

Original Research Article

Effect of *Hunteriaumbellata* methanolic seed extracts on streptozotocin-induced diabetic albino rats

Abstract

This study investigated the inhibitory effect of alpha-amylase, LD50 and antidiabetic properties of the methanolic seed extract of *Hunteriaumbellata* and its effect on biochemical parameters in streptozotocin-induced diabetic rats. Twelve albino rats were used for the acute toxicity test, while thirty-five were divided randomly into seven groups of five in each group. Group 1 served as the normal control, Group 2 served as the diabetic control, Group 3 was treated with glycinorm at 50 mg/kg body weight, Group 4 was treated with extract at 200 mg/kg body weight, Group 5 was treated with 400 mg/kg body weight, Group 6 was treated with 600 mg/kg body weight and Group 7 was treated with 800 mg/kg body weight by oral administration. Diabetes was induced in albino rats by intraperitoneal injection of streptozotocin at a single dose of 120 mg/kg body weight into groups 3 to 7 and was fed with methanolic seed extract of *Hunteriaumbellata* for a period of 28 days. The oral acute (LD₅₀) toxicity study showed that extract did not cause mortality in any experimental animals even at the highest dose of 5000 mg/kg. Body weight and glucose levels were measured on days 0, 7, 14, 21 and 28. The animal was sacrificed on day 28. Serum biochemical parameters were analysed. Additionally, renal function tests, including potassium, sodium, and chloride levels and protein and albumin levels, were performed. The study also evaluated hematological parameters in the animals that were fed the methanolic seed extract of *Hunteriaumbellata*. The animals that received different methanolic seed extracts of *Hunteriaumbellata* showed significant ($P < 0.05$) reductions in blood glucose, serum liver enzymes, renal function biomarkers, packed cell volume and platelet counts and improved body weight. Conclusively, from this study, it has been demonstrated that the methanolic seed extract of *Hunteriaumbellata* may possess weight enhancing, antihyperglycemic, hepatoprotective, improved hematological values, and cell and organ protective activities.

Keywords: *Hunteriaumbellata*, Diabetes, Glycated hemoglobin, Antihyperglycemic.

Introduction

Diabetes is a disease associated with glucose metabolism resulting from defects in insulin secretion and action (WHO, 1999). Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves. It is characterized by hyperglycemia, glucosuria and several microvascular and macrovascular complications (Brownlee, 2001; Virella-Lopes and Virella, 2003).

The quest for a scientific understanding of the etiopathogenesis of diabetes mellitus (DM) and the ultimate development of definitive curative and/or prophylactic options in its management

have stimulated great scientific research interest in recent years (Bailey, 2001). Drug management of DM without associated untoward effects has also remained a challenge for orthodox medical practice. This has necessitated the exploration and screening of medicinal plants with acclaimed therapeutic efficacies in DM management as recommended by the World Health Organization (WHO) WHO Expert Committee on DM (WHO, 1980; WHO, 2002).

Hunteriaumbellata grows as either a shrub or small tree up to 22m (22 m (72 ft) tall, with a trunk diameter of up to 40 cm (16 inches). Its flowers feature a white, creamy or pale yellow corolla. The fruit is yellow and smooth. Its habitat is forests from sea level to 600 m (2000 ft) altitude (Bouquet and Debray, 2015). *Hunteria* is a genus of plants in the family Apocynaceae first described as a genus in 1824. It is native to Africa and to South and Southeast Asia (Ballay, 2014).

Hunteriaumbellata is used for the treatment of different varieties of diseases. Its numerous medicinal uses include fever, leprosy sores, stomach and liver problems, and as an anthelmintic, especially against internal worms (Burkill, 2016). Many alkaloids have been isolated from *Hunteriaumbellata*, but little is known about the pharmacological activities showing cerebrovascular and cardiovascular activities, which merits further research (Endresset *al.*, 2017).

The study was therefore designed to determine the effect of *Hunteriaumbellata* seeds on streptozotocin-induced albino rats.

Materials and Methods

Plant Materials

Hunteriaumbellata seeds were obtained from Ihie in IsialaNgwa North L.G. A, Abia State. The plant was identified and authenticated at the Department of Plant Sciences and Biotechnology, Abia State University, Uturu, Nigeria, by Prof. I. C Ogbonna. Voucher specimens were number ABSU/PSB/68 and were deposited at the Departmental Herbarium.

Sample preparation

Hunteriaumbellata seeds were washed with distilled water to remove dirt and contaminants. The seeds of the plants were dried in a shade oven and milled using an electric blender.

Preparation of plant extract

The extracts were prepared using the method described by Jones and Kinghorn (2012) with slight modifications. *Hunteriaumbellata* seeds (1000 g) were soaked in 300 ml of methanol for 24 hours, strained with muslin cloth, and then filtered using Whatman no. 1 filter paper. The filtrate was allowed to dry in the open air, and a dark greenish residue was left, which was the extract

that gave a yield of 53.10 g after extraction. Twenty grams (20 g) of the sample was dissolved in 10 ml of 3% Tween 80 and brought up to 100 ml with distilled water.

Experimental Animals

Thirty (35) male albino rats of the same stock assumed healthy were obtained from the animal house of Abia State University, Uturu. The animals were taken to the laboratory, where they were housed in plastic cages and placed on commercial feed bought from the local market produced by Nigeria Flour Mills and were allowed food and water *ad libitum*. Ethical principles in animal handling strictly adhered to.

Induction of Diabetes:

The rats were fasted for 18 h, and diabetes was induced by a single intravenous injection of freshly prepared solution of streptozotocin (55 mg/kg of body weight) in 0.1 M citrate buffer (pH 4.5) (Ravi *et al.*, 2004). The animals were allowed water (5% glucose solution) to protect them against the diabetogenic action of streptozotocin and subsequently fasted to avoid excessive accumulation of feeding glucose, which may antagonize the streptozotocin effect. Control rats were injected with citrate buffer alone. After 24 h of injection, fasting blood glucose levels were checked, and animals with levels above 13.9 d/L were considered diabetic (Ravi *et al.*, 2004).

Determination of blood glucose

Blood glucose was determined by pricking the tail of the rats with a needle after massaging. The glucose concentration was determined using an On-Call Plus glucometer on a weekly basis for four weeks. The weight of the rats was also noted.

Measurement of Body Weight

Bodyweight was measured on days 0,7,14,21, and28.Body weight was expressed as the mean body weight (g).

Experimental design

Animal Grouping

The animals were randomly placed into seven groups of ten animals each. **Group 1:** Served as normal control and were fed with rat feed and water *ad libitum*. No diabetes was induced. **Group 2:** The diabetic control groups were fed rat feed and water *ad libitum* after inducing diabetes. **Group 3:** The diabetic group was fed rat feed and was given oral glycinorm at 80 mg/kg body weight and allowed water and feed *ad libitum* after the induction of diabetes. **Group 4:** The diabetic group was fed rat feed and aqueous extracts of *Hunteriaumbellatamethanolic* seed extract at 200 mg/kg body weight by oral administration and allowed feed and water *ad libitum*.

Group 5: The diabetic group was fed rat feed and aqueous extracts of *Hunteriaumbellatamethanolic* extract at 400 mg/kg body weight by oral administration and allowed feed and water *ad libitum*. **Group 6:** The diabetic group was fed rat feed and aqueous extracts of *Hunteriaumbellatamethanolic* seed extract at 600 mg/kg body weight by oral administration and allowed feed and water *ad libitum*. **Group 7:** The diabetic group was fed rat feed and aqueous extracts of *Hunteriaumbellatamethanolic* seed extract at 800 mg/kg body weight by oral administration and allowed feed and water *ad libitum*.

Blood Collection

After 28 days of treatment with the extract, the animals were fasted overnight, anesthetized with chloroform and sacrificed. Blood from each animal was collected by cardiac puncture, and blood samples from each animal were collected into dry test tubes. The blood sample was divided into two groups. The first was dispensed into heparinized tube (EDTA) bottles for hematological analysis. The second was allowed to stand for approximately 15 minutes to clot and was further spun in a centrifuge. Serum was separated from the clot with a Pasteur pipette into sterile sample test tubes for the measurement and evaluation of liver enzymes, antioxidant activities, and serum electrolytes.

Estimation of Other Biochemical Parameters

On the 28th day, animals were sacrificed following ether anesthesia, and blood was collected via cardiac puncture. Blood samples collected were used to estimate the following:

Glycated Haemoglobin (HBA1c)

Glycated hemoglobin (HBA1c) was estimated using the appropriate commercial kits (Randox Laboratory UK). (Little *et al.*, 2008).

Liver enzymes

Determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT)

The colorimetric method described by Reitman and Frankel (1957) was employed.

Estimation of Alkaline Phosphatase (ALP) Activity

Alkaline phosphatase (ALP) activity was estimated by using an ALP test kit (Randox Diagnostics Ltd.)(Vroon,1990).

Determination of Bilirubin

The colorimetric method described by Jendrassik and Grof (1938) was employed.

Determination of Serum Potassium

The method employed was a modification of the colorimetric method described by Terri and Sesin (1958).

Determination of Serum Sodium

The method employed was a modification of the colorimetric method described by Maruna (1958) and Trinder (1951).

Determination of Serum Chloride

A modification of the colorimetric method described by Skeegs and Hochestrasser (1964) was used.

Determination of Serum Bicarbonate

The method employed was a modification of the enzymatic procedure described by Forrester *et al.* (1976).

Haematological analysis: A portion of the blood samples collected from the rats was dispensed into an ethylene diamine-tetra-acetic acid (EDTA) anticoagulant bottle from which the red blood cell (erythrocyte) count, white blood cell (leukocyte) count, differential leucocyte count, relative volume of corpuscles to plasma (packed cell volume or haematocrit) and haemoglobin count were determined.

Results

Table 1 :Acute toxicity profile of *Hunteriaumbellata*methanolic seed extracts

Doses (mg/kg bw)	Mortality	Physical observation
10	0/3	No sign of toxicity
100	0/3	No sign of toxicity
1000	0/3	No sign of toxicity
1600	0/3	No sign of toxicity
2900	0/3	weakness
5000	0/3	Weakness, redness of the eye

TABLE 2: The inhibitory effect of alpha-amylase at different concentrations.

Samples	5 mg/ml	1 mg/ml	2 mg/ml	4 mg/ml
<i>Hunteriaumbellata</i> methanolic seed extracts	0.71±0.32 ^a	6.80±1.34 ^b	20.48±1.24 ^c	21.06±0.93 ^d

Values represent the mean \pm SD for N=5. Values in the same row bearing the same letter of the alphabet are not significantly different from each other ($P < 0.05$).

The results above, show a significant difference ($p < 0.05$) at different concentrations of the extract.

The null hypothesis states that alpha amylase inhibition above 50 indicates high toxicity and hence low inhibitory activity. *Hunteriaumbellata*methanolic seed extracts at alpha-amylase concentrations of 5 mg/ml, 1 mg/ml and 4 mg/ml have an appreciable inhibitory activity of alpha amylase enzyme and less toxic effects. The extract of orange leaves may contain herbal bioactive compounds inhibiting enzyme activity, which is helpful in diabetic thereapies (Maria *et al.*, 2011)

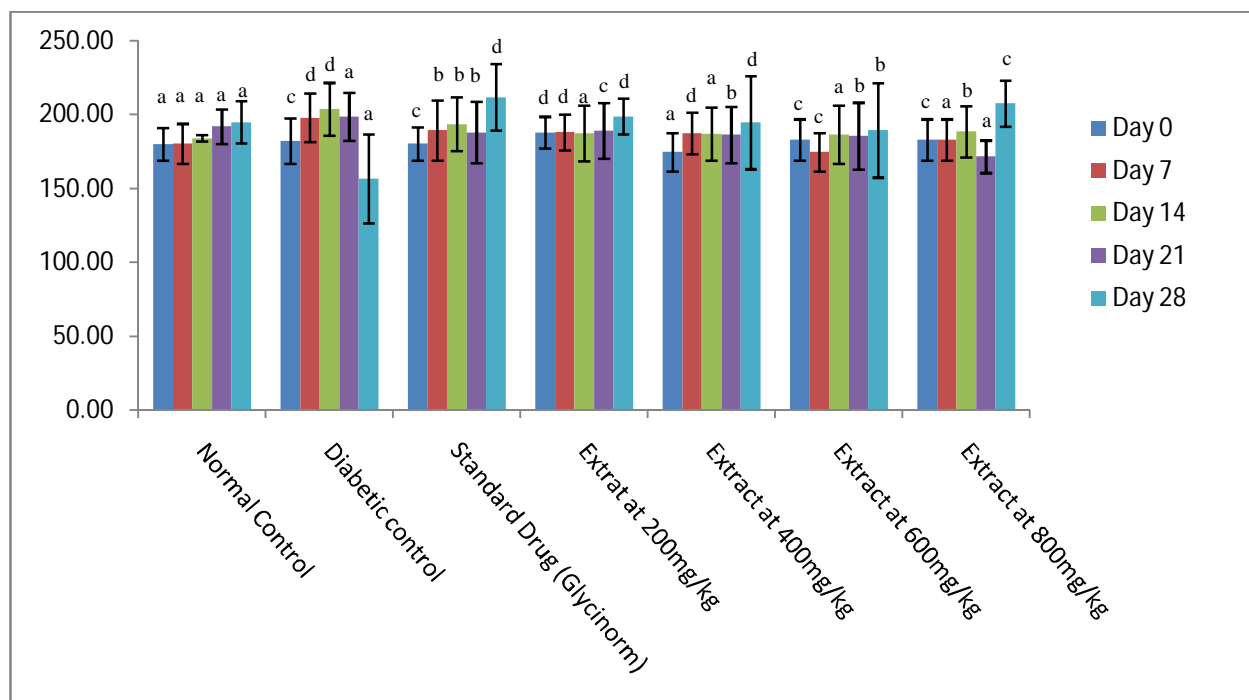


Fig 1: Effects of *Hunteriaumbellatamethanolic* seed extracts on mean body weight (g)

Values are the mean \pm SD for n=5. Values in the same column bearing the same letter of the letters are not significantly different ($P > 0.05$) from each other.

There was a significant increase ($P < 0.05$) in the body weight of animals that were fed with different diets (groups 3 to 7). There was a significant ($P < 0.05$) reduction in weight in the diabetic animals in group two (2) when compared to group one (1), which was the normal control group. Therefore, from this study, it was observed that there was a general improvement in body weight between the control diabetic group and animals in the other groups fed with different extracts from the different parts of *Hunteriaumbellatamethanolic* seed extracts. The test diabetic group had a significant ($P < 0.05$) reduction in weight, probably because of the diabetic condition. The animals from the other groups had a nonsignificant ($P > 0.05$) increase in weight, probably because of the administration of aqueous extracts of different parts of *Hunteriaumbellatamethanolic* seed extracts. (Akhere and Iyere, 2008).

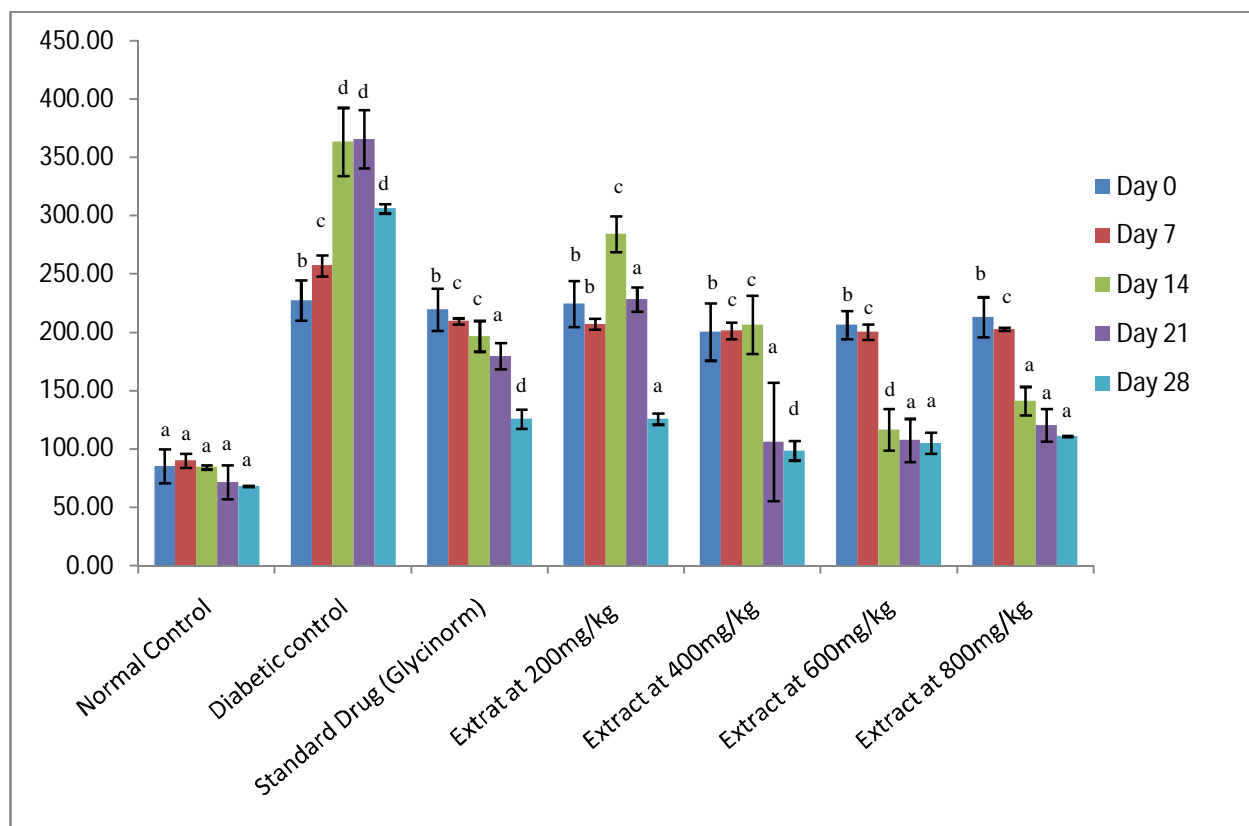


Fig 2: Effects of *Hunteriaumbellata* methanolic seed extracts on blood glucose levels (mg/dl)
 Values are the mean \pm SD, n=5. Values in the same column bearing the same letter of the letters are not significantly different ($P > 0.05$) from each other.

The results of the effect of *Hunteriaumbellata* seed methanolic extracts on the blood glucose levels of streptozotocin-induced diabetic rats are presented in Fig 2. The results revealed that STZ significantly increased ($P < 0.05$) the blood glucose level of the animals when compared to the normal control at days 7, 14, 21 and 28. Glycinorm (standard drug) and seed extract significantly ($P < 0.05$) reduced the glucose levels on different days. However, there was a significant ($P > 0.05$) difference in the blood glucose level of the animals between the normal and positive controls.

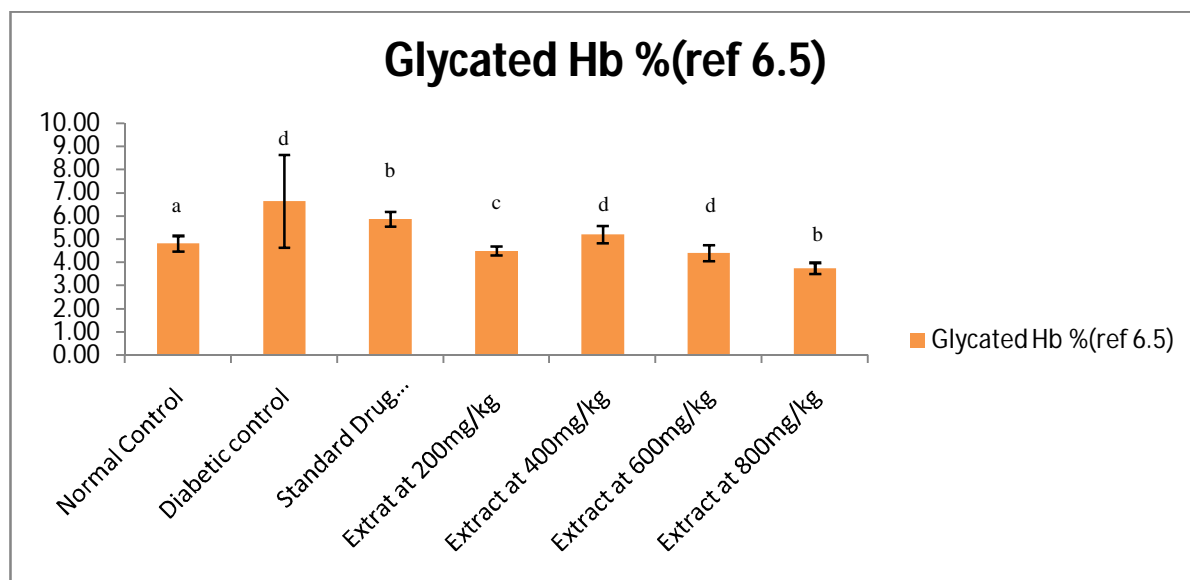


Fig 3: Effects of *Hunteriaumbellata* seed methanolic seed extracts on glycated hemoglobin (%) Day 28.

Values are the mean \pm SD for n=5. Values in the same column bearing the same letter of the letters are not significantly different ($P > 0.05$) from each other.

The glycated hemoglobin results showed elevated values in group 2 (diabetic control), while the standard drug was able to significantly ($P < 0.05$) reduce the glycated hemoglobin below those of the normal. The groups treated with different extract concentrations had significantly reduced glycated hemoglobin levels ($P < 0.05$).

It is, therefore, possible that the extract of *Hunteriaumbellata* seed methanolic extract plants may possess active substances that scavenge the free radicals of glucose oxidation protein glycation and oxidative degeneration or probably upregulate insulin secretion.

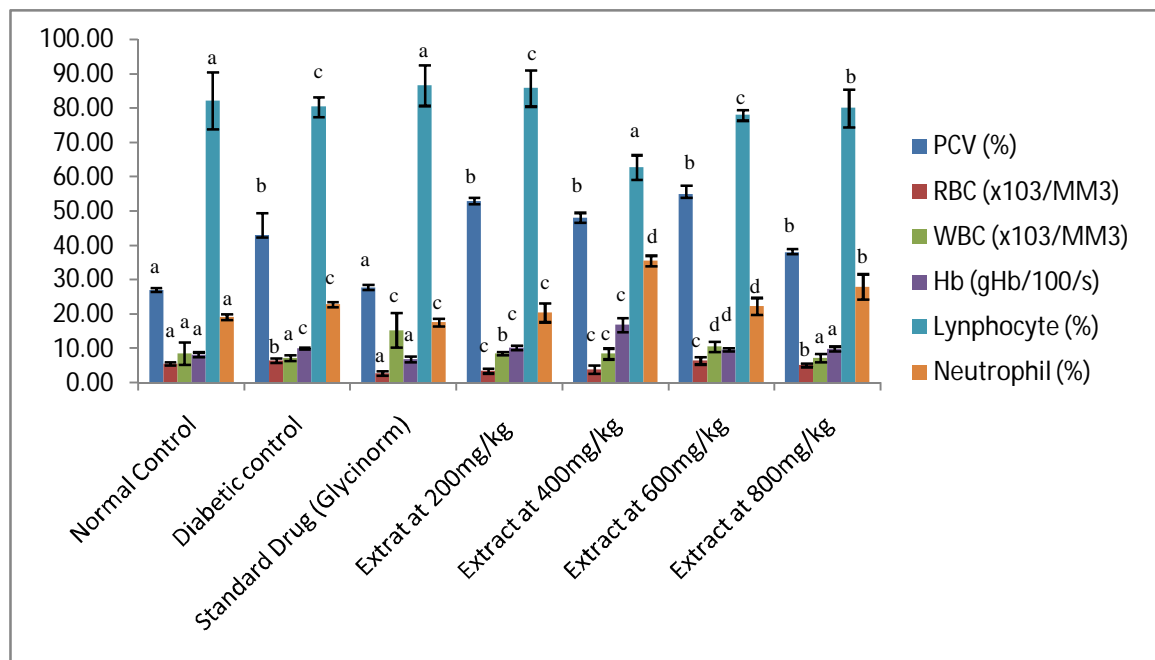


Fig 4. Effect of *Hunteria umbellata* methanolic seed extract on haematological indices in streptozotocin-induced diabetic rats

Values are means of triplicate determinations \pm standard deviation. Means along the same column with different superscripts are significantly different ($p < 0.05$).

There was a significant ($P < 0.05$) increase in Hb, RBC neutrophil and lymphocyte counts in the test animals when compared with untreated diabetic animals.

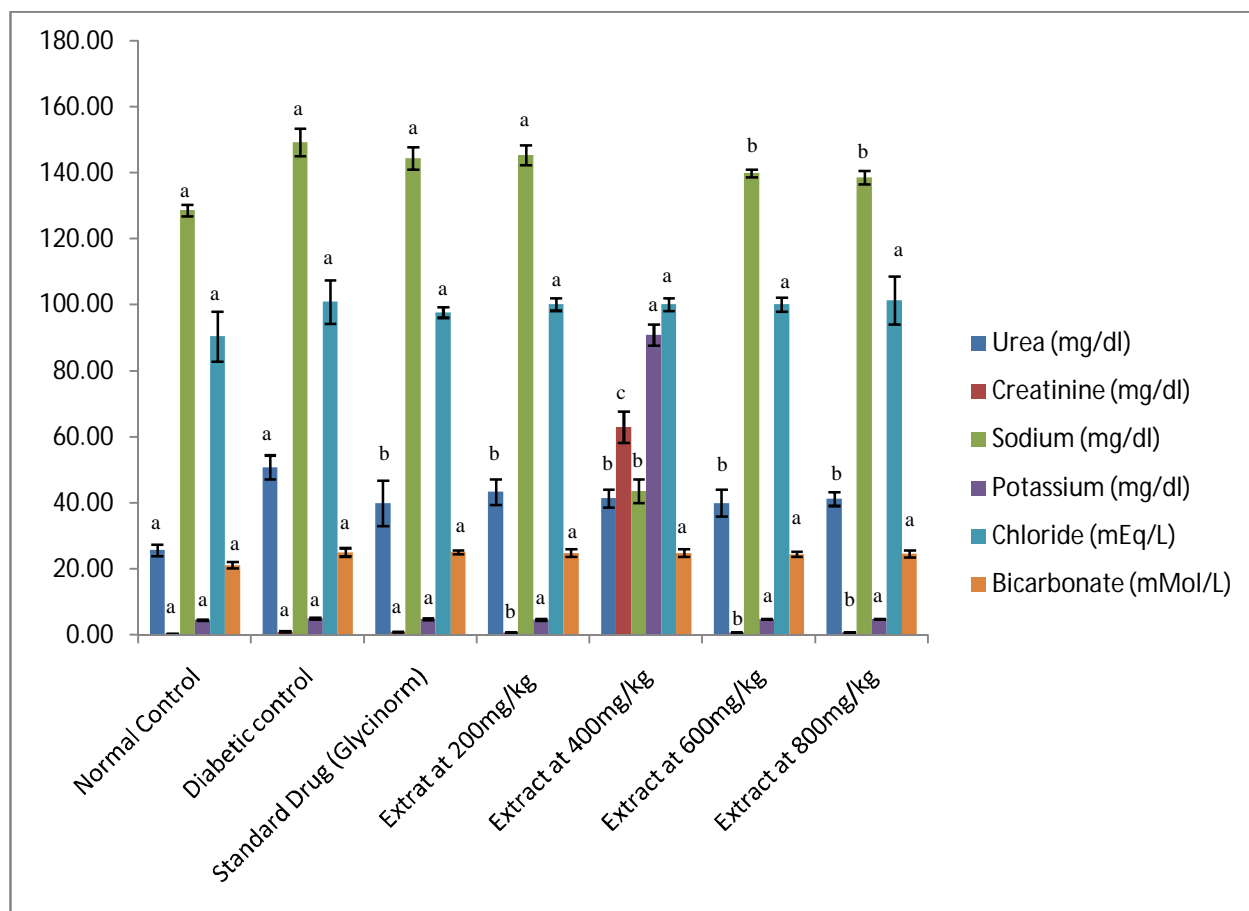


Fig 5: Effect of *Hunteriaumbellatamethanolic* seed extract on renal biomarkers of streptozotocin-induced diabetic rats

Values are means of triplicate determinations \pm standard deviation. Means along the same column with different superscripts are significantly different ($p < 0.05$)

The effect of *Hunteriaumbellatamethanolic* seed extract on the renal biomarkers of streptozotocin-induced diabetic rats. The results revealed that streptozotocin caused a significant increase ($P < 0.05$) in the renal biomarkers of the experimental animals when compared to the normal control. The administration of Glycinorm and *Hunteriaumbellatamethanolic* seed extract at varying concentrations of the extract significantly ($P < 0.05$) reduced serum levels of urea and creatinine. However, a nonsignificant difference ($P > 0.05$) was observed in the serum levels of sodium and bicarbonate following the administration of the seed extract and standard drug. The results further revealed that there was a nonsignificant difference ($P > 0.05$) in the serum levels of chloride and potassium ions among the experimental groups.

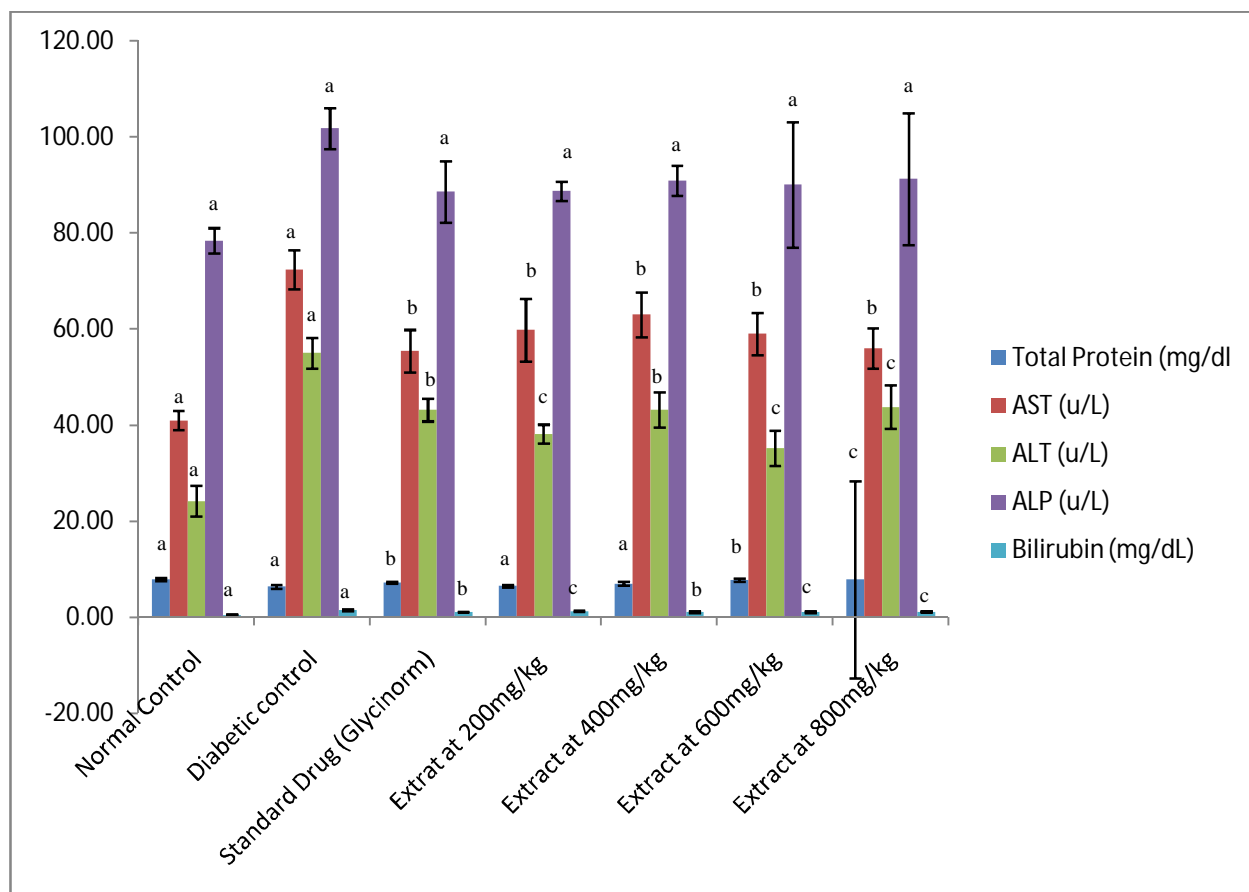


Fig 6: Effect of *Hunteriaumbellata* seed methanolic extracts on the liver enzyme indices of streptozotocin-induced diabetic rats

Values are means of triplicate determinations \pm standard deviation. Means along the same column with different superscripts are significantly different ($p < 0.05$)

The results of the effect of the aqueous extract of *Hunteriaumbellata* seed methanolic extracts on the hepatocellular indices of streptozotocin-induced diabetic rats are presented in Fig 6. The results revealed that the administration of streptozotocin resulted in a significant ($P < 0.05$) increase in serum total protein, AST, ALT, ALP, and bilirubin levels when compared to the control. This abnormality in serum hepatocellular biomarkers was significantly reduced ($P < 0.05$) following the administration of glycinorm and *Hunteriaumbellata* seed methanolic extracts at varying concentrations. There were no significant differences ($P < 0.05$) in serum levels of total protein between the normal control and *Hunteriaumbellata* seed methanolic extracts at 600 mg/kg body weight and animals that received glyphosate at 80 mg/bw.

Discussion

Alpha-amylase is an important enzyme that hydrolyzes dietary starch during carbohydrate metabolism. In the present study, the potent inhibitory effects of *Hunteriaumbellata* seed methanolic extracts on α -amylase activity are an indication that this plant would be beneficial in keeping the blood glucose level low by delaying the digestion of carbohydrates and thus reducing the concentration of postprandial plasma glucose. This inhibitory activity of the extract could be attributed to the presence of antioxidant phytochemicals, including flavonoids, tannins and saponins, which have been reported to inhibit α -amylase activity and preserve β -cell integrity, thus protecting against the development of insulin resistance in type 2 diabetic patients (Sarma *et al.*, 2010).

A preliminary toxicity study of the extract showed that in a single dose, the plant extract had no adverse effect up to a concentration of 5000 mg/kgb.wt.

The results of the effect of *Hunteriaumbellata* seed methanolic extracts on the blood glucose levels of streptozotocin-induced diabetic rats are presented in Table 2. The results revealed that STZ significantly increased ($P < 0.05$) the blood glucose level of the animals when compared to the normal control at days 7, 14, 21 and 28. Glycinorm (standard drug) and plant extract significantly ($P < 0.05$) reduced the glucose levels on different days. However, there was a significant ($P > 0.05$) difference in the blood glucose level of the animals between the normal and positive controls.

This study also showed that the *Hunteriaumbellata* seed methanolic extracts significantly reduced blood glucose levels ($p < 0.05$) in diabetic rats. This reduction is similar to that reported for other plants Perez *et al.*, (2003); Islam, (2011); Sasidharan *et al.*, (2011) and Gaamoussiet *et al.*, (2010). Such an effect may be explained in part by either a decrease in the rate of intestinal glucose absorption (Hamden *et al.*, 2011 and Gupta *et al.*, 2012) or an increase in peripheral glucose utilization, Porchezian *et al.*, (2000); Gupta *et al.*, (2012). Some authors have proposed increased catabolism of glucose due to GLUT4 translocation to the plasma membrane in muscle and brown adipose cells (Adisa *et al.*, 2011; Shen *et al.*, 2010), with upregulation of the uncoupling protein-1 in brown adipose tissue and hepatic gluconeogenesis (Gupta *et al.*, 2012), causing hyperinsulinaemia or enhancement of peripheral glucose utilization (Adisa *et al.*, 2011;

Adeneyeet *al.*, 2010). Moreover, a possible stimulatory mechanism on the few surviving β -cells has been considered, which could allow the release of more insulin (Pepeoet *al.*, 2004). Our results suggest that the *Hunteriaumbellata* seed methanolicextractsmay act by stimulating the few remaining β -cells with the subsequent release of more insulin, instead of pointing to the regeneration of β -cells of the islets as responsible for the insulin increase.

Increasing blood glucose levels in diabetes leads to the overproduction of free radicals, defined as an imbalance between oxidants and antioxidants. Glucose auto-oxidizes in the presence of transition metal ions, generating oxygen-free radicals and making the membrane vulnerable to oxidative damage (Bakirelet *al.*, 2008). Insulin and C-peptide are the products of the enzymatic cleavage of proinsulin and are secreted into the circulation in equimolar concentrations.

Glycated hemoglobin (hemoglobin A1c, HbA1c, A1C) is a form of hemoglobin that is covalently bound to glucose (WHO, 2018). Glycated hemoglobin causes an increase in highly reactive free radicals inside blood cells. Radicals alter blood cell membrane properties. This leads to blood cell aggregation and increased blood viscosity, which results in impaired blood flow (Saleh, 2015). The glycated hemoglobin results showed elevated values in group 2 (diabetic control), while the standard drug was able to significantly ($P < 0.05$) reduce the glycated hemoglobin below those of the normal. The groups treated with different plant parts had significantly reduced glycated hemoglobin levels ($P < 0.05$).

It is, therefore, possible that *Hunteriaumbellata* seed methanolicextractsmay possess active substances that scavenges the free radicals of glucose oxidation protein glycation and oxidative degeneration or probably an upregulat-regulation in insulin secretion.

The assessment of hematological parameters to determine possible alterations in the levels of biomolecules such as enzymes, metabolic products, hematology and normal function(Magalhaeset *al.*, 2008). The occurrence of anemia in diabetes mellitus has been reported to be due to the increased nonenzymatic glycosylation of RBC membrane proteins (Oyedemiet *al.*, 2011). Oxidation of these proteins and hyperglycemia in diabetes mellitus causes an increase in the production of lipid peroxides that leads to hemolysis of RBCs (Arun and Ramesh, 2002). Diabetes mellitus causes the development of hypochromic anemia due to

a decrease in the iron content of the body resulting from oxidative stress associated with the condition (Colaket *et al.*, 2012). In this study, red blood cell parameters such as Hb were studied to investigate the beneficial effect of *Hunteriaumbellata* seed methanolic extract on the anemic status of diabetic rats. These results may be attributed to infection of the normal body systems of the rats. Additionally, a significant ($P < 0.05$) increase was observed in neutrophil and lymphocyte counts in the test animals when compared with untreated diabetic animals. The presence of some bioactive compounds with the ability to stimulate the production of white blood cells in the extract could be responsible for the observed result in the treated rats. The extract at different dosages significantly increased the levels of WBCs and lymphocytes as well as a standard drug when compared with the untreated diabetic group. The neutrophils increased significantly in the standard drug group compared to the normal group. The RBC and Hb parameters are used mathematically to check the concentration of hemoglobin and to look at the restoration of oxygen-carrying capacity of the blood.

It was also observed in this study that there was a significant reduction in glycated hemoglobin (Table 3) in the test diabetic control group when compared with animals in the test groups fed with *Hunteriaumbellata* seed methanolic extracts ($P < 0.05$). It is, therefore, possible that the *Hunteriaumbellata* seed methanolic extracts may possess active substances that scavenge the free radicals of glucose oxidation, protein glycation and oxidative degeneration or probably an improvement in insulin secretion. The result of this study is, however, supported by the work of Gupta *et al.* (2001), which demonstrated that Fenugreek seeds showed improved glycemic control with a significant decrease in HBA1c on day 28 of treatment compared with n-STZ control rats.

Hepatic impairment is one problem of diabetes mellitus, and it is obvious by elevation of these liver biomarkers, such as ALT, ALP, and AST activities, so an increase in these liver biomarkers will provide a reliable or good indicator of functional integrity of the liver as well as treatment outcome (Shittuset *et al.*, 2017; Shittuet *et al.*, 2015 and Yusuf *et al.*, 2018) in diabetes conditions. In this study, the elevated levels of ALT activities in diabetic untreated are a marker of plasma membrane and hepatic impairment, which will adversely prevent amino acid and carbohydrate metabolism and thus affect ATP production (Yusuf *et al.*, 2018). This observation of ALT and

AST activities is an indication that diabetes selectively affects transaminase activities (Lawalet *al.*,2015).

The administration of the extract and the standard drug caused a significant restoration of the plasma membrane and liver functional integration, as evidenced by decreased ALP AST and ALP activities.

Bilirubin is an endogenous anion product of hemoglobin degradation in red blood cells. The improvement in the concentrations of bilirubin in rats tested with a group is an indication of increased glucose mobilization into cells leading to more efficient glucose utilization (Lin *et al.*, 2010).

Additionally, total protein plays major roles in assessing the integrity of the kidney and liver (Lawalet *al.*,2016). The observed increase in the diabetic untreated rate could be attributed to the elevation of different acute phase proteins, such as globulin and fibrinogen, in diabetes mellitus (Malawed and Usha, 2011).

This is in accordance with the finding of Ladeaet *al.*,2006, who reported increased plasma levels of acute-phase proteins in adult patients with type 1 and 2 diabetes. Therefore, the increase in total protein observed in this study could lead to dehydration, which is injurious to cellular hemostasis, and harms the normal metabolic activities of the liver and consequently the health of the animals (Shittuet *al.*,2015). The reduction in total protein involved the mechanisms responsible for alterations, including a change in the relative abundance of specific mRNAs and a decrease in total cellular RNA.

The kidneys eliminate metabolic wastes such as urea nitrogen, uric acid, creatinine and ions, and thus, the optimum chemical composition of body fluids is maintained (Shokeenet *al.*, 2008). Hyperglycemia causes renal dysfunction, such as acute glomerulonephritis, nephrosclerosis and even tubular necrosis, resulting in abnormal excretion of urea and creatinine, thereby elevating serum urea nitrogen and creatinine (Stambeet *al.*, 2003, Cheng *et al.*, 2005 and Jaramillo-Juarez *et al.*, 2008). An elevation in the serum levels of urea and creatinine in clinical analyses presupposes renal dysfunction (Sood, 2006 and Mehrdadet *al.*, 2011). Creatine is a breakdown waste product formed in the muscle in creatine phosphate metabolism. It is synthesized in the

liver, passes into circulation and is taken up almost entirely by skeletal muscle for energy production. While urea is the main end product of protein catabolism, amino acid deamination takes place in the liver, which is also the site of the urea cycle, where ammonia is converted into urea and excreted through urine. In this study, blood urea, creatinine and sodium levels were increased (Table 3) following the induction of diabetes, indicating renal dysfunction.

Creatinine and urea concentrations are useful clinical indicators of renal integrity (Bashir *et al.*,2015). Creatinine is a waste product of muscular metabolism, while urea is a byproduct of protein metabolism. During renal impairment, the excretion of these metabolites by the kidney is altered and thus accumulates in the plasma (Lawal *et al.*,2016).

The observed significant increase in urea and creatinine concentrations in diabetes is an indication of renal impairment. The disease condition must have either altered the metabolism of creatinine, leading to increased syntheses or decreased tubular excretion (Zilva *et al.*,1991).

These findings corroborated those of the studies by Aldle *et al.*, 2003 and Judgkay *et al.*, 2017, which showed that increased plasma urea levels in diabetes patients may indicate a prerenal problem. Furthermore, the significant alteration in the concentration of sodium, chloride and carbonate suggests that the integrity of renal tubules with regard to the excretion and maintenance of normal levels of these electrolytes in the system of the animal has been compromised (Bashir *et al.*,2015).

Conclusion

Hunteriaumbellata seed methanolic extracts can reverse the hyperglycemia associated with diabetes mellitus, and the ability of seed methanolic extracts to reduce the levels of glycated hemoglobin, which is a marker showing effective diabetic control and management, was also demonstrated. This study also showed a reduction in the levels of AST, ALT, ALP, and bilirubin (liver enzymes), which are markers of cellular damage, following the induction of diabetes and complications associated with the disease state by treatment with an aqueous extract of *Hunteriaumbellata* seed methanolic extracts. Additionally, this study demonstrated the renoprotective potentials of *Hunteriaumbellata* seed methanolic extracts by decreasing the levels of urea and creatinine, improving protein and albumin levels and markedly improving electrolyte levels.

The study also demonstrated marked improvement in hematological parameters, especially the red blood cell count and enhanced platelet count.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References

- Adeneye, A.A., Adeyemi, O.O. and Agbaje, E.O. (2010) Anti-obesity and antihyperlipidaemic effect of *Hunteria umbellata* seed extract in experimental hyperlipidaemia. *J Ethnopharmacol.* 2010 Jul 20; 130(2):307-14.
- Adisa, R.A., Choudhary, M.I. and Olorunsogo, O.O. (2011) Hypoglycemic activity of *Buchholziacoriacea* (Capparaceae) seeds in streptozotocin-induced diabetic rats and mice. *Exp. Toxicol. Pathol.* 2011 Nov; 63(7-8):619-25.
- Akhere, A. and O. Iyere, 2008. Effect of *Irvingiagrandidifolia*, *Urenalobata* and *Carica papaya* on the oxidative status of normal rabbit. *International Journal of Nutritional Wellness.* 6.(3): 4-10
- Arun GS, Ramesh KG (2002). Improvement of insulin sensitivity by perindopril in spontaneously hypertensive and streptozotocin-diabetic rats. *Indian Journal of Pharmacology* 34:156-164.
- Atangwho, I.J., Ebong, P.O., Eyong, M.U., Eteng, M.U. and Uboh, F.E. (2007). *Vernonia amygdalina* Del.: A potential prophylactic antidiabetic agent in lipids complication. *Global Journal of Pure and Applied Science*, 13(1): 103-106.
- Bakirel, T., Bakirel, U., Keleş, O. U., Ulgen, S. G. and Yardibi, H. (2008) " In vivo assessment of antidiabetic and antioxidant activities of rosemary (*Rosmarinus officinalis*) in alloxan - diabetic rabbits", *J Ethnopharmacol.*, 116: 64-73.
- Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. *Nature*, 414(6865):813-820

- Chauhan HVS.(2008) Veterinary clinical and laboratory diagnosis.3rd Edition Jaypee Publishers. New Delhi.
- Cheng, H., Liu, W., and Ai, X. (2005).Protective effect of curcumin on myocardial ischaemia–reperfusion injury in rats.*Zhong Yao Cai*. 28:920-922. 32.
- Colak S, GeyiKoghu F, Aslan A, Deniz GY (2012).Effects of Lichen extracts on hematological parameters of rats with experimental insulin-dependent diabetes mellitus. *Toxicology and Industrial Health*,30(10):878-87
- Forrester, J.S., Diamond, G., Chatterjee, K., et al. (1976) Medical therapy of acute myocardial infarction by application of hemodynamic subsets (second of two parts). The New England Journal of Medicine, 295, 1404-1413.
- Gaamoussi, F., Israili, Z.H., and Lyoussi B. (2010)Hypoglycemic and hypolipidemic effects of an aqueous extract of *Chamaerops humilis* leaves in obese, hyperglycemic and hyperlipidemicMerionesshawirats. *Pak J Pharm Sci*. 23(2):212-9.
- Gupta, R., Sharma, A.K., Sharma, M.C. and Gupta, R.S. (2012) Antioxidant activity and protection of pancreatic β -cells by embelin in streptozotocin-induced diabetes.*J Diabetes*.4(3):248-256.ZA.
- Hamden, K., Jaouadi, B., Zaraï, N., Rebai, T., Carreau, S., and Elfeki, A. (2011)Inhibitory effects of estrogens on digestive enzymes, insulin deficiency, and pancreas toxicity in diabetic rats. *J Physiol.Biochem*. 67(1):121-128.
- Islam MS (2011) Effects of the aqueous extract of white tea (*Camellia sinensis*) in a streptozotocin-induced diabetes model of rats. *Phytomedicine*. 19(1):25-31.
- Jaramillo-Juarez, F., Rodriguez-Vazquez, M.L., Rincon-Sanchez, A.R., Consolacion Martinez, M. and Ortiz, G.G.(2008). Acute renal failure induced by carbon tetrachloride in rats with hepatic cirrhosis. *Annals Hepatology*.7(4):331-338.
- Jendrassik, L. and Grof, P. (1938) Simplified Photometric Methods for the Determination of Bilirubin. *Biochemical Journal*, 297, 81-89.
- Lopes-Virella M. F and Virella G. (2003).Lipoprotein autoantibodies: measurement and significance.*Clin.Diagn. Lab. Immunol*.10, 499–505
- Magalhaes P, Appell H, Duart J. (2008). Involvement of advanced glycation end products in the pathogenesis of diabetes complication: the protective role of regular physical activity. *European Revive for Aging Physical Activities*5(1):17-29
- Maruna, R.F.L. (1958).Colorimetric determination of sodium in human serum and plasma.*Clin. Chem. Acta*, 2: 581-581.
- Mehrdad M. and Mozghan G.P.(2011) Effect of Zingiber Extract on Histopathologic Changes in Mice Kidneys, *Research Journal of Applied Sciences*, 6(5):1-3

- Oyedemi SO, Yakubu MT, Afolayan AJ (2011). Antidiabetic activities of the aqueous leaf extract of *Leonotis leonurus* in streptozotocin-induced diabetic rats. *Journal of Medicinal Plants Research* 5(1):119-125
- Pepato, M.T, Baviera, A.M., Vendramini, R.C. and Brunetti, I.L.(2004) Evaluation of toxicity after one-months treatment with *Bauhinia forficata* decoction in streptozotocin-induced diabetic rats. *BMC Complement Altern Med.* 8; 4(7):7-9.
- Pèrez, C., Canal, J.R. and Torres, M.D. (2003) Experimental diabetes treated with ficus carica extract: effect on oxidative stress parameters. *Acta Diabetol.* 40(1):3-8.
- Porchezian, E, Ansari S.H. and Shreedharan, N.K.(2000) Antihyperglycemic activity of *Euphrasia officinale* leaves. *Fitoterapia.* 71(5):522-6.
- Ravi, S., D'Odorico, P., Over, T.M, and Zobeck, T.M. (2004), On the effect of air humidity on soil susceptibility to wind erosion: The case of air-dry soils, *Geophys. Res. Lett.*, 31, L09501,
- Reitman, S. and Frankel, S. (1957) A Colorimetric Method for the Determination of Serum Glutamic Oxaloacetic and Glutamic Pyruvic Transaminases. *American Journal of Clinical Pathology*, 28, 56-63.
- Saleh, Jumana. (2015). "Glycated hemoglobin and its spinoffs: Cardiovascular disease markers or risk factors?". *World Journal of Cardiology.* 7 (8): 449–53.
- Sasidharan, S., Sumathi, V. Jegathambigai, N.R., and Latha, L.Y.(2011) Antihyperglycaemic effects of ethanol extracts of *Carica papaya* and *Pandanus amaryfollius* leaf in streptozotocin-induced diabetic mice. *Nat Prod Res.* 25(20):1982-7.
- Shen, Y., Fukushima, M., Ito, Y., Muraki, E., Hosono, T., Seki, T. and Ariga, T.(2010) Verification of the antidiabetic effects of cinnamon (*Cinnamomum zeylanicum*) using insulin-uncontrolled type 1 diabetic rats and cultured adipocytes. *Biosci. Biotechnol Biochem.* 74(12):2418-25.
- Shokeen, P., Anand, P., Murali, Y.K. and Tandon, V.(2008) Antidiabetic activity of 50% ethanolic extract of *Ricinus communis* and its purified fractions. *Food and Chemical Toxicology.* 46(11):3458-66 31.
- Skeggs, L.T. and Hochstrasser, H.C.(1964). Thiocyanate (colorimetric) Method of Chloride Estimation. *J. Clin. Chem.* 10: 918.
- Sood, R.(2006) Medical Laboratory Technology, Jaypee Brothers Medical Publishers Limited, New Delhi.
- Stambe, C., Atkins, R.C, Tesch, G.H., Kapoun, A.M., Hill, P.A., Schreiner, G.F. and NikolicPaterson, D.J.(2003) Blockade of p38 {alpha} MAPK ameliorates acute inflammatory renal injury in rat anti-GBM glomerulonephritis. *Journal of the American Society of Nephrology.* 14(2):338–351.

- Terri, A.E. and Sesin, P.G. (1958).Determination of serum potassium by using sodium tetraphenyl boron method.*American Journal of Clinical Pathology*, 29(1): 86–90.
- Trinder, P. (1951). Calorimetric determination of sodium in human serum and plasma. *Analyst*, 76: 596-596.
- WHO (2018) Use of GlycatedHaemoglobin (HbA1C) in the Diagnosis of Diabetes Mellitus: Abbreviated Report of a WHO Consultation. Geneva: World Health Organization. 2018. p. 2.
- World Health Organization (WHO)(1999) Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Part 1: Diagnosis and Classification of Diabetes. WHO/NCD/NCS 99, 2, *Geneva*, 19;1-58
- World Health Organization, (1980). The WHO Expert Committee on diabetes mellitus. Technical Report Series No. 646.http://whqlibdoc.who.int/trs/WHO_TRS_646.pdf
- World Health Organization, (2002). WHO launches the first global strategy on traditional and alternative medicine. WHO Press release 38. <http://www.who.int/mediacentre/news/release/release38/en>