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EFFICACY OF A SYRUP FORMULATED FROM COMBINED EXTRACTS OF *VERNONIA AMYGDALINA* AND *MUSA PARADISIACA* FOR THE MANAGEMENT OF TYPE 2 DIABETES

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ABSTRACT

Diabetes mellitus affects about 415 million people worldwide, of which 400,000 are Ugandans. *Vernonia amygdalina (VA)* and *Musa paradisiaca (MP)* are some of the major plants used in Uganda for the management of diabetes mellitus type 2 by herbalists and individual patients; though without proper dosing and safety considerations since there are no standard formulations. In this research, we evaluated the efficacy of a syrup formulated from ethanol *VA leaf* and methanol *MP flower* extracts for the management of type 2 diabetes mellitus in streptozocin induced diabetic male waster rats. Efficacy was determined by assessing the glucose lowering abilities of varying combinations of the extracts based on the Oral Glucose Tolerance (OGT) and Random Blood Glucose (RBG) tests against glibenclamide standard. In both the OGT and RBG tests, the 1:1 combination (100 mg/kg VA and 200 mg/kg MP) of the extracts showed hypoglycemic effect greater than that of either of the plants extracts used singly at their most effective doses (100 mg/kg VA or 200 mg/kg) MP; ($p<0.05$). The hypoglycemic effect of the 1:1 combination (100mg/kg VA and 200 mg/kg MP) was also comparable to glibenclamide (0.05 mg/kg) ($p<0.05$) at 60 and 120 min for the OGT tests and on day 2 for the RBG tests. The maximum observed activity of *Vernonia amygdalina (100 mg/kg)* was greater than that of *Musa paradisiaca (200 mg/kg) though not significant (p>0.05.* Aspartame- sweetened syrup was formulated from the most active combinations; it is pale brown, sweet, homogeneous, with a vanillin flavor and pH 4.27. This study showed that a combined preparation of *MA* and *VP* extracts exhibits a synergistic and clinically significant hypoglycemic activity; and that the aspartame based syrup base has not effect on that hypoglycemic effects on *MA* and *VP* extracts, made singly or as combination

KEYWORDS: *Efficacy, Vernonia amygdalina, Musa paradisiaca, Diabetes Mellitus*

INTRODUCTION

Diabetes mellitus is due to insufficient production of insulin (type 1 or child-onset diabetes mellitus) or from the inefficient use of insulin by the body (type 2 or adult on-set diabetes mellitus)[1] ; type 2 is responsible for more than 90% of the cases[2]. Inefficient production or utilization of insulin results in high plasma concentration of glucose. Thus diabetes is defined by; a random plasma glucose concentration equal to or greater than 200mg/dl (11.1 mmol/L) and a Fasting plasma glucose of greater or equal to 126mg/dl (7.0 mmol/L)[1, 2].

The disease affects more than 400 million people worldwide and contributes to about 1.6 million deaths per year [1]. About 14 million people in Africa [2] and over 400,000 people in Uganda live with diabetes [3] , and the burden will be more than double by 2040 [2].

Morbidity and mortality from diabetes, results from complications arising from damage to cells, tissues and/or whole organs by consistently high blood glucose concentrations. Thus it is common for diabetics to suffer from conditions such as renal

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failure, blindness, limb amputations and cardiovascular disorders [1-3].

Prompt control of blood sugar levels (HbA_{1c} <7%) is key in the treatment of diabetes and control of complications. Type 1 diabetes is exclusively treated by insulin while type 2 diabetes is treated mainly by medicines that increase production, uptake and/or utilization of insulin by the body. Such medicines include; sulfonyl ureas, formins, glitazones, α-glucosidase inhibitors; they are used in combinations for better therapeutic outcomes[4].

World over, many patients use herbal concoctions for managing diabetes [5, 6]. Various plants with anti-diabetic activity have been studied widely [5, 7, 8]. *Vernonia amygdalina* and *Musa paradisiaca are some of the* major plants used in Uganda for the treatment of diabetes [9]. The hypoglycemic effect of *Vernonia amygdalina* has been extensively documented [10-13]. The plant is used in different parts of the world to manage a number of conditions including malaria, hypertension, intestinal worms, sexual functionality, liver disorders, and kidney disorders, among many others [13, 14]. It has been shown to effectively lower blood glucose in diabetic rats at doses of about 100mg/kg [14].

The ethno pharmacology of *Musa paradisiaca* (banana) has been reviewed and its glucose lowering ability has also been extensively studied [6, 15-21]. A dose of about 200mg/kg has been shown to be effective [6]

In this study, we evaluated the efficacy of a syrup prepared from a combination of the aqueous extracts of *Vernonia amygdalina* leaf and *Musa paradisiaca* flower for the management of hyperglycaemia in Waster albino rats with streptozocin induced type 2 diabetes mellitus.

MATERIALS AND METHODS

MATERIALS

Chemicals

Dimethyl sulfoxide (DMSO), HPMC, Benzoic acid, Sodium benzoate, Citric acid, Dibasic sodium phosphate, Aspartame, ethanol, methanol, distilled water and glibenclamide. All were analytical grade; most were obtained from the Pharmacy Department and others from chemicals dealers from within Uganda.

Plant materials

Vernonia amygdalina leaves and *Musa paradisiaca* flowers were collected from Kabanyoro Agricultural Research Center, Makerere University; identity of the plants was confirmed by a Botanist.

Study animals

Twenty seven (27) two month's old Waster albino rats weighing between 80-120 g were obtained from the College of Veterinary Medicine-Makerere University. Only healthy male rats were selected. These were acclimatized for one week under standard conditions; 25˚C temperature and a 12 hour dark/light routine. They were given food and water regularly.

METHODS

Study sites

Extraction of the plants, phytochemical profiling and formulation of the syrup were carried out at the Pharmaceutical Chemistry Laboratory while bioassays were carried out in the College of Veterinary Medicine, Makerere University.

Extraction of *Vernonia amygdalina*

The fresh mature leaves were cleaned, dried in a shade free from wind currents to prevent destruction of the active principles. The dried leaves were then ground to a fine powder using a miller. The powdered herb was soaked in 70% ethanol (2L) for 3days and the residue filtered off and the filtrate stored at 4°C. The residue was further soaked in 70% ethanol (1L) for 3 more days and filtration done using a whatman® paper. The two filtrates were then combined and concentrated using a rotary evaporator at 80˚C. The semisolid concentrates were stored at 4˚C until time of use [22].

Extraction of *Musa paradisiaca*

Tepals were removed from bracts and oven dried for a week at 40˚C. They were crushed, ground to a fine powder using a grinder. They were soaked in 70% methanol for 3 days and then filtered using a whatman® paper. The residues were further soaked in methanol (1 L) for 3 more days and filtered. The filtrate was concentrated at 42˚C to yield a dark brown solid using a rotatory evaporator. The dried extract was weighed and dissolved in 10% DMSO phase to yield a stock solution from which lower concentrations were prepared [23].

Phytochemical screening

Phytochemicals were tested for using the standard methods i.e. saponins (Form test); tannins (ferric chloride test); flavonoids (Shinoda test); test for terpenes (Copper acetate test); test for alkaloids (Hager's test); glycosides (Legal's test); test for phenols (Lead acetate test)[24].

Preparation of the syrup

Preparation of the buffer (pH 4.2)

Freshly prepared solutions of 0.1M citric acid (29.4ml) and 0.2M dibasic sodium phosphate (20.6ml) were mixed. The final volume was adjusted to 100ml with deionized water.

Preparation of the syrup base

This was prepared by vigorously shaking 40ml of buffer with 5%w/v hydroxyl propyl methyl cellulose (HPMC) as a viscosity enhancer.

Pharmaceutical evaluation of the syrup

Visual inspection; was done using a plain white and black background to check for clarity and any microbial growth. The syrup was also checked for consistency

Physicochemical analysis

Colour: 5ml of the syrup were placed in a white tube and observed with naked eye against a white back ground.
Odour: 2ml of the syrup was smelt.

pH: 10ml of the syrup was put in volumetric flask (100 mL) and made up to volume with distilled water. The solution was sonicated for about 10 minutes. The pH was measured using a digital pH meter.

Stability testing

Three test tubes (A, B, C) were filled with 5ml of the freshly prepared syrup each and stoppered. Tube A was kept at 4˚C, Tube B at 25˚C and Tube C at 60˚C for 24hrs, 48hrs and 72hrs. The organoleptic characteristics, pH and homogeneity were assessed at these intervals.

Experiments
Induction of diabetes

The basal blood glucose levels of all the rats were obtained after fasting them overnight. Streptozotocin (60mg/kg) was injected intraperitoneally 15 minutes after intraperitoneal injection of nicotinamide (230mg/kg). The blood glucose levels were measured after 3 days and those rats with levels above 200mg/dl were considered diabetic [25].

Table 1: Formulae for test syrups

Ingredients	F1	F2	F3	F4	F5	F6
Vernonia amygdalina	500mg	0	250mg	500mg	125mg	375mg
Musa paradisiaca	0	1000mg	500mg	1000mg	750mg	250mg
Sodium benzoate	0.2%					
Benzoic acid	0.1%					
Aspartame	0.5%					
Syrup base	q.s to 50ml					

Design of the experiment

The rats were divided into nine (9) groups comprising of three (3) animals per group. The groups were generated using an online research randomizer (https://www.randomizer.org/). The respective test formulae (2ml) were administered each day for a period of 3 days as follows.
Group 1-Normal rats administered syrup base.
Group 2-Diabetic rats administered syrup base.
Group 3-Diabetic rat administered 100mg/kg *Vernonia amygdalina* (F1)
Group 4-Diabetic rat administered 200mg/kg *Musa paradisiaca* (F2)
Group 5-Diabetic rat administered 100mg/kg *Vernonia amygdalina* and 200mg/kg *Musa paradisiaca* (F3)
Group 6-Diabetic rat administered 50mg/kg *Vernonia amygdalina* and 100mg/kg *Musa paradisiaca* (F4)
Group 7-Diabetic rat administered 25mg/kg *Vernonia amygdalina* and 150mg/kg *Musa paradisiaca* (F5)
Group 8-Diabetic rat administered 75mg/kg *Vernonia amygdalina* and 50mg/kg *Musa paradisiaca* (F6)
Group 9-Diabetic rat administered glibenclamide 0.05mg/kg

Determining efficacy

Blood was drawn from the rats by making a small cut at the tip of the tail. The blood glucose was measured using a WELLION® glucometer and strips. The tests below were performed Oral glucose tolerance test: On the 1st day of the experiment fasting blood samples were drawn from all the rats. The rats were then administered glucose solution (2g/kg BW). Three blood samples were drawn at 30, 60 and 120 minute intervals.
Random Blood glucose test: The random blood glucose levels were measured on the 2nd day.

Acute toxicity studies

Graded doses of *Vernonia amygdalina* and *Musa paradisiaca* (100mg/kg: 200mg/kg and 200mg/kg: 400mg/kg.) were administered to 5 rats in each group. Observations were done for the first 30 minutes after dosing and then periodically during the first 24hrs. Observations for signs of toxicity included changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somato-motor activity and behaviour pattern. Observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep were also done (OECD guidelines for acute toxicity testing).

Data management

Data was read and recorded in mg/dl at various time intervals. The data was then entered and analysed using two-way ANOVA using a GraphPad Prism 7.0®.

Ethical Considerations

Ethical approval was sought from Institutional Review Board, School of Health Sciences (IRB No: MAKSHSREF2016-005). For the research animals, reduction was by using improved experimental techniques such as the STZ/ nicotinamide model, using improved techniques of data analysis and sharing information with other researchers. Refinement was achieved by using less invasive techniques in order to reduce suffering. The animals were housed in appropriate structures and fed daily so as to ensure good living conditions.

RESULTS

Extraction yield

The extraction yields were 7.68% for *Vernonia amygdalina* and 6.27% for *Musa paradisiaca*.

Phytochemical profiles

Table 2: Results of phytochemical profiles of *Musa paradisiaca* and *Vernonia amygdalina*

Phytochemical	Quantitative Abundance	
	Vernonia amygdalina	*Musa paradisiaca*
Saponins	+++	+
Phenols	+++	+++
Tannins	+	++
Flavonoids	++	+
Terpenes	+	+
Glycosides	++	++

(+) present in low amounts, (++) present in moderate amounts, (+++) present in large amount

Induction of Diabetes

Diabetes was successfully induced in all the rats

Efficacy of the syrups

Oral glucose tolerance

Generally there was a decrease in blood glucose levels after 2 hours in all the groups. Group 1 and group 2 that were given the syrup base (negative control) showed no significant change ($p>0.05$) in blood glucose levels.
At 30 minutes: All the groups showed an initial rise in the blood glucose levels. The rise was very significant ($p<0.0001$) in groups 2-9 but wasn't significant ($p>0.05$) in group 1 (non-diabetic rats).
At 60 minutes: Groups 3-9 showed a very significant decrease ($p<0.001$)) in blood glucose levels. There was a significant difference ($p<0.001$) in decrease in blood glucose levels in group 9 (positive control) compared to that of groups 3-8.

Table 3: Induction of diabetes mellitus

Status	Fasting Blood Glucose (mg/dl) Mean ± SD							
	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Before induction	105.7 ± 2.5	102±6.6	101±5.3	104±2.6	98±7.8	98.7±0.6	102±5.0	83.3±5.1
After induction	381.3±22.1	377.7±25.4	366.3±33.5	365.3±27.3	382.3±28	349.3±37.5	365±23.8	333±32.6

Table 4: Oral glucose tolerance

Group	Fasting Blood Glucose (mg/dl) Mean±SD			
	0 minutes	30 minutes	60 minutes	120 minutes
Group 1	92.7±5.5	112±7.2	95.3±4.2	94.3±5 ***
Group 2	381.3±22.1	437±16.7	408±15.4	400±18***
Group 3	377.7±25.4	422.7±20.6	359.7±24.6	340±26.1*
Group 4	366.3±33.5	396.7±36.7	366±30.1	342.7±31.1*
Group 5	365.3±27.3	400±26.9	345.7±19.1	306±24.9
Group 6	365.3±27.3	428±27.4	377.7±22.5	334.7±30.9*
Group 7	349.3±37.5	379±30.8	350.3±36.5	338.7±37.5*
Group 8	365±23.8	407±20.8	369.3±19.3	337±23.3*
Group 9	333±32.6	386±30.4	284.7±33.6	272±34.4

*p<0.05, ***p<0.001 statistical significance compared to the group 9 (positive control)

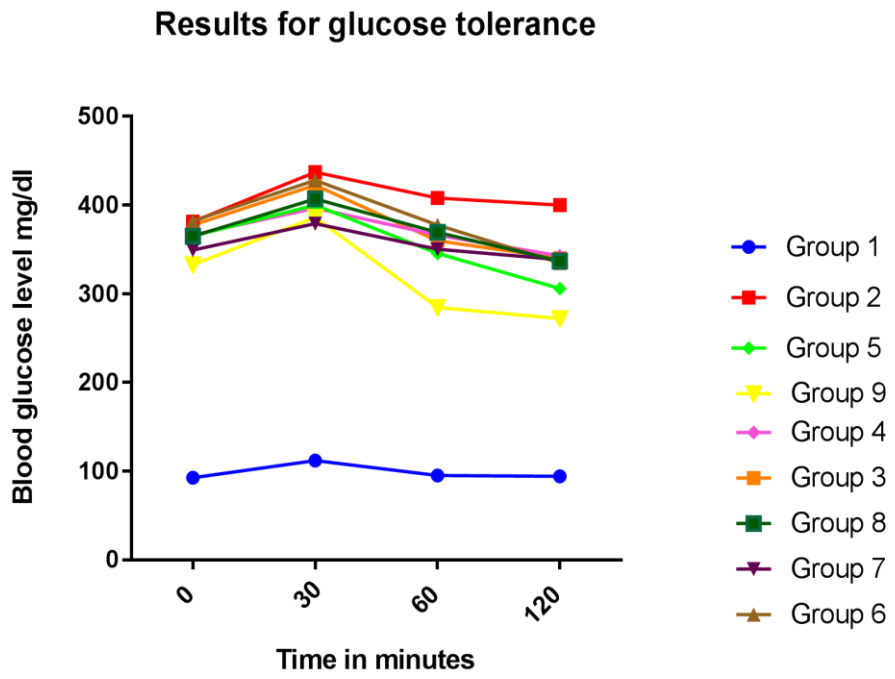

Figure 1: Glucose tolerance

Group 9 (positive Control) showed the highest percentage decrease in blood glucose levels. However, unlike other groups (2, 3, 4, 6, 7 and 8), there was no significant difference (p>0.05) in the decrease in group 9 compared to that of group 5 (with highest composition of both extracts)

Table 5: Oral acute toxicity of the formulated syrups

Parameters	Observations	
	V.A100mg/kg:M.P200mg/kg	V.A200mg/kg:M.P400mg/kg
Respiratory distress	Absent	Absent
Diarrhoea	Absent	Absent
Changes in fur	Absent	Absent
Changes in behaviour	Absent	Absent
Change in mucus membranes	Absent	Absent

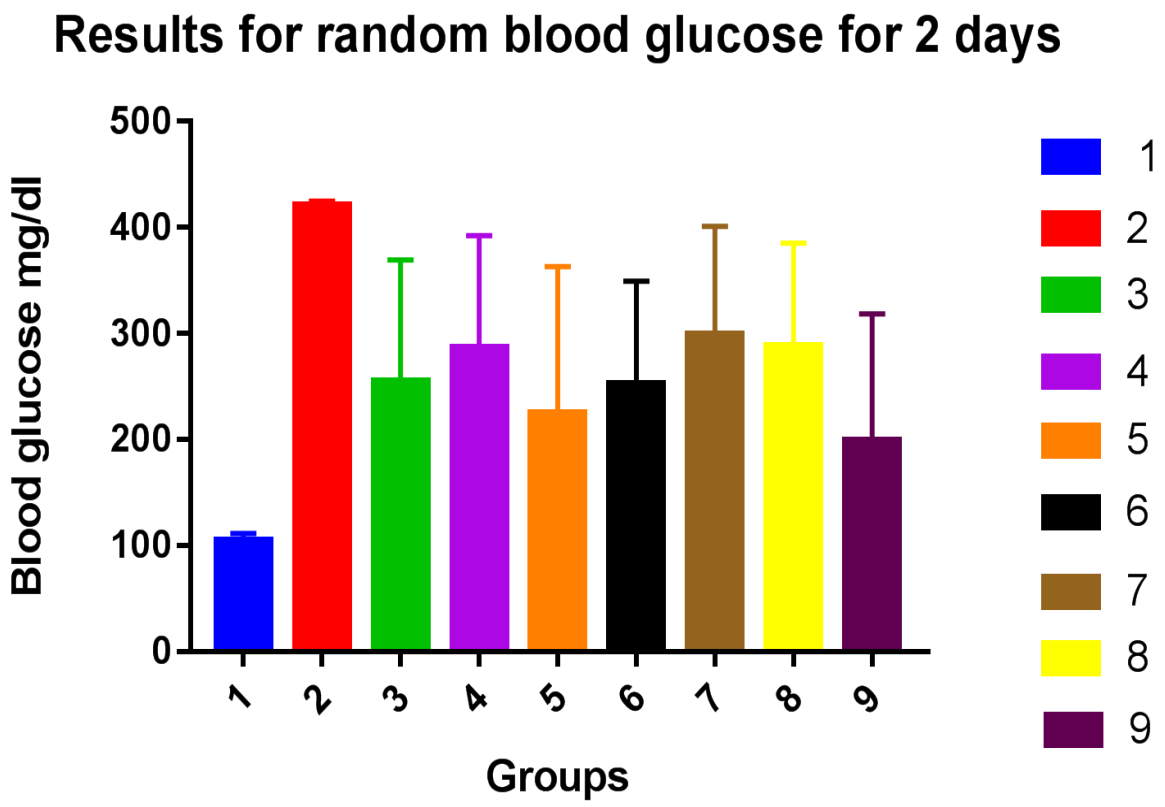

Figure 2: Random blood glucose levels in the different rats.

Acute Toxicity

There were no signs of toxicity observed for the combined extracts both at the reported most efficacious doses of each (i.e. 100mg/kg bwt *VA* and 200mg/kg bwt *MP*) and at double these doses

Syrup properties

Formulation of the most efficacious syrup

Table 6: Formula for the most efficacious syrup

INGREDIENT	AMOUNT
Vernonia amygdalina extract	1.2 g
Musa paradisiaca extract	2.4 g
HPMC	6 g
Sodium benzoate	240mg
Benzoic acid	120mg
Aspartame	600mg
Citric acid (19.21g/l)	70.6ml
Dibasic sodium phosphate (35.6g/l)	49.4ml
Vanilla flavour	2ml
Total formulation volume	100ml

Physical chemical profile of the syrup

The herbal syrup was homogeneous, pale brown, with a pH of 4.27 and having a vanilla flavor.

Random blood glucose

After two days, there was no significant difference ($p>0.05$) in the blood glucose levels of groups 3, 5, 6 and 9 (positive control) However, there was a significant difference ($p<0.05$) in the blood glucose levels of the rest of the groups (2, 4, 7 and 8) compared to that of group 9.

DISCUSSION AND CONCLUSIONS

Phytochemical screening

The results of phytochemical screening of both plants correlate well with literature. *Vernonia amygdalina* leaf extract showed presence of saponins, tannins, flavonoids, terpenes, alkaloids and glycosides as reported by other researchers [13, 26]. Phytochemical screening of *Musa paradisiaca,similar to reports from other studies,* also revealed presence of flavonoids, alkaloids, tannins, saponins, terpenes and glycosides [16, 27].

Efficacy testing

Oral glucose tolerance

The hyperglycemic state of the rats at 0 minutes confirmed that diabetes mellitus was successfully induced. The initial rise in blood glucose levels at 30 minutes is attributed to the glucose challenge that was given and the delay in onset of activity of glibenclamide and the herbal extracts. Between 30-60 minutes there was significant difference ($p<0.05$) in the reduction in blood glucose levels ($p<0.05$) in the group administered with glibenclamide compared to the other groups. This may be attributed to the delayed manifestation of the effects of the *Vernonia amygdalina* and *Musa paradisiaca* extracts. After 2 hours, the effect of combination of the extract in a ratio of 1:1 was comparable to that of glibenclamide ($p>0.05$). This extracts combination was at the doses of 100mg/kg bw and 200mg/kg bw for *Vernonia amygdalina and Musa paradisiaca respectively that are reported to have the best hypoglycemic effect [6, 14]*.

Comparison of the syrups with individual extracts (0, 30, 60 and 120 mins after glucose meal) showed that the hypoglyceamic effect of *Vernonia amygdalina* alone (Group 3) was greater than that of *Musa paradisiaca* alone (group 4. More so, as the *concentration of Vernonia amygdalina* decreased in the syrup combinations, the hypoglycemic effect also reduced; with the highest effect being observed in combination that had 100mg/kg bw (Group 5) and the lowest being that with 25mg/kg bw (group 7) (see table 4). This could also imply that the effect of *Vernonia amygdalina is* dose dependent.

All the syrups with the different combinations of both extracts (Group 5, 6, 7 and 8) showed superior hypoglycemic effect as compared with the syrups with *Vernonia amygdalina* or *Musa paradisiaca* alone *(see fig1 and table4).* This suggests a possibility that the interaction between the two extracts is synergistic.

Random blood glucose

There was a maintained decrease in blood glucose levels after 2 days. The combination in a ratio of 1:1 showed the greatest decrease in blood glucose levels compared to the other syrups with extracts. Though in this study, Random blood sugar testing was done once (after two days), *Vernonia amygdalina at doses of 200mg, 300mg and 400mg/kg bw has been reported to maintain lower Fasting Blood glucose in diabetic rats even after 14-28 days* [28].

The anti-hyperglycemic effects of *Musa paradisiaca* have been attributed to the anti-oxidant properties of preserving the pancreatic β cells. The flower extracts are reported as having insulin stimulatory properties with the mechanism of action thought to be similar to that of glibenclamide. The active principle responsible for the hypoglycemic activity is a glucoside, known as syringing [6, 16].

Stability profile of the syrup

Table 7: Stability profile

Syrup Sample code	Time (Hrs)	Temp (^{o} C)	Physicochemical properties			
			Colour	pH	Odour	Homogeneity
A	24	4	No change	No change	No change	No change
B		25	No change	No change	No change	No change
C		60	No change	No change	No change	No change
A	48	4	No change	No change	No change	No change
B		25	No change	No change	No change	No change
C		60	No change	No change	No change	No change
A	72	4	No change	No change	No change	No change
B		25	No change	No change	No change	No change
C		60	No change	No change	No change	No change

The polyphenols from *Vernonia amygdalina* leaf extracts are reported to inhibit alpha amylase (though not important in this case) and decrease glucose uptake through the action of the sodium glucose (S-GLUT) transporter at the intestinal brush border [29].It was also suggested that they increase insulin production and peripheral glucose uptake [30, 31]. *Vernonia amygdalina* doesn't affect glycolysis but rather is postulated to act through repressing glyconeogenesis and potentiation of glucose oxidation through the Pentose Phosphate pathway[28]. The bitter principle was also proposed to be responsible for the insulin production, while tannins, flavonoids and glycosides could act as alpha glucosidase inhibitors[32]. The different postulated mechanisms of action of the extracts may be responsible for the better activity of the combinations as compared to that of the individual extracts.

Toxicity

Due to limited resources, we couldn't perform extensive biochemical or histological toxicity tests. Nevertheless, the tests we carried out showed no acute toxicity signs. Besides, past studies on toxicities of *Vernonia amygdalina [13, 33]* and *Musa paradisiaca* [6, 21] individual extracts reported no acute or chronic toxicities at doses far higher those used in this study. However, high doses of Vernonia amygdalina have been shown to affect haemopoesis [13, 34]. Both plants have been shown to exhibit cell healing and protective properties. *Vernonia amygdalina* reversed streptozocin induced hepato-toxicity [13], has antioxidant properties [34], and is superior in lowering body weight as compared to Metformin[28]. The same protective abilities have been observed with *Musa paradisiaca* [21, 35] .

CONCLUSION

The study showed that the combination of the syrup containing *Vernonia amygdalina* 100mg/kg *and Musa paradisiaca* 200mg/kg exhibits a synergistic anti-hyperglycemic could be clinically significant since it was comparable to hypoglycemic effect of glibenclamide. The combination of the extracts is also safe at the doses double those reported as efficacious and for the period of the study. More so, the aspartame based syrup base doesn't alter the hypoglecemic potential of the two extracts.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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