Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.5, No.9, 2015

Hypoglycemic Effect of Ethanoic Extract of a Herbal Drugs Formulation Used by Kenyan Herbalists

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Abstract
Diabetes is a condition that is characterized by high blood sugar levels in the human body due to lack of insulin or its insufficient production. The causes are known to be genetic or occupational factors. Conventional diabetes drugs developed lower and manage the blood sugar levels. However, due to their side effects and costs, the drugs have made management of the disease be expensive. Herbal drugs, on the other hand, have been formulated as suitable alternatives. One herbal drug (Diabetic formular®) was obtained from a market in Nairobi, and its ethanolic extract was analysed for anti-diabetic properties and available phytochemicals. Swiss albino rats were used as the diabetic model in the study. The rats were induced diabetes using alloxan. Thereafter, Fasting Blood Glucose (FBG), was carried out to confirm if the rats were induced with diabetes. Rats induced with diabetes were then grouped into five groups, (n=5), corresponding to the following treatments: placebo (distilled water), conventional drug (Metformin, 500 mg/Kg) and three doses of the herbal drug extracts (500 mg/Kg, 1000 mg/Kg and 1750 mg/Kg). Oral glucose tolerance test (OGTT) carried out involved administering glucose (2 g/Kg) followed by the drugs to the various groups, after 30 minutes. This was then followed by monitoring the decrease in blood glucose, in intervals, for a period of 3 hours. The highest dose of the extract lowered the blood glucose level from a peak 373.5 ± 13.6 mg/dL to 167.4 ± 11.4 mg/d. The decrease was found to be comparable to the conventional drug, which lowered the blood glucose levels from the peak 344.7 ± 11.5 mg/dL to 144.0 ± 78.9 mg/dL. The results from phytochemical screening revealed the presence of Saponins, Flavonoids and Tannins.
Keywords: Herbal drug, Antidiabetic activity, Ethanolic extract, Blood glucose, Phytochemical screening.

INTRODUCTION

Diabetes has become an important chronic metabolic disease in the world, and especially in developing countries. It comprises a collection of heterogeneous diseases differing in their etiological, clinical and epidemiological characteristics, but have hyperglycemia and glucose intolerance in concurrence. This is attributed to either insulin deficiency or impaired effectiveness, or a combination of both.[1] Diabetes mellitus ranks highly among the top ten disorders which cause mortality and lower the life expectancy throughout the world[2]. Its complications have been shown mainly to come from hyperglycemic effects. These complications include: heart disease, Retinopathy, Neuropathy, Nephropathy Macroangiopathy and Microangiopathy.[2, 3]Ideal oral treatment and management of diabetes would be a drug that controls the glycemic level, as well as preventing the development of complications of diabetes[3]. Over the years, insulin and oral hypoglycemic have been used to keep the sugar levels down to manageable levels. However, side effects and the cost of long term treatment of this chronic disease have been hindrance to their use. Present antidiabetic agents possess side effects such as risk of hypoglycemia, anemia and choestatic jaundice[2]. This has led people to turn to herbal drugs for the management of the disease. Most of the herbal drugs that treat diabetes have been derived from medicinal plants that have been documented to treat diabetes in the past. Documentation on the use of plants for antidiabetic activity has been present since ages. Research on the use of plants in treatment of diabetes has been done. However, the challenge on the mechanism of operation of the active ingredients, whether additive or synergistic, remains to be of interest. Diabetic formula® is a herbal drug formulation from various medicinal plants. They include: *Gymnema sylvestre, Cinamon, Momordica foetida, Moringa oliefera, Azandracta indica* and *Utica masaica*. Most of these plants have been known to be active hypoglycemic, but active principles in most of these plants are little known[4 5]. Challenges of herbal formulations remain to be efficacy and quality due to changes in the phytochemicals. The variations come from the environmental factors and their mode of preparation[5]. The aim of this work was to evaluate the efficacy and to screen phytochemicals present in the formulation and to verify the claims of treatment and origin.

METHODOLOGY

Collection and preparation of plant material

Packed formulation of Diabetic formula® was bought from commercial herbalists in Nairobi. Ethanol was used

as extraction agents of the dry herbal drugs. The solution was then pre-concentrated and the solvent was liberated using a rotary evaporator at 50 ^{0}C. The extract was the kept in a refrigerator at 4 ^{0}C.

Experimental animals.

The study comprised of female rats, eight weeks old, obtained from Kenyatta University's zoology department. They were kept for two weeks to acclimatize at the animal unit of medical sciences, Jomo Kenyatta University of Agriculture and Technology. During this period, the rats were kept and were given standard laboratory diet and water *ad libitum.* Twelve 12 hour Light- dark cycle was allowed during the period of the experiment.

Experimental design.

Toxicity study

Toxicity study was done following the OECD 425 guidelines[6]. This study required a single rat be administered a starting dose of 175 mg/Kg of the ethanolic extract, in 5 % DMSO, and monitored for 48 hrs for any toxic signs, or mortality before moving to the subsequent dose. The dose followed a logarithmic progression of various set doses from which the LD 50 was to be estimated.

Antidiabetic activity.

Rats were divided into two major groups according to the treatment. One group comprised of 5 rats that were not induced diabetes, and the other groups which were induced diabetes. The groups induced diabetes were further divided according to the various treatment to be administered:-

Those induced, but not distilled water as the placebo (group B), negative control for rats induced diabetes.
Those that were treated with the conventional drug (group C), positive control.
Those that were treated with the herbal drugs at various concentrations 500 mg/Kg, group D
Those that were treated with the herbal drugs at various concentrations 1000 mg/Kg, group E
Those that were treated with the herbal drugs at various concentrations 1750 mg/Kg, groups F

Analysis of blood glucose

The blood was withdrawn from the tail vein of rats. Fasting blood glucose and the Oral tolerance glucose tests were performed by glucose oxidation method using a glucometer (prodigy® pocket glucometer).

Phytochemical Screening

Standard method for phytochemical screening were carried out to determine the presence of Alkaloids, saponins, flavonoids, triterpenoids, tannins and reducing sugars[5,7,8,9]

Alkaloids were determined as follows: 200 mg of herbal drug was boiled in 20 ml of 1% H_2SO_4 in 50% ethanol. The mixture was then filtered. To the filtrate, 5 drops of concentrated NH_4OH and 20 ml chloroform were added and later extracted to 20 ml dilute H_2SO_4. To the extract, 5 drops of Mayer's/Dragendorff's reagents were added. The solution was observed for the formation of a white precipitate with Mayer's reagent and an orange precipitate with Dragendorff's reagent to indicate the presence of alkaloids.

Frothing test was carried out to determine the presence of saponins as follows: a sample of the formulation was extracted with water and filtered. To 0.5 ml of the filtrate, 5 ml distilled water was added, and the mixture shaken for 30 s to observe persistent frothing.

Flavonoids were determined by Shinoda's Test as follows: a sample of the herbal drug (200 mg) was added to 5 ml ethanol the mixture then shaken for 2 minutes and filtered. To 1 ml of the filtrate, magnesium ribbon and concentrated hydrochloric acid (HCl) was added. The solution was observed for the presence of a pink colour to indicate the presence of flavonoids.

Liebermann - Burchard's Test was carried out to determine the presence of Terpenes/steroids as follows: 10 ml chloroform was added to 200 mg of sample in a test tube then filtered after 2 minutes. To the 2 ml filtrate, 2 ml acetic anhydride was added followed by 1 ml of conc. Sulphuric acid (H_2SO_4). A blue solution was observed to indicate the presence of steroidal saponins.

Tannins were determined by the following procedure: a small sample of the formulation was extracted with water at 40 ºC and filtered. To 2 ml of the filtrate, 1 ml $FeCl_3$ was added and, the presence of a blue-black or greenish-black precipitate was observed to indicate the presence of tannins.

Reducing sugars were determined by the following procedure: 0.2 g of each sample was warmed in a test tube with 5 ml of 10% v/v sulphuric acid (H_2SO_4) on a water bath at 100 ºC for 2 minutes. The mixture was then filtered and neutralized with 5% (w/v) solution of sodium hydroxide (NaOH). The volume of the aqueous NaOH added to each test tube was noted, and 0.1 ml of Fehling's solutions A and B was added to each followed by heating of the mixture in a water bath at 100 ºC for 2 minutes. The intensity of the red precipitate formed in each case was observed to represent the amount of reducing sugars.

Statistical analysis

The results obtained were expressed as means ± sd. Significance test was carried out on in vivo tests using the student t - test. The results were compared to the positive and negative control[10].

RESULTS AND DISCUSION

The rats were confirmed to have been induced diabetes by determining the fasting blood glucose as shown in

table 1. The rats that had blood sugar of above 138.6 mg/Kg were considered to be diabetic [11]. After administering glucose to the rats, the blood sugar rose to the peak from table 1 from which the treatment was taken, and blood glucose was determined subsequently. The results for the decrease in blood glucose were as shown in table 1.

Groups	FBG	0 min	60 min	90 min	120 min	150 min
NEGATIVE NOT INDUCED	76.5 ± 7.63	117.0 ± 2.51	128.7 ± 3.86	135.9 ± 39.55	87.3 ± 39.55	97.2 ± 22.94
INDUCED NEGATIVE	182.7 ± 11.45	374.4 ± 46.43	381.6 ± 48.4	276.3 ± 12.60	243.9 ± 20.75	239.4 ± 10.95
INDUCED POSITIVE	211.5 ± 1.28	344.7 ± 11.54	307.8 ± 7.62	270.9 ± 1.32	185.4 ± 68.17	144.0 ± 78.79
500 mg/Kg	398.7 ± 1.28	290.7 ± 22.78	212.4 ± 20.36	204.3 ± 17.44	160.2 ± 7.64	110.7 ± 29.23
1000 mg/Kg	261 ± 71.28	500.4 ± 10.44	468.9 ± 92.90	351.9 ± 57.37	212.4 ± 33.11	213.3 ± 49.66
1750mg/ Kg	450 ± 73.82	373.5 ± 13.65	219.6 ± 13.75	152.1 ± 59.84	194.4 ± 16.55	167.4 ± 11.40

Table 1: Fasting blood glucose and decrease in blood sugar in intervals of 30 minutes for a period of 2 hrs 30minutes during the experiment

The glucose tolerance tests for the herbal formulation in various concentrations including controls are as presented in Figure 1.

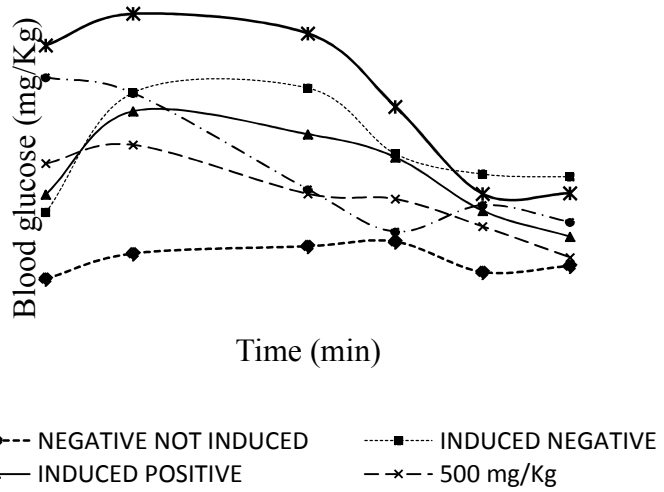

Figure 1: Fasting blood glucose and decrease in blood sugar in intervals of 30 minutes for a period of 2 hrs 30minutes during the experiment

From fig 1, the extract was administered 30 minutes after the commencement of the glucose tolerance test. All the concentration of extracts 500mg/Kg 1000 mg/Kg and 1750mg/Kg decreased the blood glucose level within 2 hours and 30 minutes on administration and monitoring against the control. This decrease showed the activity of the extract from the formulation which was comparable to that of the positive control.

Toxicity data

No effects of toxicity were observed in increasing concentration of the herbal drug from the results in table 2

Table 2: Table showing toxicity data

Test Animal Sequence	ID	Dose (mg/Kg)	Short-term Result	Long- term Result
1	A	175	0	0
2	B	550	0	0
3	C	1750	0	0
4	D	5000	0	0
5	E	5000	0	0
6	F	5000	0	0

(X = Died, O = Survived)

Table 2 shows the results on toxicity from the up and down procedure from the OECD guidelines. From the results, no rat died during the experiment. From such a case, the outcome of the LD 50 was greater than 5000 mg/Kg. Therefore, the ethanolic extract of the herbal formulation was safe for the rats even at higher dose limits. [6]

Table 3: Showing phytochemical constituents of the Diabetic Formula® herbal drug formulation.

Parameters	Results
Alkaloids	-
Saponins	+
Flavonoids	+
Steroids and Triterpenoids	++
Tannins	++
Reducing Sugars	+++

Results from the phytochemical studies showed that the plant contained a lot of reducing sugars Saponins, Tannins and Flavoinoids. Saponins and flavonoids have been shown to be active among the plants that make up the formulation[4]. Blood sugar increase comes as a result of the destruction of insulin secreting β- cells of the pancreas by alloxan, resulting in decreased endogenous insulin release. When glucose is administered to alloxan rats, they become hyperglycaemic in a short period. This is manifested by overproduction of glucose. Alloxan drug has been used, for a long time, to induce non insulin dependent diabetes mellitus in rats, and its mechanism has also been studied [12]. Alloxan is known to be selective against β cells of the pancreatic islet of the Langerhans. The islet of the Langerhans is known to have 5 different types of cells of different functions the β cells are known processes the insulin. Alloxan is known to be selective in destroying the cells by causing degranulation, hydropic degeneration and clumping of the cells. [13] This is observed by cell necrosis.[13,14,15] Generally, various classes of phytochemicals have been linked to lowering the blood sugar levels in animals. These include alkaloids, Flavonoids and Tannins. [9,13,14] Alkaloids have been linked to the regeneration of the B cells of the pancreas. [13] Flavonoids and tannins are known for their antioxidant properties. [17]

Conclusion

From the results of ethanolic extract of the herbal drug formulation (Diabetic formula®) was shown to be active against diabetes and safe from the *in vivo* studies using Rats as the animal model for diabetes. However, further research needs to be done to identify the active ingredients and evaluate their mode of action.

Acknowledgements

We acknowledge the following institutions for their support in the research: National Council of Science and technology for funding and The Medical Laboratory Science department in JKUAT for technical support.

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