World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

# EVALUATION OF BIOCHEMICAL INDICES AND HYPOGLYCERMIC EFFECT OF AZADIRACHTA INDICA ON ALLOXAN INDUCED DIABETIC RATS

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**Article Received on 14/06/2020** **Article Revised on 05/07/2020** **Article Accepted on 26/07/2020**

## ABSTRACT
Antidiabetic activity of *Azadirachta indica*, was checked. Twenty-five rats were used for the research and were grouped into five of five rats each. Groups 1 was the untreated diabetic group while groups 2, 3 and 4 were the treatment which received 200, 400 and 800 mg/kg body weight of the *A. indica* extract respectively. Group 5 was the positive control and was administered known antidiabetic drug Glibenclamide. Diabetes was induced in the rats with alloxan monohydrate. *A. indica*, extract was administered to rats for 14 days orally by intubation, thereafter were sacrificed and blood collected from heart for analysis. Effect of *A. indica*, extract was checked on blood glucose level for possible hypoglycermic potential and Biochemical parameters. All results in treatment groups were compared with the untreated diabetic group at statistical confidence of $p<0.05$. Hypoglycermic effect was recorded at Day 1, Day 3 and Day 7 of the treatment. Result shows that *A. indica* extract reduced blood glucose level in the test groups as dose of extract increased. *A. indica*, demonstrated hypoglycermic effect. Biochemical indices indicated liver, kidney and cardiac protective effect.

**KEYWORD:** Alloxan, Blood glucose, *Azadirachta indica*, Diabetes, Glucose oxidase, Leaf.

## INTRODUCTION

Many plants extracts and drugs have been reported to cause diabetes by destruction of B- cells of islet of Langerhans of the pancreas, leading to elevated blood glucose level above normal reference range (Lenzen 2008).

*Azadirachta indica* (Family, Meliaceae) known by common name Neem tree is native to Asian countries. It has long been used in India as remedy for sickness, Tiwari et al (2014). The leaves, bark, stem, root have medicinal properties Biswas et al (2002).

The leaf extract is reported to be anthelmintic, antibacterial, antifungal, antihyperglycermic anti-inflammatory, antiviral, antipyretic, insecticidal, hypercholesteremic and hypoglycermic agent (Parotta 2001; Maragathavalli et al 2012).Chemical compounds in plants mediate their effect on the human/ animal body through processes identical with compounds in conventional drugs, thus herbal medicine do not differ greatly from conventional drug with reference to their mechanism of action, Vickers et al (2001).*Azadirachta indica* is called '*Ogwu akom*' by Ibo tribe in Nigeria meaning literally malaria drug. The Hausa tribe in Nigeria call it *Dogo yaro.* AST and ALT elevation in conditions of hepatocye damage in inflammatory condition of the liver, hypoxic states, hepatotoxicity by toxicants, trauma and some plant extracts, Reitman and Frankel, (1957). Liver ALP elevation is seen in hepatocyte and biliary epithelial damage. They could also be ALP elevation in osteoblast, intestinal epithelial and corticosteroid stimulation when used for treatment (Klein et al 1960; Babson et al 1966). Hyperproteinaemia is associated with dehydration occasioned by vomitting, diarrhoea, impaird renal concentration ability, excessive sweating or decreased water intake (Doumas et al 1971; Doumas and Peters, 1997; Lubran, 1978). Elevated urea production is associated with intestinal haemorrhage, increased dietary urea or increased protein catabolism, Fawcett and Scott, (1960). Elevated creatinine occure in pathological processes that cause a decrease in glomerular filration rate which could be pre-renal, renal or post renal, Blass et al, (1974). Hyperbilirubinaemia occur in diseases associated with haemolyses of blood as seen in babesiosis, anaplasmosis, trypanosomiasis, snake bite and some plant toxicants, Doumas et al (1973). This research is aimed at checking the effect of *A. indica* on blood glucose and biochemical indices for liver and kidney status after treatment. The disease diabetes

mellitus, usually just 'diabetes' is a metabolic disease characterized by raised levels of blood glucose and elevated fat and protein metabolism. It is one of the most prevalent human metabolic defects ADS (2007), Guyton (2006). It is caused by failure of the β-cell, of islet of Langerhans of the pancreas to produce insulin. Lensen (2008). It can be treated successfully by regulating the diet alone otherwise it is necessary to give daily injection of insulin or treat with sulphonylurea drugs.Dietary management is very important in the control of diabetes Bantle et al (2006). Diabetes is a risk factor for cardiovascular diseases such as hypertension, heart failure and nephropathy Bantle et al (2006). Nutritional therapy, counselling and the use of specialized nutritional supplement are recommended for diabetic cases Pastor et al (2002).Chronic complications of diabetes results from elevated blood glucose levels and associated with impairment of lipid and other metabolic pathways Sheetz and King (2003), Diabetic Control Trial (1993).Diabetic nephropathy is the leading cause of chronic kidney disease/failure, ADS (2007), Guyton (2006). The term diabetic nephropathy is used to describe the combination of lesions that occur concurrently in the diabetic kidney Sheetz and King (2003)**.** Diabetic retinopathy which is a leading cause of blindness is closely linked to elevation in blood glucose and hyperlipidemia seen in people with uncontrolled diabetes. Seen in cataracts and glaucoma ADS (2004). Diabetic neuropathies which can affect the somatic and autonomic nervous systems, results from uncontrolled diabetes Boulten (2004), ACCE (2003). Macrovascular disorder such as coronary heart disease, stroke and peripheral vascular disease reflect the combined effects of unregulated blood glucose levels, elevated blood pressure and hyperlipidemia due to metabolic disorder Martin (2007), Grundy and Panel (2007). Diabetic foot ulcers has been associated with diabetic patients, causing ulceration, infection and eventually the need for amputation, ADS (2004). The chronic complications of diabetes are best prevented by measures aimed at tight control of glucose levels, maintenance of normal lipid levels and control of hypertension Lenzen, (2008). Hypoglycermia occur when an agent, drug or plant extract causes lowering of blood glucose level below normal reference range Masharani and Gitelman (2007), ADS Workgroup (2005). Igwe and his co-workers sceenedfor toxic effect of leaf extract of plants usingliver and kidney marker enzymes on *Ficus capenses* Igwe *et al* (2019) and on *Telferia occidentalis*, Igwe *et al* (2019a). This study is aimed to investigate the hypoglycermic potentials of leaves of *A. indica* and to disprove or otherwise the natives claim that *A. indica* leaf extract can be used to reduce the blood glucose level of diabetic patients and Biochemical indices.

## MATERIAS AND METHODS

**Fig. 1: Picture of *Azadirachta indica* tree.**

### Plant Materials

Fresh leaves of *A.indica* were collected from Obi-Ngwa, Abia State Nigeria on 24th May, 2020. Sample of plant was identified by a Botanist at the Department of Plant Science and Biotechnology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria.

### Preparation of Plant Extract

The identified leaves of *A. indica* was shade dried for 10 days and pulverized to a coarse powder using mechanical grinder (Corona-Landers C 1A SA). The plant extract was prepared using Soxhlet method described by Jensen, (2007). Thirty five grams (35g) of coarse powdered sample was introduced into the extraction chamber of the Soxhlet extractor using ethanol as solvent. Temperature was maintained at $70^0$C throughout the extraction period of 48 hours. At the end of the extraction period the extract was concentrated using oven at $30^0$C to obtain dried extract which was weighed and kept in a well labelled sterile specimen bottle.

### $LD_{50}$ and Dose selection

A preliminary acute toxicity test was done using rats to determine the $LD_{50}$ (lethal dose that kills 50% of the rats) Acute toxicity ($LD_{50}$) was determined according to Lorke method, Lorke (1983). At 5000 mg/kg body weight of administered leaf extract, the treated rats were still healthy and active. This observation shows that the extract is safe at dose below 5000 mg/kg b.w. Based on this safety determination, different doses of 200, 400 and 800 mg/kg body weight was prepared and administered to rats in group 2, 3, and 4 respectively. These doses were calculated from a stock solution dissolved in distilled water.

### Chemicals

Alloxan was used in this study and was obtained from Sigma and Alderich USA. Other reagents/chemicals used were obtained within Nigeria and were of analytical grade.

**Experimental Animals**

Adult albino rats (148 to 253 g) were purchased from University Farm. Approval was obtained from College of Vet Medicine, Michael Okpara University of Agriculture Umudike, Nigeria, in line with the guidelines for the care and use of laboratory animals as given by the National Research Council NRC, (1985). The rats were acclimatize and fed *ad libitum.*

**Experimental Design**

Twenty-five rats were used for the research were grouped into five of five rats each. Groups 1 was the untreated diabetic group, 2, 3 and 4 were the treatment groups which received 200, 400 and 800 mg/kg body weight of the *A. indica* leaf extract. Group 5 was the positive control and was administered known antidiabetic drug Glibenclamide. Diabetes was induced in the rats with alloxan monohydrate. *A. indica* extract was administered to rats for 14 days orally by intubation, thereafter were sacrificed and blood collected from heart for analysis.

**Experimental Diabetes Induction**

The method of Lenzen (2008) was adopted. The animals were fasted for 16–18 hours with free access to water before the induction of diabetes. Induction of diabetes was carried out by single intraperitoneal injection of Alloxan Monohydrate (Sigma St Louis, M.O., USA) dissolved in 0.9% v/v normal saline solution at a dose of 150 mg/kg body weight Katsumat *et al.,* (1999). The diabetes was assessed in alloxan induced rats by determining the blood glucose concentration using one touch glucometer and Accu-check strips at day 1 and day 3 after injection of alloxan. The rats that recorded elevated blood glucose level above 240 g/dL were considered diabetic and were selected for the study.

**Blood Glucose Levels determination**

The procedure of Aziz (1983), Bergman (1984) based on the glucose oxidase principle was adopted in the determination of blood glucose level of the experimental rats.The enzyme glucose oxidase reacts with glucose, water and oxygen to form gluconic acid and hydrogen peroxide. The hydrogen peroxide can then be used to oxidize a chromogen or the consuption of oxygen measured to estimate the amount of glucose present. Glucose oxidase is specific for B-D-glucose so cross reaction with other sugars is not a problem Aziz (1983), Bergman (1984), Howanitz and Howanitz (1984). The blood samples were collected by cutting the tip of the tail artery of the rats, and a drop allowed touching the sensor part of one touch glucometer strips. The values obtained were recorded in mg/dl. The blood glucose levels were sampled at intervals of day 1, day 3 and day 7 of treatment.

**Biochemical Investigation**

Biochemical investigation was performed using ELISA reagent kits. The measure included alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST), using serum enzyme levels to determine liver and kidney state [ELISA]. They were determined by method of Reitman and Frankel, (1957)**.** Total protein was determined by Biuret method as described by Lubran (1978). Samples were analysed immediately to avoid artifactual changes, Ihedioha and Onwubuche, (2007).

**Statistical Analysis**

Data obtained were analysed using Statistical Package for Social Sciences (SPSS) version 20. All values were expressed as mean ± Standard Error of Mean (SEM). Data were further subjected to one - way analysis of variance (ANOVA) to compare the different doses of 200, 400 and 800 mg/kg body weight with the normal control. Duncan post-hoc test was used to separate the meam with a significant difference. The statistical confidence was set at 95% ($p<0.05$).

**RESULTS AND DISCUSSION**

Hypoglycemic result of leaf extract of *A. indica* on alloxan induced diabetic wistar rats after Day 3 of treatment.

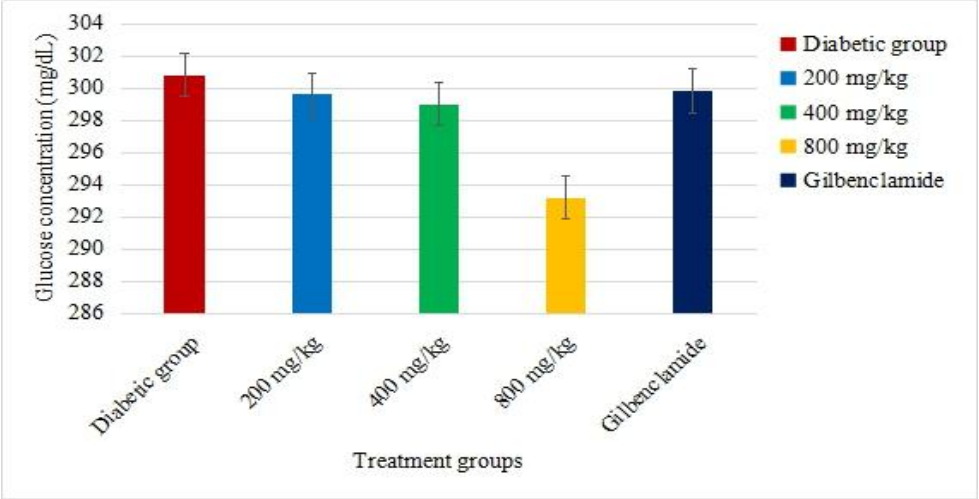


**Figure 2: Represent glucose level at Day 1.**

Graph in Fig 2 represent hypoglycermic result of leaf extract of *A. indica* on alloxan induced diabetic rats at Day 1 of treatment. Values are presented as Mean ± Standard Error of Mean (S.E.M.)

There was mild reduction of glucose in the treatment groups 299.60±16.85, 299.00±10.25 and 293.20±10.91 when compared to the diabetic untreated group 300.80±14.54. The reference standard drug was 299.80±8.13 was used as a check. The graph shows no significant reduction of glucose level at $p<0.05$ on Day 1 treatment.

Hypoglycemic result of leaf extract of *A. indica* on alloxan induced diabetic Wistar rats after Day 3 of treatment.

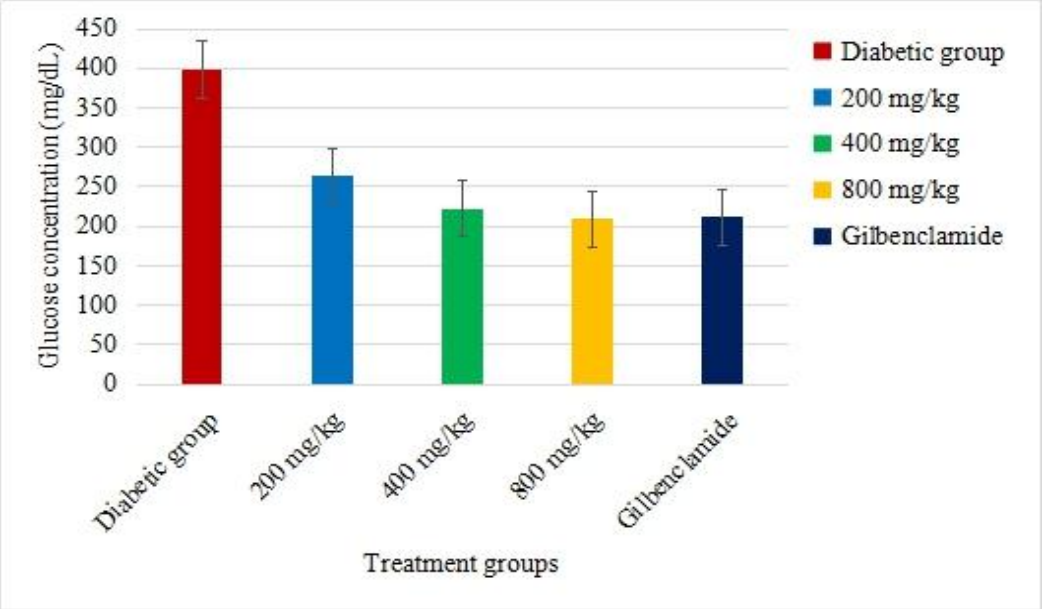


**Figure 3: Represent glucose level at Day 3.**

Graph in Fig 3 represent hypoglycermic result of leaf extract of *A. indica* on alloxan induced diabetic rats at Day 3 of treatment. Values are presented as Mean ± Standard Error of Mean (S.E.M.)

There was mild reduction of glucose in the treatment groups 263.40±12.69, 222.40±6.54 and 209.60±5.50 when compared to the diabetic untreated group 398.00±25.05. The reference standard drug was 211.80±3.18 was used as a check. There was significant reduction of glucose level at $p<0.05$ on Day 3 treatment.

Hypoglycemic result of leaf extract of *A. indica* on alloxan induced diabetic wistar rats after Day 7 of treatment.

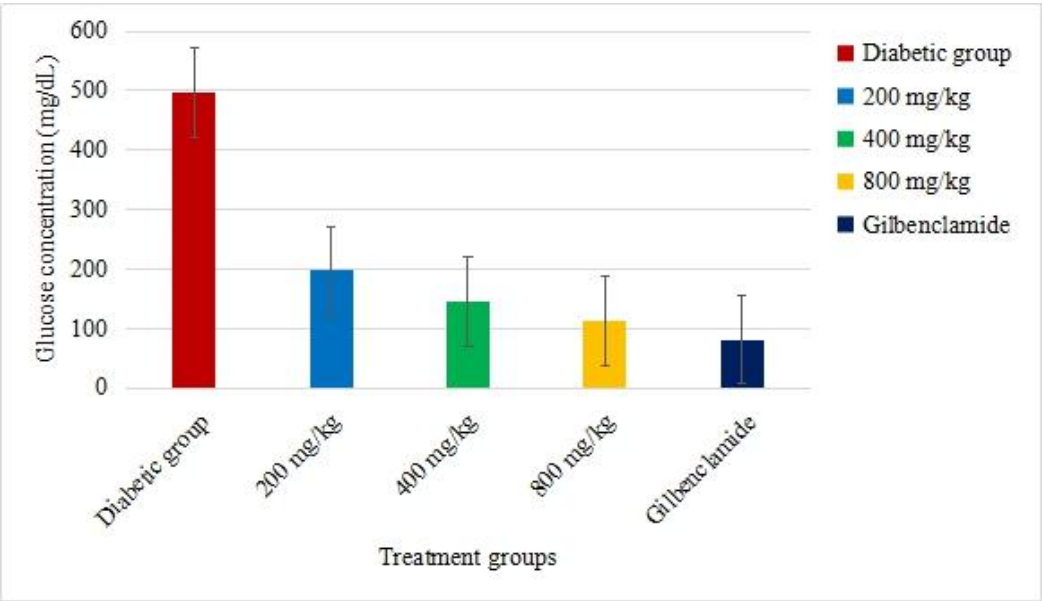


**Figure 4: Represent glucose level at Day 7.**

Graph in Fig 4 represent hypoglycermic result of leaf extract of *A. indica* on alloxan induced diabetic rats at Day 7 of treatment. Values are presented as Mean ± Standard Error of Mean (S.E.M.)

There was high reduction of glucose in the treatment groups 197.20±8.45, 197.20±8.45 and 113.60±4.70 when compared to the diabetic untreated group 496.80±4.74. The reference standard drug was 81.80±1.52 was used as a check. A significant reduction of glucose level at $p<0.05$ on Day 7 treatment was observed.

**Table 1: Percentage Reduction of leaf extract of *A.indica* on mean fasting blood glucose concentration (mg/dl) of alloxan induced diabetic Wistar rats after seven days of treatment.**

| Treatment groups | Value before induction | Day 1 | Day 3 | Day 7 | Percentage reduction after Day 7 |
|---|---|---|---|---|---|
| 5 mg/kg Gilbenclamide | 60.60±2.24 | 289.60±16.85 | 232.20±5.47$^{b}$ | 81.80±1.52$^{e}$ | 72.32 |
| Untreated | 60.80±1.39 | 315.17±3.16 | 398.00±25.05$^{a}$ | 496.80±4.74$^{a}$ | 0.00 |
| 200 mg/kg *A. indica* leaf extract | 61.80±0.91 | 289.08±19.08 | 263.40±12.69$^{b}$ | 197.20±8.45$^{b}$ | 32.77 |
| 400 mg/kg *A. indica* leaf extract | 62.40±0.67 | 283.14±11.32 | 237.80±20.69$^{b}$ | 146.20±15.21$^{c}$ | 51.52 |
| 800 mg/kg *A. indica* leaf extract | 64.00±1.30 | 280.08±10.42 | 234.80±14.84$^{b}$ | 113.60±4.70$^{d}$ | 62.00 |

Values are presented as mean ± S.E.M. Different superscripts represent significant differences at $p<0.05$.

Table 1 shows that the reference drug glibenclamide reduced the blood glucose of thr rats by 72.32%. The untreated diabetic group administered distilled water did not reduce the blood glucose (0.00%). In the treatment groups 200 mg/kg reduced 32.77% ; 400 mg/kg reduced 51.52% while 800 mg/kg reduced 62.00%. This shows that *A. indica* leaf has antidiabetic potential.

**Table 2: Effect of *A. indica* leaf extract on lipid profile of alloxan-induced diabetic rats.**

| Treatment | Total cholesterol (mg/dl) | Triglycerides | LDL (mg/dl) | HDL (mg/dl) | VLDL (mg/dl) |
|---|---|---|---|---|---|
| Normal control | 6.54±0.37$^{b}$ | 0.72±0.18$^{b}$ | 3.96±0.47$^{b}$ | 1.81±0.05$^{a}$ | 0.12±0.03$^{b}$ |
| Untreated group | 8.34±0.45$^{a}$ | 1.58±0.33$^{a}$ | 6.01±0.58$^{a}$ | 1.47±0.10$^{b}$ | 0.27±0.06$^{ab}$ |
| 200 mg/kg) | 8.49±0.17$^{a}$ | 1.46±0.06$^{a}$ | 4.26±0.09$^{b}$ | 1.57±0.02$^{b}$ | 0.26±0.01$^{ab}$ |
| 400 mg/kg | 9.08±0.61$^{a}$ | 1.41±0.04$^{a}$ | 3.56±0.05$^{bc}$ | 1.50±0.12$^{b}$ | 0.37±0.06$^{a}$ |
| 800 mg/kg | 7.68±0.46$^{ab}$ | 1.34±0.01$^{a}$ | 2.70±0.12$^{c}$ | 1.81±0.08$^{a}$ | 0.20±0.05$^{b}$ |
| Glibenclamide (5 mg/kg) | 8.15±0.42$^{a}$ | 1.34±0.19$^{a}$ | 6.02±0.55$^{a}$ | 1.51±0.01$^{b}$ | 0.25±0.05$^{ab}$ |

Values are presented as means ± Standard Error of Mean. Means with different superscript along columns are significantly different at $p<0.05$.

The result in Table 2 above shows that there was significant reduction of Total cholesterol, triglyceride, LDL, HDL and VLDL when compared with the untreated diabetic group. The treatment groups showed more reduction in lipid profile data than the reference drug.

**Table 3: Effect of *A. indica* leaf extract on the liver markers of alloxan-induced diabetic rats.**

| Treatment | ALP (IU/L) | AST (IU/L) | ALT (IU/L) | Total protein (g/dL) | Albumin (mg/dL) | Globulin (mg/dL) | Total Bilirubin (mg/dL) |
|---|---|---|---|---|---|---|---|
| Normal group | 156.34±6.21$^{b}$ | 186.64±7.60$^{b}$ | 66.08±3.78$^{b}$ | 6.12±0.19$^{b}$ | 2.69±0.13$^{b}$ | 3.80±0.31$^{ab}$ | 0.65±0.02$^{c}$ |
| Untreated group | 156.70±8.53$^{b}$ | 236.52±14.60$^{a}$ | 78.72±4.39$^{a}$ | 7.00±0.21$^{a}$ | 3.61±0.22$^{a}$ | 3.38±0.08$^{b}$ | 0.75±0.03$^{abc}$ |
| 200 mg/kg) | 151.56±0.65$^{bc}$ | 144.00±1.41$^{c}$ | 53.04±1.41$^{c}$ | 6.93±0.03$^{a}$ | 3.21±0.07$^{ab}$ | 3.66±0.04$^{ab}$ | 0.79±0.01$^{ab}$ |
| 400 mg/kg | 136.26±6.31$^{c}$ | 125.20±1.01$^{c}$ | 42.60±0.87$^{d}$ | 7.03±0.25$^{a}$ | 3.11±0.22$^{ab}$ | 3.91±0.14$^{a}$ | 0.72±0.04$^{bc}$ |
| 800 mg/kg | 111.80±3.35$^{d}$ | 98.40±0.81$^{d}$ | 38.00±0.69$^{d}$ | 6.26±0.17$^{b}$ | 2.82±0.15$^{b}$ | 3.39±0.13$^{b}$ | 0.71±0.03$^{bc}$ |
| Glibenclamide (5 mg/kg) | 192.44±1.98$^{a}$ | 235.42±2.86$^{a}$ | 79.38±2.00$^{a}$ | 7.08±0.09$^{a}$ | 3.44±0.11$^{a}$ | 3.63±0.05$^{ab}$ | 0.85±0.02$^{a}$ |

Values are presented as means ± Standard Error of Mean. Means with different superscript along columns are significantly different at $p<0.05$. AST: Aspartate Transaminase, ALT: Alanine Aminotransferase; ALP: Alkaline Phosphatase.

The result in Table 3 above shows that ALP, AST and ALT showed mild reduction.Total protein, albumin, globulin and total bilirubin were not significantly affected. This indicates hepatoprotective potential of *A. indica* leaf.

**Table 4: Effect of *A. indica* leaf extracts on the kidney markers of alloxan-induced diabetic rats.**

| Treatment | Urea (mMol/L) | Creatinine (µMol/L) | $Na^+$ (mMol/L) | $Cl^-$ (mMol/L) | $K^+$ (mMol/L) | $HCO_3^-$ (mMol/L) |
|---|---|---|---|---|---|---|
| Normal group | $32.20\pm1.85^{cd}$ | $0.60\pm0.02$ | $137.54\pm5.44^{a}$ | $95.12\pm3.09^{ab}$ | $4.57\pm0.29^{bc}$ | $16.42\pm0.81^{bc}$ |
| Untreated group | $41.32\pm1.78^{a}$ | $0.65\pm0.03$ | $135.24\pm1.93^{a}$ | $101.40\pm2.76^{a}$ | $5.21\pm0.37^{ab}$ | $18.58\pm1.09^{ab}$ |
| 200 mg/kg) | $35.04\pm1.75^{bcd}$ | $0.55\pm0.05$ | $114.80\pm3.77^{b}$ | $89.40\pm0.87^{b}$ | $4.12\pm0.07^{cd}$ | $20.62\pm0.46^{a}$ |
| 400 mg/kg | $37.42\pm2.46^{abc}$ | $0.60\pm0.01$ | $130.80\pm1.59^{a}$ | $97.34\pm2.26^{a}$ | $4.18\pm0.09^{cd}$ | $19.56\pm1.12^{a}$ |
| 800 mg/kg | $29.52\pm2.12^{d}$ | $0.57\pm0.01$ | $137.60\pm0.74^{a}$ | $88.82\pm3.60^{b}$ | $3.43\pm0.16^{d}$ | $14.82\pm1.03^{c}$ |
| Glibenclamide (5 mg/kg) | $38.90\pm0.53^{ab}$ | $1.86\pm1.19$ | $134.28\pm0.65^{a}$ | $101.66\pm0.73^{a}$ | $5.54\pm0.37^{a}$ | $19.26\pm0.31^{a}$ |

Values are presented as means ± Standard Error of Mean. Means with different superscript along columns are significantly different at $p<0.05$.

In Table 4 above, Urea and Creatinine were reduced mildly compared with the untreated diabetic rats while $Na^+$, $Cl^-$, $K^+$ and $HCO_3^-$ were within normal reference range. This indicate kidney safety.

## DISCUSSION

Liver the primary site of biotransformation and detoxification of xenobiotics is vulnerable to xenobiotics (Lee, 1993; Sturgill and Lambert, 1997). Similarly, kidneys as the principal organ for the excretion of xenobiotics and their metabolites are prone to toxic effects (Subcommittee on Biologic Markers in Urinary Toxicology, 2002). Damage to these organs often results in elevation in clinical biochemistry parameters such as serum enzymes; AST, ALP and ALT and/or creatinine as a marker of impairment of renal filtration (Ferguson *et al.*, 2008). The increase in these enzymes is indicated in hepatic and nephrotic disorders.

The result as presented in Table 3 showed that the liver enzymes Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) showed mild decrease but was not significant ($p<0.05$).This observation suggests that *A. indica* extract did not cause any significant damage to liver or the kidney which are responsible for its metabolism, biotransformation, and elimination.

Since the ethanol extract of *A. indica* leaves did not cause any significant ($p<0.05$) increase in the levels of serum total protein (TP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), of the treated Wistar rats compared with the untreated diabetic rats, therefore it could be suggested that the administration of this *A. indica* extract at dose <800 mg/kg body weight has no significant hepatotoxic or nephrotoxic effect on the liver and kidney respectively. This findings lay credence to the traditional claim of safety of the extract of *A. indica* to liver and kidney when used in the treatment of diabetes at moderate doses (< 800 mg/kg body weight).

The mild decrease in value of total protein by this plant extract had no significant ($p<0.05$), effect indicating its safety to the hepatosynthetic cells of the liver.

Diabetes mellitus is among the most common disorder in developed and developing countries (Makund *et al.*, 2008). The disease is increasing rapidly in most parts of the world (Kumar *et al.*, 2008). Hyperglycaemia in diabetic patients is associated with alterations in glucose and lipid metabolism and modification in liver enzyme levels (Jenson and Stender, 1998). Despite the presence of known anti diabetic medicines in the pharmaceutical market, screening for new anti-diabetic sources from plants is a good alternative because they contain substances which are safer for use on diabetes mellitus (Alli Smith, and Adanlawo, 2012). Other plant extracts have been known to show antidiabetic effects, Uhuegbu and Ogbuehi (2002), Nwanjo and Nwokoro (2004).

In this study diabetes was induced in rats by a single intra-peritoneal injection of alloxan monhydrate at 150 mg/kg body weight. Alloxan is a cytotoxic agent known to induce diabetes in a wide variety of animal species by damaging insulin secreting β-cell, resulting in decrease of insulin release. This results in decrease utilization of glucose by the tissues leading to hyperglycermia, Lenzen, (2008). The findings indicate that administration of *A. indica* leaf extract at the graded dosage on alloxan-induced diabetic rats caused a significant ($p < 0.05$) reduction of the elevated glucose level. The extract from the leaf of *A. indica* caused a significant decrease of blood glucose in a dose dependent manner and hypoglycermic effect was highly pronounced at day 7, treatment (Fig 4). We suspect that the anti-hyperglycemic activity of this extract may be partly due to insulin release from the existing cells of the pancreas, stimulation of insulin secretion and release, regeneration of β-cell of Langerhans islets or activation of enzymes responsible for glucose utilization, Spasov *et al.*, (2008). This findings is not in isolation as it is in agreement with other studies reported by various researchers, Ezeja *et al* (2015), Lensen (2008) who demonstrated that antidiabetic activities of plant extract may be due to its

multiple effects involving both pancreatic and extra-pancreatic mechanisms.Figure 2 showed that reduction of blood glucose was not significant at Day 1 treatment. Figures 3 and 4 shows significant reduction of the leaf extract of *A. indica* at Day 3 and Day 7 treatment respectively. The hypoglycermic response was in a dose dependent manner when compared to the diabetic untreated group. The extract competed favourably with the reference drug Gilbenclamide. This suggest that the at higher doses the leaf extract of *A. indica* can compete favourably with known antidiabetic drugs, hence a good alternative for diabetic cases.

Acute toxicity test shows that <5000 mg/kg of *A. indica* leaves was safe and was used for the study. The hypoglycermic potential of *A. indica* was evaluated by checking its ability to reduce the FBS of rats induced with alloxan monohydrate (150 mg/kg).

Elevated serum lipids observed in diabetes mellitus are suspected to cause coronary heart disease in diabetic cases, Murugan et al (2009).

There was mild decrease in total cholesterol which was not significant. Triglyceride, LDL, HDL and VLDL showed reduction when compared with untreated diabetic rats. Though these reduction was not significant at $p<0.05$ (Table 2). This reduction could be beneficial in preventing diabetic complications. We suspect that the reduction is due to a control in lipid metabolism (Cho et al, 2002). Therefore *A. indica* leaf could be useful in preventing cardiac diseases associated with diabetes.

Induction of diabetes with alloxan monohydrate can lead to leakage and elevated levels of liver enzymes ALT, AST, ALP into the blood, Edet et al (2011) as seen in untreated diabetic group (Table 3). The significant dose-dependant reduction in the elevated serum ALT, AST and ALP after administration of *A. indica* leaf suggest hepatoprotective effect. This could be due to membrane stabilization and repair of tissue damage, Argawal et al (2012).

There was mild reduction in total protein of treated rats when compared with the untreated diabetic rats. This effect was not significant and bilirubin level was not affected (Table 3).

There was mild reduction in urea and creatinine levels which is an indication of kidney protective effect of *A. indica* leaf extract. Na+, Cl-, K+ and $HCO_3^-$ were within normal reference range (Table 4)

There is plan for further studies to find the bioactive compounds responsible for this antidiabetic activity in *A. indica* leaf.

## CONCLUSION

Leaf extract of *A. indica* has a potent antidiabetic activity which is comparable with the known drug, Gilbenclamide in alloxan - induced diabetic rats and hence may be a good alternative in the treatment of diabetes. Further studies are hereby recommended to isolate and characterize the active ingredients responsible for the hypoglycermic effect in *A. indica* leaf extract. Biochemical indices indicated liver, kidney and cardiac protective effect at dose <800 mg/kg body weight.

## ACKNOWLEDGEMENT

We are grateful to Abia State Government, Nigeria for their grant to support this Research.

**Declaration of Interest**
The authors report no declaration of conflict of interest.

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