

Research Article

Effect of Methanolic Extract of *Cassia occidentalis* L. Root Bark on Body Weight and Selected Biochemical Parameters in Alloxan Induced Diabetic Rats

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Abstract: The effect of the methanolic root extract of *Cassia occidentalis* L. on body weight and selected biochemical parameters were investigated. Diabetes was induced by intraperitoneal administration of a single dose of alloxan (150 mg/kgbw), after the confirmation of diabetes, extracts at three different dose levels (300, 400, 600 mg/kgbw) and Metformin (Standard drug) were administered daily to alloxan induced diabetic rats for the period of 14 days. The experiment lasted for 21 days and within this period both blood glucose and body weight were measured at day 0, 7, 14 and 21. The body weight of all animals except those in the normal control group reduced significantly ($p < 0.05$) throughout the experiment. Blood glucose levels and serum levels of Triglyceride, Total Cholesterol, Low Density Lipoprotein (LDL) significantly increased ($p < 0.05$), while the serum levels of Total protein, Albumin and High Density Lipoprotein (HDL) decreased significantly ($p < 0.05$) in diabetic rats compared to the normal control. Treatment of the diabetic rats with the extracts was able to significantly reduce Blood glucose, Triglyceride, Total cholesterol and LDL and significantly increase Total protein, Albumin and HDL. Based on the result of this study optimum hypoglycaemic activity was observed in the animal group treated with 400mg/kgbw of *C.occidentalis* root extract and this did not differ significantly ($p > 0.05$) from that of metformin-treated rats. The present study shows that the Methanolic extract of *C.occidentalis* root has hypoglycaemic potential and is able to correct the dyslipidemia associated with hyperglycaemia. This finding scientifically proves its use in traditional medicine for the treatment of diabetes.

Keywords: Alloxan, blood glucose, diabetes, hypoglycaemic, hyperglycaemia

INTRODUCTION

Diabetes is a chronic non communicable metabolic disorder which results from insulin deficiency or reduced effectiveness of insulin activity (Karau *et al.*, 2013; Noor *et al.*, 2008). The disease is characterized by elevated level of glucose in the blood and associated with long term damage, dysfunction and failure of various organs mainly the eyes, kidney, liver and heart (Pedrini *et al.*, 2006).

Diabetes constitutes a global burden as its incidence is considered to be on the increase, coupled with the disease being the fifth leading cause of death in the World (Rother, 2007). According to WHO, around 171 million people worldwide were suffering from diabetes in 2000 and this figure is predicted to double by 2030 (Wild *et al.*, 2004; WHO, 2002). Nature provides an abundant source of medicinal plants which are known to be used in the treatment of a wide range of diseases right from the word go. In Africa and elsewhere, plant extracts are still widely used in the treatment of diabetes and other ailments and up to 80% of the African population use traditional medicines for primary health care (WHO, 2002). *Cassia occidentalis*

Linn. belong to the family *Caesalpinaceae* (*Leguminosae*). Synonyms include *Senna occidentalis*, *Cassia carolinian*, *Cassiacyliata*, *Cassia foetida*, *Cassia frutescens*, *Cassiageminiflora* and *Cassia linearis*. It is commonly called Coffee Senna in English (Yadav *et al.*, 2010) and locally called *Akidi agbara* (Igbo,) *Abo rere* (Yoruba) and *Rai Dore/Sanga sanga* (Hausa) (Eghareva *et al.*, 2010). *Cassia occidentalis* (*C.occidentalis*) is extensively used in the indigenous and folklore medicine systems as an antidote of poisons, blood purifier, expectorant, anti-inflammatory agent and a remedy for the treatment of liver diseases (Emmanuel *et al.*, 2010). Its roots, flowers, seeds and leaves have been employed in herbal medicine around the world for a variety of purposes such as laxative, expectorant, analgesic (Sini *et al.*, 2011), anti-malarial (Tona *et al.*, 2001), hepatoprotective (Usha *et al.*, 2007), relaxant (Ajagbonna *et al.*, 2001), anti-inflammatory (Vijayabhaskar *et al.*, 2013), antibacterial (Daniyan *et al.*, 2011) and wound healing (Sheeba *et al.*, 2009). The roots are considered as a diuretic, a tonic for dysmenorrhea (menstrual problem), tuberculosis, anemia, liver complaints and fever reducer.

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Though many studies have been carried out on the leaf, stem and seed extracts of this plant, less work has been done on the root bark in the treatment of diabetes mellitus as claimed by the traditional healers in Nigeria. This study is therefore aimed at investigating the effect of methanolic extracts of *C.occidentalis* root bark on body weight and selected biochemical parameters in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Collection of plant material and extraction preparation: Fresh samples of the root of *C. occidentalis* were collected from New Bussa in Niger State, Nigeria in April 2014. The plant was identified and authenticated by Dr. Jemilat A. Ibrahim at the Department of Medicinal Plant Research and Traditional Medicine Herbarium and Ethnobotany Unit, National Institute for Pharmaceutical Research and Development, Idu Abuja, Nigeria and voucher number NIPRD/H/6629 was obtained.

The root bark of *Cassia occidentalis* after collection was rinsed with clean water, cut into smaller pieces and air dried at room temperature. The dried root bark were grinded to coarse powder using a blender (Excella, Marlex Electrolite LBD 4872) and passed through a sieve to obtain a fine powder. The powdered root bark (50 g) was weighed and extracted in 400mls of absolute methanol and heated for 3 h at 65°C in a reflux extractor, after which it was filtered using mushin cloth. The filtrate was concentrated in rotary evaporator and then Water bath to a semi solid mass. The extract obtained thereafter were kept in airtight sample bottles and stored in the refrigerator.

Phytochemical screening: The methanolic root extract of *Cassia occidentalis* was subjected to qualitative test for the presence of bioactive components; Alkaloids, Flavonoids, Tanins, Phenol, Steroid, Terpene, Anthraquinone, Cardiac glycoside and Saponin by carrying out phytochemical screening as described by Sofowara (1993), Trease and Evans (1989) and Harbone (1973).

Experimental animals: A total of twenty four wistar albino rats of either sex weighing between 150-250 g were obtained from National Veterinary Research Institute Vom in Jos, Nigeria. The rats were kept in clean plastic cages and housed under suitable conditions in the Department of Biochemistry Laboratory, Federal University of Technology Minna, Niger State. They were provided with food and water ad libitum and allowed to acclimatize to the laboratory environment for two weeks before proceeding with the experiment. The cages in which the rats are kept were cleaned regularly throughout the period of the

experiment. The study was conducted according to the Principles of Laboratory Animal Care (WHO, 2000).

Experimental induction of diabetes: Healthy Albino rats of either sex were fasted overnight (16 h) and hyperglycaemia was induced by a single intraperitoneal injection of 150 mg/kg body weight alloxan monohydrate freshly dissolved in normal saline immediately before use to overnight-fasted albino rats and feeding was resumed an hour after alloxan injection. Seventy two hours after Alloxan induction the blood glucose of each animal was checked using a glucometer (AccuCheck Active, Roche Diagnostics Mannheim, Germany). Each animal with a blood glucose level of 250 mg/dl and above were considered to be diabetic and used for the study (Verma *et al.*, 2010). In order to overcome the hypoglycaemia which might occur during the first 24 h following the alloxan administration, diabetic rats were given 5% glucose solution orally.

Animal grouping and treatment: After the confirmation of diabetes in the induced animals, 24 rats were randomly selected and divided into six groups each containing (3) animals:

- Group I:** Normal control (Saline) (Not induced)
- Group II:** Diabetic control (Alloxan-150 mg/kgbw) (induced not treated)
- Group III:** Standard Drug (Metformin-5 mg/kg)
- Group IV:** Dose-1 300 mg/kgbw of extract (induced and treated)
- Group V:** Dose-2 400 mg/kgbw of extract (induced and treated)
- Group VI:** Dose-3 600 mg/kgbw of extract (induced and treated)

A moderate to high dose were chosen randomly for the study with respect to the LD50 of the whole plant and that of the roots as reported in literatures (Verma *et al.*, 2010; Tona *et al.*, 2001).

Both the standard drug and the extracts were administered orally by gavages to the experimental rats. Animals were treated once daily for fourteen days.

Determination of fasting blood glucose: Fasting blood glucose level measured at day 0, 7, 14, 21 of treatment. Prior to these days the animals were fasted overnight (16 h) and only allowed access to clean water. Blood samples were collected by a snip-cut at the tip of the tail and blood glucose levels were measured using an autoanalyzer-AccuCheck Active glucose kit.

Determination of body weight: The weight of the rats in each group were determined prior to treatment and

during treatment with the extracts and the standard drug at an interval of seven days. The animals were fasted overnight (16 h) but given free access to clean water before taking their weight using a weighing balance.

Blood collection and serum preparation: Twenty four hours after the final treatment, the animals were anaesthetized using diethyl ether and blood samples were collected through jugular puncture. The blood samples were allowed to stand at room temperature for 30 min and then centrifuged at 1500 rpm for 15 min.

Biochemical analysis: Biochemical Analysis was carried out using Randox Diagnostic Kits. Lipid profile (Total Cholesterol, Triglyceride, HDL and LDL), Total Protein and Albumin were estimated as described by Tietz (1990), Roeschlau *et al.* (1974), Richmond (1973), Tietz (1995) and Doumas *et al.* (1971) respectively.

Statistical analysis: Data obtained from the study were subjected to one way analysis of variance (ANOVA) followed by post-hoc Duncan Test for comparison using SPSS version 16.0. Significance was accepted at $p < 0.05$ and results were expressed as mean \pm standard error of mean.

Table 1: Phytochemical constituents of the methanolic extract of *Cassia occidentalis* root

Compounds	Inference
Alkaloids	-
Flavonoids	++
Tannins	++
Phenols	++
Steroids	++
Terpenes	+
Anthraquinones	++
Cardiac glycosides	+
Saponins	+

(-) Absent, (+) Moderately present, (++) Highly present

RESULTS

Phytochemical screening: The result of the phytochemical screening (Table 1) revealed the high presence (++) of Flavonoids, Tannins, Phenols, steroids and anthraquinones, a moderate presence (+) of Terpenes, Cardiac glycosides and Saponins and the absence (-) of Alkaloids.

Hypoglycaemic Effects of the Extract in Alloxan Induced Diabetic Rats: Figure 1 shows the effect of the various dose levels (300, 400 and 600 mg/kgbw) of methanolic root extract of *Cassia occidentalis* and Metformin (standard drug 5 mg/kgbw) on fasting blood glucose of alloxan induced diabetic rats. Serum

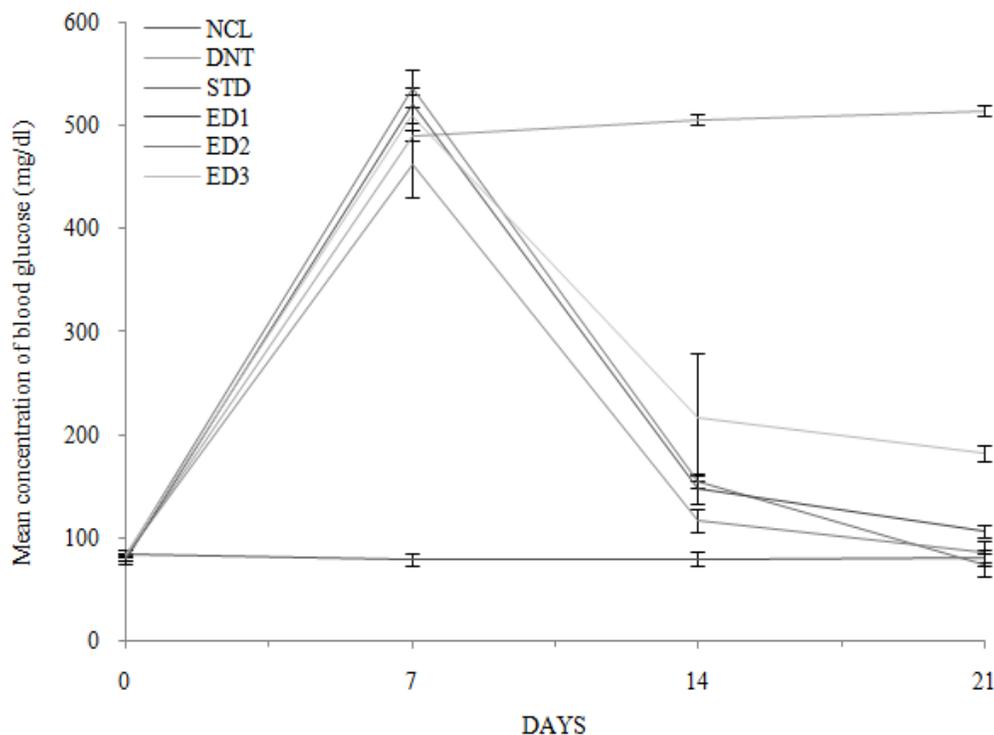


Fig. 1: Effect of methanolic root extract of *Cassia occidentalis* and standard drug on blood glucose levels of alloxan induced diabetic rats; NCL: Normal Control, DNT: Induced but not treated, STD: Induced but treated with standard drug (Metformin 5 mg/kgbw), ED1: Induced but treated with methanolic root extract (300 mg/kgbw), ED2: Induced but treated with methanolic root extract (400 mg/kgbw), ED3: Induced but treated with methanolic root extract (600 mg/kgbw)

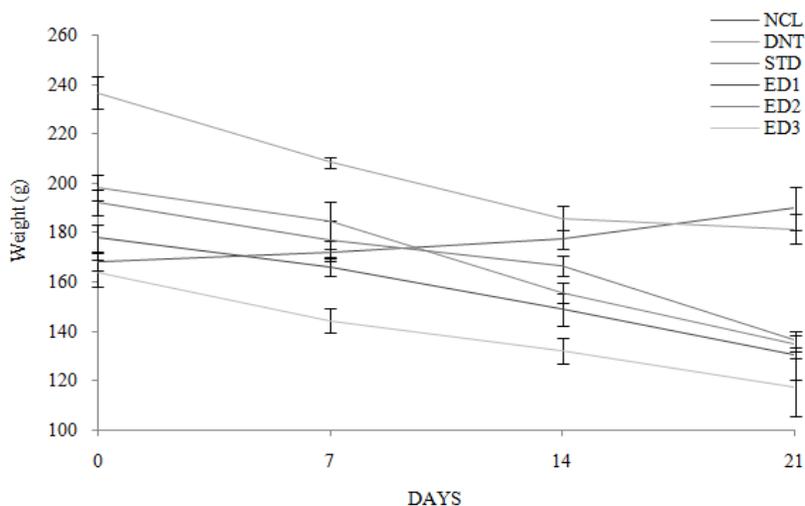


Fig. 2: Effect of methanolic extracts of *Cassia occidentalis* on body weight of rats; NCL: Normal control, DNT: Induced but not treated, STD: Induced but treated with standard drug (Metformin 5mg/kgbw), ED1: Induced but treated with methanolic root extract (300mg/kgbw), ED2: Induced but treated with methanolic root extract (400mg/kgbw), ED3: Induced but treated with methanolic root extract (600mg/kgbw)

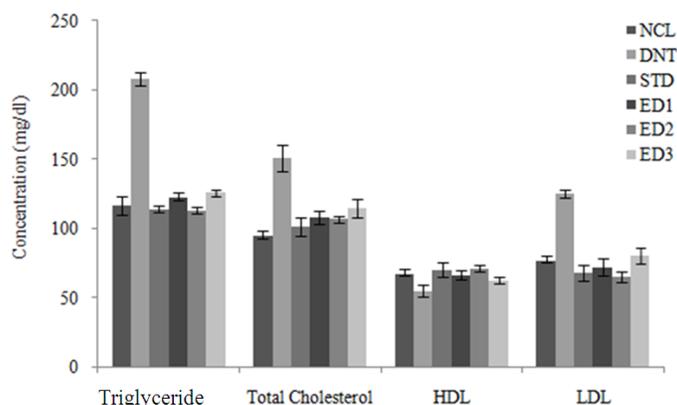


Fig. 3: Effect of methanolic extracts of *Cassia occidentalis* on serum lipid profile of rats; NCL: Normal control, DNT: Induced but not treated, STD: Induced but treated with standard drug (Metformin 5mg/kgbw), ED1: Induced but treated with methanolic root extract (300 mg/kgbw), ED2: Induced but treated with methanolic root extract (400mg/kgbw), ED3: Induced but treated with methanolic root extract (600mg/kgbw)

blood glucose level increased significantly ($p < 0.05$) in untreated diabetic rats when compared with the normal control. However, following the oral administration of the extract at various dose levels and the standard drug there was significant reduction ($p < 0.05$) in the fasting blood glucose levels of the diabetic rats when compared to the diabetic control. The baseline glucose level for the various dose levels starting from the lowest dose and that of the standard drug was 519.67 ± 17.61 , 462.67 ± 31.87 , 508.00 ± 22.30 and 535.33 ± 18.11 but was reduced to 106.67 ± 5.93 , 87.67 ± 10.59 , 182.33 ± 8.45 and 74.67 ± 10.67 , respectively. The reduction in blood glucose levels was not dose dependent, the highest dose administered (600 mg/kgbw) though was able to reduce the blood glucose significantly ($p < 0.05$) is incomparable with the normal control but differs

significantly from the diabetic control. Other dose levels (300 and 400 mg/kgbw) administered including the standard drug reduced blood glucose to levels comparable to the normal control.

Effect of methanolic extracts of *Cassia occidentalis* on body weight of rats: Figure 2 shows the effect of both the extract and the standard drug on the body weight of the treated and untreated diabetic rats. Body weight of the diabetic control group and that of the treated diabetic groups was reduced significantly ($p < 0.05$) throughout the duration of the study.

Effect of methanolic extracts of *Cassia occidentalis* on serum lipid profile of rats: Figure 3 shows the hypolipidemic effect of both the extract at various doses

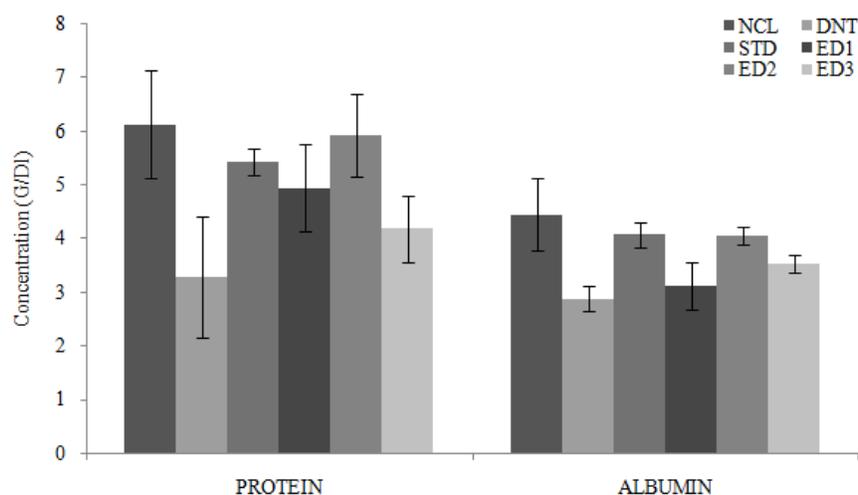


Fig. 4: Serum levels of total protein and Albumin in treated and untreated diabetic rats; NCL: Normal control, DNT: Induced but not treated, STD: Induced but treated with standard drug (Metformin 5mg/kgbw), ED1: Induced but treated with methanolic root extract (300mg/kgbw), ED2: Induced but treated with methanolic root extract (400mg/kgbw), ED3: Induced but treated with methanolic root extract (600mg/kgbw)

and Metformin on the serum lipid profile. Oral administration of Metformin and the various doses of methanolic root extract of *Cassia occidentalis* caused significant ($p < 0.05$) reductions in the serum Concentrations of Triglyceride, Total Cholesterol and Low Density Lipoprotein (LDL) but caused an opposite effect on the serum concentration of High Density Lipoprotein (HDL).

Effect of methanolic extracts of *Cassia occidentalis* on total protein and Albumin: Figure 4 shows the serum concentration of Total protein and Albumin for the various groups. The diabetic control group and the group treated with 600 mg/kgbw showed significantly low concentrations for total protein while for albumin the diabetic control and the group kept on 300 mg/kgbw treatment had significantly ($p < 0.05$) low concentrations.

DISCUSSION

Diabetes comprises of diverse group of disorders which are characterized by hyperglycaemia which is the hallmark of diabetes mellitus and it is one of the many metabolic abnormalities present in diabetes that has been used to define the presence of the disease. The deficiency of insulin coupled with poor diet, lack of exercise and elevated counter-regulatory hormone lead to the various metabolic abnormalities observed in diabetes which results into damage, dysfunction and failure of various organs (Olaitan, 2012).

Medicinal plants have been used as an alternative to orthodox medicine in many countries including Nigeria. Despite their widespread use, few scientific studies have been undertaken to ascertain the efficacy

of traditional remedies (Graca *et al.*, 2007). In line with the recommendations of the WHO Expert Committee (1985) on diabetes mellitus, it is important to investigate and ascertain the hypoglycaemic action of plants which are traditionally used in traditional medicine (WHO Expert Committee, 1985). *Cassia occidentalis* (*C. occidentalis*) is found in many tropical areas around the world, where it is used as a medicinal plant for decades (Distasi and Hiruma-Lima, 2002). The biologically active components of plants with hypoglycaemic actions include; Flavonoids, alkaloids, glycosides, polysaccharides, peptidoglycans, steroids and terpenoids (Rahman and Zaman, 1989). In this study, phytochemical screening of methanolic root extract of *C. occidentalis* revealed the presence of high amount of sterols, flavonoids, tannins, phenols, terpenes and anthraquinones with moderate amount of cardiac glycoside and saponin while the test for alkaloid showed negative result (Table 1). In addition to these Usha *et al.* (2007) reported the presence of alkaloids in the aqueous root extract of the same plant. The presence of these various chemical compounds might be responsible for the physiological/biological activity of the plant. Alkaloids are found to have antimicrobial activity by inhibiting DNA topoisomerase (Bonjean and De Pauw-Gillet, 1998). Tannins reduce the risk of coronary heart diseases (Ranjithkumar *et al.*, 2010). Saponin, present in plants, have been suggested as possible anti-carcinogens. Flavonoids and phenols are excellent sources of natural antioxidant (Ali *et al.*, 2008). Steroids have been reported in clinical studies as anti-inflammatory and analgesic agents (Singh, 2006)

and also they are used as treatment for congestive heart failure (Saidu *et al.*, 2012). Phlobatannins are known to exhibit diuretic property (Awoyinka *et al.*, 2007).

Intraperitoneal administration of 150 mg/kgbw of Alloxan monohydrate induced hyperglycaemia in rats after 72 h. Alloxan has been reported to induce diabetes mellitus by forming highly reactive superoxide radicals which destroy the insulin producing beta cells in the pancreas (Szkudelski, 2001).

Oral administration of standard drug (Metformin), 300, 400 and 600 mg/kgbw of the methanolic root extract of *C. occidentalis* led to significant reduction ($p < 0.05$) in blood glucose levels in alloxan induced diabetic rats when compared to the diabetic control after the period of treatment (Fig. 1). Similar effects have been documented in other studies using the leaves and stem of the same plant (Singh *et al.*, 2011), the whole plant (Verma *et al.*, 2010) and also using other plants from either the same family (Shiradkar *et al.*, 2011) who reported the antidiabetic effect of *cassia auriculata* bark extract and Elaikim-Ikechukwu *et al.* (2013) who reported the effect of *cassia alata* leaf extract in diabetic wistar rats or different families (Ihedioha *et al.*, 2010; Stalin *et al.*, 2012) in terms of plant taxonomy while Stalin *et al.* (2012) and Subrahmanyam *et al.* (2011) also found Metformin to significantly reduce blood glucose which is in agreement with the observation made in this present study. The reduction exhibited by the groups treated with standard drug, 300 and 400 mg/kgbw is higher than those in groups treated with 600 mg/kgbw, that is to say the reduction in blood glucose was not dose dependent. In this study it was observed that at the highest dose (600 mg/kgbw) used in the study there was less but significant reducing effect of the extract at that dose on blood glucose of the rats. This result was in agreement with the previous reports in literature (Karau *et al.*, 2013; Gopalakrishnan *et al.*, 2011). Karau *et al.* (2013) in their work hypoglycaemic effect of aqueous and ethyl acetate extracts of *Senna spectalis* in alloxan induced diabetic male mice observed that on administration of the ethyl acetate fraction of the stem of the plant used at 50 mg, 100 mg and 200 mg/kgbw, highest activity was observed at 50 mg/kgbw and the activity was seen to decline with increased concentration which was explained to be as a result of the extract at higher concentrations could inhibit its absorption by saturating the cells in the Gastrointestinal Tract. Also Karau *et al.* (2012) in their study hypoglycaemic activity of aqueous and ethylacetate leaf and stem bark of *Pappea capensis* in alloxan induced diabetic BALB/c mice observed that the ethylacetate extract of both the leaf and stem at doses of 100 mg and 200 mg/kgbw induced hypoglycaemia in a dose independent manner this according to the authors suggests that the extract may

have been absorbed in the cell system through active transport where a particular concentration saturation of the extracts occurred resulting to the rest being excreted. This observation could also be attributed to the fact that there are certain drugs/plant extracts whose bioavailability are affected by their concentrations i.e the higher the dose the less bioavailable they are (Foster, 1998; Karam, 1998). In addition to this, at higher doses they either form complexes with polyvalent ions, undergo hydrolysis by gastric acid or digestive enzymes and conjugation in the intestinal wall or metabolism by luminal micro flora thus reducing the amount of drug that is absorbed into circulation. Several mechanisms have been proposed by which plant extracts are able to exert their hypoglycaemic effect which include; stimulation of insulin release from beta cells (Kawano *et al.*, 2009; Gray and Flatt, 1999), decreasing hepatic glucose production (Edduoks *et al.*, 2003), decreasing the utilization of ingested carbohydrate (Musabayane *et al.*, 2006) and increasing peripheral tissue utilization of glucose (Zambare *et al.*, 2011). More so Metformin is amongst the group of anti diabetic drugs called insulin sensitizers as a result of its ability to stimulate insulin release (Karam, 1998). Furthermore, it was reported that plants exert their hypoglycaemic effect by interfering with carbohydrate absorption (Nelson *et al.*, 1991). The presence of phytochemicals in plants has being implicated to be responsible for their hypoglycaemic effect. Earlier studies have established that saponin and flavonoids in plant exhibit hypoglycaemic effect by increasing insulin release from pancreatic beta cells, increasing peripheral glucose uptake and by reducing glucose absorption (Saravanan and Pari, 2008; Luo *et al.*, 2005). Tannins and phenols are insulin like substances (Tullo, 2008) and they mimic the effect of insulin on glucose metabolism and enhance its secretion. Interestingly, these above mentioned phytochemicals were found present in the methanolic root extract of *C. occidentalis* in this study and may be the explanation for the hypoglycaemic effect which was observed.

Another possible mechanism by which *C. occidentalis* might exert its hypoglycaemic effect may be by buttressing the body's antioxidant system. Phytochemicals such as flavonoids and tannins are known to scavenge free radicals (Berenguer *et al.*, 2006) and build up immunity respectively, thus taking care of the stimulation of free radicals and oxidative stress as a result of high glucose levels. In addition, Usher *et al* have reported that the root of *C. occidentalis* is a rich source of antioxidants (Usha *et al.*, 2007). In this study the hypoglycaemic effect of the methanolic root extract of *C. occidentalis* could be attributed to the presence of phytochemicals like flavonoids and tannins which are known to serve as antioxidants.

The result presented in Fig. 2 shows that the body weight of the test animals (the diabetic treated and untreated) except for the normal control were found to decrease significantly ($p < 0.05$) when compared throughout the duration of the experiment. This result is in agreement with reported studies where tannin were implicated to be involved in growth regulation (Kadam *et al.*, 1990; Muthusamy *et al.*, 2008), the tannins present in the extract could potentially inhibit the activity of lipases found in rats, thereby lowering their fat content (Chichioco-Hernandez and Leonido, 2011). Also previous studies carried out using plants (*Cassia fistula* and *Cassia alata*) from the same family with *Cassia occidentalis* showed that they exhibited weight lowering effect which was also attributed to the presence of tannins in the plant (Khan *et al.*, 2010; Elaikim-Ikechukwu *et al.*, 2013). *Senna/cassia* are considered natural laxatives for decades and this effect is a characteristic of cassia species as described by Elujoba *et al.* (1999). Its laxative effect can be attributed to be the source of where weight loss is experienced due to the body fluid lost when it activates the colon (Xing and Softer, 2001). Anthraquinone derivative have been identified as the chemical constituents of cassia species responsible for the laxative and purgative activities (Akomolafe *et al.*, 2003). According to Haraguchi *et al.* (2003) anthraquinones were considered the secondary metabolites responsible for effect such as weight loss in rats administered with *Cassia occidentalis* seeds, also Ilodigwe *et al.* (2013) reported that oral administration of the stem bark of *Khaya senegalensis* and seed extract of *Picralima nitida* lead to weight loss in rats, which was attributed to the presence of tannins and concluded that it might have contributed to the reduced feeding ability and thus weight loss noticed in the treatment groups.

Thus the weight loss observed in this study could be attributed to the presence of anthraquinones and tannins in the methanolic root extract of *Cassia occidentalis* administered to the diabetic treated groups. On the other hand, studies of Silva *et al.* (2011) which evaluated the effect of the oral acute and sub acute treatment with hydroalcoholic extract of *Cassia occidentalis* L. stem and leaves (0.1, 0.5, 2.5 g/kgbw) on the mean body weight of wistar rats observed an increase in the body weight of the animals which was attributed to the low amount of anthraquinones observed by the chromatographic analysis of *Cassia occidentalis* stem and leaves. Also previous studies have reported that the use of different plant extracts in the treatment of diabetes led to weight gain which is contrary to the result obtained from this study (Stalin *et al.*, 2012; Akah *et al.*, 2009).

Diabetes mellitus is associated with dyslipidemia (marked alterations in the level of serum lipid, triglycerides and lipoprotein levels) (Maghrani *et al.*,

2004). This abnormally elevated concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots (adipose tissue) to the blood (Shukla *et al.*, 2003), since insulin is known to inhibit the hormone sensitive lipase (Daisy and Feril, 2013). In the present study, Alloxan induced diabetic rats had an elevation in the serum lipids. The dyslipidemia observed in the diabetic rats in this present study is in agreement with earlier reports in experimentally induced diabetes mellitus (Dolui *et al.*, 2012; Akah *et al.*, 2009). Oral administration of methanolic root extracts of *C.occidentalis* significantly decreased the serum cholesterol, triglyceride and LDL and increased the HDL-cholesterol (Fig. 3). The implication of this effect being that the root extracts at the said doses are able to correct the abnormalities resulting from increased blood glucose level. The results reported in this study are comparable to previous studies that have been reported (Johnkenedy *et al.*, 2012; Sriram, 2011; Akah *et al.*, 2009). The serum lipid lowering effect of *C. occidentalis* root extract observed may be attributed to inhibition of endogenous synthesis of lipids probably by enhancing the secretion of insulin, inhibition of hormone sensitive lipase as a result of insulin availability or by decreasing the mobilization of free fatty acids from the adipose tissue. More so, lowering of lipid levels in rats by plant extracts have been reported to be due to antioxidant activity of phytochemical compounds such as flavonoids (Igarasi and Onhuruma, 1995) which is present in the extract used in this study.

As regards to the effect of alloxan-injection on serum total protein and albumin, there was a significant decrease in the diabetic control group as compared with the normal control (Fig. 4). The decreased rate of total protein may be due to several reasons like increased rate of amino acids conversion to glucose (Chu *et al.*, 2002), decreased amino acids uptake (Sayed *et al.*, 2011) and increased conversion rate of glucogenic amino acids to CO₂ and H₂O (Ahmed, 2000). Other reports associated protein decrease to a decrease in the amount and availability of mRNA (Ahmed *et al.*, 2006), a loss of transitional factor, reduction of ribosomal protein synthesis as a result of insulin deficiency and decreased defensive mechanism (Mahboob *et al.*, 2005). Results in Fig. 4 showed that, the treatment of diabetic rats with Metformin and *C. occidentalis* methanolic root extract resulted in marked improvement of serum protein contents. These results correlated with the study of Sayed *et al.* (2011), Owolabi *et al.* (2011) and Kamalakkanan and Prince, 2005) which reported that serum total protein concentration was increased in STZ-diabetic rats treated with different plants extracts. According to Sayed *et al.* (2011) the effect could be attributed to increased protein synthesis, increasing incorporation of certain

amino acids as a result of increasing insulin secretion, increase of hepatic uptake of glucogenic amino acids, stimulation of amino acid incorporation into protein and decreased proteolysis by activating the enzyme that catalyzing amino acids transamination while Owolabi *et al.* (2011) attributed it to the ability of the plant used in his study to enhance the protein synthesizing function of the liver. Also, Nahla and Ismail (2006) reported a good correlation between protein synthesis and insulin level.

Albumin is necessary for appropriate distribution of body fluids between body tissues and intravascular compartments and functions as a plasma carrier by binding several hydrophobic hormones (Zunszain *et al.*, 2003). Albumin also keeps the blood from leaking out of blood capillaries. Administration of alloxan decreases serum albumin levels due to increased non-enzymatic glycosylation of protein (Gupta *et al.*, 2012). In the present study, *C. occidentalis* root treatment significantly ($p < 0.05$) elevated serum albumin concentrations (Fig. 4), which could be possibly associated with a reduced affinity of albumin towards glucose. This is in agreement with earlier studies (Santhan and Zuber, 2013).

CONCLUSION

The findings of the present study indicate that the root of *Cassia occidentalis* can be a source of potent antidiabetic agent and is able to ameliorate the metabolic abnormalities associated with Diabetes Mellitus.

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REFERENCES

- Ahmed, O.M., 2000. The hypoglycemic effect of curcumin and esculetin and their probable mechanism of action in STZ-induced diabetic albino rats. *J. Egypt Ger. Soc. Zool.*, 46:351-375.
- Ahmed, O.M., H. Abdel-Hamid, M. Bastawy and N.A. Hasona, 2006. Antihyperglycemic effects of *plantago ispaghula* seeds aqueous extracts in diabetic and hypercholesterolemic rats. *J. Egypt Ger. Soc. Zool.*, 51:371-393.
- Ajagbonna, O.P., F.B.O. Mojiminiyi and O.A. Sofola, 2001. Relaxant effects of the aqueous leaf extract of *Cassia occidentalis* rat aortic rings. *Afr. J. Biomed. Res.*, 4(2): 120-127.
- Akah, P.A., J.A. Adeniji, O.A. Salawu, T.C. Okoye and N.V. Offiah, 2009. Effects of *Vernonia amygdalina* on biochemical and hematological parameters in diabetic rats. *Asian J. Med. Sci.*, 1(3): 108-113.
- Akomolafe, R.O., I.O. Adeosun, A.A., Elujoba, E.O. Iwalewa and A.O. Ayoka, 2003. Effects of *Cassia sieberiana* leaf extracts on the intestinal motility of rat. *Afr. J. Biomed. Res.*, 6: 141-145.
- Ali, S.S., N.Kasoju, A. Luthra, A. Singh, H. Sharanabasava, A. Sahu and U. Bora, 2008. Indian medicinal herbs as source of antioxidants. *Food. Res. Int.*, 41:1-15.
- Awoyinka, O.A., I.O. Balogun and A.A. Ogunnowo, 2007. Phytochemical screening and in vitro bioactivity of *Cnidioscolus aconitifolius* (*Euphorbiaceae*). *J. Med. Plants Res.*, 1(3): 63-65.
- Berenguer, B., L.M. Sanchez, A. Quilez, M.B. Lopez, O.D. Haro, J. Galvez and M.J. Martin, 2006. Protective and antioxidant effects of *Rhizophora mangle* L. against NSAID induced gastric ulcers. *J. Ethnopharmacol.*, 103: 194.
- Bonjean, K., M.C. De Pauw-Gillet, M.P. Defreone, P. Colson, C. Houssier, L. Dassonneville, C. Bailly, R.C. Gremers, J. Quertinleclercq, M. Tits and L. Angenot, 1998. The DNA intercalating alkaloid cryptolepine interferes with topoisomerase II and inhibits primarily DNA synthesis in B16 melanoma cells. *J. Ethnopharmacol.*, 69: 241-246.
- Chichioco-Hernandez, L.C. and F.M.G. Leonido, 2011. Weight-lowering effects of *caesalpinia pulcherrima*, *cassia fistula* and *senna alata* leaf extracts. *J. Med. Plants Res.*, 5(3): 452-455.
- Chu, C.A., S.M. Sherck, K. Igawa, D.K. Sindelar, D.W. Neal, M. Emshwiller and A.D. Cherrigton, 2002. Effects of free fatty acids in hepatic glycogenolysis and gluconeogenesis in conscious dogs. *Am. J. Physiol. Metab.*, 282:402-411.
- Daisy, P. and G.J.K. Feril, 2013. Hypolipidemic and hepatoprotective effect of *Cassia auriculata* Linn bark extracts on streptozotocin induced diabetics in male wistar albino rats. *Asian J. Pharmaceut. Clin. Res.*, 6(2): 43-48.
- Daniyan, S.Y., J.B. Oloruntimelehin and O. Ifeadi, 2011. Antibacterial activity of *Cassia occidentalis* flower vegetable extract on selected bacteria. *Asian J. Biomed. Pharmaceut. Sci.*, 1(1): 23-27.
- Distasi, L.C. and C.A. Hiruma-Lima, 2002. *Plantas Mediciniais na Amazônia e na Mata Atlântica*. 2nd Edn., Editora UNESP, SAO Paulo, pp: 604.
- Dolui, A., S. Das and A. Kharat, 2012. Antihyperglycemic effect of *Cassia sophera* leaf extracts in rats with alloxan induced diabetes. *Asian J. Tradit. Med.*, 7(1): 8-13.

- Doumas, B.T., W.A. Watson and H.G. Biggs, 1971. Albumin standards and the measurement of serum albumin with bromocresol green. *Clin. Chim. Acta*, 31: 87.
- Edduoks, M., H. Jouad, M. Maghrani, A. Lemhadri and R. Burcelin, 2003. Inhibition of endogenous glucose production accounts for hypoglycaemic effects of *Spergularia purpurea* in streptozotocin mice. *Phytomedicine*, 10: 544-599.
- Eghareva, H., A.C. Odigwe, M.S. Abdullahi, S.K. Okwute and J.I. Okogun, 2010. Phytochemical analysis and broad spectrum antimicrobial activity of *Cassia occidentalis* L. (whole plant). *New York Sci. J.*, 3(10):74-81.
- Elaikim-Ikechukwu, C.F., A.A. Edem, M.U. Williams, S.O. Okori and C.J. Ihentuge, 2013. Phytochemical composition of *Cassia alata* leaf extract and its effect on the histology of the pancreas of diabetic wistar rats. *IOSR J. Pharm. Biol. Sci.*, 5(5):7-13.
- Elujoba, A.A., A.T. Abere and S.A. Adelusi, 1999. Laxative activities of *Cassia* pods sourced from Nigeria. *Niger. J. Nat. Prod. Med.*, 3:51-53.
- Emmanuel, S., M.S. Rani and M.R. Srekanth, 2010. Antidiabetic activity of *Cassia occidentalis* Linn in streptozotocin-induced diabetic rats: A dose dependent study. *Int. J. Pharma Biosci.*, 1(4): 14-25.
- Foster, D.W., 1998. Diabetes Mellitus. In: Isselbacher, K.J., E. Braunwald, J.D. Wilson, J.B. Martin, A.S. Fauci and D.L. Kasper (Eds.), *Harrison's Principles of Internal Medicine*. 14th Edn., McGraw-Hill Inc., Health Professions Division, pp: 2060-2080.
- Gopalakrishnan, K.V., M. Sunitha, G. Akila, U. Beulah and K. Devaki, 2011. Hypoglycaemic activity of *Passiflora edulis Sims* leaf extract in wistar albino rats. *Int. Res. J. Pharm.*, 2(9): 170-172.
- Graca, C., C.S. Freitas, C.H. Baggio, P.R. Dalsenter and M.C. Marques, 2007. *Mikania laevigata* syrup does not induce side effects on reproductive system of male Wistar rats. *J. Ethnopharmacol.*, 72: 215-219.
- Gray, A.A. and P.R. Flat, 1999. Insulin releasing like activity of the traditional Antidiabetic plant *Coriander sativum* (coriander). *J. Nutr.*, 81: 203-208.
- Gupta, R., M. Mathur, V.K. Bajaj, P. Katariya, S. Yadav, R. Kamal and R.S. Gupta, 2012. Evaluation of antidiabetic and antioxidant activity of *Moringa oleifera* in experimental diabetes. *J. Diabetes*, 4:164-171.
- Haraguchi, M., M.L.Z. Dagli, P.C.F. Raspantini and S.L. Górnjak, 2003. The effects of low doses of *Senna occidentalis* seeds on broiler chickens. *Vet. Res. Commun.*, 27: 321-328.
- Harbone, J.B., 1973. *Phytochemical Methods*. Chapman and Hall Ltd., London, pp: 49-188.
- Igarasi, K. and M. Ohmuma, 1995. Effect of rhamnetin and quercetin on concentration of cholesterol and lipoperoxidase in the serum and liver and the blood and liver antioxidative enzyme activities of rats. *Biosci. Biochem.*, 59: 595-601.
- Ihedioha, T.E., V.U. Omoja and I.U. Asuzu, 2010. Effects of Methanolic stem bark extract of *Cassia sieberiana* DC on fasting blood glucose and serum Lipid profile of alloxan induced diabetic rats. *Anim. Res. Int.*, 11(1): 1871-1880.
- Ilodigwe, E.E., I.C. Urukwen, D.L. Ajaghaku, I.S. Mbagwu and C.A. Agbata, 2013. Acute and subchronic toxicities of aqueous antidiabetic herbal decoction commonly taken in South Eastern Nigeria. *Int. J. Res. Pharma Biomed.*, 4(4): 1256-1263.
- Johnkennedy, N., A. Emejulu, A. Ihim and H.I. Udujih, 2012. Influence of *Gongronema latifolium* on some biochemical parameters in alloxan induced diabetes. *Int. Anal. Pharm. Biomed. Sci.*, 1(1): 13-17.
- Kadam, S.S., D.K. Salunkhe and J.K. Chavan, 1990. *Dietary Tannins: Consequences and Remedies*. CRC Press, Boca Raton, pp: 177.
- Kamalakkanan, N. and P.S. Prince, 2005. The effect of *Aegle marmelos* fruit extract in STZ-diabetes: a histopathological study. *J. Herb. Pharmacother.*, 5: 87-96.
- Karam, J.H., 1998. Pancreatic Hormones and Antidiabetic Drugs. In: Katzung, B.G. (Ed.), *Basic and Clinical Pharmacology*. Appleton-Lange, pp: 684-703.
- Karau, G.M., E.N.M. Njagi, A.K. Machocho, N.L. Wangai, N.P. Kamau and B.P. Karau, 2012. Hypoglycaemic effect of aqueous and ethyl acetate leaf and stem bark extracts of *Pappea capensis* in alloxan induced diabetic BALB/c mice. *Brit. J. Pharm. Toxicol.*, 3(5): 251-258.
- Karau, G.M., E.N.M. Njagi, A.K. Machocho, N.L. Wangai, N.P. Kamau and B.P. Karau, 2013. Hypoglycaemic effect of aqueous and ethyl acetate extracts of *Senna spectabilis* in alloxan induced diabetic male mice. *J. Pharmaceut. Biomed. Sci.*, 31(31): 1089-1095.
- Kawano, A., H. Nakamura, S.I. Hata, M. Minakawa, Y. Miura and K. Yagasaki, 2009. Hypoglycaemic effect of *aspalathin*, a rooibos tea component from *Aspalathus Linearis*, in type 2 diabetic model db/db mice. *Phytomedicine*, 16(5): 437-443.
- Khan, Z.I., B. Nahir, Md. Abu Jakaria, S. Rahman, H.M. Chowdhury and M. Rahimatullah, 2010. An evaluation of antihyperglycaemic and antinociceptive effects of methanolic extract of *Cassia fistula* L. (*Fabaceae*) leaves in swiss albino mice. *Adv. Nat. Appl. Sci.*, 4(3): 305-310.

- Luo, L., H.J. Yin, Y. Zhang, Y.R. Jiang, Y. Liu and D.Z. Shi, 2005. Effect of Gineseng fruit saponins on insulin sensitivity index in high fat-fed rats. *J. Chinese Integr. Med.*, 3(6): 463-465.
- Maghrani, M., A.Lemhadri, N.A.Zeggwagh, M.El Amraoui, M.Haloui, H.Jouad, M. Eddouks, 2004. Effects of an aqueous extract of *Triticum repens* on lipid metabolism in normal and recent-onset diabetic rats. *J. Ethnopharmacol.*, 90(2-3): 331-337.
- Mahboob, M., M.F. Rahman and P. Grover, 2005. Serum lipid peroxidation and antioxidant enzyme levels in male and female diabetic patients. *Singapore Med. J.*, 46:322-324.
- Musabayane, C. T., P. T., bwititi and J. A. O. Ojewole, 2006. Effect of oral administration of some herbal extracts on food consumption and blood glucose levels in normal and streptozotocin treated diabetic rats. *Methods and Findings in Experimental and Clinical Pharmacology*, 28 (4), 223-228.
- Muthusamy, V.S., S. Anand, K.N. Sangeetha, S. Sujatha, B. Arun and B.S. Lakshmi, 2008. Tannins present in *Cichorium intybus* enhance glucose uptake and inhibit adipogenesis in 3T3-L1 adipocytes through PTP1B inhibition. *Chem. Biol. Interact.*, 174(1): 69-78.
- Nahla, S.E.S. and M.A.N. Ismail, 2006. Hypoglycemic effect of *Cleome droserifolia* ethanolic leaf extract in experimental diabetes and on non-enzymatic antioxidant, glycogen, thyroid hormones and insulin levels. *Diabetol Croat*, 35-36.
- Nelson, R.W., S.L. Ihle, L.D. Lewis, S.K. Salisburg, T. Miller, Bergdall and G.D. Bottoms, 1991. Effects of dietary fibre supplementation on glycemic control in dogs with alloxan-induced diabetes mellitus. *Am. J. Vet. Res.*, 52(12):2060-2066.
- Noor, A., S. Gunasekaran, A.S. Manickam and M.A. Vijayalakshmi, 2008. Antidiabetic activity of Aloe vera and histology of organs in streptozotocin-induced diabetic rats. *Curr. Sci.*, 94: 1070-1076.
- Olaitan, O.L., 2012. Patient's knowledge of causes effects and complications of diabetes mellitus in Illorin, Kwara State, Nigeria. *J. Biotechnol. Pharam. Res.*, 3(6): 112-117.
- Owolabi, O.A., D.B. James, K.M. Arugo and I.I. Olaiya, 2011. Combined effects of aqueous extracts of *phyllanthus amarus* and *vitex domiana* stem bark on blood glucose and some liver biochemical parameter. *Brit. J. Pharmacol. Toxicol.*, 12(3): 143-147.
- Pedrini, M.T., A.S. Levy, J. Lau, T.C. Chalmers and P.H. Wang, 2006. The effect of dietary protein restriction on the progression of diabetic and non diabetic renal diseases: A meta analysis. *Ann. Intern. Med.*, 124(7): 627-32.
- Rahman, A.U. and K. Zaman, 1989. Medicinal plants with hypoglycemic activity. *J. Ethnopharmacol.*, 26: 1-55.
- Ranjithkumar, J., 2010. Secondary metabolite investigation. *J. Chem. Pharm. Res.*, 2(4):371-377.
- Richmond, N., 1973. The use of cholesterol oxidases following saponification. *Clin. Chem.*, 19: 1350-1356.
- Roeschlau, P., E. Berntand W.A. Gruber, 1974. Enzymatic determination of total cholesterol. *J. Clin. Chem. Clin. Bio.*, 12: 403.
- Rother, K.I., 2007. Diabetes treatment-bridging the divide. *New Engl. J. Med.*, 356(15): 1499-1501.
- Saidu, A.N., A. Mann and C.D. Onuegbe, 2012. Phytochemical screening and hypoglycemic effect of aqueous *Blighia sapida* root bark extract on normoglycemic albino rats. *Brit. J. Pharm. Res.*, 2(2): 89-97.
- Santhan, J. M. and A.M. Zuber, 2013. Evaluation of anti-diabetic and nephroprotective activity of 95% ethanolic extract of *canthium dicocum* whole plant by using albino rats. *J. Chem. Pharm. Sci.*, 6(4): 218-222.
- Saravanan, G. and I. Pari, 2008. Hypoglycemic and anti hyperglycemic effect of *Syzygium cumini* bark in streptozotocin-induced diabetic rats. *J. Pharmacol. Toxicol.*, 3: 1-10.
- Sayed, M.R., M.M. Iman and A.S. Dawlat, 2011. Biochemical changes in experimental diabetes before and after treatment with *mangifera indica* and *psidium guava* extracts. *Int. J. Pharmaceut. Biomed. Sci.*, 2(2): 29-41.
- Sheeba, M., S. Emmanuel, K. Revathi and S. Ignacimuthu, 2009. Wound healing activity of *Cassia occidentalis* L. in Albino wistar rats. *Int. J. Integr. Biol.*, 8(1): 1-6.
- Shiradkar, M., G. Pawankumar and K. Shah, 2011. Pharmacological evaluation of *Cassia auriculata* Bark extract. *Int. J. Pharm. Biosci.*, 2(2):758-766.
- Shukla, N., G.D. Angelini and J.Y. Jeremy, 2003. Homocysteine as a risk factor for nephropathy and retinopathy in type 2 diabetes. *Diabetologia*, 46:766-772.
- Silva, M.G., T.P. Aragão, C.F. Vasconcelos, P.A. Ferreira, B.A. Andrade, I.M. Costa, J.H. Costa-Silva, A.G. Wanderley and S.S.L. Lafayette, 2011. Acute and subacute toxicity of *Cassia occidentalis* L. stem and leaf in Wistar rats. *J. Ethnopharmacol.*, 136: 341-346.
- Singh, A.P., 2006. Short review: Distribution of steroid like compounds in plant flora. *Pharmacogn. Mag.*, 2(6):87-89.
- Singh, M., K. Nagori, S. Iyer, G. Khare, G. Sharwan and D.K. Tripathi, 2011. Ethnomedicinal traditional and pharmacological aspects of *Plumbago Zeylanica* Linn. *Pharmacology online*, 3: 684-700.

- Sini, K.R., B.N., Sinha, M. Karpakavalli and P.T. Sangeetha, 2011. Analgesic and antipyretic activity of *Cassia occidentalis* Linn. Ann. Biol. Res., 2(1): 195-200.
- Sofowara, E.A., 1993. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, pp: 289.
- Sriram, N., 2011. Antidiabetic and antihyperlipidemic activity of bark of *casuarinas equisetifolia* in streptozotocin induced diabetic rats. Int. J. Pharm. Rev. Res., 1(1): 4-8.
- Stalin, C., P. Dineshikumar and K. Nithiyanthan, 2012. Evaluation of antidiabetic activity of methanolic leaf extract of *Ficus carica* in alloxan-induced diabetic rats. Asian J. Pharm. Clin. Res., 5(3): 85-87.
- Subrahmanyam, G.V., M. Sushma, A. Alekya, C. Neeraja, H. Sai Sri Harsha and J. Ravindra, 2011. Antidiabetic activity of *Abelmoschus esculentus* fruit extract. Int. J. Res. Pharm. Chem., 1(1): 17-20.
- Szkudelski, T., 2001. The mechanism of Alloxan and streptozotocin action in beta cells of the rats pancreas. Physiol. Res., 50: 536-546.
- Tietz, N.W., 1990. Clinical Guide to Laboratory Tests. 2nd Edn., W.B. Saunders Company, Philadelphia, USA, pp: 554-556.
- Tietz, N.W., 1995. Clinical Guide to Laboratory Tests. 4th Edn., W.B. Saunders Company, Philadelphia, PA, pp: 518-519.
- Tona, L., K.Mesia, N.P.Ngimbi, B. Chrimwami, O. Ahoka and K. Cimanga, 2001. In-vivo antimalarial activity of *Cassia occidentalis*, *Morinda morindoides* and *Phyllanthus niruri*. Ann. Trop. Med. Parasit., 95: 47-57.
- Trease, G.E. and W.C. Evans, 1989. Pharmacognosy. 11th Edn., Brailliar Tiridel Can, Macmillian Publishers, London.
- Tullo, A.H., 2008. Anutty chemical. Chem. Eng. News, 86(36): 26-27.
- Usha, K., G.M. Kasturi and P. Hemalata, 2007. Hepatoprotective effect of *Hygrophila spinosa* and *Cassia occidentalis* carbon tetrachloride induced liver damage in experimental rats. Indian J. Clin. Biochem., 22(2): 132-135.
- Verma, L., K. Anirudh, K. Basant, K.P. Umesh and S.P. Rajesh, 2010. Antidiabetic activity of *Cassia occidentalis* (Linn) in normal and alloxan-induced diabetic rats. Indian J. Pharmacol., 42(4): 224-228.
- Vijayabhaskar, K., K. Chaitanyaprasad, K. Srisailam, N.M.Arunadevi, S. Swathi and R. Subhashini, 2013. Analgesic and anti-inflammatory activities of the extract of *Cassia occidentalis* (Linn.) animal model. Int. J. Res. Pharm. Chem., 3(4): 759-762.
- WHO, 2002. Traditional Medicine Strategy 2002-2005. WHO Publications, pp: 1-6.
- WHO Expert Committee, 1985. Diabetes mellitus. WHO Technical Report Series 727, Geneva.
- Wild, S., G. Roglic and A. Green, 2004. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care, 27(5): 1047-1053.
- Xing, J.H. and E.E. Softer, 2001. Adverse effects of laxatives. Dis. Colon Rectum., 44(8): 1202-1209.
- Yadav, J. P., V. Arya, M. Panghal and S. Karma, 2010. *Cassia occidentalis* L: A review on its ethnobotany, phytochemical and pharmacological profile. Fitoterapia, 81: 223-230.
- Zambare, M.R., U.A. Bhosale, R.S. Somani, R.A. Yegnanarayan and K.A. Talpate, 2011. *Achyranthes aspera* (Agadha). Herb that improves pancreatic function in alloxain-induced diabetic rats. Asian J. Pharmaceut. Biol. Res., 1(2): 99-104.
- Zunzain, P.A., G.J. Jamie, T. Komatsu, E. Tsuchida and S. Curry, 2003. Crystal structural analysis of human serum albumin complexed with hemin and fatty acid. BMC Struct. Biol., 3: 6.