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Hypoglycemic effects of selected herbal drug formulations from the Kenyan market

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ABSTRACT

Five herbal drugs were obtained from a market in Nairobi and analyzed for anti-diabetic properties. Swiss albino rats were used as the animal model in the study. Metformin (500mg) was used as the positive control. The rats were induced with diabetes using alloxan. Thereafter, Fasting Blood Glucose (FBG) was carried out to confirm induction of diabetes. Rats induced with diabetes were then grouped into five groups, (n=5), corresponding to treatments with various herbal drugs: Commercial Diabetic formula®, Ganotech® herbal powder, Prunus africana herbal powder, Flax seeds herbal powder and Stevia rebudiana ground leaves powder. Herbal flax seeds at concentrations of 30, 40 and 50% recorded percentage blood glucose reduction of 44.9%, 9.4% and 31.1% respectively compared with the group administered with rat pellets only where the blood sugar increased by 9.5%. The group administered with metformin as positive control showed 75 % reduction of blood sugar. Stevia powder extract concentrations of 20, 30 and 40 % recorded 29.7, 32.7 and 66.7% reduction respectively compared to the group administered with water only which recorded a reduction of 30.3% compared to the group administered with 500 mg/Kg metformin which recorded a reduction of 64.3%. Aqueous and ethanolic extracts of Diabetic Formula® reduced the blood sugar by 56 and 44 % respectively while aqueous and ethanolic extract of Ganotech® herbal powder reduced the blood sugar by 61 and 72% respectively. The aqueous and ethanolic extracts of Prunus africana herbal treatment resulted in reduction of blood sugar by 21.71 and 38.5% respectively compared to the rats treated with the 5% DMSO which reduced by 24.0% and the group treated with 500mg/Kg metformin reduced blood sugar by 53.3%. The herbal drugs used in this study were found to be active against diabetes when compared to the commercial drug Metformin and should be exploited as possible alternative sources of medicine and dietary supplements for diabetes.

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 [1]. Diabetes affects quit a number of populations in the world about 200 million and it is the emerging epidemic in Africa which threatens to overwhelm the health care system [2]. It is one of the non communicable diseases on the rise because of changes in occupation, and lifestyle in Africa affecting 3.2% of the adult population. It is increasingly affecting those in the productive

range 15 to 49 years [3]. Estimates in 2006 showed that 10.8 million people had diabetes in sub-Saharan Africa and the figure is projected to increase by a rate of 80%, exceeding the predicted worldwide increase of 55% and it is expected to double by 2030 [4]. In Kenya, the prevalence of diabetes has been noted at 3.5% however it is believed that it represents small fraction of the prevalence since there are many untreated cases of diabetes [5]. Just like in most African countries since only 15% of those affected are diagnosed [5]. In most cases the clinical diagnosis is made when the person presents to the clinic with complication which are acute or chronic. Several oral medications have been developed to manage the blood sugar levels of the disease. However still there has not been and medication to cure the disease [6]. The burden of the diabetes also affects the population since many people living in endemic areas do not have access to the medications due to low purchasing power [7]. Plants have been a source of novel compound for the drugs against diabetes. In a review by Hussein *et al.* (2012) several compounds have been isolated from plants known to treat diabetes [8].

Plants also have been investigated and found to possess numerous mechanisms for the management of the blood sugar and easing the stress as single compounds or a group of compounds in an additive or synergistic format. There is a provision of cheap alternative and available drugs associated with little side effects are being explored for the potential sources of drugs [5, 7]. In most of the developing countries herbal medicine still provides an alternative therapy for the disease. Despite of this, it still remains unregulated. Therefore there are quite a number of herbal drugs medicinal compound that remain unevaluated for their activity. This study therefore sought to sample herbal drugs used by traditional and herbal practitioners in Nairobi and evaluating for their anti-diabetic activity.

MATERIALS AND METHODS

Collection and preparation of plant material

Packaged formulation of Commercial Diabetic formula® herbal drug, commercial Ganotech® herbal powder, *Prunus africana* herbal powder, Flax seeds herbal powder and stevia tea were bought from commercial herbalists in Nairobi. The modes of preparation were noted to guide in the means of extraction and administration.

Experimental animals

Eight weeks old rats, female rats were obtained from Zoology department of Jomo Kenyatta University of Agriculture and Technology. The rats were kept for two weeks to acclimatize. During this period, the rats were given standard laboratory diet, rat pellets from Labchow, Unga feeds, water was allowed *ad libitum*. Twelve 12 hour Light-dark cycle was also allowed in the entire period of the experiment.

Preparation of herbal drugs

Herbal drugs were extracted in two portions the first portion using Ethanol at a ratio 1:10 and the second portion was extracted with water at the same ratio. Ethanolic extracts were concentrated *in vacuo* using a rotary evaporator at 50°C while the water extracts were freeze dried. The extracts were then kept in a refrigerator for further the tests.

Preparation of doses

Herbal mixtures of 30%, 40% and 50% (w/w) herbal flax seeds and rat pellets were prepared by mixing rat pellets and herbal flax seeds powder and different at ratios. The powders were mixed and water was added and they were molded back in pellets of the same size as original pellets then dried under shade.

Preparation of Stevia herbal powder extracts

Different ratios of powder 20% ,30% and 40% were extracted with water in the ratio of 1:30 three times water was the evaporated to concentrate the solution. 2ml of the extract was administered to the animals.

Experimental design for *in-vivo* antidiabetic activity

Dose was calculated with the formula:

$$Dose = \frac{Dose\ rate \times Body\ weight}{concentration\ of\ the\ drug\ in\ water}$$

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).

	Set up 1	Set up 2	Set up 3		
	Flux seed powder	Stevia powder	Commercial Ganotec powder	Commercial Diabetic formula	Prunus africana powder
Group 1	30% herbal flax seeds + rat pellets	20% stevia solution	250mg/Kg aqueous extract powder		
Group 2	40% herbal flax seeds + rat pellets	30% stevia solution	250mg/Kg ethanolic extract powder		
Group 3	50% herbal flax seeds + rat pellets	40% stevia solution	250mg/Kg aqueous extract of powder		
Group 4	rat pellets only	water only	250mg/Kg ethanolic powder		
Group 5	pellets + with 500 mg/Kg metformin	500 mg/Kg metformin	250mg/Kg aqueous extract powder		
Group 6			250mg/Kg ethanolic extract powder		
Group 7	5% DMSO in H ₂ O				
Group 8	500 mg/Kg mertformin				

Statistical analysis

Results were expressed as SEM. Statistical divergence between the treatments and the controls were tested by one way analysis of variance (ANOVA) using the SAS version 9.1. Individual comparisons were done by Students Newman Keul’s Test (SNK) analysis.

RESULTS AND DISCUSSION

Hypoglycemic effect of herbal flax seeds (*Linum usitatissimum*) on alloxan induced diabetic rats

Glucose levels after 12 hours overnight fasting was significantly higher (P<0.05) in the group treated with 30%, 40% and 50% herbal flax seeds mixture with rat pellets at 15.040 ± 2.961, 14.500 ± 3.675 and 21.060 ± 2.0 mMol/L respectively compared to rats fed with pellets only at (6.860 ± 1.070 mMol/L) and rats administered with pellets and 500mg/Kg metformin (5.620 ± 0.185 mMol/L).

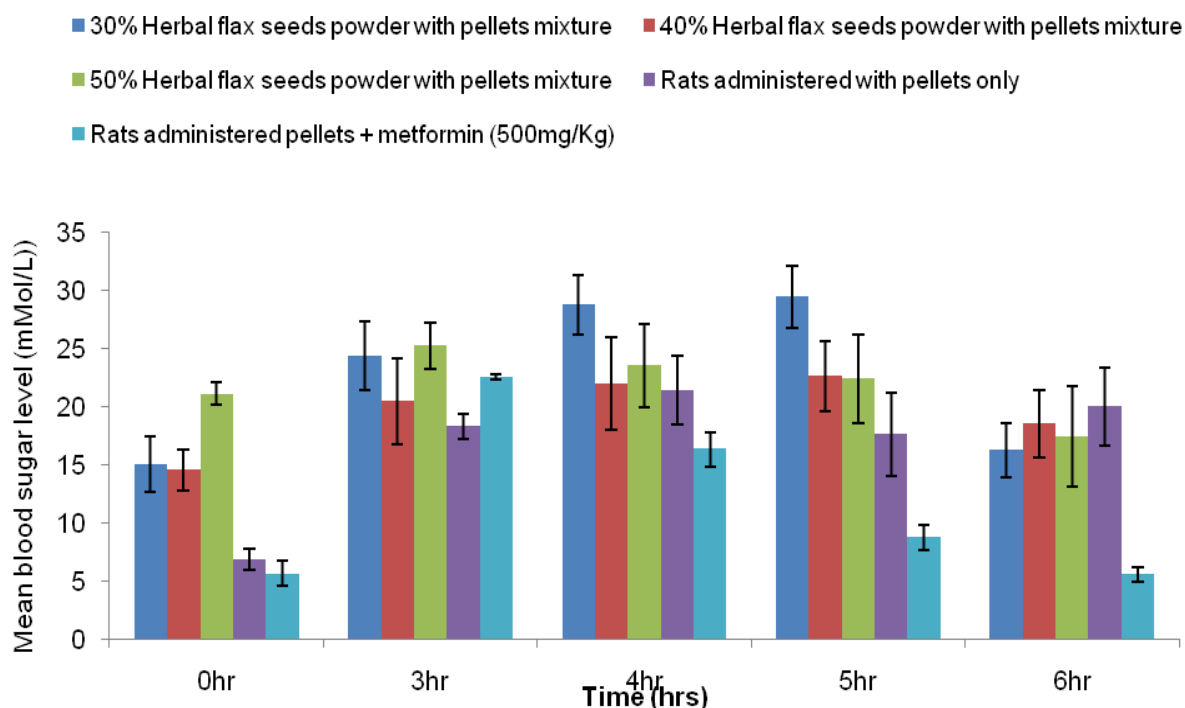


Figure I: Blood glucose levels in diabetic rats administered with Flax seed powder at various concentrations

Glucose levels increased significantly ($P < 0.05$) three hours later after administration of mixture of 30, 40 and 50% herbal flax seeds powder and rat pellets (24.320 ± 2.585 , 20.420 ± 3.986 and 25.200 ± 3.582 mMol/L) against rats fed with pellets only (18.260 ± 2.936 mMol/L) but were slightly higher ($P > 0.05$) in rats fed with 500 mg/Kg metformin at 22.520 ± 1.479 mMol/L.

The peak blood sugar was observed after 5 hours treatment with 30% and 40% mixture of herbal flax seeds with rat pellets at 29.440 ± 2.288 and 22.600 ± 2.898 while in 50% mixture of herbal flax seeds with rat pellets it was observed at 25.200 ± 3.582 mMol/L against the against rats fed with pellets only (18.260 ± 2.936 mMol/L, $P < 0.05$) observed at 4 hrs after and in rats fed with 500 mg/Kg metformin at 22.520 ± 1.479 mMol/L observed after 3 hrs of treatment.

Six hour after treatment, there was a significant drop of blood sugar ($P < 0.05$) from the peak values in the treatment groups at 16.220 ± 3.792 , 18.500 ± 3.094 and 17.384 ± 4.360 mMol/L compared to the rats fed with pellets only (20.000 ± 3.929 mMol/L) but were higher ($P > 0.05$) in rats fed with 500 mg/Kg metformin at 22.520 ± 1.479 mMol/L. The percentage reduction for 30, 40 and 50% herbal flax seed powder and rat pellet mixture was 44.9%, 9.4% and 31.1% respectively compared with the group administered with rat pellets only where the blood sugar increased by 9.5% and group administered with rat pellets and administered with 500mg/Kg metformin by which showed 75 % reduction (Figure I).

The finding were in agreement with other studies on antidiabetic activity of herbal flax seeds. *L. usitatissimum* has been shown to be active against diabetes and its activity has been attributed to complex phenol such as lignans [9]. Flax seeds contain anti-oxidants and have high dietary fiber that can help diabetics by inhibiting lipid peroxidation and scavenging of hydroxyl radicals. Whole flax seed have been reported to reduce serum and hepatic lipid levels in rats induced with type 2 diabetes mellitus [10]. A mixture of herbal flax seed powder and pumpkin seeds was able to make alloxan induced diabetic rats recover after 21 one days of treatment with the dietary supplement. Also the rate of storage of hepatic blood glucose to hepatic glycogen has been reported [11]

Hypoglycemic effect of Stevia herbal powder on alloxan induced diabetic rats.

The glucose levels after 12 hour fasting was significantly higher ($P < 0.05$) in the group to be administered 20% stevia at 10.0 ± 1.3 mMol/L, compared to rats to be administered with 2ml water (4.2 ± 0.6 mMol/L) and the rats to be treated with 500mg/Kg metformin (6.1 ± 0.9 mMol/L).

The blood glucose levels of groups treated with 30 and 40% stevia was recorded at 5.0 ± 0.6 mMol/L and 5.2 ± 0.6 mMol/L respectively after 12 hours fasting ($P > 0.05$) compared to rats fed with 2g/kg of glucose monohydrate in 2ml water (4.2 ± 0.6 mMol/L), and rats fed with 500mg/Kg metformin (6.1 ± 0.9 mMol/L).

On administration of 2g/Kg dose of glucose monohydrate to the rats, the glucose levels rose to 15.1 ± 0.8 mMol/L ($P > 0.05$) compared to rats fed with 500mg/Kg metformin (17.1 ± 3.5 mMol/L) and rats fed with water (19.1 ± 1.9 mMol/L). Increase in the blood sugar was significant ($P < 0.05$) in rats to be administered with 20% stevia and 40% stevia at 22.7 ± 1.0 mMol/L and 13.6 ± 0.2 mMol/L compared to rats fed with 500mg/Kg metformin (17.1 ± 3.5 mMol/L) and rats fed with water (19.1 ± 1.9 mMol/L).

The peak blood sugar levels was observed at the during the administration of the treatments except the group treated with 20 % stevia and 30 % stevia observed 30 minutes treatment (27.6 ± 2.9 , $P < 0.05$) and (17.1 ± 3.3 , $P > 0.05$) against the rats fed with 2g/kg of glucose monohydrate in 2ml water (19.1 ± 1.9 mMol/L), and rats fed with 500mg/Kg metformin (17.1 ± 3.5 mMol/L).

Two hours after administration, of 20 % stevia at 19.4 ± 2.7 ($P < 0.05$), 30 % stevia at 11.5 ± 0.8 ($P < 0.05$) and 4.6 ± 8.0 ($P > 0.05$) against rats fed with 500mg/Kg metformin (6.1 ± 1.2 mMol/L) while they were significantly different compared to the rats fed with 2g/kg of glucose monohydrate in 2ml water (13.3 ± 2.5 mMol/L). The blood sugar reduction from the peak of rats treated with 20, 30 and 40 % stevia was 29.7, 32.7 and 66.7% respectively compared to group administered with water only 30.3% and the group administered with 500 mg/Kg metformin 64.3% (Figure II).

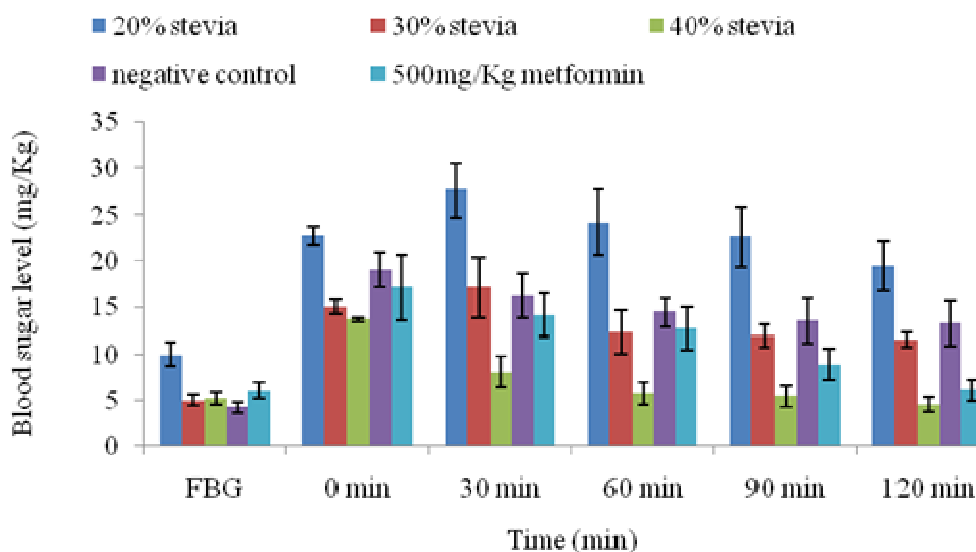


Figure II: Blood glucose levels in diabetic rats fed with Stevia powder extracts

Stevia rebusiana also known as Stevia is a widely renowned plant used in making beverages. A separated study done on antidiabetic activity of herbal drugs in the market found out that stevia was able to reduce diabetes by a significant fraction compared to metformin in a period of 8 hours [12]. Based on *invitro* tests stevia contains steviol glycosides that were good radical O_2^- and $\cdot OH$ radical scavengers and thus enhance their role in counteracting oxidative stress caused by hyperglycemia [13].

Hypoglycemic effect of aqueous and ethanolic extracts of Diabetic formula® herbal drug on alloxan induced diabetic rats

The glucose levels after 12 hour fasting in rats to be treated with aqueous extracts of commercial Diabetic Formula® herbal drug was significantly higher ($P < 0.05$), 10.7 ± 2.2 compared to rats fed with water only (4.3 ± 0.5 mMol/L) and rats treated with the 500mg/Kg metformin (9.6 ± 3.1 mMol/L). On the other hand, the glucose levels in rats to be treated with ethanolic extracts of commercial Diabetic Formula® was noted at 9.6 ± 1.6 mMol/L against the group treated with 500 mg/Kg metformin (9.6 ± 3.1 mMol/L) and rats administered with water only (4.3 ± 0.5 mMol/L, $P > 0.05$) after 12 hours of fasting.

The levels rose to 16.6 ± 1.6 mMol/L and 17.7 ± 4.7 mMol/L ($P > 0.05$), 30 minutes after administration of 2g/Kg of glucose compared to rats fed with 500mg/Kg metformin (15.2 ± 3.1 mMol/L) and 19.6 ± 1.4 mMol/L in rats administered water only. The peak blood sugar was observed 30 minutes after treatment at 16.7 ± 4.1 and 18.0 ± 4.3 mMol/L ($P > 0.05$) against the rats treated with 500mg/Kg metformin (12.0 ± 2.6 mMol/L) and the rats administered with water only (17.8 ± 2.3 mMol/L).

The levels declined after 120 minutes to 7.2 ± 2.4 and 9.9 ± 3.5 mMol/L ($P > 0.05$), compared to a rats treated with 500mg/Kg metformin (7.1 ± 3.0 mMol/L) and rats administered with water (14.9 ± 2.4 mMol/L) fig 9. Rats treated with aqueous and ethanolic extracts of commercial Diabetic Formula® reduced the blood sugar by 56 and 44 % respectively compared to compared tot the rats treated with the 5% DMSO which reduced by 24.0% and the group treated with 500mg/Kg metformin which reduced by 53.3% from the peak (Figure III).

Hypoglycemic effect of aqueous and ethanolic extracts of Ganodema® herbal drug on alloxan induced diabetic rats.

After 12 hour fasting, rats to be treated the rats with aqueous extract of Commercial Ganotech® herbal powder, had glucose levels of significantly lower ($P < 0.05$) at 4.6 ± 0.9 mMol/L compared to rats fed with 500mg/Kg metformin (9.6 ± 3.1 mMol/L) while it was slightly higher than 5% DMSO in water (4.3 ± 0.5 mMol/L). Rats to be treated with ethanolic extract of Commercial Ganotech® herbal powder had a glucose level slightly lower than those treated with

metformin 500mg/Kg (9.6 ± 3.1 mMol/L) and significantly higher than the group administered %5 DMSO water at 4.3 ± 0.5 mMol/L.

The levels rose to the peak of 15.0 ± 1.4 mMol/L and 17.2 ± 2.3 ($P > 0.05$) in groups to be treated the rats with aqueous and ethanolic extract of Commercial Ganotech® herbal powder respectively immediately after administration of 2g/Kg solution of glucose monohydrate compared to 500mg/Kg metformin (15.2 ± 3.1 mMol/L) and those administered with water (19.6 ± 1.4 mMol/L).

The blood sugar levels decreased to 5.8 ± 0.9 ($P < 0.05$) and 4.8 ± 1.3 mMol/L ($P > 0.05$) against group administered with 500mg/Kg metformin (7.1 ± 3.0 mMol/L) and those administered with water (14.9 ± 2.4 mMol/L). Aqueous and ethanolic extract of Commercial Ganotech® herbal powder reduced the blood sugar by 61 and 72% respectively compared tot the rats treated with the 5% DMSO which reduced by 24.0% and the group treated with 500mg/Kg metformin which reduced by 53.3% from the peak (Figure III).

Hypoglycemic effect of aqueous and ethanolic extracts of *Prunus africana* on alloxan induced diabetic rats.

After 12 hours fasting, glucose level of the rats was registered at 12.1 ± 3.2 mMol/L ($P < 0.05$) compared to rats treated with 500mg/Kg metformin (9.6 ± 3.1 mMol/L) and the rats administered with 2ml water (4.3 ± 0.5 mMol/L). Glucose levels of rats after 12 hours overnight fasting was noted at 9.6 ± 1.6 mMol/L compared to the rats treated with 500mg/Kg metformin (9.6 ± 3.1 mMol/L, $P > 0.05$) while it was significantly higher compared to rats administered with 2ml water (4.3 ± 0.5 mMol/L, $P < 0.05$).

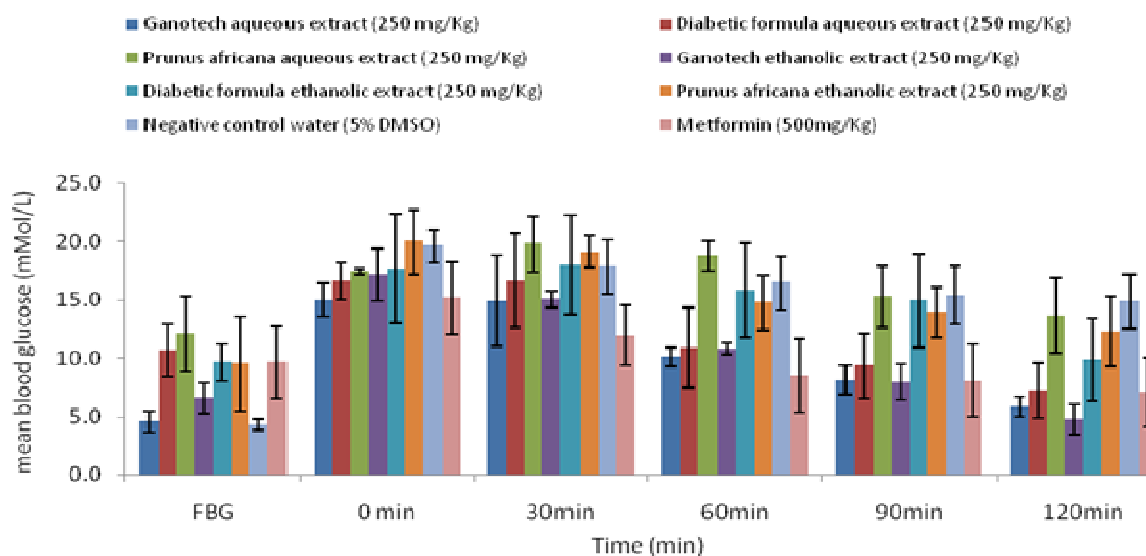


Figure III. Blood glucose levels in diabetic rats administered with aqueous and ethanolic extracts of Ganotech®, Diabetic formular ® and *Prunus africana* herbal powders.

The blood sugar dropped to 17.5 ± 0.2 mMol/L in rats to be administered with 250mg/Kg *Prunus africana* herbal powder whereas the blood sugar of rats to be administered with ethanolic extract of *Prunus africana* herbal drug rose to the peak of 20.0 ± 2.8 mMol/L compared to compared to rats treated with 500mg/Kg metformin (12.0 ± 2.6 mMol/L) and rats administered with 2ml water (17.8 ± 2.3 mMol/L, $P < 0.05$).

The peak blood sugar, was observed 30 minutes after administration after rats were treated with 250 mg/Kg aqueous extract of *Prunus africana* herbal powder at 19.8 ± 2.4 mMol/L compared to the rats treated with 500mg/Kg metformin (12.0 ± 2.6 mMol/L) and rats administered with 2ml water (17.8 ± 2.3 mMol/L, $P < 0.05$).

Significant glucose reductions ($P < 0.05$) was also observed after 120 minutes of administration of *Prunus africana* herbal extract (13.7 ± 3.2 mMol/L) compared to rats fed with 500mg/Kg metformin (7.1 ± 3.0 mMol/L) and rats

administered 2ml water (14.9±2.4 mMol/L). Glucose reductions of rats treated with ethanolic extract of *Prunus africana* herbal drug was also observed at 12.3±3.0mMol/L compared to compared rats fed with 500mg/Kg metformin (7.1±3.0 mMol/L) and rats administered 2ml water (14.9±2.4 mMol/L). Aqueous and Ethanolic extract of *Prunus africana* herbal treatment resulted in reduction of blood sugar by 21.71 and 38.5% respectively compared to the rats treated with the 5% DMSO which reduced by 24.0% and the group treated with 500mg/Kg metformin which reduced by 53.3% from the peak (Figure III).

Active component of herbal drugs are in the class of alkaloids, saponins, flavonoids and phenolic, compounds which act through different mechanisms [8]. Herbal drugs with antidiabetic activity are blended together with other herbal drugs to produce poly herbal drugs for synergy [14][15]. The resulting multi component herbal drug has been found to be active against diabetes and other diseases [15] [16].

Blood sugar increase observed after rats were administered with glucose monohydrate was in agreement with several studies. This confirmed the destruction of insulin secreting β - cells of the pancreas by alloxan, resulting in decreased endogenous insulin release. 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 [17]. Alloxan is known to be selective against β cells of the pancreatic islets known to processes the insulin. Alloxan generates free radicals that destroying the cells by causing degranulation, hydropic degeneration and clumping of the cells [18, 19].

CONCLUSION

Herbal drugs tested had hypoglycemic effect against Wistar albino rats and should be exploited as possible sources of medicine for diabetes .

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