Acute Toxicity and Hypoglycemic Effect of a Polyherbal Formulation on Blood Glucose in Oral Glucose Tolerance Test (OGTT) and Alloxan-Induced Diabetic Rats

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Abstract

Madam F. Kayes Bitters® is an herbal formulation commonly used in Nigeria and some African countries in the management of diabetes mellitus and other diseases conditions. This study evaluated the in-vivo hypoglycaemic activity, as well as acute toxicity of the polyherbal formulation to provide its efficacy and safety. Healthy albino mice (20-30 g) and Sprague Dawley female rats (90-130 g) were used for this study. Acute toxicity study (LD_{50}) of the herbal formulation was determined by methods originally described by Miller and Tainter in 1994. Following oral dosing with glucose (2 g/kg) in normal fasted animals, herbal formulation (HF) at various doses was administered and blood glucose levels at 30 minutes, 60 minutes, 90 minutes, and 120 minutes were taken and recorded. Diabetes was induced using alloxan 150 mg/kg and diabetic rats were given the HF at doses of 50, 100, and 200 mg/kg with glibenclamide 2.5 mg/kg used as standard drug treatment. Blood glucose level was determined on 1st day, 7th day, 14th day and 21st day. The LD_{50} was greater than 5g/kg with oral administration. The oral glucose tolerance test showed that the group that received 100 mg/kg HF showed a significant reduction ($p<0.05$) in glucose level after 120 minutes when compared to the basal level of glucose recorded. All treated diabetic groups showed a significant decrease in glucose level on the 21st day. The herbal formulation of *Hydrastis canadesis Aloe capensis, Echinacea angustifolia* and honey exhibited a significant glucose-lowering activity in alloxan-induced diabetic rats.

Keywords: alloxan monohydrate; diabetes mellitus; herbal formulation; LD50 value.

INTRODUCTION

Diabetes mellitus (DM, diabetes) is a chronic disease characterized by persistent elevation of blood glucose level of an individual which resulting from defects in insulin secretion, insulin action, or both (ADA, 2009). This disease is also marked by altered lipids, carbohydrates and protein metabolism (Ozougwu et al., 2013; Ezuruike and Prieto, 2014). The hyperglycaemic condition takes place due to the inappropriate secretion of insulin hormone or the inappropriate use of insulin hormone by the body itself. Insulin hormone is secreted by the Islet of Langerhans located in the beta cells of pancreas and it helps to maintain the glucose level in the blood (Xavier, 2018). Besides the storage of glucose, insulin also inhibits the secretion of glucagon and lowers the concentration of serum fatty acids leading to a decline in liver glucose production (Asmat et al., 2016). Insufficient insulin or resistance to insulin in the body results in reduced tissue uptake of glucose that results in intracellular hypoglycemia and extracellular hyperglycemia. The intracellular hypoglycemia causes glucogenesis and gluconeogenesis that leads to fats breakdown (causing diabetic ketoacidosis) and decreases protein synthesis and gamma globulins (causing cachexia, polyphagia, and impaired wound healing), while the extracellular hyperglycemia leads to hyperglycemic coma and osmotic dieresis (Ozougwu et al., 2013). Based on the requirements of insulin, diabetes is classified into insulin-dependent diabetes mellitus (Type 1), and non-insulin-dependent diabetes mellitus (Type 2) (ADA, 2009).

Madam F. Kayes Bitters® is an herbal formulation commonly used in Nigeria and some African countries in the management of diabetes mellitus and other diseases conditions. This formulation is listed to be composed of *Aloe capensis, Hydrastis canadesis, Echinacea angustifolia* and honey. In spite of significant advancements in the development of conventional drugs, herbal medicines remain useful in the management of diseases, especially in developing countries (Ekor, 2014; Erhirhie et al., 2015; Moke et al., 2021). This may be due to the adverse effects and high cost of therapy

associated with conventional drugs (Gurib-Fakim, 2006; Ekor, 2014).

Medicinal plants are plants whose parts contain substances of therapeutic important which can be constituted into drug and used for treatment of various disease (Sofowora et al., 2013). Many conventional drugs such as quinine, aspirin, digoxin, amongst others, are of plant origins (Vickers et al., 2001; Veeresham, 2013). Presently, there is an urgent need to develop safer drugs for the management of chronic diseases such as diabetes mellitus. Consequently, assessment of various medicinal plants used in traditional systems have grown (Lahlou, 2013; Sofowora et al., 2013; Ekor, 2014, Anachuna et al., 2018; Okafo et al., 2019; Moke et al., 2020). However, high cost of production is a factor, developmental processes for newer drugs is a way of evaluating of efficacy and adverse effects of chemical substances. The present study aimed to evaluate the in-vivo hypoglycaemic activity, as well as acute toxicity of the polyherbal formula extracts (Madam F. Kayes Bitters®) on experimental animals.

MATERIAL AND METHODS

Herbal Formulation
The herbal formulation used was purchased from a local pharmacy. It was stored in a cool dry place during the period of the study.

Experimental Animals
Healthy albino mice (20-30 g) and Sprague Dawley female rats (90-130 g) were used for this study. These were procured from the Animal House of the College of Medicine, University of Lagos, Nigeria. The animals were kept in polypropylene cages with wire mesh for proper ventilation throughout the study. The animals were given free access to standard diet (Lifestock Feed Plc, Ikeja, Nigeria) and water *ad libitum*. The animals were kept in room temperature with a 12:12 day and night cycle and maintained at temperature of 27 °C. The animals were allowed to acclimatize for two weeks before commencing the study. The maintenance and treatment of the animals was according to the principles of the guide for care and use of laboratory animals in research and teaching prepared by the National Academy of Science and Published by the National Institute of Health (NIH) publication 86-23 revised in 1985.

Acute Toxicity Study
Acute toxicity study of the herbal formulation was determined by methods originally described by Miller and Tainter of 1994 (Erhirhie et al., 2018). Mice (20-25 g) were fasted for 12 hours and divided into four groups of 5 mice each. In order to determine the LD_{50} of the formulation, different doses 1000 mg/kg, 2500 mg/kg and 5000 mg/kg were administered orally to different groups of mice while the control group received 10 ml/kg distilled water (pH = 6.9). All animals were closely observed for symptoms of toxicity and the mortality noted. Based on data obtained, the LD_{50} was determined.

Oral Glucose Tolerance Test (OGTT)
Fifteen adult female rats (100-126 g) were fasted overnight. The animals were divided into five different groups with three animals each. Blood samples were obtained from each rat by gently nipping the tail with a lancet, and then gently squeeze the tail to let out 2 drops of fresh venous whole blood on the glucometer strip inserted and the readings recorded for the 0 minute. Different doses of the herbal formulation were orally administered. Glucose (2 g/kg) was orally administered 30 minutes after extract administration to each animal according to their body weight. Blood glucose level at 30 minutes, 60 minutes, 90 minutes, and 120 minutes were taken and recorded.

Alloxan-Induced Diabetic Study
Eighteen healthy female rats were randomly divided into 6 groups of 3 animals each. The rats were fasted for 24 hours after which group I rats were given 10 ml/kg normal saline while groups II-VI were made hyperglycemic with a single intraperitoneal injection of 150 mg/kg of alloxan monohydrate (Sigma Chemicals Company, St. Louis, Mo., U.S.A) (Gbolade et al., 2008; Moke et al., 2015). The baseline fasting blood glucose was determined prior to the administration of alloxan. After 48 hours, blood was collected from the tail vein of each rat and the fasting blood glucose level determined. A blood glucose ≥ 200 mg/dl was considered diabetic. Animals were further tested after 72 hours and animals with stable hyperglycemia were selected for the study. The treatment schedule of the rats is as follows:
- Group I (Control) – Normal Saline 10 ml/kg
- Group II (diabetic and untreated) – Normal Saline 10 ml/kg
- Group III – Herbal Formulation 50 mg/kg
- Group IV – Herbal Formulation 100 mg/kg
- Group V – Herbal Formulation 200 mg/kg
- Group VI – Glibenclamide 2.5 mg/kg

Determination of Fasting Blood Glucose in Diabetic Rats
The blood glucose level was determined using the tail tipping method after 12-16 hours of fast. The tail was gently squeezed to let out 2-3 drops of blood which is placed on the test spot of the glucose strip after which the test strip was gently inserted into the digital glucometer. Blood glucose level was determined on 1st day, 7th day, 14th and 21st day.

Data Analysis
Data were presented as mean ± standard error of mean (SEM) using Graph Pad Prism. Test of statistical

significance was carried out using a one-way ANOVA. P-values lesser than 0.05 (p<0.05) were considered statistically significant.

RESULTS AND DISCUSSION

Acute Toxicity Test of Herbal Formulation
When the herbal formulation was administered orally, there was no death of experimental animals even at a dose of 5g/kg. Hence, for the oral route, the LD_{50} is greater than 5g/kg. (Table 1)

Table 1. Oral acute toxicity of Herbal Formulation.

Group/Dose (mg/kg)	Number of Mice	Number of Death
Control	5	Nil
1000	5	Nil
2500	5	Nil
5000	5	Nil

$LD50_{oral} > 5000mg/kg$

Body Weight of Animals
There was no statistically significant change in body weights of the animals (p>0.05) when compared to control as shown in Table 2.

Table 2. Herbal Formulation treatment on body weights.

Treatment	Day 1 (g)	Day 7 (g)	Day 14 (g)	Day 21 (g)
Control (NS)	103.70±4.91	105.00±5.29	107.3±3.84	115.30±2.60
Untreated (NS)	106.70±4.49	105.00±3.06	99.67±2.60	97.00±3.22
GLB 2.5 mg/kg	100.70±6.36	101.00±7.21	99.67±2.60	68.83±28.67
50 mg HF	107.00±6.56	106.00±5.69	103.70±3.48	105.0±2.52
100 mg HF	107.00±6.08	104.30±6.94	105.00±3.61	108.00±2.31
200 mg HF	105.00±5.57	105.70±6.84	105.30±7.42	113.30±16.05

Values are expressed as mean ± SEM (n=3). p<0.05 statistically significant when day 1 is compared to other respective groups using one way-ANOVA followed by Tukey's post hoc multiple comparison tests. Key: NS = Normal Saline; GLB = Glibenclamide; HF =Herbal Formulation

Oral Glucose Tolerance Test of Herbal Formulation.
The oral glucose tolerance test showed a sharp decrease in the glucose level after 30 minutes when compared to the level at 0 minute. This was followed by a gradual reduction in glucose level after 60, 90 and 120 minutes. The control group showed a statistically significant reduction in glucose level at the 90th minute (p<0.01) when compared with the basal level recorded (78.33±0.88). Glibenclamide showed a significant reduction (p<0.01) in glucose level after 120 minutes (26±13.01). Also the group that received 100 mg/kg HF showed a significant reduction (p<0.05) in glucose level after 120 minutes (39.67±2.33) when compared to the basal level of glucose recorded. (Table 3)

Table 3. Oral Glucose Tolerance Test (OGTT) of Herbal Formulation.

Treatment	Basal (mg/dL)	0 minute (mg/dL)	30 minutes (mg/dL)	60 minutes (mg/dL)	90 minutes (mg/dL)	120 minutes (mg/dL)
Control (NS)	85.33±1.86	104.33±6.57	91.67±5.90	77.33±1.86	87.54±0.88	90.67±2.96
GLB 2.5 mg/kg	90.33±7.86	106.67±6.77	87.67±12.25	66.00±10.82	43.67±6.64	26.00±13.01**
50 mg HF	83.33±6.23	104.67±4.41	85.33±9.70	52.67±12.03	67.67±6.94	54.00±13.58
100 mg HF	87.00±5.86	114.00±10.60	88.33±7.84	60.00±5.29	61.00±4.58	39.67±2.33*
200 mg HF	82.67±4.48	116.67±8.84	110.33±8.29	74.33±5.36	71.00±1.53	63.67±6.36

*Values are mean ±SEM (N=3): *p<0.05, **P<0.01 statistically significant compared to basal within group with other respective minutes using one way-ANOVA followed by Tukey's post hoc multiple comparison tests. Key: NS = Normal Saline; GLB = Glibenclamide; HF = Herbal formulation*

Blood Glucose Level of alloxan-induced Diabetic Animals
The 21 days treatment of alloxan-induced diabetic rats with herbal formulation resulted in statistically significant reduction in blood glucose (Table 4). There was a statistically significant difference between all treated groups and the untreated diabetic group when compared with the non-diabetic control indicating initial hyperglycemic condition upon induction of diabetes.

On the 7th day, there was a statistical significant difference in glucose level in the untreated group (p<0.05) and groups administered glibenclamide (p<0.01) and 200 mg/kg HF (p<0.001) compared to non-diabetic control. On the 14th day, there was no statistically significant difference in all the treatment groups when compared to both diabetic untreated group and non-diabetic control group. However, the untreated control showed a significant difference (p<0.01) in

glucose level when compared with non-diabetic control. All treated groups showed a significant decrease in glucose level on the 21st day. The p values were as follows: glibenclamide ($p < 0.001$), 50 mg/kg HF ($p < 0.001$), 100 mg/kg HF ($p < 0.001$) and 200 mg/kg HF ($p < 0.01$).

Table 4. Blood glucose level of alloxan-induced diabetic animals.

Treatment	Baseline (mg/dL)	Day 1 (mg/dL)	Day 7 (mg/dL)	Day 14 (mg/dL)	Day 21 (mg/dL)
Control (NS)	53.33±7.31	54.67±7.31	59.33±8.29	48.33±1.20	55.67±5.90
Untreated (NS)	66.67±11.79	307.70±56.69*	335.30±51.27*	338.30±49.97**	360.00±55.08****
GLB 2.5 mg/kg	52.00±1.53	386.70±51.57***	207.00±67.35**	126.00±62.98	78.67±18.49[b]
50 mg HF	78.67±7.75	336.00 ±64.29*	289.30±35.97	116.00±52.81	83.33±13.42[b]
100 mg HF	67.00±16.50	383.30±52.03**	260.00±60.65	124.70±11.05	55.00±4.73[b]
200 mg HF	72.33±22.72	399.67±25.11**	284.00±44.23**	192.33±39.07	106.70±8.74[a]

Values are mean ±SEM (N=3): *p<0.05, **P<0.01, ***P<0.001, ****p<0.0001 versus control; [a]P<0.001, [b]P<0.0001 versus untreated control. Analysis by one way ANOVA followed by Tukey's post hoc multiple comparison tests. Key: NS = Normal Saline; GLB = Glibenclamide; HF = Herbal formulation

Over centuries, herbal products of medicinal value have been recruited in traditional medicine for the management and treatment of several ailments including diabetes mellitus (Jung et al., 2006). Although there have been a significant advancement in development of conventional drugs in recent times, herbal remedies are still used especially in developing countries in the treatment of diseases (Ekor, 2014; Moke et al., 2021). Numerous medicinal plants are widely used in Nigeria to manage diabetes mellitus (Abo et al., 2008; Ezuruike and Prieto, 2014; Moke et al., 2015; Abubakar et al., 2017; Okafo et al., 2019). The crude extracts of these plants may be used alone or mixed with extracts from other plants or other sources. Madam F. Kayes Bitters® is a poly-herbal formulation of *Aloe capensis, Hydrastis canadesis and Echinacea angustifolia* plus honey.

Diabetes mellitus is characterized by persistent elevations of fasting blood glucose above 200 mg/dl due to insufficient or complete cessation of insulin synthesis or secretion and/or peripheral resistance to insulin action (Adeneye and Agbaje, 2008; Kitabchi et al., 2009). In this study, acute toxicity test revealed that the polyherbal formulation is quite safe for oral consumption. The oral glucose tolerance test (OGTT) showed a gradual reduction in the glucose level from 0 minute to 120 minutes. This decrease was found to be significant for the groups administered the standard treatment (glibenclamide) and HF 100mg/kg which suggests that the herbal formulation has a glucose lowering effect. Following 21 days treatment of alloxan-induced diabetic rats with the herbal formulation, there was a significant reduction in blood glucose level in all groups receiving treatment compared to the untreated diabetic control. The reduction in glucose level was gradual in all groups with glucose level brought to below 150 mg/dL on the 14th day with the exception of the group receiving 200mg/kg. The hypoglycemic effect was more profound in the group receiving 100 mg/kg than that of 50 mg/kg and 200 mg/kg which suggests that the hypoglycemic effect is not dose-dependent.

The result obtained is consistent with earlier reports in scientific literature demonstrating the hypoglycemic effect of *Aloe barbadensis*/*Aloe vera* (used synonymously with *Aloe carpensis*) in alloxan-induced diabetic rats (Nwanjo, 2006; Choudhary et al., 2014; Pothuraju et al., 2016; Hammeso et al., 2019). Also honey has been shown to exert a dose-dependent hypoglycemic effect in diabetic rats (Erejuwa, 2014). Honey is sweet and rich in sugars. It is therefore surprising that it has a hypoglycemic effect. It has been hypothesized that fructose and oligosaccharides present in honey may in some way contribute to its observed hypoglycemic effect (Erejuwa et al., 2012). Hence, the hypoglycemic effect of the herbal formulation investigated is consisted with scientific reports on the constituents.

CONCLUSION

The polyherbal formulation of *Hydrastis canadesis Aloe capensis, Echinacea angustifolia* and honey exhibited a significant glucose-lowering activity in alloxan-induced diabetic rats. This suggests that this formulation may be useful for the management of diabetes mellitus. This study therefore corroborates the claim that this herbal formulation is effective in managing diabetes mellitus.

Conflict of Interest: The authors declare that there are no conflicts of interest concerning the publication of this article.

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