Trop J Nat Prod Res, January 2019; 3(1):10-16

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Antidiabetic Potentials of Aqueous Leaf Extracts of *Momordica balsamina* Linn and *Leptadenia hastata* (Pers) Decne Alone and in Combination in Streptozotocin-Induced Diabetic Rats

Nafisatu Kabir[1]*, Ismail A. Umar[2], Dorcas B. James[2], Hajiya M. Inuwa[2], Muhammad K. Atiku[3]

[1]*Department of Biochemistry, Federal University Dutse, Jigawa State-Nigeria.*
[2]*Department of Biochemistry, Ahmadu Bello University Zaria, Kaduna State-Nigeria.*
[3]*Department of Biochemistry, Bayero University Kano State-Nigeria.*

ARTICLE INFO

Article history:
Received 19 December 2018
Revised 22 January 2019
Accepted 27 January 2019
Published online 04 February 2019

ABSTRACT

Metabolic derangements associated with Diabetes Mellitus (DM) require multiple therapeutic approaches to improve glycaemia outcomes and thus prevent onset of long-term complications. Composite antidiabetic plants mixture appears to be more therapeutically effective than the individual components through their synergistic or additive effect. The present study evaluated the antidiabetic potentials of the aqueous leaf extracts of *Momordica balsamina* (MB) and *Leptadenia hastata* (LH) alone and in combination in streptozotocin (STZ)-induced diabetic rats. Acute oral toxicity study was conducted at 2000 mg/kg body weight as per the OECD guidelines to determine the safe dose range of each plant extract alone and in combination. Graded doses of LH, MB and their combination (100-400 mg/kg) were screened acutely for antidiabetic activity at specific time intervals in rats made diabetic with a single intraperitoneal injection of STZ (60 mg/kg) to determine their effective doses (ED). Antidiabetic activities of the ED were sub-acutely (28 days) evaluated by measuring biochemical parameters in blood, serum and liver homogenate of STZ-induced rats. The ED at 400 mg/kg (LH), 200 mg/kg (MB) and 400+200 mg/kg (LH+MB) mg/kg proved to significantly decrease levels of blood glucose, glycated heamoglobin (HbA_{1c}), glucose-6-phosphatase (G6P) and increase levels of serum insulin, total heamoglobin (Hb) and hexokinase (HK) in STZ-induced diabetic rats when compared to normal rats. These results demonstrated the antidiabetic activity of the aqueous leaf extracts of *L. hastata* and *M. balsamina* and their combination, thus validating their ethno-medicinal usage in the management of DM and its complications.

Keywords: *Momordica balsamina*, *Leptadenia hastata*, aqueous extract, antidiabetic, streptozotocin.

Introduction

The increased prevalence of diabetes in Africa is threatening the developmental gains Africa has achieved.[1] There were more than 1.7 million cases of diabetes sufferers in Nigeria in 2015 and the prevalence of adults with diabetes in Nigeria aged between 20-79 years was estimated at 2.0%.[2] The most prevalent case of diabetes mellitus in Nigeria is type 2 diabetes mellitus resulting from heterogeneous etiologies. While type I diabetes develops from an absolute deficiency of insulin as a result of pancreatic β-cell destruction,[3] the more prevalent type 2 diabetes mellitus occurs mostly due to a combination of insulin resistance and an inadequate compensatory insulin secretory response.[4] However, both types of diabetes mellitus share the common characteristic of hyperglyceamia and associated long term microvascular and macrovascular complications.[5] Persistent hyperglyceamia of diabetes mellitus associated with derangements of major metabolic pathways of carbohydrate, lipid and protein metabolism enhances the irreversible production of glycated hemoglobin, which is a reliable marker of long term glyceamic control.[6] The main therapeutic strategies for the achievement of good glyceamic control could include the reduction of the demand for insulin, stimulation of endogenous insulin secretion, enhancement of the action of insulin at the target tissues and the inhibition of degradation and absorption of oligo and disaccharides.[7]

The adverse effects, high cost and non-availability of conventional hypoglyceamic drugs in sub-Saharan Africa has driven many patients to opt for herbal anti-diabetic treatments alone or in combination with the conventional drugs in an attempt to provide a multitherapeutic approach for targeting the multifactorial nature of diabetes.[8] In Nigeria, the use of traditional dietary herbal plants for their therapeutic values is widespread due to their availability and assumed safety. In response to this challenge, world health organization (WHO) expert committee on diabetes recommended further evaluation of folkloric methods of managing diabetes and its complications.[8] Vast number of medicinal plants have been used by the Nigerian traditional herbal healers with ethno-medicinal claims ascertaining their antihyperglyceamic effects. *Leptadenia hastata* (Pers) Decne and *Mormodica balsamina* Linn; commonly used for dietary purposes in Northern Nigeria, are traditionally reported to have diverse hypoglyceamic activities in many countries especially in Asia, Latin America and Africa.[9] The hypoglyceamic effects of the fruits and seeds of momordica species in rats and the anti-inflammatory and analgesic properties of the leaves

*Corresponding author. E mail: kabirnafisa0@yahoo.com
Tel: +2348142100902

Citation: Kabir N, Umar IA, James DB, Inuwa HM, Atiku MK. Antidiabetic Potentials of Aqueous Leaf Extracts of *Momordica balsamina* Linn and *Leptadenia hastata* (Pers) Decne Alone and in Combination in Streptozotocin-Induced Diabetic Rats. Trop J Nat Prod Res. 2019; 3(1):10-16. doi.org/10.26538/tjnpr/v3i1.3

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

extract have been reported.[10, 11] *Leptadenia hastata (LH)* (Pers.) Decne (Asclepiadaceae) is considered beneficial against milk drying, cough, sexual-impotency,[12] trypanosomiasis,[24] acute rhinopharyngitis, hypertension and wounds in human beings.[13] The hypoglyceamic, hypolipidemic and carbohydrate hydrolysis inhibitory effects of the water and methanol extracts of the fresh leaves of *L. hastata* in normal and alloxan induced diabetic rat models have been documented in previous studies.[14, 15] The leaves, fruits, seeds, and bark of *M. balsamina* and *L. hastata* have been reported to contain resins, alkaloids, flavonoids, glycosides, steroids, terpenes, cardiac glycoside, tannins, proanthocyanidins, alkaloids and saponin each having various pharmacological activities.[16, 17] Zhang *et al*. have identified the leaves of momordica species as important sources of triterpenoids.[18]

Experimental evidences have demonstrated that the different physiological effect on biological system of traditional herbal medicines is due to their composite bioactive phytochemicals that are more therapeutically effective than their individual components through their synergistic or additive effect.[11, 14, 18] Most traditional herbal healers use combination of indigenous plant parts with the expectation of an enhanced anti-hyperglyceamic effect. However, the rationale for the combination and the mechanism of action of these plant parts alone and in combinations has not been thoroughly investigated despite their wide usage and consequences which may be mortality or severe morbidity. Medicinal plants could therefore serve as valid alternatives in drug development which may give new antidiabetic drug leads. In this perspective, this study seeks to evaluate the antidiabetic potentials of the aqueous leaf extracts of *L. hastata* and *M. balsamina* alone and in combination in streptozotocin-induced diabetic rats.

Materials and Methods

Plant Material

Fresh plant leaves of *Momordica balsamina* Linn and *Leptadenia hastata* (Pers) Decne were collected from Wudil and Kumbotso Local Government Areas of Kano State, Nigeria in March 2013. The plants were taxonomically authenticated at the Department of Biological Sciences, Ahmadu Bello University, Zaria. Voucher specimens were deposited at the Herbarium of the same department with number LH (No.900220) and MB (No.1139) for future reference.

Preparation of Extracts

The fresh plant leaves of *L. hastata* and *M. balsamina* were separated from undesirable plant parts, washed and air dried in shade. The dried plant leaves were grounded into a fine powder and a portion of the dried leaf powders (500 g) of each plant material was extracted with distilled water (1500 mL) by cold maceration. Combination of the aqueous leaf extracts of *L. hastata* and *M. balsamina* were formulated to obtain LHMB.

Drugs and Chemicals

Streptozotocin was purchased from Tocoris Systems, UK and Glucovance (glibenclamide 2.5 mg/metformin 500 mg) was purchased from Merck, Germany, insulin and HbA1c were purchased from Fortress Inc., UK, glucose-6-phosphate (G6P) and hexokinase (HK) kits were purchased from Mybiosource Inc., US.

Experimental Animals

Apparently healthy Wistar rats of both sexes of average weight 250 ± 5 g were obtained from the animal house of the Department of Biological sciences, Bayero University, Kano. The animals were acclimatized for one week, housed in a wire meshed laboratory cage and fed with commercial pelleted feeds (Vital feeds®, Jos, Nigeria) and distilled water *ad libitum*. They were handled under standard conditions in accordance to the requirements of the guide for care and use of laboratory animals.[19] The study protocol was approved by the Department of Biochemistry, Faculty of life sciences, Ahmadu Bello University, Zaria (PhD/SCIE/3691/2011-2012).

Acute Oral Toxicity Study

The acute oral toxicity study was carried out as described by the Organization for Economic Cooperation Development (OECD) guidelines number: 425.[20] Apparently healthy normal female albino rats were randomly assigned into four groups of five (5) rats each after an overnight fast (food but not water was withheld overnight) prior to dosing and were sequentially dosed with a single oral dose of distilled water (2 mL) and 2000 mg/kg b.w of LH, MB and LHMB, respectively. The animals were observed continuously for behavioural and neurological changes for the first 30 minutes to 4 hours after dosing then occasionally up to 24 hours and thereafter, daily up to 14 days.

Induction of Diabetes

The experimental animals were made diabetic by a single intraperitoneal injection of freshly prepared solution of streptozotocin (60 mg/kg body weight in 0.1 M cold citrate buffer (pH 4.5), after an overnight fast.[21] The rats were kept for the next 24 hours on five (5%) percent glucose solution (4 hours post STZ induction) to prevent drug induced hypoglyceamia.[22] Diabetes was confirmed by the determination of blood glucose level by tail prick using a glucometer (Roche one touch glucometer, Germany), and rats with blood glucose level of ≥ 250 mg/dL were selected for the experiment.[23]

Acute Antidiabetic Study (Effective antihyperglyceamic dose (ED) determination)

Since the safe dose range was found to be up to 2000 mg/kg, the effective antihyperglyceamic dose was determined by screening $1/5^{th}$, $1/10^{th}$ and $1/20^{th}$ of 2000 mg/kg i.e. 400, 200 and 100 mg/kg, respectively of *L. hastata* and *M. balsamina* and their combination in STZ-induced diabetic rats, randomly assigned into six groups of four (4) rats each as follows:

Group 1, 2 and 3 were diabetic rats treated orally with graded doses of LH (100, 200, 400 mg/kg body weight, respectively).

Groups 4, 5, 6 were diabetic rats treated orally with graded doses of MB (100, 200, 400 mg/kg body weight, respectively).

Groups 7, 8, 9 were diabetic rats treated orally with graded combination dose of LH and MB (100+100, 200+200, 400+400 mg/kg body weight, respectively).

Group 10-13 served as normal, diabetic and positive controls and were normal and diabetic rats treated orally with water and standard drug (glucovance) (500+2.5 mg/kg body weight), respectively.

After determination of effective dose (ED) of LH and MB, the combination of the ED of LH and MB was also screened for antihyperglyceamic effect.

The effective antihyperglyceamic dose was defined as the dose that produced the highest percentage decrease in blood glucose 6 hours post extract administration.

Blood samples were collected from rat tail vein for blood glucose level measurement prior to extract administration; at 0 hour and at one hour interval up to six hours post oral extract administration using one-touch glucometer (Roche, Germany) after an overnight fast. At the end of the experiment, percentage decrease in blood glucose (BG) level of the experimental animals was calculated using the formula given below:

$$\text{Percentage decrease in FBG} = \frac{\text{FBG before treatment} - \text{FBG after treatment}}{\text{FBG before treatment}} \text{ X } 100$$

Sub-Acute Antidiabetic Activity

The sub-acute antidiabetic activity (28 days treatment) of the ED of LH, MB and their combination were evaluated in normal and diabetic Wistar rats randomly assigned into nine groups of six rats each as follows: Group 1 served as normal control treated with distilled water only; Group 2 served as diabetic control, treated orally with distilled water only; Group 3 served as positive controls treated with standard drug (glucovance); Group 4 were normal rats treated orally with ED of LH; Group 5 were diabetic rats, treated orally with ED of LH; Group 6 were normal rats, treated orally with ED of MB; Group 7 were diabetic rats treated orally with ED of MB; Group 8 were normal rats treated orally with ED of LHMB; Group 9 were diabetic rats treated orally with ED of LHMB. The percentage decrease in FBG was calculated at the end of the experimental period.

Collection and Preparation of Samples

At the end of the sub-acute antidiabetic study (28 days), the experimental animals were fasted overnight, sacrificed by cervical dislocation under light anaesthesia and fasting blood samples were collected from the sacrificed animals from the jugular vein and the liver was harvested. The blood collected was divided into two parts, one half was placed into plain sample container and the other half was placed into an EDTA containing sample container. Serum was harvested after

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

the blood in the plain sample container was allowed to clot and after clot retraction it was centrifuged at 3000 x g for 10 minutes. Whole blood in the EDTA container was used for the determination of glycated haemoglobin (HbA1c) and total haemoglobin (Hb) levels. The liver harvested was washed using chilled saline solution to remove excess blood, weighed and homogenized in phosphate buffered saline (PBS) solution for hepatic carbohydrate metabolizing enzymes determination (glucose-6-phosphatase (G6P) and hexokinase (HK).

Biochemical Determinations and Data Analysis

Serum, blood and liver homogenates were analyzed for serum insulin, HbA1c, G6P and HK levels, respectively using commercial kits following manufacturer's instructions while Hb was analyzed using haematology autoanalyser (α-Swelab Automated Haematology Analyser, Sweden). Results obtained were expressed as mean ± Standard Deviation (SD) for all biochemical parameters. Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test using R studio software. Differences were considered significant at $p < 0.05$.

Results and Discussion

The acute oral toxicity study of LH, MB and their combination demonstrated their non-toxic nature, as all the animals displayed normal behavioural and neurological symptoms and survived up to 14 days of single oral dose administration of 2000 mg/kg b.w as shown in Table 1. The LD_{50} of LH, MB and their combination was found to be >2000 mg/kg and the safe dose ranges were therefore considered to be up to 2000 mg/kg b.w.

Evaluating the safety of polyherbal antidiabetic formulations is a major step in discovering safer antihyperglyceamic agents. Herbal medicines have the advantage of having lesser side effects and been more readily available.[8] Contrary to the Asian and North American countries where the fruits of *Momordica balsamina* are the most commonly used part for management of diabetes mellitus; the leaves are the most commonly utilized parts in northern Nigeria.[9] *Leptadenia hastata* has been observed to possess diverse therapeutic properties and recently have been noted for its antidiabetic potentials in Hausa speaking Northern areas of Nigeria. In an attempt to overcome the multifactorial nature of diabetes, combination therapies have recently been opted for to provide multitherapeutic approach to reduce the onset of complications. Additionally, it has been observed that most traditional herbal healers use polyherbal formulations with the knowledge of which plant parts might have hypoglyceamic effects. Aqueous extract was employed in the present study in consonance with the most common solvent used by traditional herbal healers in Northern Nigeria. The non-toxic nature and safe dose range of the *L. hastata* and *M. balsamina* alone and as a composite mixture was demonstrated in the present study. The safe dose range of 2000 mg/kg observed in the present study agrees with that of Tamboura *et al.*[24] where the safe dose range of LH was reported to be between 1000-2000 mg/kg body weight due to its high LD quotient dose of 0.78.[24] The safety of the plant leaves can further be emphasized by the fact that traditionally, LH and MB have been used for culinary and dietary purposes for ages and are medicinal plants commonly used by traditional herbal healers without any noticeable side effects. The leaf of *M. balsamina* is especially appreciated for its bitter taste, while *L. hastata* leaves are considered as famine foods consumed with ground groundnut cake in Northern Nigeria.

Determination of Effective Antihyperglyceamic Dose (ED)

Figure 1 presents the dose- and time-dependent antihyperglyceamic activities of the graded doses of LH, MB and their combinations in STZ-induced diabetic rats. All the doses screened displayed effective antihyperglyceamic effect with varying potency. The doses of 400, 200 and 400 +200 mg/kg body weight of LH, MB and LHMB were most effective with percentage decrease in blood glucose of 45.17±7.28, 53.52±3.43, 59.49 ± 3.19%, respectively at 6 hours post oral extract administration as shown in Table 1.

Sub-Acute Antidiabetic Study

The blood glucose levels of the normal and STZ-induced diabetic rats treated for 28 days with ED of LH, MB and their combination are presented in Figure 2. The results displayed a persistently elevated fasting blood glucose (FBG) level in the untreated diabetic rats throughout the experimental period when compared to the normal controls. However, 28 days treatment of the diabetic rats with ED of LH, MB and combination resulted in a significant ($p < 0.05$) time-dependent decrease in the level of blood glucose when compared to the untreated diabetic rats. Combination of LH and MB (63.89%) and MB (60.69%) alone showed comparable effective antidiabetic activity to that of the standard drug (glucovance) (66.94%). However, LH treatment alone displayed a lower antidiabetic effect than the combination treatment. With respect to the normal animals, significant ($p < 0.05$) decrease of fasting blood glucose (FBG) was observed in animals treated with the combination dose when compared to normal controls.

Achievement of good glyceamic control and preventing the onset of both micro and macrovascular complications of diabetes, are the major challenges in the treatment of diabetes. It is a well-documented fact that aggressive control of hyperglycemia in patients with diabetes can attenuate the development of chronic complications.[5] STZ-induced diabetic animals are considered as good models for screening of traditional hyperglyceamic agents. Induction of diabetes with streptozotocin is accompanied with a significant increase in the level of blood glucose as such its wide usage to induce diabetes in experimental animal models. STZ selectively causes necrosis of pancreatic β-cells *in vivo* through its nitric oxide donor property as a result of alkylation of DNA and reduction of nicotinamide adenine dinucleotide (NAD+) and consequently reduced release and synthesis of insulin.[25, 26] This fact further confirms the aetiology of the almost three fold rise in blood glucose level and a diminished serum insulin level observed in the STZ-induced diabetic rats as a reflection of abnormalities in beta-cell function in the present study.

Effect of LH, MB and their Combination on Insulin, HbA1c and Hb

As presented in figure 3, in addition to the persistent hyperglyceamia observed in diabetic control rats, STZ-induction resulted in a significantly elevated ($p<0.05$) levels of HbA_{1c} and decreased level of serum insulin and Hb when compared to normal controls. The results showed that daily treatment (28days) with LH, MB and their combination resulted in a significantly decreased HbA_{1c} level and increased levels of serum insulin and Hb which correlated with a decreased blood glucose level when compared to the untreated diabetic rats. However, combination of LH and MB demonstrated a more significant ($P < 0.05$) ameliorative effect than LH and MB treatments alone which was similar to the standard drug (glucovance) ($P > 0.05$).

Table 1: Acute oral toxicity study and LD_{50} determination.

TREATMENT	1ST HOUR	4 HOURS	7 DAYS	14 DAYS	LD_{50} Value
Leptadenia hastata (2000 mg/kg b.w) n=5	Normal	Normal behaviour	No symptoms of toxicity	Survived	>2000 mg/kg b.w.
Mormordica balsamina (2000 mg/kg b.w) n=5	Increased Irritability	Normal behaviour	No symptoms of toxicity	Survived	>2000 mg/kg b.w.
Leptadenia hastata + *Mormordica balsamina* (2000 mg/kg b.w) n=5	Irritable	Normal Behaviour	No symptoms of toxicity	Survived	>2000 mg/kg b.w.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

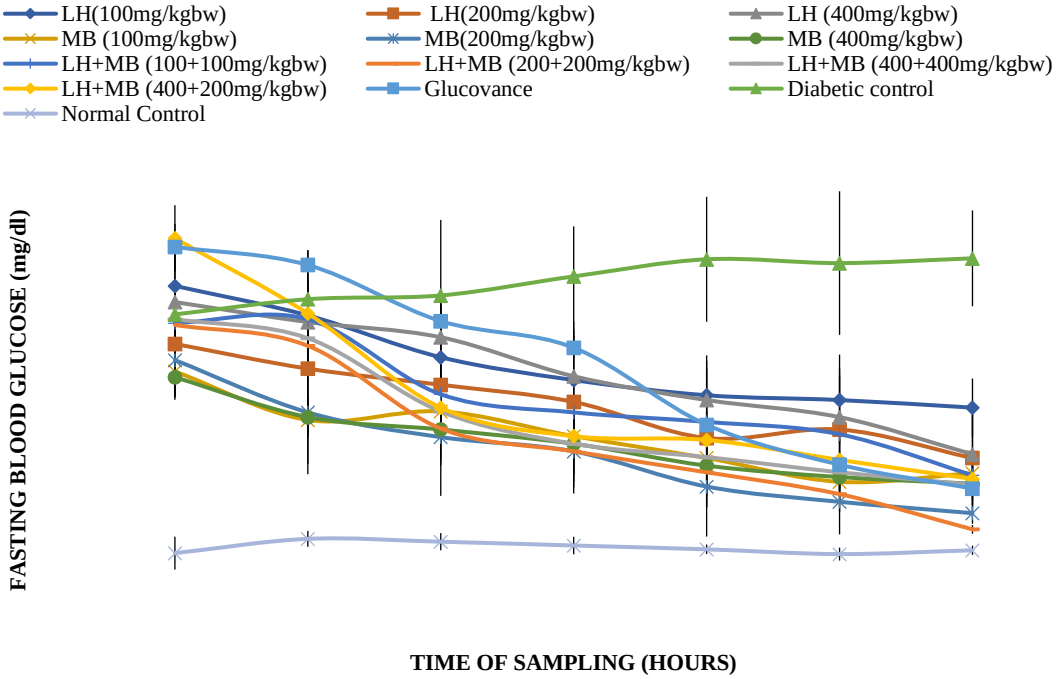

Figure 1: Anti-hyperglycemic activities of graded doses of LH, MB and their combination in STZ-Induced diabetic rats.
Values are expressed as Mean ± SD of four replicates. LH and MB represent *Lepatadenia hastata* and *Momordica balsamina* oral treatments alone, respectively. LH+MB represents oral treatment of the combination of *Lepatadenia hastata* and *Momordica balsamina*.

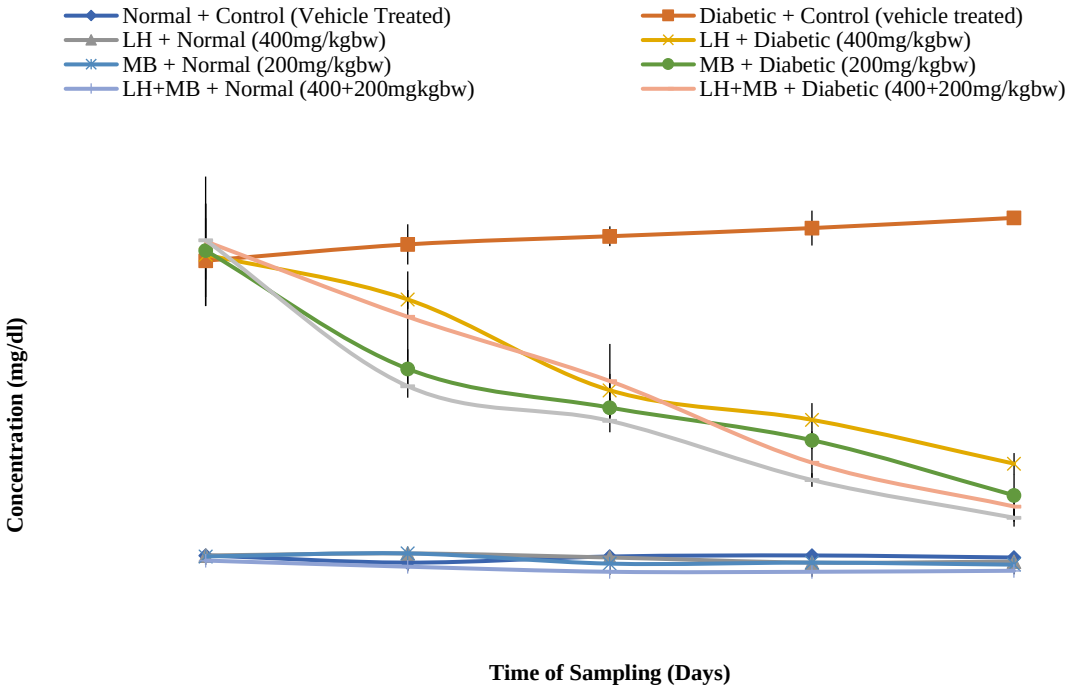

Figure 2: Effect of 28 days Administration of the Combination of LH and MB and Glucovance on Blood Glucose Levels of Normal and STZ-Induced Diabetic Rats.
Values are expressed as mean ± SD of six replicates. LH, MB represents *Lepatadenia hastata* and *Momordica balsamina* oral treatments alone respectively. LH+MB represent oral treatment of the combination of *Lepatadenia hastata* and *Momordica balsamina.*

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Antidiabetic effect of antidiabetic plants have been shown to be achieved through the enhancement of insulin secretion from beta cells, increasing glucose uptake by tissues, inhibition of glucose production in liver and increased pancreatic tissue regeneration and/or presence of insulin like agents in plants.[27, 28, 29] The *L. hastata* and *M. balsamina* alone and in combination could have caused a marked insulin increase through protective effect conferred by the phytoconsituents present in both extracts on the remnant pancreatic beta cells thereby preventing them against further free radical STZ-induced pancreatic beta cell damage. These findings are in agreement with that of Ayoub *et al.*[30] where the authors attributed the antihyperglyceamic effect of *Momordica charantia* to its ability to enhance insulin production from pancreatic beta cells and improving peripheral glucose utilization resulting in increased glycogen storage by the liver. The presence of phytoconstituents such as glycosides, phenolic compounds and triterpenoids in *L. hastata* and *M. balsamina* have been documented to portray potent antioxidant and antidiabetic activities.[31, 14] Consequently, combination of the phytoconstituents of *L. hastata* and *M. balsamina* as a formulation in the present study may have acted synergistically to enhance the antidiabetic activity by targeting different pathologies of diabetes.

Persistent hyperglycaemia of diabetes mellitus is often accompanied with an increased non-enzymatic reaction of the excess glucose present in the blood with haemoglobin to form glycated haemoglobin (HbA1c). The level of glycated haemoglobin at any given period of time is dependent on the life span of haemoglobin molecule and blood glucose level. HbA_{1C} therefore provides a simple reliable index to measure medium and long term glycaemic control in diabetes and thus its recent widespread use for monitoring diabetic treatments. In the present study, the extent of increase of glycated haemoglobin (HbA1c) was found to be proportional to the blood glucose levels, where significantly higher levels of glycated haemoglobin indicated poor glycaemic control. The low haemoglobin levels observed in the present study could be due to its involvement in the formation of glycosidic bonds with glucose as reported by Latha and Pari.[32] The authors reported a 16% increase in HbA_{1c} levels in diabetic patients. Furthermore, these findings also agree with studies that reported that various proteins such as haemoglobin, albumin, collagen, LDL, or crystalline proteins undergo non-enzymatic glycation in diabetes with the rate of glycation being proportional to the concentration of blood glucose level.[30] Aqueous extract of *L. hastata* and *M. balsamina* alone possess potentials to improve glyceamic status of diabetic patients and consequently cause an increment in levels of total heamoglobin. Improved glyceamic status impacted by the combination of *L. hastata* and *M. balsamina* was similar to that of glucovance (LHMB/glucovance; 7.2 ±1.58/6.9 ±0.79; $P>0.05$). Non-enzymatic glycation is also observed in normoglycaemic individuals, however, after the 120 days life span of haemoglobin, it is destroyed and the levels of HbA_{1c} and total haemoglobin are thus maintained within reference limits, thus the non-significant difference ($P>0.05$) in the level of HbA1c and significantly increased ($P<0.05$) Hb level observed in the present study. The HbA_{1c} levels in normal red cells have been reported to be between 3.4 to 5.8% .[32]

Effect of LH, MB and their combination on hexokinase and glucose-6-phosphatase.

Effects of LH, MB and combination on hexokinase and glucose-6-phosphate dehydrogenase are illustrated in figure 4. A significantly decreased level of hexokinase ($p<0.05$) and increased glucose-6-phosphatase ($p<0.05$) was observed in the diabetic rats when compared to the untreated normal rats. However, 28 days administration of LH, MB and their combination significantly decreased ($p<0.05$) the hepatic glucose-6-phosphatase and increased ($p < 0.05$) hexokinase levels when compared to untreated diabetic rats. Moreover, LHMB treatment exhibited a more effective hepatic antidiabetic effect than LH and MB treatments alone. The study revealed no significant difference ($p>0.05$) in the levels of hepatic glucose-6-phosphatase and hexokinase of normoglycemic treated rats when compared to the untreated normal rats.

Liver plays a critical role in glucose homeostasis in the diabetic states. Diminished glycolysis, hindered glycogenesis and expanded gluconeogenesis are a progressive part of glucose synthesis in the diabetic liver.[33] Hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate while glucose-6-phosphatase is a regulatory gluconeogenic pathway enzyme.[34] Glucose uptake by the liver via promotion of glucose-6-phosphate dehydrogenase and declining glucose-6-phosphatase activities observed in STZ-induced diabetic rats could therefore be attributed to an increased gluconeogenesis and inhibited glucose utilization. Diminished insulin levels observed in STZ-induced rats prompts the impedance in the activity of hexokinase, consequently glucose-6-phosphatase increase in the liver, facilitating glucose release into the blood as a result the liver continues to produce glucose even in severe hyperglycaemic states.[34] In correlation with studies by McCarthy and Shelia and James,[35, 34] treatment with the combination of *L. hastata* and *M. balsamina* exhibited a significant amelioration of the deranged hepatic hexokinase and glucose-6-phosphatase. This is suggestive of the fact that the combination of *L.*

Table 2: Percentage decrease in fasting blood glucose (FBG) of STZ-induced diabetic rats, 6 hours post oral extract administration of graded doses of LH, MB and their combination.

Treatments	Dose (mg/kg b.w.)	Percentage Decrease in FBG
LH	100	36.72 ± 5.97^{a}
LH	200	39.42 ± 10.96^{a}
LH	400	45.17 ± 7.28^{a}
MB	100	36.94 ± 5.17^{a}
MB	200	53.52 ± 3.43^{b}
MB	400	39.84 ± 0.96^{a}
LH+MB	100+100	47.45 ± 2.93^{a}
LH+MB	200+200	55.42 ± 4.09^{b}
LH +MB	400+400	51.02 ± 2.06^{b}
LH+MB	400+200	59.49 ± 3.19^{b}
GLUCOVANCE	500/2.5	60.75 ± 2.43^{b}
Diabetic Control	water	0.18 ± 0.19^{c}
Normal Control	water	-3.13 ± 0.20^{d}

Values are expressed as Mean ± SD of four replicates. Values with different superscripts within a column are significantly different (Tukey-Kramer post hoc test $P<0.05$).
LH, MB represents *Lepatadenia hastata* and *Momordica balsamina* oral treatments alone respectively. LH+MB represent oral treatment of the combination of *Lepatadenia hastata* and *Momordica balsamina*.

Table 3: Percentage decrease in fasting blood glucose (FBG) of normal and STZ-induced diabetic rats after 28 days treatment with the ED of LH, MB and their combination.

Treatment	Dose (mg/kg b.w.)	Percentage Decrease in FBG
Normal Control (Water)	--	2.31 ± 2.31^{a}
Diabetic Control (Water)	--	-3.88 ± 5.65^{b}
Normal + LH	400	6.14 ± 2.23^{a}
Diabetic + LH	400	51.85 ± 4.99^{c}
Normal + MB	200	8.34 ± 2.62^{a}
Diabetic + MB	200	60.69 ± 1.06^{d}
Normal + LH+MB	400+200	10.85 ± 5.90^{e}
Diabetic + LH+MB	400+200	63.89 ± 2.77^{d}
Diabetic + Glucovance	500+2.5	66.94 ± 0.66^{d}

Values are expressed as Mean ± SD for Groups of six replicates. Values with different superscripts within the column are statistically significant ($P < 0.05$). ED represents the effective antihyperglycaemic dose of *Leptadenia hastata* (LH), *Momordica balsamina* (MB) and their combination (LH + MB).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

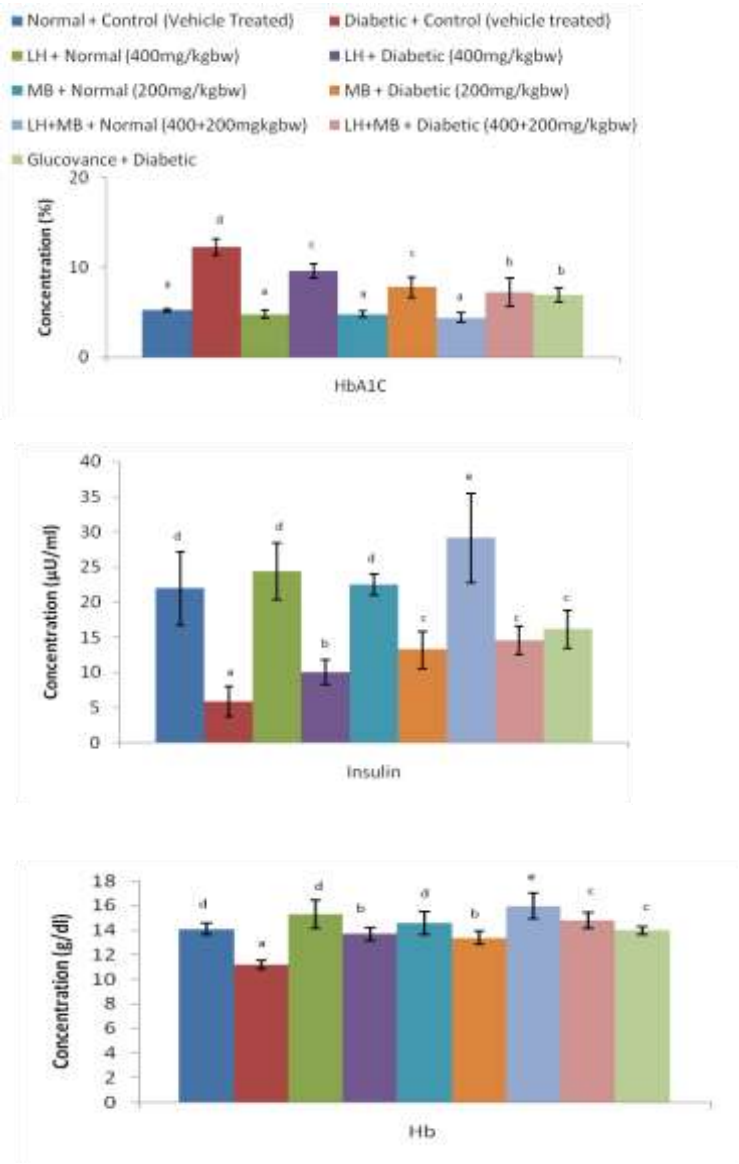

Figure 3: Effects of LH, MB, their combination and Glucovance on levels of HbA1c, Hb and Insulin in Normal and STZ-induced diabetic rats.
Values are expressed as mean ± SD for groups of six replicates. Values with different superscript are statistically different ($P < 0.05$). LH, MB represents *Lepatadenia hastata* and *Momordica balsamina* oral treatments alone respectively. LH+MB represent oral treatment of the combination of *Lepatadenia hastata* and *Momordica balsamina*.

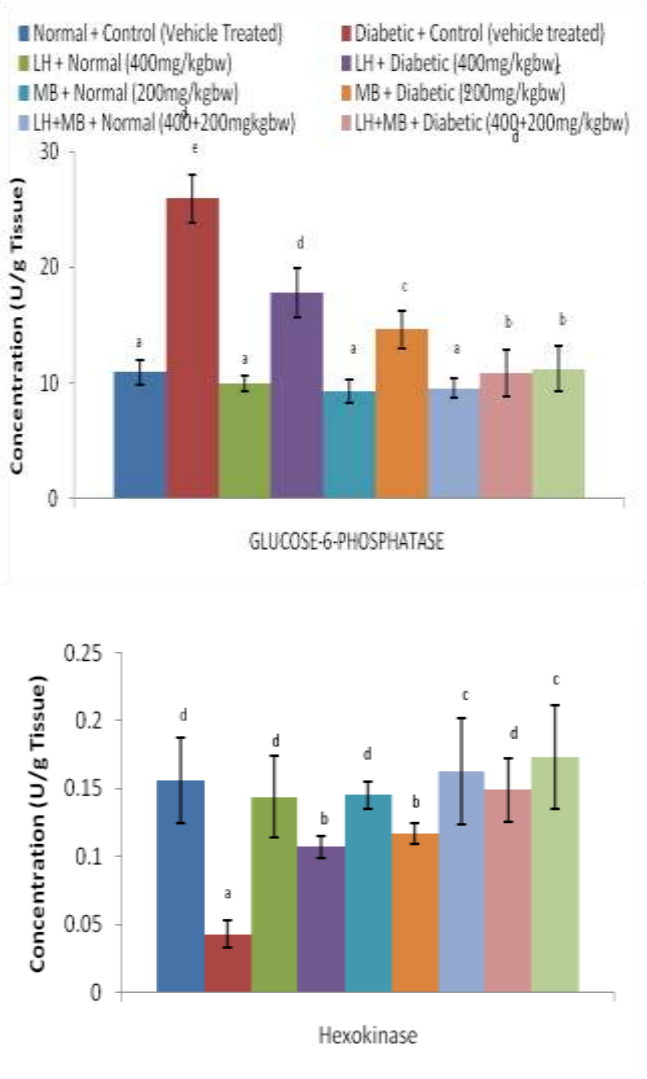

Figure 4: Effect of LH, MB and their combination on Glucose-6-phosphatase and Hexokinase in the Liver of Normal and Streptozotocin Induced Diabetic Rats.
Values Expressed are mean ± SD for groups of six animals each. Values differently superscripted for each set of data is statistically significant. LH, MB represents *Lepatadenia hastata* and *Momordica balsamina* oral treatments alone respectively. LH+MB represent oral treatment of the combination of *Lepatadenia hastata* and *Momordica balsamina.*

hastata and *M. balsamina* possesses the potential to stimulate the deposition of glycogen in the liver; which is dependent upon the stimulatory release of insulin from pancreas and significant hepatic increase in the activity of three key enzymes involved in carbohydrate metabolism; hexokinase, phosphofructokinase and glucokinase.[35] Activity based isolation and characterisation of the bioactive constituents of *L. hastata* and *M. balsamina* is currently being carried out for further validation.

Conclusion

Aqueous leaf extracts of *L. hastata* and *M. balsamina* alone and in combination possesses anti-diabetic potentials that may contribute to the achievement of good glycaemic control. The anti-diabetic effects of the composite formulation on STZ-induced diabetic rats may be exerted through the protection conferred on the remnant pancreatic β cells as evidenced by an increased level of serum insulin, stimulation of the deposition of glycogen in the liver which is dependent upon the stimulatory release of insulin from pancreas and significant hepatic increase in the activity of hexokinase and decreased glucose-6-phosphate. Consequently, this translates into an improved glycaemic status. These findings validate the use of the combination of *L. hastata* and *M. balsamina* for the treatment of diabetes which could prevent the onset of diabetic complications. Further study on bioactive principles of the studied plant extracts is recommended.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors wish to thank Mr Yakubu Otaro of the Department of Biological Sciences, Bayero University, Kano for animal care and handling.

References

1. International Diabetes Federation. Diabetes Atlas (7th ed.). Brussels, Belgium: International Diabetes Federation. 2016. Available from: http://www.diabetesatlas.org/index.php. Accessed online 26th September 2016.
2. International Diabetes Federation. IDF atlas. [online]. 2018. Available from: http://www.idf.org/our-network/regions-members/africa/members/20-nigeria.html. Accessed online on 1st May 2018.
3. World Health Organization. Definition, Diagnosis and Classification of Diabetes Mellitus and Its Complication. Part 1: diagnosis and clasification of diabetes mellitus. Department of Non-Communicable Disease Surveillance, Geneva. Report Number: WHO/NCD/NCS/99.2. 1999. 2-23.
4. Kumar S, Kumar V, Rana M, Kumar D. Enzymes inhibitors from plants: an alternate approach to treat diabetes. Pharmacogn Commun. 2012; 2(2):257-267.
5. Ramachandran S, Rajasekaran A, Manisenthilkuma KT. Investigation of hypoglycemic, hypolipidemic and antioxidant activities of aqueous extract of *Terminalia paniculata* bark in diabetic rats. Asian Pac J Trop Biomed. 2012; 2(4):262-268.
6. Marx J. Unraveling the causes of diabetes. Science. 2002; 296:686-689.
7. Funke I and Melzing MF. Traditionally used plants in diabetes therapy-phytotherapeutics as inhibitors of α-amylase activity. Rev Bras Pharmacogn, 2006; 16:1-5.
8. Chinenye S and Ogbera AO. Socio-cultural aspects of diabetes mellitus in Nigeria. J Soc Health Diabet. 2013; 1(1):15-21.
9. Thakur GS, Bag M, Sanodiya BS, Bhadouriya P, Debnath M, Prasad GB, Bisen PS. *Mormodica balsamina*: A medicinal and neutraceutical plant for healthcare management. Curr Pharm Biotechnol. 2009; 10(7):667-82.
10. Karumi Y and Bobboi A. Hypoglycemic effects of balsam apple (*Momordica balsamina*) in alloxan diabetic male rabbits. Biochem. 1999; 9:795-808.
11. Karumi Y, Onyeyili P, Ogugbuaja OV. Antinflammatory and anti inoceptive (Analgesic) properties of *Momordica balsamina* Lin (Balsam apple) leaves in rats. Pak J Biol Sci. 2003; 6:1515-1518.
12. Neuwinger HD. African ethno botany: poisons and drugs. Chapman and Hall. 1996. 280-296 p.
13. Aliero AA and Wara SH. Validating the medicinal potential of *Leptadenia hastata*. Afr J Pharm Pharmacol. 2009; 3(6):335-338.
14. Bello A, Aliero AA, Saidu Y, Muhammad S. Phytochemical Screening, Polyphenolic content and Alpha-Glucosidase Inhibitory potential of *Leptadenia hastata* (pers) Decne. Nig J Basic Appl Sci. 2011a; 19:181-186.
15. Gwarzo MY and Ameen ZS. Assessment of hypolipidemic effect of *Leptadenia hastata* leaves in albino rats. Adv J Food Sci Technol. 2015; 7(1):1-5.
16. Bhardwaj N, Gauttam V, Kalia, AN. Evaluation of antidiabetic activity of *Momordica balsamina* Linn seeds in experimentally-induced diabetes. J Chem Pharm Res. 2010; 2(5):701-707.
17. Bello A, Aliero AA, Saidu Y, Muhammad S. Hypoglycaemic and hypolipidemic effects of *Leptadenia hastata* (pers) decne in alloxan-induced diabetic rats. Nig J Basic Appl Sci. 2011b; 19:187-192.
18. Zhang YB, Huang Y, Kikachi T. *et al.* Cucurbitane triterpenoids from the leaves of *M. charantia* and their cancer chemopreventive effects and cytotoxicites. Chem Biodiv. 2012; 9:428-440.
19. National Research Council (NRC). Guide for the care and use of laboratory animals. Washington, DC: The National Academies Press. https://doi.org/10.17226/5140. 1996. Accessed online on 6th September, 2013.
20. Organisation for Economic Co-operation and Development (OECD). Test No 425: Acute Oral Toxicity: Up and Down Procedure. OECD Guidelines for the Testing of Chemicals. (Section 4). Health effects. 2008. 2-7 p.
21. Sankar N and Pari N. Influence of thymoquinone on glycoprotein changes in experimental hyperglycemic rats. Neuro Dis. 2011; 1:51-5.
22. Stanley A, Mainzen P, Venugopal MP. Antioxidant action of *Tinospora cordifolia* root extract in alloxan diabetic rats. Phytother Res. 2001; 15:213-218.
23. Zulfiker AH, Ripa FA, Rahman M, Ullah MD, Hamid K, Khan MR, Rana S. Antidiabetic and Antioxidant Effect of *Scoparia dulcis* in Alloxan induced Albino Mice. Int J Pharm Tech Res. 2010; 2(4):2527-2534.
24. Tamboura HH, Bayala B, Lompo M, Guissou IP, Sawadogo L. Ecological distribution, morphological characteristics and acute toxicity of aqueous extracts of *Holarrhena floribunda* (G. Don) Durand & Schinz, *Leptadenia hastata* (Pers.) Decne and *Cassia sieberiana (d c)* used by veterinary healers in Burkina Faso. Afr J Trad CAM. 2008; 2(1):13 – 24.
25. Bennett C and Pegg M. Alkylation of DNA in rat tissues following administeration of streptozotocin. Cancer Res. 1981; 41:2786.
26. Weis M. Streptozotocin: A review of its pharmacology, effective and toxicity. Cancer Treat Rev. 1982; 66:427.
27. Barnes J. Herbal therapeutics: an introduction to herbal medicinal products. The Pharm J. 2002; 268:803.
28. Goldman P. Herbal medicines today and the roots of modern pharmacology. Ann Int Med. 2001; 135:594-600.
29. Cheng D, Liang B, Li Y. Antihyperglycemic effect of *Ginkgo biloba* extract in streptozotocin-induced diabetes in rats. Biomed Research International. 2013; Article ID 162724, 7 pages.
30. Ayoub SM, Rao S, Byregowada SM, Satyanarayana L, Bhat N, Shridhar NB, Shridhar BR. Evaluation of hypoglycemic effect of *Momordica charantia* extract in distilled water in streptozotocin-diabetic rats. Braz J Vet Pathol. 2013; 6(2):56-64.
31. Bhardwaj N, Gauttam V, Kalia AN. Evaluation of antidiabetic activity of *Momordica balsamina* Linn seeds in experimentally-induced diabetes. J Chem Pharm Res. 2010; 2(5):701-707.
32. Latha M and Pari L. Effect of an aqueous extract of Scoparia dulcis on blood glucose, plasma insulin and some polyol pathway enzymes in experimental rat diabetes. Braz J Med Biol Res. 2004; 37:577-586.
33. Banquer NZ, Gupta D, Raja J. Regulation of metabolic pathways in liver and kidney during experimental diabetes: effects of antidiabetic compounds. Ind J Clin Biochem. 1998; 13:63-80.
34. Shelia S and James D. Diseases of the liver and biliary system. London: oxford blackwell science publication. 1993. 414 p.
35. McCarthy MF. Does bitter melon contain an activator of AMP activated kinase? Med Hypotheses. 2004; 63:340-343.