, Wara SH. Validating the medicinal potential of *Leptadenia hastata*. Afr J Pharm Pharmacol. 2009;3:335-8.
11. NRC. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory

Animals. Guid Care Use Laboratory Animals. Vol. 8. Washington, (DC): National Research Council; 2011. p. 1-246.
12. Harborne. Phytochemical Methods of Extraction. Vol. 2. London: Chapman and Hall; 1993. p. 135-203.
13. Harris R. Trease and Evans' pharmacognosy. Int J Aromather 2003;13:55.
14. Sofowora A. Research on medicinal plants and traditional medicine in Africa. J Altern Complement Med 1996;2:365-72.
15. Zhou JY, Zhou SW, Zeng S, Zhou J, Jiang M, He Y. Hypoglycemic and hypolipidemic effects of ethanolic extract of *Mirabilis jalapa* L. Root on normal and diabetic mice. Evid Based Complement Alternat Med 2012;2012:257374.
16. Etuk EU. Animals models for studying diabetes mellitus. Agric Biol J North Am 2010;1:130-4.
17. Chika A, Onyebueke DC, Bello SO. Phytochemical analysis and evaluation of antidiabetic in alloxan-induced diabetic rats treated with aqueou leaf extract of *Acanthospermum hispidum*. Afr J Biomed Res 2018;21:81-5.
18. Bello A, Aliero AA, Saidu Y, Muhammad S, Musa U, Katsina PM. Phytochemical screening, polyphenolic content and alpha-glucosidase inhibitory potential of *Leptadenia hastata* (Pers.) Decne. Niger J Basic Appl Sci 2011;19:181-6.
19. Ukwuani AN, Igbokwu MO. *In vitro* antidiabetic effect of *Leptadenia hastata* leaves fractions. Biosci Res Support 2016;1:40-6.
20. Maina VA, Sandabe UK, Williams A, Mahre MB. Phytochemical analysis and acute toxicity of *Leptadenia hastata* Pers (Decne) leaf in albino. Sahel J Vet Sci 2015;14:43-7.
21. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res 2001;50:537-46.
22. Maqbool M, Dar MA, Gani I, Mir SA. Animal models in diabetes mellitus : An overview. J Drug Deliv Ther 2019;9:472-5.
23. Yang J, Klaidman LK, Nalbandian A, Oliver J, Chang ML, Chan PH, *et al*. The effects of nicotinamide on energy metabolism following transient focal cerebral ischemia in Wistar rats. Neurosci Lett 2002;333:91-4.
24. Melo SS, Arantes MR, Meirelles MS, Jordão AA, Vannucchi H. Lipid peroxidation in nicotinamide-deficient and nicotinamide-supplemented rats with streptozotocin-induced diabetes. Acta Diabetol 2000;37:33-9.
25. Kuchmerovska T, Shymanskyy I, Bondarenko L, Klimenko A. Effects of nicotinamide supplementation on liver and serum contents of amino acids in diabetic rats. Eur J Med Res 2008;13:275-80.
26. Panuel B, Nadro MS. Anti-diabetic and hypolipidemic effects of aqueous and ethanolic extracts of *Leptadenia hastata* on streptozotocin-induced diabetic albino rats. Int J Sci Res Eng Stud 2015;2:23-8.
27. Sanda KA, Sandabe UK, Auwal MS, Bulama I, Bashir TM, Sanda FA, *et al*. Hypoglycemic and antidiabetic profile of the aqueous root extracts of *Leptadenia hastata* in albino rats. Pak J Biol Sci 2013;16:190-4.
28. Weng Y, Yu L, Cui J, Zhu YR, Guo C, Wei G, *et al*. Antihyperglycemic, hypolipidemic and antioxidant activities of total saponins extracted from Aralia taibaiensis in experimental Type 2 diabetic rats. J Ethnopharmacol 2014;152:553-60.
29. Babby A, Elanchezhiyan C, Suhasini S, Chandirasegaran G. Antihyperglycemic effect of tannic acid in streptozotocin induced diabetic rats. Int J Curr Res 2014;6:5396-8.
30. Hule AK, Shah AS, Gambhire MN, Juvekar AR. An evaluation of the antidiabetic effects of Elaeocarpus ganitrus in experimental animals. Indian J Pharmacol 2011;43:56-9.
31. Konaté K, Yomalan K, Oksana S, Patrice Z, Marian B, Van-Damme P, *et al*. Free radicals scavenging capacity, antidiabetic and antihypertensive activities of flavonoid-rich fractions from leaves of *Trichilia emetica* and *Opilia amentacea* in an animal model of Type 2 diabetes mellitus. Evid Based Complement Alternat Med 2014;2014:867075.
32. Ferdosi MF, Khan IH, Javaid A, Munir A. Biochemical profile of *Calotropis procera* flowers. J Weed Sci Res 2021;27:341-9.
33. Dhumaskara K, Meenab S, Ghadi S, Tilve S. Graphite catalyzed solvent free synthesis of dihydropyrimidin-2(1H)-ones/thiones and their antidiabetic activity. Bioorg Med Chem Lett 2014;24:2897-9.
34. Keerthana G, Kalaivani MK, Sumathy A. *In-vitro* alpha amylase inhibitory and anti-oxidant activities of ethanolic leaf extract of *Croton bonplandianum*. Asian J Pharm Clin Res 2013;6(Suppl 4):32-6.
35. Mahmood R, Kayani WK, Ahmed T, Malik F, Hussain S, Ashfaq M, *et al*. Assessment of antidiabetic potential and phytochemical profiling of *Rhazya stricta* root extracts. BMC Complement Med Ther 2020;20:293.
36. Heendeniya SN, Keerthirathna LR, Manawadu CK, Dissanayake IH, Ali R, Mashhour A, *et al*. Therapeutic efficacy of Nyctanthes arbor-tristis flowers to inhibit proliferation of acute and chronic primary human leukemia cells, with adipocyte differentiation and *in silico* analysis of interactions between survivin protein and selected secondary meta. Biomolecules 2020;10:165.
37. Khatik GL, Datusalia AK, Ahsan W, Kaur P, Vyas M, Mittal A, *et al*. A retrospect study on thiazole derivatives as the potential antidiabetic agents in drug discovery and developments. Curr Drug Discov Technol 2018;15:163-77.
38. Husni A, Sulistyo RP, Rahma SA, Nugraheni PS, Budhiyanti SA. *In vitro* antidiabetic activity of *Sargassum hystrix* extract and its ethyl acetate fractions. Syst Rev Pharm 2020;11:859-65.
39. Griffin SJ, Leaver JK, Irving GJ. Impact of metformin on cardiovascular disease: A meta-analysis of randomised trials among people with Type 2 diabetes. Diabetologia 2017;60:1620-9.
40. Kenny BJ, Preuss CV, McPhee AS. ED50. National library of medicine. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2022. p. 1-3.
41. Dimmitt S, Stampfe H, Martin JH. When less is more-efficacy with less toxicity at the ED50. Br J Clin Pharmacol 2017;83:1365-8.
42. Foucquier J, Guedj M. Analysis of drug combinations: current methodological landscape. Pharmacol Res Perspect 2015;3:e00149.
43. Veeresham C, Samala S. Andrographolide pretreatment en hances the bioavailability and hypoglycaemic action of glimepiride and metformin. Int J Phytomed 2015;7:254-64.
44. Elango H, Ponnusankar S, Sundaram S. Assessment of pharmacodynamic and pharmacokinetic interaction of aqueous extract of *Cassia auriculata* L. and metformin in rats. Pharmacogn Mag 2015;11:423-6.
45. Raja P, Thejaswini B, Gurupadayya S, Mruthyunjaya C. Evaluation of influence of gymnema tea on antidiabetic activity of metformin in diabetic rats. Indo Am J Pharm Res 2013;3:1262-8.

How to cite this article: Aluefua OF, Chika A, Ishaka A, Abubakar K. Influence of concurrent administration of the methanol leaf extract of *Leptadenia hastata* (Pers) decne (*Apocynaceae*) with metformin on blood glucose in diabetic rats. Indian J Physiol Pharmacol 2022;66:241-50.