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Anti-diabetic potentials of stem-bark extracts of *Terminalia avicennioides* on alloxan-induced diabetic rats

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Abstract

This study evaluated the extracts of *Terminalia avicennioides* stem-bark for their effect on alloxan-induced diabetes mellitus in male wistar rats. The powdered stem-bark of the plant was extracted with 70% methanol to yield crude methanol extract (CME). The CME was dissolved in distilled water to obtain the aqueous methanol (AME), then partitioned using ethyl acetate and hexane to obtain ethyl acetate (EAE) and hexane (HEX) extracts respectively. Fifty five alloxan induced diabetic rats were randomly divided into 11 groups of five rats each. Rats in groups1 and 2 received distilled water (DW) and 1% Tween 80 (TW80) at 5 ml/kg, respectively. Rats in group 3 received glibenclamide (GLB) 10 mg/kg. Rats in groups 4, 5, 6 and 7 were administered with 100 mg/kg of CME, AME, EAE and HEX, respectively. Similarly, rats in groups 8, 9, 10 and 11 were given the extracts at 200 mg/kg, respectively. In addition, three normoglycaemic rats were used as non-diabetic non-treated control (group 12). All treatments and diabetic inductions were done intraperitoneally. Treatments started 72 hours after induction of diabetes which served as day 1, then on day 4, 7, 14 and 21. Blood glucose level in all the rats was monitored weekly for three weeks. All animals were sacrificed by jugular venipuncture and serum levels of total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides (TGL) were determined. The extracts significantly ($p<0.05$) decreased the levels of blood glucose, serum total cholesterol, serum triglyceride and serum low density lipoprotein in diabetic rats when compared with the negative controls. However, HDL level was significantly ($p<0.05$) increased in the HEX, EAE and CME (100 mg/kg) treated rats. In conclusion, the extracts exhibited an anti-hyperglycaemic and anti-hyper lipidaemic effect which validates the use of the plant in traditional treatment of diabetes mellitus.

Keywords: Alloxan, anti-diabetic, extracts, rats, *Terminalia avicennioides*

Introduction

Diabetes mellitus is a metabolic disease characterized primarily by high blood glucose levels (Baena-Diez *et al*., 2016), resulting from defects in insulin secretion, insulin action or both (American

Diabetes Association, 2010). It is the most common metabolic disorder. Among adults aged 20-79 years, it had a global prevalence of about 8.8% in the year 2017, and estimated to hit 9.9% by the year 2045 (IDF, 2017). It is a common disease in dogs and cats. The most common form of diabetes in dogs resembles type 1 diabetes in humans whereas the most common form in cats resembles type 2 diabetes in humans (Nelson & Reusch, 2014). It has an estimated incidence as high as 1: 66 (1.52%) for dogs and 1:800 for cats. The disease in dogs occurs most frequently in the mature or older female, and is often associated with estrus. In contrast, male cats appear to be more commonly affected than female (Kaneko *et al*., 2008).

Although, insulin and oral hypoglycaemic drugs have been the mainstay of the management of diabetes mellitus, there are many proven side effects for these compounds (Cole *et al*., 2013). Insulin treatment in type 2 diabetics can lead to weight gain (Jansen *et al.,* 2014), pain and hypoglycaemia (Petznick, 2011). Many animals appear to be resistant to insulin, while others, especially cats are very sensitive to its effects and therefore prone to bouts of hypoglycaemia (Wallace & Kirk, 1990). Also, there is an issue of rapid insulin metabolism, whereby insulin wears off quickly in some animals. This leads to the animal requiring a second injection during the day or even additional injections during the day (Brooks, 2010; Ramsey & Maclauchlan, 2013).

Currently, the use of herbal drugs is being explored in the management of diabetes mellitus (Amraie *et al.*, 2015).

Terminalia avicennioides is a yellowish brown, hard and durable wood, commonly found in the savannah region of West Africa (Mann *et al*., 2011). It is reported to have been used traditionally to treat a variety of diseases in both animals and humans, such as tuberculosis and cough (Mann *et al*., 2012) and also diabetes mellitus.

This study was thus aimed at evaluating the anti-diabetic effects of the stem-bark extracts of *T. avicennioides* in alloxan-induced diabetes mellitus in male Wistar rats.

Materials and Methods

Plant collection

Fresh stem-bark of *Terminalia avicennioides* was collected from the wilds around Kufena, Zaria, Nigeria, in the month of November. The flower, leaves and seeds of the plant were also collected and sent to the Herbarium, Department of Botany, Ahmadu Bello University, Zaria, Nigeria for identification, where a voucher specimen number 900239 was allocated for reference purpose.

Plant extraction and partitioning

The stem-bark of *T. avicennioides* was air-dried at room temperature and then pulverized. About 1kg was extracted with three litres of 70% aqueous methanol and evaporated to dryness in a water-bath at 45ºC. Fifty grams of the crude methanol extract (CME) were dissolved in 500 ml of distilled water to form an aqueous methanol extract (AME). The solution was transferred to a 1L separating funnel and 600 ml of hexane were added. The funnel was agitated gently, then allowed to stand for some hours after which the lower denser aqueous portion was collected into a conical flask. The remaining upper portion was dispensed into a clean conical flask to obtain the hexane extract (HEX). The procedure was repeated 3 more times and the HEX was pooled together. The remaining aqueous portion was then transferred to a separating funnel and 600 ml of ethyl acetate were added to obtain the ethyl acetate extract (EAE). The procedure was then repeated as described for the hexane fraction.

Phytochemical screening

The crude methanol (CME), aqueous methanol (AME), ethyl acetate (EAE) and hexane (HEX) extracts were all subjected to phytochemical screening using standard procedures (Evans, 2009).

Experimental animals

Adult male wistar rats weighing an average of 170 grams were obtained from the Animal House, Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, and maintained on standard feed. Water was provided *ad libitum*. The animals were kept in clean iron cages at room temperature throughout the study. The beddings were changed twice every week. Approval for the use of rats was obtained from Ethical Committee on Animal Use and Care, Ahmadu Bello University, Zaria. The approval number MSc/VET MED/ 29391/2012-2013 was allocated.

Acute toxicity studies of the extracts

Acute toxicity studies of the extracts of *T. avicennioides* were carried out using the OECD fixed dose procedure (OECD, 2001). Sixteen rats were used for the toxicity study. Four rats were selected at random and were individually administered through the intraperitoneal route with 5mg/kg of CME, AME, EAE and HEX. The same procedure was repeated for the extracts using 50, 300 and 2000

mg/kg respectively on other groups of rats. All the rats were observed for any sign of toxicity during the first four hours and thereafter for 24 and 48 hours. All surviving animals were kept under observation for 14 days.

Experimental design

Fifty five adult male Wistar rats were randomly allocated into 11 groups of five rats each. Diabetes was induced in the experimental rats by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg) dissolved in 0.9% cold normal saline solution (Tanko *et al.*, 2014). Seventy two hours after alloxan injection, rats with plasma glucose levels of >140 mg/dl were included in the study (Owoyele *et al.,* 2005; Ravichandra & Paarakh, 2013; Aleem *et al.*, 2014). Rats in groups 1 and 2 received DW (5 ml/kg) and TW80 (5 ml/kg), respectively and they served as negative controls. Rats in group 3 received standard drug glibenclamide (10 mg/kg). Rats in groups 4, 5, 6 and 7 were administered with 100 mg/kg of CME, AME, EAE and HEX respectively. Similarly, rats in groups 8, 9, 10 and 11 received CME, AME, EAE and HEX at 200 mg/kg. Three normoglycaemic rats were also included in the study and they served as group 12. All treatments were administered intraperitoneally. Treatments were done on days 1(72 hours after induction of diabetes), 4, 7, 14 and 21 of the experiment. The weight of each rat was monitored and taken on each treatment day, while blood glucose level was monitored weekly using a glucometer. At the end of the experiment (day 22), the animals were sacrificed under light chloroform anaesthesia. Blood was collected from the severed jugular vein of each rat into a centrifuge tube and centrifuged at 314 *g* for 10 min.

Evaluation of the effect of treatments on serum lipid profile

Serum total cholesterol (TC), serum high density lipoproteins (HDL), serum low density lipoproteins (LDL) and serum triglycerides (TGL) were analysed using an auto-analyser Vitalab Selectra XL, Flexor Company, The Netherlands.

Gross and histopathological examination

At the end of the experiment, each rat was sacrificed and the organs examined for visible gross lesions, which were recorded. The liver and pancreas were dissected out and preserved in 10% buffered formalin. Embedding was carried out a week later and the tissues were prepared for histopathology using the modified method described by Luna (1960). The slides were stained with haematoxylin and eosin and viewed under light microscope at different magnifications for any tissue or cellular morphological changes.

Statistical analysis

Data obtained were expressed as mean ± standard error of mean (±SEM). Analysis of variance (ANOVA) was used, followed by Tukey's post-hoc test for multiple comparisons of groups using Graph pad prism version 5. Values of $p<0.05$ were considered significant.

Results

Phytochemical screening

Carbohydrates, cardiac glycosides, saponins, triterpenes, flavonoids, tannins and alkaloids were detected in the crude methanol extract, aqueous methanol extract, and ethyl acetate extract. However, hexane extract contained only carbohydrates and cardiac glycosides.

Acute toxicity test

The extracts did not cause mortality on rats during the 14 days observation period. The extracts were considered safe up to a dose of 2000 mg/kg bodyweight.

Body weight changes of diabetic rats

There was a decrease in body weight of all alloxan-induced diabetic rats within the first few days of treatment, and it was significant ($p< 0.05$) on day 4 in all the extract treated groups, TW80, and the GLB groups when compared with the DW group. From day 4 to 7, the groups experienced a significant ($p< 0.05$) increase in body weight, except AME 100 and HEX 200 groups that experienced a decrease in body weight, when compared with DW control. Similarly, on day 14 there was significant ($p< 0.05$) increase in body weights, except those in CME 200 and GLB group that experienced a decrease in body weight by day 21 when compared to the DW group (table 1).

Blood glucose levels of diabetic rats

Alloxan induced diabetic rats had blood glucose levels greater than 140 mg/dl. By week 1, there was a decrease in blood glucose levels across the groups which was statistically significant ($p< 0.05$) in CME (100, 200 mg/kg), EAE (100 mg/kg), AME and HEX (200 mg/kg) respectively. By week 2, this decrease was sustained in the treated and GLB control groups and was statistically significant ($p< 0.05$) when compared to the DW group except in AME (200 mg/kg). At week 3, there was a non-significant increase in blood glucose levels across the groups except in the CME 200 group where decrease was sustained. CME (100 mg/kg) and HEX (200 mg/kg)

still maintained values below 140 mg/dl, and showed statistically significant ($p< 0.05$) difference from the non-treated diabetic control group. Normoglycaemic rats maintained normal blood glucose levels throughout the experiment (table 2).

Serum total cholesterol levels of diabetic rats

There was a significant decrease ($p< 0.05$) in total cholesterol levels in rats treated with all the extracts and glibenclamide (10 mg/kg). However, rats treated with CME (100 mg/kg) showed a non-significant decrease when compared to the DW group (figure I).

Serum high density lipoprotein levels of diabetic rats

The HDL levels in rats treated with AME, EAE and HEX (200 mg/kg) respectively showed a non-significant decrease, AME (100 mg/kg) and CME (200 mg/kg) showed a non-significant increase. However, rats treated with HEX, EAE and CME (100 mg/kg) showed significant ($p< 0.05$) increase in HDL levels when compared to DW group (figure II).

Serum low density lipoprotein levels of diabetic rats

There was a significant ($p< 0.05$) decrease in serum low density lipoprotein in the extract treated groups and glibenclamide (10 mg/kg) group when compared to the DW group except in EAE (100 mg/kg) where the decrease was not significant (figure III).

Table 1: Mean body weight of alloxan-induced diabetic rats treated with extracts of *Terminalia avicennioides* for three weeks

Treatment (mg/kg)	Day				
	1	4	7	14	21
Neutral control	190.40 ± 2.25	179.80 ± 1.07***	179.00 ± 2.10***	182.80 ± 1.16***	188.60 ± 0.40***
Distilled water (5 ml/kg)	143.00 ± 2.37	106.00 ± 1.58	93.00 ± 1.14	85.00 ± 1.84	105.00 ±1.84
Glibenclamide (10)	141.80 ± 1.20	132.80 ± 1.20***	137.80 ± 2.20***	147.00 ± 1.64***	140.80 ± 3.25***
1 % TW80 (5 ml/kg)	157.60 ± 2.66	154.60 ± 2.40***	160.00 ± 2.02***	161.60 ± 2.11***	164.00 ±1.70***
CME (100)	147.80 ± 0.97	130.60 ± 0.68***	145.00 ± 2.47***	143.00 ± 1.73***	149.40 ± 0.51***
CME (200)	143.00 ± 1.79	135.00 ± 1.30***	135.80 ± 1.24***	146.60 ± 1.63***	144.00 ±2.07***
AME (100)	141.60 ± 2.80	138.00 ± 1.41***	133.40 ± 1.33***	134.00 ± 0.83***	137.80 ±1.77***
AME (200)	146.80 ± 2.08	123.00 ± 1.00***	134.00 ± 1.58***	144.00 ± 1.41***	146.20 ±2.15***
EAE (100)	142.20 ± 0.86	134.80 ± 2.18***	139.40 ± 1.08***	138.20 ± 1.24***	153.60 ±1.72***
EAE (200)	155.40 ± 2.60	127.60 ± 1.03***	131.40 ± 1.75***	123.00 ± 2.00***	145.60 ± 1.29***
HEX (100)	148.00 ± 2.02	131.00 ± 0.71***	132.40 ± 1.12***	139.00 ± 2.28***	159.00 ±2.02***
HEX (200)	166.40 ± 2.29	134.00 ± 0.54***	130.60 ± 1.03***	144.00 ± 3.85***	149.80 ± 1.43***

* - Statistically significant ($0.01<P<0.05$)
** - Highly significant ($0.001<P\leq0.01$)
*** - Very highly significant ($P\leq0.001$)

Table 2: Mean blood glucose levels of alloxan-induced diabetic rats treated with extracts of *Terminalia avicennioides* for three weeks

Treatment (mg/kg)	Week			
	0	1	2	3
Neutral control	112.00 ± 6.53	126.00 ± 1.97***	120.20 ± 4.13***	119.40 ± 4.47**
Distilled water (5 ml/kg)	542.60 ± 23.83	445.00 ± 1.58	404.00 ± 0.00	413.00 ± 19.66
Glibenclamide (10)	454.60 ± 44.24	363.40 ± 10.84	298.00 ±20.26***	394.00 ± 21.17
1% TW80 (5 ml/kg)	528.40 ± 25.97	359.60 ± 63.91	305.80 ± 2.33***	365.00 ± 10.49
CME (100)	473.60 ± 36.14	88.60 ± 6.16***	99.80 ± 3.01***	138.00 ± 5.38**
CME (200)	500.00 ± 25.82	82.00 ± 5.65***	239.60 ± 11.99***	223.80 ± 2.87
AME (100)	545.40 ± 23.32	401.40 ± 12.17	288.00 ±20.08***	311.00 ± 9.8
AME (200)	517.80 ± 21.00	217.80 ± 4.03***	375.00 ± 6.96	365.80 ± 10.91
EAE (100)	472.80 ± 24.15	316.20 ± 17.79*	277.20 ±14.41***	318.80 ± 18.10
EAE (200)	537.00 ± 9.21	445.60 ± 21.16	298.00 ± 3.89***	422.00 ± 3.11
HEX (100)	551.40 ± 36.80	415.00 ± 4.11	123.00 ± 0.00***	124.00 ± 0.00**
HEX (200)	510.00 ± 9.42	308.80 ± 2.46**	232.20 ± 7.76***	280.20 ± 3.77

* - Statistically significant ($0.01<P<0.05$)
** - Highly significant ($0.001<P\leq0.01$)
*** - Very highly significant ($P\leq0.001$)

Serum triglyceride levels of diabetic rats

There was a significant ($p< 0.05$) decrease in all the extract treated groups when compared to the DW group except HEX (100 mg/kg) where there was an increase (figure IV).

Histopathology

In the CME treated rats, photomicrographs of the liver showed vascular congestion at (100 mg/kg) and congested sinusoids at 200 mg/kg (plate I). The pancreas showed partial regeneration of the islet cells (plate IV) at 100 mg/kg and no lesions at 200 mg/kg. In the AME treated rats, photomicrographs of the liver showed congested central vein (plate II), while the pancreas showed no observable lesions. Photomicrographs of the liver and pancreas of the rats treated with EAE (100 and 200 mg/kg) showed no observable lesions. In the HEX treated rats, photomicrographs of the liver showed slight focal hepatocyte necrosis at 100 mg/kg (plate II) and congested central vein at 200 mg/kg. The pancreas showed no observable lesions.

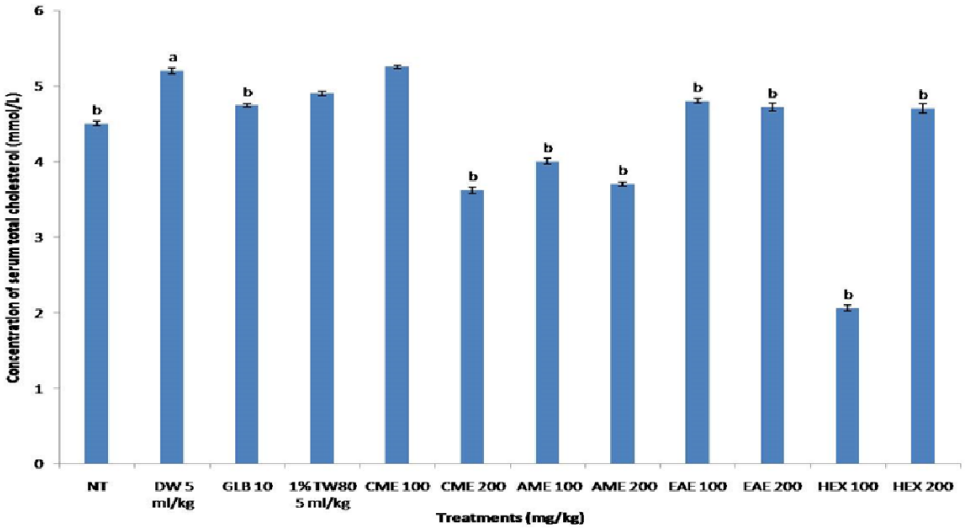

Figure I: Effect of crude methanol (CME), aqueous methanol (AME), ethyl acetate (EAE) and hexane (HEX) extracts of *Terminalia avicennioides* on concentration of serum total cholesterol level of diabetic rats. Glibenclamide (GLB) was used as treated control, while distilled water (DW) and tween-80 (1 %) were used as untreated control. Non-diabetic and non-treated group (NT) was used as neutral.

Means having different superscript letters (a,b) are significantly different ($p< 0.05$)

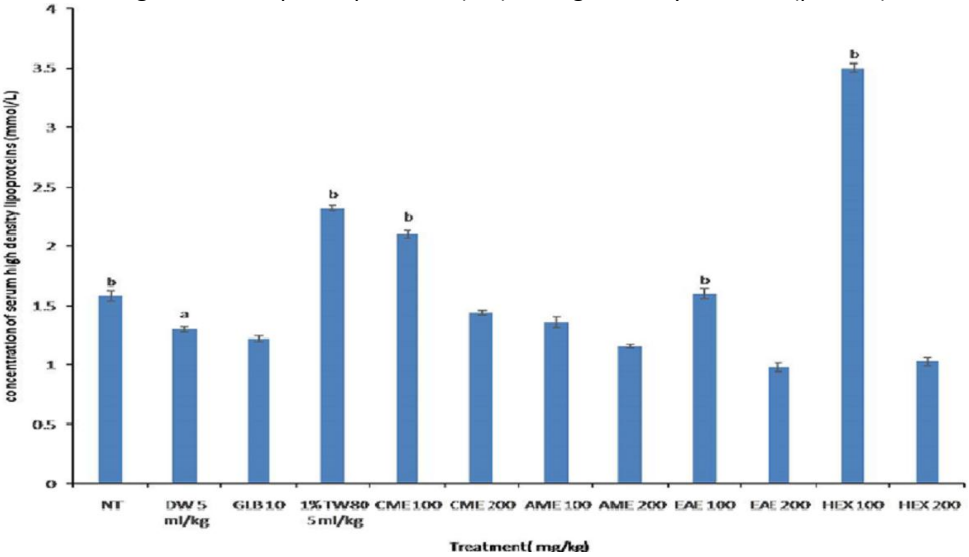

Figure II: Effect of crude methanol (CME), aqueous methanol (AME), ethyl acetate (EAE) and hexane (HEX) extracts of *Terminalia avicennioides* on concentration of serum high density lipoprotein level of diabetic rats. Glibenclamide (GLB) was used as treated control, while distilled water (DW) and tween-80 (1 %) were used as untreated control. Non-diabetic and non-treated group (NT) was used as neutral.

Means having different superscript letters (a, b) are significantly different ($p< 0.05$)

Discussion

The extracts of *T. avicennioides* stem- bark showed no toxicity up to 2000 mg/kg. This result did not agree with the work of Abdullahi *et al*. (2001) where the intra peritoneal LD_{50} was found to be between 871 – 917 mg/kg. A number of factors could be responsible for this variation. These include age of the plant, altitude and ecological factors. Age (Riet-Correa *et al*., 2011) and altitude (Ganzera *et al*., 2008) could affect the contents of active

ingredients in the plant. Liu *et al*. (2015), in a study confirmed that ecological factors such as annual precipitation, annual sunshine duration, soil pH, organic matter and also readily available potassium which vary based on geographical location significantly affect active ingredient contents of a plant. Similarly, Stefanucci *et al*. (2018), in a study using *Capparis spinosa* showed that geographical, climatic variations and also extraction techniques could have effect on the phytochemical composition of a plant.

In the present study, diabetes mellitus was induced using alloxan at a dose rate of 150 mg/kg as animals in the study had a blood glucose level of above 140 mg/dl. This is in agreement with the works of Owoyele *et al*. (2005); Aziz (2009); Ravichandra & Paarakh (2013).

Loss of body weight is a major consequence of diabetes in rats (Ramachandran *et al*., 2012). The decrease in body weight of experimental rats in the present study is in conformity with the findings of Aziz (2009) in rats. The reduction of body weight in diabetic rats is due to dehydration and catabolism of fats and proteins (Hakim *et al*., 1997); increased catabolic reaction leading to muscle wasting can be the cause of the reduced body weight gain in diabetic rats

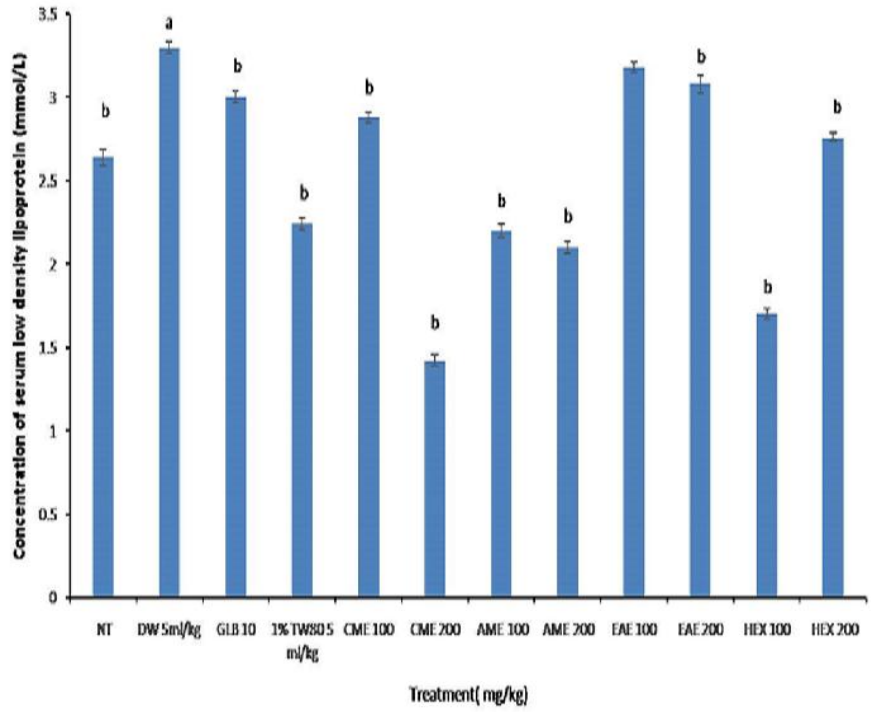

Figure III: Effect of crude methanol (CME), aqueous methanol (AME), ethyl acetate (EAE) and hexane (HEX) extracts of *Terminalia avicennioides* on concentration of serum low density lipoprotein level of diabetic rats. Glibenclamide (GLB) was used as treated control, while distilled water (DW) and tween-80 (1 %) were used as untreated control. Non-diabetic and non-treated group (NT) was used as neutral.
Means having different superscript letters (a, b) are significantly different ($p< 0.05$)

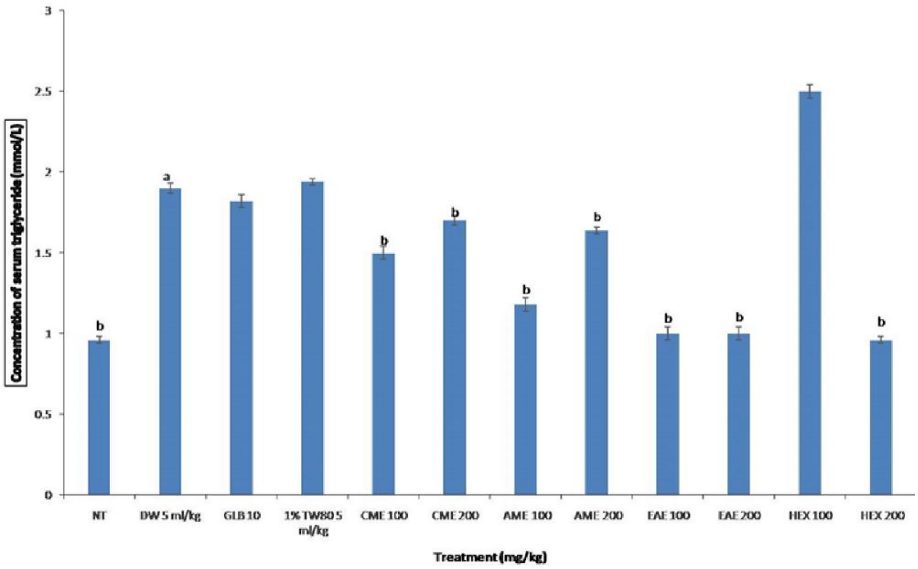

Figure IV: Effect of crude methanol (CME), aqueous methanol (AME), ethyl acetate (EAE) and hexane (HEX) extracts of Terminalia avicennioides on concentration of serum triglyceride level of diabetic rats. Glibenclamide (GLB) was used as treated control, while distilled water (DW) and tween-80 (1 %) were used as untreated control. Non-diabetic and non-treated group (NT) was used as neutral.
Means having different superscript letters (a, b) are significantly different ($p< 0.05$)

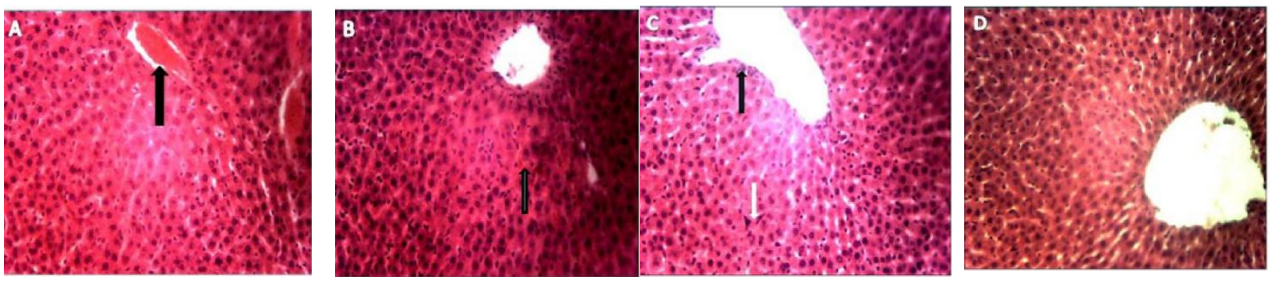

Plate I: Photomicrograph of liver of rats induced with diabetes mellitus using alloxan (150 mg/kg) and treated with CME (100 mg/kg) showing vascular congestion (arrow A) liver of rat treated with CME (200 mg/kg) showing congested sinusoids (arrow B), liver of rat treated with DW (5 ml/kg) showing lymphocytes (black arrow C) and kupfer cells hyperplasia (white arrow C), liver of rat in neutral group showing normal histology (D) (H and E × 400)

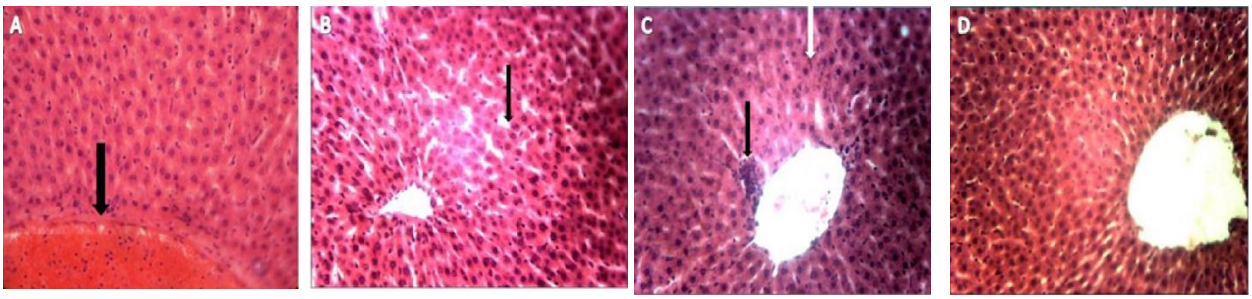

Plate II: Photomicrograph of liver of rats induced with diabetes mellitus using alloxan (150 mg/kg) and treated with AME (100 mg/kg) showing vascular congestion (arrow A), b. diabetic rat liver treated with HEX (100 mg/kg) showing focal hepatocyte necrosis (arrow B), diabetic rat liver treated with 1% TW80 (5 ml/kg) showing lymphocytes (big arrow C) and kupfer cell (small arrow C) hyperplasia, liver of rat in neutral group showing normal histology (D) (H and E × 400)

(Rajagopal & Sasikala, 2008). Also, glucose is prevented from gaining access to cells due to lack of insulin which leads to a deficiency of energy for cells (Holm, 1997). The improvement in body weight in the extract treated groups showed that the stem bark of *T. avicennioides* improved glucose utilisation by the cells. This improvement in body weight could be as a result of increased glucose uptake in peripheral tissues or inhibition of catabolism of fat (Ambika *et al*., 2013).

The anti-hyperglycaemic action of *T. avicennioides* extracts was better than that of the standard drug. Similar results were reported in the works of Pareek *et al*. (2009) and Petchi *et al*. (2013) using *Tridax procumbens* which produced better anti hyperglycaemic effect than the standard drug. They proposed that the possible mechanism by which *Tridax procumbens* produced anti-diabetic activity might be due to individual or synergistic activity of flavonoids and other active phyto-constituents of the plant. In this study,

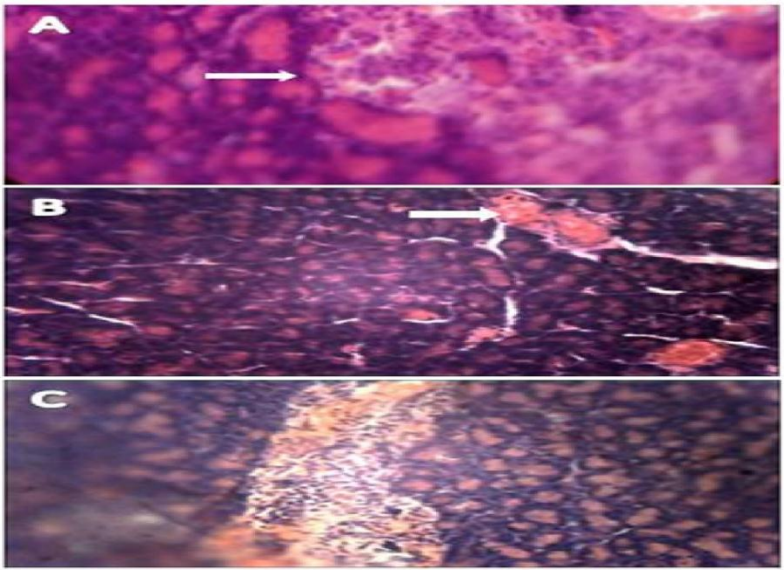

Plate III: Photomicrograph of pancreas of rats induced with diabetes mellitus using alloxan (150 mg/kg) and treated with CME (100 mg/kg) showing partial regeneration of islet cells (arrow A), DW (5 ml/kg) showing severe necrosis of islet cells (arrow B),pancreas of rat in neutral group showing normal histology (C) (H and E × 200)

flavonoids have been isolated as part of the phytoconstituents of *T. avicennioides* stem-bark extracts. Hyperglycaemia generates glucose auto-oxidation and auto-oxidative glycosylation of proteins which leads to increased oxidative stress by increasing reactive oxygen species (Shabeer *et al*., 2009). The increased oxidative stress results to depletion of majority of plasma antioxidants (Price *et al*., 2001; Valabhji *et al*., 2001; Mooradian, 2006). Expression of antioxidant enzymes is known to be very low in islet cells compared with other tissues and cells (Tiedgar *et al*., 1997), therefore, once beta cells are exposed to oxidative stress, they are rather sensitive to its damaging effects suggesting that oxidative stress, may in part mediate toxicity of hyperglycaemia. Flavonoids, saponins and alkaloids have potent antioxidant activities. Flavonoids can exert their antioxidant activity by various mechanisms, e.g., by scavenging or quenching free radicals, by chelating metal ions, or by inhibiting enzymatic systems responsible for free radical generation (Bláha *et al*., 2004; Dias *et al*., 2005). Flavonoids are also known to regenerate the damaged beta cells in the alloxan induced diabetic rats and act as insulin secretagogues (Alagammal *et al.,* 2012). In another study, ellagic acid which is also found in *T.avicennioides* (Shuaibu *et al*., 2007) exhibited anti hyperglycaemic activities. Its activity has been demonstrated in rats and the proposed mechanism of action was by increasing the peripheral utilisation of glucose and inhibiting glucose transporter activity from the intestine (Jadhav & Puchchakayala, 2012).

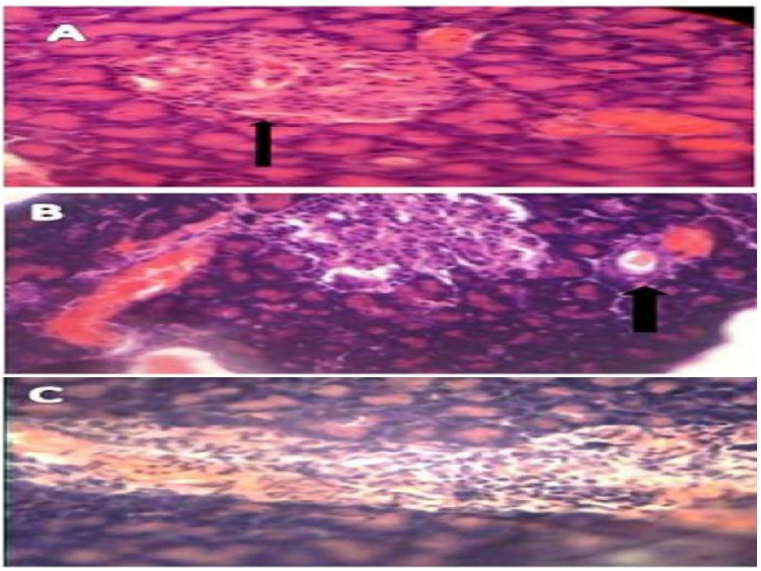

Plate IV: Photomicrograph of pancreas of rats induced with diabetes mellitus using alloxan (150 mg/kg) and treated with GLB (10 mg/kg) showing regeneration of islet cells (arrow A), 1% TW80 showing necrosis of islet cells (arrow B) and EAE (100 mg/kg) showing normal pancreas histology (arrow C)(H and E × 200)

Hyperlipidaemia, a common complication of both clinical and experimental diabetes mellitus, is characterised by increase in serum total cholesterol, triglycerides, low density lipoprotein and a decreased high density lipoprotein (Balamurugan *et al*., 2014). This marked hyperlipidaemia may be regarded as uninhibited actions of lipolytic hormones on fat depots (Patel *et al.*, 2012). In the present study, the stem bark extracts of *T. avicennioides* exhibited anti hyperlipidaemic effects. Similar anti hyperlipidaemic effects to that of *T. avicennioides* have been reported in studies using other members of the combretacae family such as *Terminalia arjuna* (Morshed *et al.*, 2011) *Terminalia catappa*(Ahmed *et al*., 2005), *Terminalia paniculata*, (Ramachandran *et al*., 2013), *Terminalia superba* (Desire *et al*., 2014). These effects could be attributed to the action of phytochemicals of plants. Many studies have demonstrated that saponins exert anti hyperlipidaemic effects such as the works of Han *et al*. (2000) and Patel *et al*. (2012), and the proposed mechanism of action of saponins is by inhibition of pancreatic lipase enzyme (Han *et al*., 2000). Saponins have also been reported to alleviate alloxan-induced diabetes by decreasing the level of lipid peroxidation and increasing the antioxidant defence system in the serum, liver and pancreas (Elekofehinti *et al*., 2013). Triterpenes have also been reported to have anti hyperlipidaemic activities. Gutierrez (2013) and Machaba *et al.* (2014) demonstrated this and proposed that the mechanism of action was by decreased degradation of glycogen and decreased rate of gluconeogenesis. Saponin and flavonoids isolated from plants exhibit hypoglycaemic effect by increasing insulin release from pancreatic beta cells, increasing peripheral glucose uptake and by reducing glucose absorption (Luo *et al*., 2005; Saravanan & Pari, 2008; Zambare *et al*., 2011). Tannins are insulin – like substances (Mota *et al*., 1985; Tullo, 2008) and mimic the effect of insulin on glucose metabolism and enhanced insulin secretion. These substances have been isolated from *T. avicennioides* stem bark in this study. *T. avicennioides* stem bark could also possibly

have exerted its anti hyperglycaemic effect by attenuating the body antioxidant system.

Photomicrographs of the liver showed congested central vein in some of the extract treated groups as well as the GLB group which is similar to the result in an anti-diabetic study by Oyebadejo *et al*. (2014). Photomicrographs of the pancreas revealed necrosis of islet cells in diabetic control and partial or complete restoration of islet cells in extract treated groups, which is similar to results obtained by Natarajan *et al*. (2012). Similar results were also obtained by Ahmed *et al*. (2005) using *Terminalia catappa*.

In conclusion, the results of this study showed that the stem-bark extracts of *T. Avicennioides* possess anti-diabetic effects in rats, the crude methanol and hexane extracts at 100 mg/kg were able to reduce blood glucose levels below 140 mg/dl. They also caused significant increase in serum high density lipoprotein, significant decrease in serum low density lipoprotein levels and also serum triglyceride levels. This validates the use of the plant traditionally for the management of diabetes mellitus.

Conflicts of Interest

The authors declare no conflicts of interest.

References

Abdullahi AL, Agho MO, Amos S, Gamaniel KS & Wambebe C (2001). Antidiarrhoeal activity of aqueous extract of *Terminalia avicennioindes* roots. *Phytotherapy Research*, **15**(5) : 431-434.

Ahmed SM, Vrushabendra SBM, Dhanapal PGR & Chandrashekara VM (2005).Anti-diabetic activity of *Terminalia catappa* Linn. leaf extracts in alloxan-induced diabetic rats. *Iranian Journal of Pharmacology and Therapeutics*, **4**(1): 36-39.

Alagammal M, Agnel RA & Mohan VR (2012). Anti-diabetic & anti hyperlipidaemic effect of *Polygala javana* DC on alloxan-induced diabetic rats. *International Research Journal of Pharmacology*, **3**(9): 231-234.

Aleem MA, Asad BS, Mohammed T, Khan RA, Ahmed MF, Anjum A & Ibrahim M (2014). Anti-diabetic activity of hydro alcoholic extracts of *Nardostachys jatamansi* in alloxan-induced diabetic rats. *British Journal of Medicine and Medical Research*, **4**: 4665-4673.

Ambika S, Saravanan R &Thirumavalavan K (2013). Anti-diabetic and anti hyperlipidaemic effect of *p*-hydroxycinnamic acid on streptozotocin-induced diabetic wistar rats. *Biomedical Aging Pathology*, **3**(4): 253-257.

American Diabetes Association (2010). Diagnosis and classification of diabetes mellitus. *Diabetes Care*, **33**(S1): S62-S69.

Amraie E, Farsani MK, Sadeghi L, Khan TN, Babadi VY & Adavi Z (2015). The effects of the aqueous extracts of alfalfa on blood glucose and lipids in alloxan-induced diabetic rats. *Interventional Medicine and Applied Science*, **7**(3): 124-128.

Aziz HO (2009). Effect of soybean seeds alone or in combination with insulin or glibenclamide on serum lipid profiles in alloxan-induced diabetic rats. *Iraqi Journal of Veterinary Sciences*, **23**(1): 17-23.

Baena-Díez JM, Peñafiel J, Subirana I, Ramos R, Elosua R, Marín-Ibañez A, Guembe MJ, Rigo F, Tormo-Díaz MJ, Moreno-Iribas C, Cabré JJ, Segura A, García-Lareo M, Gómez de la Cámara A, Lapetra J, Quesada M, Marrugat J, Medrano MJ, Berjón J, Frontera G, Gavrila D, Barricarte A, Basora J, García JM, Pavone NC, Lora-Pablos D, Mayoral E, Franch J, Mata M, Castell C, Frances A & Grau M (2016). Risk of cause-specific death in individuals with diabetes: A competing risks analysis. *Diabetes Care*, **39**(11): 1987–1995.

Balamurugan K, Nishanthini A & Mohan VR (2014). Anti-diabetic and anti hyperlipidaemic activity of ethanol extract of *Melastomamal abathricum Linn*. leaf in alloxan-induced diabetic rats. *Asian pacific Journal of Tropical Biomedicine*, **4**(1): S442-S448.

Bláha L, Kopp R, Šimková K & Mareš J (2004). Oxidative stress biomarkers are modulated in silver carp (*Hypophthalmichthys molitrix* Val.) exposed to microcystin-producing cyanobacterial water bloom. *Acta Veterinaria Brno,* **73**(4): 477-482.

Brooks W C (2010). Diabetes mellitus center.http://www.veterinarypartner.com/content.plx., retrieved 04-06-2015.

Cole JB, Stellpflug SJ, Ellsworth H, Anderson CP, Adams AB, Engebretsen KM & Holger JS (2013). A blinded, randomized, controlled trial of three doses of high-dose insulin in poison-induced cardiogenic shock. *Clinical Toxicology*, **51**(4): 201–207.

Desire DDP, Benoit NI, Jacqueline A, Gerard C, Pierre K & Theophile D (2014). Anti- diabetic activity of *Terminalia superba*

(*Combretaceae*) stem-bark extract in Streptozotocin-induced diabetic rats. *British Journal of Pharmaceutical Research,* **14**(11): 1300-1310.

Dias AS, Porawski M, Alonso M, Marroni N, Collado PS & González-Gallego J (2005). Quercetin decreases oxidative stress, NF-B activation, and iNOS over expression in liver of streptozotocin-induced diabetic rats. *Journal of Nutrition*, **135**(10): 2299-2304.

Elekofehinti OO, Kamdem JP, Kade IJ, Rocha JBT & Adanlawo IJ (2013). Hypoglycaemic, antiperoxidative and antihyperlipidaemic effects of saponins from *Solanum anguivi* Lam. fruits in alloxan-induced diabetic rats. *South African Journal of Botany*, **88**: 56-61.

Evans CW (2009). Pharmacognosy. Fifteenth edition. Saunders/ Elsevier, Edinburgh. Pp 257.

Ganzera M, Guggenberg M, Stuppner H & Zidorn C (2008). Altitudinal variation of secondary metabolite profiles in flowering head of *Matricana chamomila*.v. BONA *Planta Medica*, **74**(4): 453-457.

Gutierrez RMP (2013). Evaluation of the hypoglycaemic and hypolipidaemic effects of triterpenoids from *Prosthecheam ichuacana* in streptozotocin-induced type 2 diabetic mice. *Pharmacologia*, **4**: 170-179.

Hakim ZS, Patel BK & Goyal RK (1997). Effect of chronic ramipril treatment in streptozotocin-induced diabetic rats. *Indian Journal of Physiology and Pharmacology*, **41**(4): 353-360.

Han L, Xu B, Kimura Y, Zheng Y & Okuda H (2000). *Platycodi radix* affects lipid metabolism in mice with high fat diet induced obesity. *Journal of Nutrition*, **130**(11): 2760-2764.

Holm B. (1997). Diabetes mellitus in the dog. (Part 1). *European Journal of Companion Animal Practice*, **7**(2): 61-66.

IDF *Diabetes Atlas*. Eighth Edition (2017). http://www.diabetesatlas.org, retrieved 03-03-2019.

Jadhav R & Puchchakayala G (2012). Hypoglycaemic and anti-diabetic activity of Flavonoids; boswellic acid, ellagic acid, quercetin, rutin on streptozotocin-nicotinamide induced type 2 diabetic rats. *International Journal of Pharmacy and Pharmaceutical Sciences*, **4**(2): 251-256.

Jansen HJ, Vervoort GM, de Haan AF, Netten PM, de Grauw WJ & Tack CJ (2014). Diabetes-related distress, insulin dose, and age contribute to insulin-associated weight gain in patients with type 2 diabetes: results of a prospective study. *Diabetes Care*, **37**(10): 2710-2717.

Kaneko JJ, Harvey JW & Bress ML (2008). *Clinical Biochemistry of Domestic Animals*.P. 60.http://books.google.com.ng/booksdiabetes+mellitus+in+domestic+animals, retrieved 04-06-2015.

Liu W, Liu J, Yin D & Zhao X (2015). Influence of ecological factors on the production of active substances in the anti cancer plant *Sinopodo phylum hexandrum* (Royle). *PLos One*, **10**(4): 1-22.

Luo L, Yin HJ, Zhang Y, Jiang YR, Liu R & Shi DZ (2005). Effect of Ginseng fruit saponins on insulin sensitivity index in high fat-fed rats. *Journal of Chinese Integrative Medicine*, **3**(6): 463 – 465.

Luna LG (1960). *Manual of Histologic and Special Staining Techniques of the Armed Forces Institute of Pathology*. McGraw-Hill, New York. Pp 140-152.

Machaba KE, Cobongela SZZ, Mosa RA, Oladipupo LA, DjarovaTG & Opoku AR (2014). *In vivo* anti-hyperlipidemic activity of the triterpene from the stem-bark of *Protorhus longifolia* (Benrh) Engl. *Lipids in Health and Disease*, **13**(1): 131.

Mann A, Ajiboso OSO, Ajeigbe S, Gbate M & Isaiah S (2011). Evaluation of the wound healing activity of ethanol extract of *Terminalia avicennioides* root bark on two wound models in rats. *International Journal of Medicinal Aromatic Plants*, **1**(2): 95-100.

Mann A, Ibrahim K, Oyewale AO, Amupitan JO, Fatope MO & Okogun JI (2012). Isolation and elucidation of three triterpenoids and antimicrobial activity of *Terminalia avicennioides. American Journal of Organic Chemistry*, **2**(2): 14-20.

Mooradian AD (2006). Antioxidants and diabetes. *Nestle Nutrition Workshop Series. Clinical and Performance Program*, **11**: 107-122.

Morshed AD, Haque A, Rokeya B & Ali L (2011). Anti-hyperglycemic and lipid lowering effect of *Terminalia arjuna* bark extract on streptozotocin-induced type 2 diabetic model rats. *International Journal of Pharmacy and Pharmaceutical Sciences*, **3**(4): 450-454.

Mota ML, Thomas GJ & Barbosa M (1985). Anti-inflammatory actions of tannins isolated

from the bark of *Anacardium occidentale*. *Journal of Ethnopharmacology*, **13**(3): 289-300.

Natarajan A, Ahmed KSZ, Sundaresan S, Sivaraj A & Devi K (2012). Effect of aqueous flower extract of *Catharanthus roseus* on alloxan induced diabetes in male albino rats. *International Journal of Pharmaceutical Sciences and Drug Research*, **4**(2): 150-153.

Nelson RW & Reusch CE (2014). Animal models of disease: Classification and etiology of diabetes in dogs and cats. *Journal of Endocrinology*, **222** (3): T1-9.

OECD (2001). Guideline for testing of chemicals. Acute oral toxicity: fixed dose procedure. https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl420.pdf, retrieved 04-06-2015.

Owoyele VB, Adeyemi FM & Soladayo AO (2005). Effects of aqueous leaves extract of *Occimumgratissimum* (sweet basil) on alloxan induced diabetic rats. *Pharmacognosy Magazine,* **1** (2): 62-64.

Oyebadejo S, Bassey E, Oyewunmi A, Archibong V & Usoro E (2014). Histopathological study of the liver of alloxan-induced diabetic rats and macerated *Allium sativum* (garlic) ameliorative effect. *Asian Journal of Biomedical and Pharmaceutical Sciences*, **4**(34): 72-77.

Pareek H, Sharma S, Khajja BS, Jain K & Jain GC (2009). Evaluation of hypoglycemic and anti-hyperglycemic potential of *Tridax procumbens*(Linn.). *BioMed Central Complementary and Alternative Medicine*, **9**(1): 48.

Patel SB, Santani D, Shah MB & Patel VS (2012). Anti hyperglycaemic anti hyperlipidaemic effects of *Bryonia lacinosia* seed extract and its saponin fraction in streptozotocin- induced diabetes in rats. *Journal of Young Pharmacists*, **4**(3): 171-176.

Petchi RS, Parasuraman S &Vijaya C. (2013). Anti diabetic and anti hyperlipidemic effects of an ethanolic extract of the whole plant of *Tridax procumbens* (Linn) in streptozotocin-induced diabetic rats. *Journal of Basic and Clinical Pharmacy*, **4**(4): 88-92.

Petznick A (2011). Insulin management of type 2 diabetes mellitus. *American Family Physician*, **84**(2): 183-190.

Price KD, Price CS & Reynolds RD (2001). Hyperglycaemia-induced ascorbic acid deficiency promotes endothelial dysfunction and the development of atherosclerosis. *Atherosclerosis*, **158**(2): 1-12.

Rajagopal K & Sasikala K (2008). Anti hyperglycaemic and anti hyperlipidaemic effects of *Nymphaea stellata* in alloxan-induced diabetic rats. *Singapore Medical Journal*, **49**(2): 137.

Ramachandran S, Naveen K, Rajinikanth B, Akbar M & Rajasekaran A (2012). Anti-diabetic, anti hyperlipidemic and *in vivo* antioxidant potential of aqueous extract of *Anogeissus latifolia* in type 2 diabetic rats. *Asian Pacific Journal of Tropical diseases*, **2**: S596-S602.

Ramachandran S, Rajasekaran A & Adhirajan N (2013). *In vivo* and *in vitro* anti-diabetic activity of *Terminalia paniculata* bark: An evaluation of possible phytoconstituents and mechanisms for blood glucose control in diabetes. *ISRN Pharmacology*, 2013: 1-10.

Ramsey I & Mclauchlan C (2013). Unstable diabetes mellitus in dogs and cats: Approach and treatment. *Veterinary Times*, 1-9.

Ravichandra VD & Paarakh PM (2013). Evaluation of anti-diabetic potentials of methanolic extracts of *Ficus microcarpa* leaves in alloxan-induced diabetic rats. *International Journal of Pharmacy and Pharmaceutical Sciences*, **5**(3): 369-373.

Riet-Correa B, Marcia Castro MB, Lemos RAA, Riet-correa G, Mustafa N & Riet-Correa F (2011). *Bracharia spp* poisoning of ruminants in brazil. *Pesquisa Veterinaria Brasileira*, **31**(3): 183-192.

Saravanan G & Pari I (2008). Hypoglycemic and anti hyperglycemic effect of *Syzygium cumini* bark in streptozotocin–induced diabetic rats. *Journal of Pharmacology and Toxicology*, **3**(1): 1-10.

Shabeer J, Srivastava RS & Singh SK (2009). Anti-diabetic and antioxidant effect of various fractions of *phyllanthus simplex* in alloxan-diabetic rats. *Journal of Ethnopharmacology,* **124**(3): 34-38.

Shuaibu MN, Wuyep PTA, Yanagi T, Hirayama K, Ichinose A, Tanaka A & Kuono I (2007). Trypanocidal activity of extracts and compounds from the stem-bark of *Anogeissus leiocarpus* and *Terminalia avicennioides*. *Parasitological Research*, **102**(4): 697-707.

Stefanucci A, Zengin G, Locatelli M, Macedonio G, Wang CK, Novellino E, Mahomoodally MF & Mollica A (2018). Impact of different geographical locations on varying profile of bioactives and associated functionalities of caper (*Capparis spinosa* L.). *Food and Chemical Toxicology*, **118**: 181-189

Tanko Y, Garkuwa UA, Jimoh A, Sada NM, Mohammed KA, Muhammad A & Makarfi TA (2014). Hypoglycaemic effects of methanol crude leaves extract and aqueous fraction of *Accacia nilotica* on blood glucose levels on experimental animals. *Annals of Biological Sciences*, **2**(1): 23-27.

Tiedger M, Lortz M, Drinkgern J & Lenzen S (1997). Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin producing cells. *Diabetes*, **46**(11): 1733-1742.

Tullo AH (2008). A nutty chemical. *Chemical and Engineering News*, **86**: 26 – 27.

Valabhji J, McColl AJ, Ridmond W, Schachter M, Rubens MB and Elkeles RS (2001). Total antioxidant status and coronary artery calcification in type 1 diabetes. *Diabetes Care*, **24**(9): 1608-1613.

Wallace MS and Kirk CA (1990). The diagnosis and treatment of insulin-dependent and non-insulin-dependent diabetes mellitus in the dog and cat. *Problems of Veterinary Medicine,* **2**(4): 573-590.

Zambare MR, Bhosale UA, Somani RS, Yegnanarayan R & Talpate KA (2011). *Achyranthes aspera* (Agadha). A herb that improves pancreatic function in alloxan-induced diabetic rats. *Asian Journal of Pharmaceutical and Biological Research,* **1**(2): 99-104.