# Effects of 'ZPC' Polyherbal Formulation on Diabetic-Dyslipidemic Wistar Rats

## ABSTRACT

In this study the antidiabetic effect of a polyherbal formulation- ZPC was investigated in Wistar rats. Diabetes was induced by intraperitoneal injection of streptozotocin at a dose of 50 mg/kg. Rats having Fasting Blood Sugar (FBS) level above 250mg/dl after 72 hrs were considered diabetic and used for the studies. Five rats served as non- diabetic control (Group 1) while twenty diabetic rats were randomized into 4 groups of 5 rats each. The four groups (Groups 2,3,4 and 5) received 1ml (diabetic control, 250 mg/kg chlorpropamide and ZPC at doses of 250 and 500ng/kg respectively for 28 days. During the treatment period, the FBS and bodyweight of rats were monitored weekly and on day 28, the rats were euthanized and blood samples collected for serum lipid profile analysis. Results obtained indicated that following administration of streptozotocin, there was a significant ($p<0.05$) increase in the FBS, total cholesterol, triglycerides and LDL concentration with a corresponding significant ($p<0.05$) decrease in HDL concentration compared to non- diabetic control. However, following the treatment with the polyherbal formulation, there was a significant ($p<0.05$) reduction in the FBS level and a significant ($p<0.05$) increase in the body weight of rats compared to the diabetic control. The polyherbal formulation also produced a significant ($p<0.05$) reduction in total cholesterol, triglycerides and LDL concentration with a corresponding significant ($p<0.05$) increase in HDL concentration compared to diabetic control. It was concluded that, ZPC might serve as a good alternative or as an adjunct to the oral hypoglycaemic agents in the management of diabetes.

**Key words: Polyherbal, Formulation, Diabetic, Dyslipidemic, Wistar Rats**

## INTRODUCTION

Diabetes mellitus is chronic metabolic disorders that affect human body in terms of physical, psychological and social health. It is defined as a group of disorders characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins [1], [2]. It is becoming the third "killer" of the health of mankind along with cancer, cardiovascular and cerebrovascular diseases [3]. Among all the cases of diabetes, type 2 diabetes was found to be more prevalent [4]. Knowledge about diabetes mellitus existed in ancient Egypt and Greece. The word "diabetes" is derived from the Greek word "Diab" (meaning to pass through, referring to the cycle of heavy thirst and frequent urination); "mellitus" is the Latin word for "sweetened with honey" (refers to the presence of sugar in the urine) [2]. According to ancient Hindu physicians, "Madhumeha" is a disease in which a patient passes sweet urine and exhibits sweetness all over the body, such as in sweat, mucus, breath, and blood. It was recommended that the low carbohydrate diet and almost total withdrawal of animal fats should be taken by the patients suffering from Madhumeha, whereas obese adults should live on low calorie diet.

There are two major types of Diabetes: Type 1, previously known as "Juvenile onset diabetes mellitus" (Insulin dependent diabetes mellitus), is hereditary and is managed via insulin injection, and Type 2, "Adult type" previously known as non-insulin dependent diabetes mellitus, occurs mostly in elderly people and is usually, managed via life style modification and the use of oral hypoglycemic drugs [2].

Plants have always been a good source of drugs. The ethno-botanical information reports about 800 plants that may possess anti-diabetic potential [5], [6]. The beneficial uses of medicinal plants in traditional system of medicine of many cultures are extensively documented. Several plants have been used as dietary adjuvant and in treating the number of diseases even without any knowledge on their proper functions and constituents. This practice may be attributed to the uncompromised cost and side effects of synthetic hypoglycemic agents [4]. Although numerous synthetic drugs were developed for the treatment of diabetes mellitus but the safe and effective treatment paradigm is yet to be achieved. Medicinal foods are prescribed widely even when their biologically active compounds are unknown, because of their safety, effectiveness, and availability [7]. The World Health Organization (WHO) has recommended the evaluation of traditional plant treatments for diabetes as they are effective, non-toxic, with less or no side effects and are considered to be excellent candidates for oral therapy [8].

Polyherbal extracts, which are combinations of different herbal extracts/fractions, are also used for the treatment of diseases. Many people believe that polyherbal extracts are just effective as drugs. Herbalists suggest that nature provide other ingredients that may act as buffers, synergists or counterbalances, working in harmony with the more powerful ingredients. Therefore, by using herbal combination in their complete form, the body's healing process utilizes a balance of ingredients provided by nature [9]. In this study one of such polyherbal formulations, ZPC ~~is~~ has been evaluated for anti-hyperglycaemic and hypolipidemic properties. ZPC is made from the aqueous extracts of *Zingiber officinale* (Ginger) and the leaves of *Phyllantus spp*, and *Camellia sinensis*.

*Zingiber officinale* commonly referred to as Ginger is widely used around the world as a spice. It is also widely used in traditional alternative medicine in the treatment and management of various disorders including catarrh, rheumatism, nervous diseases, gingivitis, toothache, asthma, stroke, constipation and diabetes [10], [11]. *Phyllantus* spp is widely cultivated in Africa. Its parts are considered to have antibiotic properties and also useful in the treatment of hemorrhage, diarrhoea, dysentery, anaemia, jaundice, diabetes, fever, dyspepsia, bronchitis and cough [12]. *Camellia sinensis* commonly known as tea plant is probably the most widely consumed beverage in the world [13]. Even though the tea plant is cultivated all over the world, it grows best in tropical and subtropical areas with adequate rainfalls, good drainage, and a slightly acidic soil [14].

## 2.0 MATERIALS

### 2.1 Collection and Identification of Plant Materials

The plant materials were collected from Ajaka, Igalamela/Odolu Local Government Area of Kogi State, Nigeria. The identities of the five plants were confirmed at the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, as *Zingiber officinale* (Voucher No.2261), *Phyllantus* spp (Voucher No. 900351) and *Camellia sinensis* (Voucher No.217).

### 2.2 Preparation of Aqueous Extract

The leaves were rinsed with distilled water and shade- dried for 5 days and thereafter pulverized, using electric blender. The crude powders obtained from the plants materials were mixed in the following proportion: *Zingiber officinale* (500g), *Phyllantus spp* (1000g) and *Camellia sinensis* Stem bark (500g) and extracted with 5000 ml (5L) of distilled water. After 48 hours, the mixture was filtered using muslin sieve followed by Whatmann filter paper (No 1). The filtrate was then dried and the extract was stored in the refrigerator for subsequent analysis. The extract will henceforth be referred to as ZPC.

### 2.3 Chemicals and Materials

Chlorpropamide (Diabenese) was purchased locally, Streptozotocin was purchased from the country representative of Sigma Chemical, St. Loius USA while a digital glucometer and corresponding test strips (ACCU- CHECK) ~~was~~ were purchased from a pharmacy store. All other chemicals used were of ~~analar~~ analaR-grade and obtained commercially.

**2.4 Animals**

Twenty Male Wistar rats weighing between 120-200g were used for this study. They were fed daily with growers mash diet and were given free access to water, during the experimental period. The food and water ~~was~~ were replaced each day except on days prior to testing for their fasting glucose level. The rats were housed in well ventilated plastic cages which were cleaned once in three days, with naturally illuminated condition of 12 hour light and 12 hour dark.

**2.5 Experimental Design**

**2.5.1 Acute toxicity study**

The oral median lethal dose ($LD_{50}$) of the extract was determined in rats according to the method described by [15]. The study was carried out in two phases. In the first phase, nine rats were randomized into three groups of three rats which were given 10, 100, and 1000mg extract/kg body weight. The rats were kept under the same conditions and observed for signs of toxicity which included but were not limited to paw- licking, stretching, respiratory distress and mortality for the first 4h and thereafter daily for two weeks. Based on the results of the initial phase, the following doses- 1600, 1900 and 5000mg extract/kg body weight were administered to another set of three groups of three rats in the second phase. These rats were also monitored closely for the first 4 h after treatment and subsequently daily for~~1~~ 4 days for signs of toxicity and/or mortality. The results obtained in the second phase were used to calculate the $LD_{50.}$

**2.5.2 Induction of diabetes**

The animals were injected intramuscularly with a single dose of 50mg/kg of the body weight streptozotocin. Diabetes was confirmed by the presence of fasting plasma glucose level above 250mg/dl on the third day post administration of streptozotocin.

### 2.5.3 Grouping and treatment

Twenty five (25) diabetic rats were weighed and randomly divided into five (5) groups of five rats each and treated daily for 28 days as follows:

Group I (Non- diabetic control): Normal saline only

Group II: (Diabetic control) Normal saline only

Group III: 250mg/kg b.w of chlorpropamide (an anti-diabetic drug)

Group IV: 250 mg/kg b.w of ZPC

Group V: 500 mg/kg b.w of ZPC

### 2.5.4 Assay of Fasting Blood Glucose Level

The ACCU-CHEK Glucometer with its corresponding test strips was used to determine the fasting blood glucose levels of the rats.

### 2.5.5 Estimation of Body Weight

The body weight of the rats was monitored weekly throughout the duration of the study.

### 2.5.6 Preparation of plasma for assays

After 28 days, the animals were fasted for 12 hours (overnight), after which they were sacrificed by cervical dislocation and blood collected by cardiac puncture using 5 ml syringes. A portion of the blood was dispensed into EDTA anticoagulant bottles for the estimation of haematological parameters (like pack cell volume, haemoglobin concentration, white blood cell count etc) using an automated haemoglobin machine. Another portion of the blood was dispensed into plain bottles, allowed to clot and centrifuged at 3600rpm for 15 minutes and the clear sera aspirated off using a Pasteur pipette and stored at – $4^0$c in a refrigerator.

### 2.6.1.1Assay for serum total cholesterol:

The serum level of total cholesterol was quantified after enzymatic hydrolysis and oxidation of the sample as described by the method of [16].

**2.6.1.2Assay for serum triglyceride:**

The serum triglyceride level was determined after enzymatic hydrolysis of the sample with lipases as described by the method of [17]

**2.6.1.3Assay for serum high density lipoprotein cholesterol:**

The serum level of HDL-C was estimated by the method of [18].

**2.6.1.4 Determination of serum low-density lipoprotein cholesterol:**

The serum level of (LDL-C) was calculated according to the method of [19] using the equation below:

~~LDL-C = TG/5-HDL-C~~ LDL-C = Tchol - TG/5 - HDL-C

Comment [FA1]: ?

Comment [FA2]: This is the correct Friedewald equation

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## 2.7 Statistical Analysis

Statistical analysis was carried out using SPSS version 20.0. All the data were expressed as mean ± SEM and the statistical differences between the means were determined by one way analysis of variance (ANOVA) which was followed by Duncan test and difference between means at $P > 0.05$ were considered significant.

## 3.0 RESULTS

### 3.1 Acute Toxicity Study

The results of acute toxicity studies showed no mortality or physical changes in skin and fur, eyes and mucus membrane, respiratory rate, circulatory signs, autonomic and central nervous system effects up to a dose of 5000 mg/kg of ZPC. The oral $LD_{50}$ of the extract was then taken to be > 5000 mg/kg.

### 3.2 Effect of ZPC on Fasting Blood Sugar (FBS) (mg/dl) of Streptozotocin Induced Diabetic Albino Rats

The effect of the polyherbal formulation and chlorpropamide on the FBS of diabetic Wistar rats is presented in Table 1. Administration of Streptozotocin ~~alloxan~~ significantly ($P<0.05$) elevated the FBS as seen on day 0 when the diabetic control and treatment groups are compared to the non-diabetic control. When compared to each other there was no significant ($P>0.05$) difference between the groups. Treatment with chlorpropamide and ZPC showed time and dose- dependent significant ($P< 0.05$) reduction in FBS on days 7, 14, 21 and 28 compared to Group 2 (diabetic control).

**Comment [FA3]:** ?
Diabete has been induced by streptozotocin treatment

**Table1: Effect of ZPC on Fasting Blood Sugar (FBS) (mg/dl) of Streptozotocin- Induced Diabetic Albino Rats**

| **Treatment** | **FBS (mg/dl)** | | | | |
|---|---|---|---|---|---|
| | **DAY 0** | **DAY 7** | **DAY 14** | **DAY 21** | **DAY 28** |
| **NDC (1ml dist. $H_2O$)** | 83.42± 3.65$^{a}$ | 86.1 ± 4.18$^{a}$ | 83.6 ± 6.33$^{a}$ | 81.5 ± 5.32$^{a}$ | 87.3 ± 4.51$^{a}$ |
| **DC (1ml dist. $H_2O$)** | 368.5±77.24$^{c}$ | 375.4±92.71$^{c}$ | 392.6±90.76$^{c}$ | 373.4±84.24$^{c}$ | 386.4±77.32$^{c}$ |
| **CHLOR (250 mg/kg)** | 348.4±67.25$^{c}$ | 300.4±61.23$^{b}$ | 290.4±73.45$^{b}$ | 289.2±73.35$^{b}$ | 279.3±62.33$^{b}$ |
| **ZPC (250 mg/kg)** | 352.2±71.21$^{c}$ | 325.4±58.29$^{bc}$ | 320.2±43.13$^{bc}$ | 273.4±31.32$^{b}$ | 253.0±45.01$^{b}$ |
| **ZPC (500 mg/kg)** | 349.5±45.11$^{c}$ | 275.2±41.46$^{b}$ | 207.2±53.45$^{ab}$ | 187.1±45.23$^{ab}$ | 161.3±36.44$^{ab}$ |

DC= diabetic control, NDC= non- diabetic control, CHLOR= Chlorpropamide, Data are presented as mean ± SD of FBS. Data was analysed by one- way ANOVA followed by Duncan post- hoc test for multiple comparisons, (n=5). Mean values having different lower case alphabets as superscripts are considered significant ($p< 0.05$) across the columns

### 3.3 Effect of ZPC on Body weight BW) (g) of Streptozotocin Induced Diabetic Albino Rats

Table 2 shows the effect of PZC and Chlorpropamide on the body weight of the Streptozotocin ~~alloxan~~- induced diabetic rats. Following alloxan administration, the body weight of rats in the treatment groups was significantly ($p< 0.05$) reduced compared to the non- diabetic control. The body weight of rats in the treatment groups showed no statistically significant ($P>0.05$) difference on days 7 and 14compared to diabetic control. However, there was no significant ($P<0.05$) difference between ZPC- treated and chlorpropamide- treated the groups when compared to the nondiabetic control on days 21 and 28.

**Comment [FA4]:** ?
Diabete has been induced by streptozotocin treatment

**Table 2: Effect of ZPC on Body weight (BW) (g) of Streptozotocin Induced Diabetic Albino Rats**

| Treatment | BW (g) | | | | |
|---|---|---|---|---|---|
| | DAY 0 | DAY 7 | DAY 14 | DAY 21 | DAY 28 |
| **NDC (1ml dist. $H_2O$)** | 162.2±11.33$^{b}$ | 168.2±13.46$^{b}$ | 172.7±12.15$^{b}$ | 175.5±11.19$^{b}$ | 179.4±14.52$^{b}$ |
| **DC (1ml dist. $H_2O$)** | 133.2±21.44$^{a}$ | 135.3±30.21$^{a}$ | 130.1±25.66$^{a}$ | 131.9±11.51$^{a}$ | 139.2±26.77$^{a}$ |
| **CHLOR (250 mg/kg)** | 139.3±26.42$^{a}$ | 141.2±15.26$^{a}$ | 144.1±26.77$^{a}$ | 155.4±22.33$^{ab}$ | 159.2±99.23$^{ab}$ |
| **ZPC (250 mg/kg)** | 140.1±12.78$^{a}$ | 139.6±25.44$^{a}$ | 145.4±19.16$^{a}$ | 151.3±19.44$^{ab}$ | 158.2±19.16$^{ab}$ |
| **ZPC (500 mg/kg)** | 138.9±34.33$^{a}$ | 142.3±9.97$^{a}$ | 143.3±12.12$^{a}$ | 150.7±11.14$^{ab}$ | 159.4±15.44$^{ab}$ |

DC= diabetic control, NDC= non- diabetic control, CHLOR= Chlorpropamide, Data are presented as mean ± SD of body weight (g). Data was analysed by one- way ANOVA followed by Duncan post- hoc test for multiple comparisons, (n=5). Mean values having different lower case alphabets as superscripts are considered significant ($p< 0.05$) across the columns.

### 3.4 Effect of ZPC on Serum Lipid Profile of Streptozotocin Induced Diabetic Albino Rats

Table 3 shows the effect of treatment with ZPC and chlorpropamide on the serum lipid profile of the Streptozotocin ~~alloxan~~- induced diabetic rats. Streptozotocin ~~Alloxa~~n caused a significant ($p<0.05$) elevation in total cholesterol, triglycerides and LDL concentrations and a corresponding significant ($p< 0.05$) difference in HDL concentration compared to non-diabetic control. ZPC at 250 and 500mg/kg and Chlorpropamide produced a significant ($p< 0.05$) decrease in the concentrations of cholesterol, triglyceride and LDL and a significant ($p< 0.05$) increase in the HDL concentration compared to diabetic control. The action of chlorpropamide and ZPC were comparable.

**Comment [FA5]:** ? Diabete has been induced by streptozotocin treatment

**Comment [FA6]:** ? Diabete has been induced by streptozotocin treatment

**Table 3: Effect of ZPC on Serum Lipid Profile of Streptozotocin Induced Diabetic Albino Rats**

| Treatment | Tchol (mg/dl) | TAG (mg/dl) | HDL ( mg/dl) | LDL (mg/dl) |
|---|---|---|---|---|
| **NDC (1ml dist. $H_2O$)** | $86.43\pm11.23^{a}$ | $74.43\pm5.37^{a}$ | $47.11\pm2.44^{c}$ | $24.20\pm3.44^{a}$ |
| **DC (1ml dist. $H_2O$)** | $160.43\pm88.44^{c}$ | $140.56\pm8.45^{c}$ | $13.33\pm5.66^{a}$ | $118.55\pm44.34^{c}$ |
| **CHLOR (250 mg/kg)** | $110.34\pm16.33^{b}$ | $85.26\pm7.77^{ab}$ | $39.41\pm6.87^{b}$ | $53.88\pm2.45^{b}$ |
| **ZPC (250 mg/kg)** | $117.14\pm13.11^{b}$ | $100.45\pm11.55^{b}$ | $37.24\pm3.23^{b}$ | $59.81\pm6.33^{b}$ |
| **ZPC (500 mg/kg)** | $115.48\pm8.66^{b}$ | $91.21\pm5.72^{ab}$ | $30.55\pm2.16^{b}$ | $66.68\pm8.45^{b}$ |

DC= diabetic control, NDC= non- diabetic control, CHLOR= Chlorpropamide, Data are presented as mean ± SD of body weight (g). Data was analysed by one- way ANOVA followed by Duncan post- hoc test for multiple comparisons, (n=5). Mean values having different lower case alphabets as superscripts are considered significant ($p< 0.05$) across the columns.

## 4.0 DISCUSSION

Diabetes mellitus is possibly the world's highest metabolic disorder, and as knowledge of its heterogeneity is advancing, the need for more appropriate therapy increases [20]. This disease causes many chronic complications such as vascular disease, retinopathy, neuropathy, kidney disease and heart disease. There is an increase demand to use natural products (herbs) with anti-diabetic activity due to the side effects associated with the use of insulin and oral hypoglycaemic agent [21]. The available literature shows that there are more than 1000 plant species showing hypoglycaemic activity [22]. In order to mimic the diabetic state Streptozotocin (50mg/kg) was used to induce albino rats intramuscularly as experimental models. Streptozotocin is known to selectively destroy the β-cells of the islet of Langerhans of the pancreas that functions in the regulation of insulin secretion and thus leads to increase in the blood concentration of glucose and type 1 diabetes mellitus [23]. Hence, there was evident hyperglycaemia (250-600 mg/dl) consequent to establishing the diabetic state in the animals.

The result of this study shows that the polyherbal formulation exhibited time and dose-dependent effect on the FBS of the rats. The anti-hyperglycaemic activity of the polyherbal formulation might be due to the high antioxidant content of the component plants. The hypoglycaemic activity of these antioxidants is due to their ability to scavenge the free radicals generated by alloxan hence, regenerating the destroyed beta-cells and subsequently, release of insulin [24]. ZPC might have also produced anti-hyperglycaemic activity through direct release of insulin by inhibiting the ATP- sensitive potassium channels in the membrane of the residual beta-cells just like sulfonylureas and meglitinides. It is also possible that the extract might have potentiated the action of insulin to stimulate glucose uptake and utilization by tissues, especially by the liver, skeletal muscle, and adipose tissue [25]. The goal of management of diabetes is to avoid or minimized chronic diabetic complications, as well as to avoid acute problems of hyperglycemia [26]. Hence ZPC might serve as a good alternative or as an adjunct to the oral hypoglycaemic agents.

**Comment [FA7]:** Author used streptozotocin instead of alloxan. Streptozotocin acts with a different mechanism of action (mainly DNA damage) and not increasing ROS as alloxan. So this sentence is uncorrect and need to be removed or reformulated.
A suggestion:
"It has been reported that the co-administration of ethanolic leaf extract of Moringa oleifera and metformin can be useful in ameliorating symptoms of diabetes in alloxan-induced diabetic rats [24]"

In this study, the body weights of diabetic rats decreased following streptozotocin treatment. This is in agreement with the symptoms of diabetes as stated by [27] to include unexplained weight loss. In diabetes mellitus, the gluconeogenic pathway is activated as a result of the inability of the cells to utilize glucose for energy production. Thus the weight loss in diabetes mellitus is linked to the utilization of muscle protein and excessive mobilization of fats from the adipose tissues for energy production in the gluconeogenic [28]. However, after treatment with chlorpropamide and ZPC, probably with improvement in glucose uptake by cells and subsequent reversal of gluconeogenesis, the body weights of the treated diabetic groups showed a steady increase throughout the course of the experiment.

Dyslipidemia which includes not only quantitative but also qualitative abnormalities of lipoprotein plays a significant role in the proatherogenesis of vascular complications in diabetes mellitus [29]. Lowering of serum lipid levels through herbal or drug therapy seems to be associated with a decrease in the risk of vascular disease in diabetes [30]. In this study, following streptozotocin treatment, there was an elevation in serum concentration of total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C) and a decrease in HDL-C in rats. [31], [32] also reported increased plasma cholesterol, triglycerides, LDLC and decreased HDL-C in streptozocin induced hyperglycemic rats. Similar observations were reported by [24], [33], [34], [26]. According to [35], the observed increase in serum cholesterol level results from increased intestinal absorption and synthesis of cholesterol. [36] suggested that diabetes-induced hyperlipidemia is attributable to excess mobilization of fat from the adipose due to under utilization of glucose. Insulin deficiency and elevations of the counter-regulatory hormones lead to activation of enzymes (hormone-sensitive lipase) that stimulate lipolysis and enhanced release of free fatty acids from adipose tissues which are mobilized for energy purpose [29]. The excess fatty acids are afterwards accumulated in the liver and converted to triglyceride [37]. The unregulated action of lipolytic hormones on the fat depots is therefore responsible for the hyperlipidemia that characterizes diabetes [30]. In this study, treatment with the polyherbal formulation reduced cholesterol, triglyceride and LDL concentration with a corresponding increase in HDL concentration. This dyslipidemic activity of the plant might be as a result of high phenolic compound of the component plants. Flavonoids are known to increase HDL biosynthesis in the liver and the increase in HDL concentration possibly ~~enhanced~~ enhances the excretion of cholesterol. ~~While~~ Instead, a decrease in LDL concentration could possibly be due to enhanced reverse cholesterol transport and bile acid excretion through inhibition of apo B production, needed for LDL-C production, transport and binding [38]. Triglyceride concentration is also reduced following treatment with ZPC. ZPC might have acted through a number of ways to achieve this and this include an alteration in the level of interleukin-6 (IL-6) which mediates energy mobilization in the muscles and fat tissues [39].

Polyherbal formulations due to the synergy between the components are potent scavenger of free radicals helpful in combating the progression of various diseases with oxidative stress components such as atherosclerosis, diabetes mellitus among others [40]. This study has lent credence to this statement by proving the effectiveness of ZPC in controlling the hyperglycaemia and dyslipidemia that are usually associated with diabetic conditions. [41] also reported that medicinal plants, individually or as a polyherbal formulation, could be useful in the management of diabetic complications. Hence ZPC might serve as a good alternative or as an adjunct to the oral hypoglycaemic agents in the management of diabetes/ diabetic complications.

## 5.0 CONCLUSION

In conclusion, ZPC polyherbal formulation may serve as a good candidate for alternative and/or complimentary medicine in the management of diabetes as it possesses anti- hyperglycaemic and anti-dyslipidemic activities.

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