

HYPOGLYCEMIC EFFECTS OF *CAESALPINIA VOLKENSII* ON ALLOXAN-INDUCED DIABETIC MICE

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ABSTRACT

Diabetes mellitus is a chronic disorder caused by inherited and/or acquired deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced by the target cells. Most conventional therapies for the management of type II diabetes include oral hypoglycemic drugs, exercise, diet and physical intervention therapies such as Acupuncture. Insulin is used in the management of type I diabetes mellitus. Insulin and oral hypoglycemic drugs are expensive and have numerous side effects. Through ages different communities have used medicinal herbs for diabetes mellitus management. Today herbal remedies are gaining popularity because the efficacy of conventional medicine is on the wane. This study was designed to bioscreen aqueous leaf extracts of *Caesalpinia volkensii* for its hypoglycemic potential. Its *in vivo* toxicity was also evaluated. Ethnobotanical and pharmacological information on the plant was gathered from the local traditional herbal practitioner. The three tested dose levels (50, 100, and 150mg/kg body weight) lowered blood glucose levels appreciably. Phytochemical screening results show that the aqueous extract has phytochemicals associated with antidiabetic activity. The analysis of trace metal composition of the aqueous extracts showed that it contained Manganese, Iron, Copper, Zinc, Magnesium, Molybdenum, Chromium and Vanadium, all of which aid in lowering blood glucose levels. Preliminary *in vivo* histopathological analysis established that the extracts had no toxic effects on the organs and tissues. The study has established that the aqueous leaf extracts of *Caesalpinia volkensii* are effective and safe for management of diabetes mellitus.

Keywords: *Caesalpinia volkensii*, *in vivo* toxicity, antidiabetic activity

INTRODUCTION

Diabetes mellitus is a chronic endocrinologic disorder caused by inherited and/or acquired deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced. It is characterized by high blood levels of glucose, which in turn damages many of the body systems particularly the blood vessels and nerves (Chen, 1998; WHO, 2003). This is caused by disturbances in the regulatory systems responsible for the storage and utilization of the chemical energy from food. This includes the metabolism of carbohydrates, fats and proteins resulting from defects in insulin secretion, insulin action, or both (Shillito, 1988; Votey and Peters, 2004).

According to WHO, more than 190 million people suffer from diabetes mellitus worldwide. The disease incidence is increasing rapidly and it is estimated that the figure will double by the year 2025. Most people with diabetes in developed countries will be aged 65 years or more by year 2025 yet, in developing countries the affected age bracket will be in the 45-65 year and in their most productive years (WHO, 2003). It is expected that the prevalence of diabetes will continue to increase in Africa and Asia as a result of changes in lifestyles and urbanization (Diabetes Control and Complications Trial Research Group, 1993). 49.0 million people suffer from the disease in South-East Asia; India alone accounts for almost a quarter of all patients in this region, with an estimate of 15 million people (Agrawal, 2004).

In 2000 there were 7.5 million cases of diabetes in Africa and the figure is expected to rise to around 18.2 million by the year 2030 with about 190,000 sufferers from Kenya alone (Mngola, 2004). All African countries are struggling to care for a large number of diabetic patients, yet more than 80% of the cases are undiagnosed (Mngola, 2004).

The combination of rising prevalence of diabetes and the high rate of long term complication especially in Africa will lead to drastic increase of the burden of diabetes on the health systems of African countries most of which face economic difficulties. Possible prevention strategies have been hindered by the scarcity of data on

diabetes in Africa. Many of the conventional drugs that are available for the management of the disease are not only expensive but also have numerous side effects, which lead to complications.

Herbal medications, on the other hand, are cheaper and locally available. Many of the plant species growing throughout the world contain active constituents that offer benefits that conventional drugs lack, helping combat illness while supporting the body's efforts to regain good health. Many plants have been traditionally used to manage diabetes without authentication on their antidiabetic properties and assessment of their safety. The use of crude extracts without pharmacological and toxicological evaluation could lead to serious complications and even death. Since many of the conventional drugs have originated from medicinal herbs, a study of these plants may lead to the discovery of drugs with higher efficacy and low toxicity. It is against this background that this study was designed and undertaken.

MATERIALS AND METHODS

Collection of Plant Materials

The leaves of the plant *Caesalpinia volkensii* (15kg) were collected from its natural habitat in Makunguru village, Siakago Division of Mbeere North district (Embu County) in Eastern province, Kenya based on the folklore reports from practicing herbalists, Mr. Mugeki and Mr. Njuki wa Maringa on its hypoglycemic activity. The Mbeere people call the plant *Mubuthi*. An acknowledged authority identified the plant and voucher specimen number; *Caesp. Volk. 12/2010* was deposited at the National Museum of Kenya herbarium. The leaves were air-dried at room temperature for three weeks. The dried leaves were then crushed into powder by use of an electric mill (Christy and Norris Ltd England).

Preparation of Extracts

The extracts were prepared by boiling 100 g of crushed material in one liter of distilled water for two hours with frequent stirring. The mixture was left to cool at room temperature and then decanted into a dry clean conical flask through folded cotton gauze stuffed into a funnel. The decanted extract was then filtered using filter papers by

use of a vacuum pump. The filtrate was freeze-dried in 150 ml portion using a freeze drier for 72 hours. Afterwards the powder was pooled together and stored at 4°C in airtight containers.

Preparation of Extracts for Injection into Mice

Physiological saline was prepared by dissolving 0.85g of analytical grade sodium chloride in 100 ml of distilled water. The extracts for injection were prepared as follows: the 50mg/kg body weight dose was prepared by dissolving 12.5mg in 1ml of physiological saline; the 100mg/kg body weight dose was prepared by dissolving 25mg in 1ml of physiological saline; and the 150mg/kg body weight dose was prepared by dissolving 37.5mg in 1ml of physiological saline. The animals were given 0.1 ml of the extract solutions. Insulin was also reconstituted and animals in group two were given 0.1 ml. The animals in groups one and three were also injected with 0.1 ml physiological saline intraperitoneally.

Pharmacological Testing

Animals

Health Swiss albino male mice were used for the study. The animals were 4-6 weeks old and weighed 23-27 g, and were fed on the standard mice diet and allowed free access to water *ad libitum*. For experimental purposes, the animals were fasted overnight and allowed free access to water. The animals were divided into 4 groups of four animals each. The animals in group two, three and four were alloxanized using alloxan 4-6 days before the start of the experiment. Group one was not alloxanized and were given 0.1ml of normal saline; Group two was given 0.1 ml of normal saline; the third group of animals were given insulin at the dose of 1 unit per kg body weight; group four animals was treated with plant extracts at three dose levels: 50mg/kg body weight, 100mg/kg body weight and 150 mg/kg body weight. Each dose level consisted of four animals. Group one and two served as the experimental controls while group three served as the reference. Alloxan is a chemical that kills the pancreatic islets cells that are responsible for insulin production. Once the β - cells are destroyed, the pancreas cannot produce insulin and the animal becomes diabetic. The alloxan monohydrate 10 g used in this experiment was obtained from Fluka chemie GmbH ch 9471 Switzerland and injected intraperitoneally to mice at a dose of 150mg/kg body weight. Insulin was given to mice at a dose of one unit per kg/body weight (1IU/kgbw) intraperitoneally. Before administration of the different treatments the animals were bled and blood glucose level in the animals was measured. This was the initial measurement at time zero. The animals were again bled hourly until the fourth hour. As a prerequisite to the use of the animals for bioassay, ethical permission was sought from the Association of Animal Rights in Kenya and it was granted.

Collection of Blood Samples

Blood samples were collected from the tails of the animals after wiping the tail with surgical spirit. The tail was nibbled by use of a pair of sharp scissors; a drop of blood was squeezed into a Supreme hypoguard meter. After collection of blood, the nibbled side of the tail was rubbed with cotton wool soaked in absolute alcohol to protect the animal from infection and to arrest bleeding.

Blood Glucose Level Determination

The principle of the test is based on a glucose oxidase/peroxidase reaction, which is specific for β -D-glucose. The Hypoguard machine was used together with GB Supreme blood glucose test strips. The Supreme Test Strip is a disposable plastic strip containing a chemically treated test area used to measure the amount of blood glucose. The test area is designed in such a way that when a drop of blood is placed on the top surface, color change occurs which is determined by a Supreme hypoguard meter. The Supreme Test Strip was fully inserted into the meter before applying a drop of blood to fully cover the test area inside the grey target. The Supreme Test Strips and the Supreme Hypoguard Meter were procured from Hypoguard Ltd, United Kingdom through Chemoquip Ltd, Kenya.

Trace Elements Determination

Energy Dispersive X-ray Fluorescence (EDXRF)

The different trace elements present in the extracts were investigated by use of Energy Dispersive X-ray fluorescence analytical technique. 0.3g of the freeze-dried material was weighed and made into pellets of 2.5 cm in diameter and weighing 100-200 mg/cm². The pellets were made using a pellet press machine. The pellets were weighed and their weights recorded. The EDXRF system consists of an X-Ray spectrometer and a radioisotope excitation source. The radiation from the radioactive source, Cd¹⁰⁹ ($T_{1/2}$ = 453 days and activity = 10 mCi) are incident on the sample, which emits the characteristic X-Rays. These X-Rays are detected by Si (Li) detector (EG&G Ortec, 30mm²x10mm sensitive volume, 25 μ m Be window) with energy resolution of 200eV at 5.9keV Mn K α - line. The spectral data analysis was collected using personal computer based Cariberra S-100 multichannel analyser (MCA). The acquisition time applied in the EDXRF measurements was 1000seconds. The X-Ray spectrum analysis and quantification was done using IAEA QXAS software (QXAS, 1992), which is based on the Fundamental Parameters Method (FPM) (Sparks, 1975; Giauque *et al.*, 1973,1977).

Atomic Absorption Spectrophotometry (AAS)

This technique was used for analysis of magnesium, chromium and vanadium. The machine used was Buck model 210 VGP Atomic absorption spectrophotometer. The VGP AAS is designed to measure the concentration of elemental metals in solution. It provides integrated measurements in absorbance or emission intensity as well as sample concentration in comparison to standard solutions. Readings can be integrated over a period from 0.5 seconds to 10 seconds.

Preparation of Standard Solutions

Standard stock solutions of 1000 parts per million (ppm) for AAS were used as supplied by the manufacturers (Aldrich Chemical Co. Inc).

Preparation of Working Standards

Suitable aliquots of standard stock solutions of each element were taken in a series of 100ml volumetric flasks. The solutions were diluted to volume using distilled-deionised water, mixed thoroughly and transferred into plastic beakers. This was done for each element when its analysis was to be undertaken. For each element, working standard solutions were prepared within a given range (1 ppm, 5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm). The relationship between concentration and absorbance was linear. In case of magnesium, 2 ml of 5% Lanthanum solution was added to each series of the working standard solution before diluting them to volume. Standard blank reagents for each element were prepared by adding all the used reagents, except the target element being determined.

20% Hydrochloric Acid (w/w)

The solution was prepared by transferring 548 ml of concentrated hydrochloric acid (36%) of analar grade carefully into 300ml of distilled- deionised water contained in 1 litre volumetric flask. The solution was then diluted to one litre, mixed thoroughly and kept in plastic bottles.

Lanthanum Solution

Lanthanum solution (50mg/ml) was prepared by dissolving 12.6263g of Lanthanum chloride in distilled-deionised water. The solution was then diluted to 250ml with distilled-deionised water in a volumetric flask. After mixing thoroughly, the solution was kept in clean plastic bottle and used during the determination of magnesium in the plant materials.

Digestion of Plant Materials

Each plant material that was collected for the study was brought to solution by wet oxidation/digestion. The procedure was repeated two times. Wet oxidation for determination of magnesium (Mg),

vanadium (V), and chromium (Cr) was done as follows: The dried samples of known weights were transferred into 100 ml Pyrex beakers and to each beaker, 10 ml of concentrated Nitric acid (HNO₃) was added, then allowed to soak thoroughly. 3 ml of 60% Perchloric acid (HClO₄) was added to each beaker, then warmed on hot plate slowly at first, until frothing ceased. Heating was then intensified until all Nitric acid was evaporated. When charring occurred, the mixture was cooled, 10 ml of Nitric acid was added and heating continued until white fumes of Perchloric acid were observed. The final solution was cooled and 25 ml of 20% Hydrochloric acid (HCl) was added. The solution was then transferred into 100 ml volumetric flask by filtering through Whatman filter paper No.1. The solutions were then made to volume and shaken well to allow proper mixing before the contents were transferred to plastic sample bottles. The samples were kept in a freezer awaiting analysis.

Total Elemental Content Determination

Wet digests of the plant materials were analyzed for Mg, V and Cr. Working standards were prepared according to the previously described procedure. Sample solutions for analysis of magnesium were prepared by withdrawing 1 ml of the digested sample solution into 100 ml volumetric flasks. 5% Lanthanum solution was added in each flask and the mixture diluted to volume using distilled-deionised water. However, for analysis of vanadium and chromium, the digested sample solutions were analyzed without dilution.

After setting the AAS instrument to the right conditions for each element, the respective standard and sample solutions were aspirated into the flame in turns to determine their respective absorbance. At least four standard solutions were aspirated between 6-10 samples to monitor the stability of the working conditions. Distilled-deionised water was always flushed into the flame to re-establish the zero absorbance. For each sample and element, the above procedure was repeated two times. The mean absorbance for each sample solution and standard solutions were calculated and recorded.

To prepare a calibration curve for each element, a graph of mean absorbance against corresponding concentrations of the standard solutions was plotted. In all cases, the graphs were linear; the best fitting straight line was obtained by using Microsoft Excel computer software (Microsoft Office 2000), which also helped to convert absorbance readings to concentrations of elements in each sample analyzed with better accuracy than manual graphical method.

The programme gave concentrations of the diluted and undiluted samples directly. Concentration values obtained for the diluted samples were corrected by multiplying with the respective dilution factors. The final values were expressed as µg/g dry matter, were recorded. These values were obtained by using the expression below:

$$\text{Elemental content} = a/w \times 1000$$

where, a is the amount of element (mg) in 100 ml sample analyzed
w is the dry weight (g) of the material analyzed

Phytochemical Screening

Phytochemical screening was undertaken to determine the class of compounds present in the plants extracts using the method

described by Houghton and Raman (1998). Plants extracts were screened for the presence of alkaloids, saponins, tannins, terpenoids, sterols, flavonoids and anthraquinones.

Preliminary In Vivo Histopathological Examination

The tissues were removed from formalin and trimmed and then labeled using a pencil. To remove the excess formalin, the tissues were washed in running water overnight. Dehydration was then done in an automatic tissue processor for three hours for each stage starting from 50%, 70%, 80% then 90%. The sections were then washed in a bath of absolute alcohol twice to ensure that there was no trace of water. The next step was to clear the alcohol from the sections, which was done twice by using xylene. The next step involved infiltration with paraffin wax which was carried out at 2^o C below the melting point of wax for three hours. The tissues were then embedded in molten paraffin wax and allowed to dry. The embedded tissues were then sectioned at 4-5µm thickness and floated in warm water bath to spread out the tissues, which were then attached to clean microscopic slides. After holding in hot oven for at least 15 minutes, the sections were dewaxed in xylene and then stained with haematoxylin and eosin dyes using standard histological procedures. The stained tissues were coverslipped with DPX, dried and examined microscopically for pathological changes.

Data Analysis

In the *in vivo* hypoglycemic assays, student's t-test was used to evaluate the significance between means of extract treated animals and the diabetic control, insulin control and the normal control. The data was represented as mean ± SEM. P<0.05 was considered statistically significant. InStat statistical computer software was used for data analysis.

RESULTS

In Vivo Hypoglycemic Activity Assay

All the three dose levels (50, 100, and 150mg/kg body weight) of the aqueous leaf extracts of *Caesalpinia volkensii* lowered blood glucose levels appreciably (Table 1; Figure 1). In the first hour, the three doses lowered blood glucose levels by 5%, 38%, and 51%, respectively. The 50mg/kg body weight dose insignificantly lowered blood glucose levels as opposed to the other two doses. The 100 and 150 mg/kg body weight doses significantly lowered blood glucose levels at the 1st hour but not as effectively as insulin (^ap<0.05; ^bp<0.05). The 150mg/kg body weight dose range lowered the blood sugar level to normal. In the 2nd hour, the percent reduction of blood sugar levels at the three dose levels was 29%, 48%, and 63%, respectively. At this hour, the two higher doses lowered the blood glucose levels significantly to normal. The 50mg/kg body weight dose lowered blood glucose levels of the extract treated animals when compared to the diabetic controls but not as effectively as insulin (^ap<0.05). The 150mg/kg body weight dose range exhibited hypoglycemic activity as effectively as insulin. During the 3rd hour, the percent glucose level reduction by the three dose ranges was 63%, 54%, and 69%, respectively. At this time all the three dose levels lowered blood glucose to normal and as effectively as insulin (^bp<0.05). This trend was repeated during the 4th hour, the extract lowered glucose levels by 58%, 63%, and 72 %, respectively. Here, the three dose ranges lowered blood sugar levels to normal and as effectively as insulin (^bp<0.05).

Table 1: Effects of *Caesalpinia volkensii* extract on blood glucose levels in alloxan-induced diabetic mice (mg/dl)

Animal Group	Treatment	0 hr	1 hr	2 hr	3 hr	4 hr
Normal	Saline	59.8±1.9	56.5±2.0	56.0±2.4	54.8±1.1	54.8±3.8
Diabetic	Saline	130.0±3.5	142.8±3.8	181.3±5.3	195.8±3.6	210.0±3.1
Diabetic	Insulin (1IU/kgbw)	135.0±28.1	50.3±6.2	54.0±1.4	54.5±1.3	51.8±2.7
Diabetic	<i>C.volkensii</i> (50mg/kgbw)	140.5±19.7	123.3±12.3 ^{b*}	93.3±6.2 ^{ab*}	49.8±0.3 ^{ab*}	56.3±2.5 ^a
Diabetic	<i>C.volkensii</i> (100mg/kgbw)	145.5±17.8	84.8±7.7 ^{ab*}	72.0±5.0 ^{ab*}	63.8±3.1 ^{ab*}	52.3±1.1 ^a
Diabetic	<i>C.volkensii</i> (150mg/kgbw)	171.0±16.1	86.3±12.2 ^{ab}	63.5±6.4 ^a	53.0±2.9 ^a	47.5±2.2 ^a

^{*}P<0.05 with respect to normal control; ^aP<0.05 with respect to diabetic control; ^bP<0.05 with respect to insulin. The data was analyzed using student's 't'- test. All the results were expressed as mean ± SEM. All blood glucose levels were recorded in mg/dl.

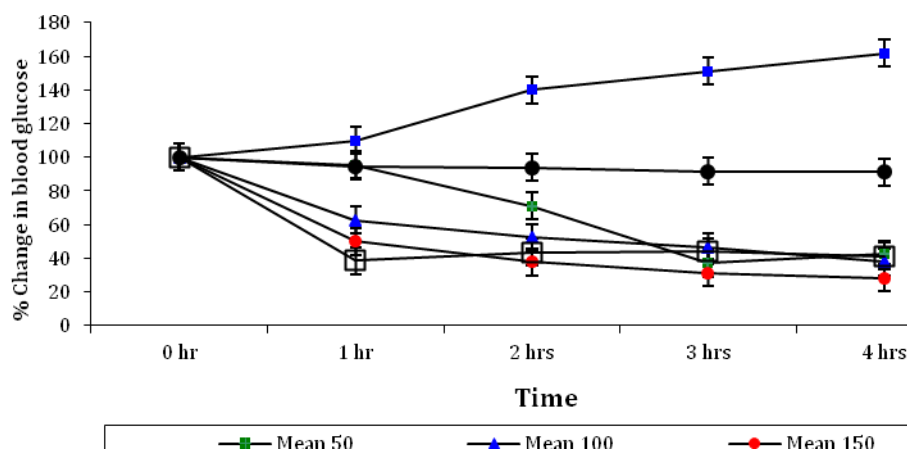


Figure 1: Percentage reduction in blood glucose by varying doses of *Caesalpinia volkensii* in diabetic mice

* $P < 0.05$ with respect to normal control; ^a $P < 0.05$ with respect to diabetic control; ^b $P < 0.05$ with respect to insulin. The data was analyzed using student's t- test

Trace Metal Analysis

The different trace elements in the aqueous extracts of *Caesalpinia volkensii* were investigated using EDXRF and AAS technique. The trace elements analyzed by EDXRF technique were Manganese, Iron, Nickel, Copper, Zinc, Strontium, Molybdenum and Lead (Table 2). Manganese, Iron, Copper, Zinc, and Molybdenum were detected at concentrations of $8.4 \pm 1.2 \mu\text{g/g}$, $210.0 \pm 27.0 \mu\text{g/g}$, $13.2 \pm 2.2 \mu\text{g/g}$, $20.2 \pm 3.3 \mu\text{g/g}$ and $24.4 \pm 2.8 \mu\text{g/g}$ respectively. Nickel, Strontium and Lead were below the detection limit by EDXRF technique. Magnesium, Chromium and Vanadium were analyzed by Atomic Absorption Spectrophotometry (AAS). Magnesium and Chromium were detected at concentrations of $762.1 \pm 5.9 \mu\text{g/g}$ and $105.1 \pm 2.9 \mu\text{g/g}$ respectively. Vanadium concentrations were below the detection limit by AAS (Table 3).

Table 2: Trace metals present in the aqueous leaf extracts of *Caesalpinia volkensii* as analyzed by EDXRF

Element	Elemental Quantity ($\mu\text{g/g}$)
Mn	8.4 ± 1.2
Fe	210.0 ± 27.0
Ni	<5
Cu	13.2 ± 2.2
Zn	20.2 ± 3.3
Sr	<1
Mo	24.4 ± 2.8
Pb	2.1 ± 0.5

Table 3: Trace metals present in the aqueous leaf extracts of *Caesalpinia volkensii* as analyzed by AAS

Element	Elemental Quantity ($\mu\text{g/g}$)
Mg	762.1 ± 5.9
Cr	105.1 ± 2.9

Preliminary In Vivo Histopathological Analysis

In vivo toxicity assay was undertaken on six mice that were divided into 2 groups of three. The first group of animals was treated with saline and served as control. The second group was treated with the aqueous extracts of *Caesalpinia volkensii* at a dose of 450mg/kg body weight as earlier explained. Aqueous leaf extract of *Caesalpinia volkensii* plant extract had no toxic effects on tissues examined. The liver tissue showed no signs of damage; the hepatocytes were intact apart from mild inflammation in the liver. Kidney examination showed isolated perivascular accumulation of lymphocytes but the

renal cells were normal. The spleen and heart tissues showed no signs of pathology.

Phytochemical screening

Results of phytochemical screening of the aqueous extracts of *Caesalpinia volkensii*, show that they contained sterols, flavonols/flavones or chalcones and flavonoids (Table 4).

Table 4: Phytochemistry of the aqueous leaf extracts of *Caesalpinia volkensii*

Phytochemical	Observed Result
Alkaloids	-
Sterols	+
Terpenoids	-
Saponins	-
Flavonols/Flavones	+
Chalcones	+
Flavonoids	+
Tannins	-
Anthraquinones	-

Key: +Ve → Compound present -Ve → Compound absent

DISCUSSION

The aqueous leaf extracts of *Caesalpinia volkensii* showed appreciable hypoglycemic activity in alloxan-induced diabetic mice. Similar work carried out by Tsuneki *et al.* (2004) demonstrated hypoglycemic activity on streptozotocin induced diabetic mice on administration of aqueous leaf extracts of *Camellia sinensis*. That *Caesalpinia volkensii* extracts demonstrated a dose dependent response on blood glucose lowering effect on alloxan induced diabetic mice is consistent with Jouad *et al.* (2004) regarding studies done on streptozotocin induced diabetic rats orally administered with leaf extracts of *Eucalyptus globules*.

In another related study, Nammi *et al.* (2003) demonstrated a dose dependent hypoglycemic activity on alloxan-induced diabetic rabbits on administration of fresh leaf extracts of *Catharanthus roseus* Linn. Similar results were observed by Aguiyi *et al.* (2000) who demonstrated hypoglycemic activity on normal and alloxan-induced diabetic rats intraperitoneally injected with methanolic extracts of *Ocimum gratissimum* leaves. This indicates that these plant extracts might have been absorbed through the cell lipids membrane through facilitated diffusion. The ions might have been transported in the direction of its electrochemical gradient. This trend is in agreement with expectations seen in administration of higher concentration of

drug. *C. volkensii* extracts also lowered blood sugar to below insulin levels. This suggests the possibility of islet repair or mimicry of insulin action by elements from the extract.

The hypoglycemic effect of the plant can also be attributed to sterols, flavonols, flavones, chalcones and flavonoids observed in the aqueous plant extracts. Several studies have shown the hypoglycemic activity of such compounds. Flavonols, sterols, flavones and chalcones in *Trigonella foenum graecum* plant extract have been shown to lower blood glucose in diabetic animals (Marles and Farnsworth, 1994; Duke, 1997). Methylhydroxy chalcone polymer (MHCP) from the extract of cinnamon has been shown to increase glucose metabolism of cells 20-fold *in vitro* in the epididymal fat cell assay (Broadhurst, 1997). Flavonoids present in *Pterocarpus marsupium* bark extracts have been shown to prevent beta cell damage in rats (Chakravarthi et al., 1981).

Flavonoids present in the aqueous extract of *Boerhavia diffusa* leaves produced non-dose dependent related decrease in blood glucose levels in alloxan induced rats (Chude et al., 2000). Methanolic root extracts of *Clitoria ternatea* Linn contains flavonoids and steroids which are responsible for the glucose lowering ability in both normal and streptozotocin induced diabetic rats (Boominathan et al., 2004).

Medicinal herbs used in indigenous medicines for the management of diabetes mellitus contain both organic and inorganic constituents. Some of these inorganic trace elements possess antidiabetic properties. The blood glucose lowering effect by the aqueous extracts of *C. volkensii* could also have been caused by the trace elements. Such trace elements isolated in appreciable amounts from the plant extracts were Manganese, Copper, Magnesium, Zinc, Molybdenum and Chromium.

It has been shown that Manganese reduces glucose intolerance present in deficiency cases (Keen and Zidenberg-Cherr, 1996). Similarly Copper deficiency is associated with disturbed carbohydrate metabolism. Zinc is involved in all aspects of insulin metabolism: synthesis, secretion and utilization, while Zinc deficiency plays a role in the development of diabetes in humans. Zinc also has a protective effect against beta cell destruction, and has well-known anti-viral effects (Lehman and Spinas, 1996).

Molybdenum stimulates glycolysis and accelerates glycogen degradation in the hepatocytes. It also increases receptor autophosphorylation and phosphorylation of its substrate and augment glucose transport (Li et al., 1995). Magnesium deficiency is the most evident disturbance of metal metabolism in diabetes mellitus. Hypomagnesemia increases the risk of ischemic heart disease and severe retinopathy (Tuvemo and Gebre-Medhin, 1985; Matsumoto, 1994; Singh, 1995; Frank et al., 2000).

Chromium enhances the body's sensitivity to insulin and increases the number of insulin receptors, to enhance receptor binding. It also potentiates insulin action (Anderson et al., 2001). Experimentally, Chromium deficiency is associated with impaired glucose tolerance, which is improved with supplementation (Trow et al., 2000). The chromium, manganese and magnesium salts present in the saltbush *Atriplex halimus* L., are believed to prevent diabetes from occurring in sand rats who feed regularly on the plant and who have a genetic predisposition to diabetes (HerbalGram, 1997). Studies undertaken by Ravi et al. (2004) showed that inorganic trace elements such as zinc, chromium and vanadium which were present in the seeds of *E. jambolana* had hypoglycemic activity in streptozotocin-induced diabetic rats.

The aqueous leaf extracts of *C. volkensii* did not alter the normal cell structure of the heart, kidney, liver and spleen as indicated. This suggested the safety of these plants when used to manage diabetes mellitus.

The dose ranges used in this study were within the dose ranges used by Tsuneki et al. (2004) and Jouad et al. (2004). Tsuneki et al. (2004) while examining the hypoglycemic effect of green tea on blood glucose levels used a dose range of 30-300mg/kg body weights in rats. Jouad et al. (2004) used a dose range of 150 and 300 mg/kg body weight while evaluating the hypoglycemic activity of aqueous

extract of *Eucalyptus globulus* in normal and streptozotocin-induced diabetic rats. Jouad et al. (2004) used a dose of 4.5 g/kg body weight of *Eucalyptus globulus* leaf extract to evaluate toxicity of this plant extract.

CONCLUSION

The aqueous leaf extracts of *Caesalpinia volkensii* showed significant antidiabetic property. The results of this study confirm its suitability for the management of diabetes mellitus. However, its modes of hypoglycemic action is still obscure.

The different trace elements and phytochemical compounds present in the aqueous plant extracts are associated with the hypoglycemic activity. Histopathological responses induced by the extracts indicated no overt pathology attributable to the plant extracts after intraperitoneal administration. This might indicate their low acute toxicity after intraperitoneal administration. Further toxicity tests using the oral route of administration at standardized dosage levels are needed to comprehensively evaluate the safety of these extracts in animal models after administration through the conventionally used route of treatment for the management of diabetes mellitus. Herbal medicines are complex mixtures of different compounds that often act in a synergistic manner and exert their full beneficial effect as total extracts. Since herbalists use the extracts in combination for the management of diabetes mellitus, incidences of toxicity are greatly arrested. In this study, the null hypothesis is accepted and the study justified.

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