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Evaluation of Anti-diabetic Effect and Liver Function Indices of Ethanol Extracts of *Moringa oleifera* and *Cajanus cajan* Leaves in Alloxan Induced Diabetic Albino Rats

P.M. Aja, I.O. Igwenyi, P.C. Ugwu Okechukwu, O.U. Orji and E.U. Alum

Department of Biochemistry, Faculty of Science,
Ebonyi State University P.M.B 053 Abakaliki, Nigeria

Abstract: This work is aimed at evaluating the anti-diabetic effect and liver function indices of ethanol extract of *Moringa oleifera* and *Cajanus cajan* leaves in alloxan induced diabetic albino rats. Thirty two albino rats were randomly grouped into A, B, C and D. Groups A and B had four rats each while groups C and D were further sub-grouped into C1, C2, C3, D1, D2 and D3 with four rats each respectively. Diabetes was induced in all the groups except A which served as the positive control. Group B served as the negative control and was not treated while group C was treated with ethanol extract of *Moringa oleifera* leaf and group D was treated with ethanol extract of *Cajanus cajan* leaf which were administered orally to the animal once daily for one week at varying doses of 200, 400 and 800 mg/kg body weight. The glucose level and liver function indices were determined using glucometric and spectrophotometric methods. The results revealed a significant ($P<0.05$) reductions in glucose levels of rats treated with ethanol extract of *Moringa oleifera* and *Cjanus cajan* leaves. The result showed significant ($P<0.05$) increase in ALT, AST and ALP levels in alloxan induced diabetic albino rats treated with *Cajanus cajan* ethanol leaf extract when compared with those treated with *Moringa oleifera* ethanol leaf extract which also showed a significant ($P<0.05$) increase in dose dependent manner. The result showed significant ($P<0.05$) reduction in albumin level in rats treated with *Cajanus cajan* and *Moringa oleifera* ethanol leaf extracts except for 400mg/kg of both extract which showed no significant ($p<0.05$) decrease in albumin level. Also a significant ($p<0.05$) increase in albumin level in rats treated with 200 mg/kg of *Moringa oleifera* ethanol leaf extract was observed. The result also showed a significant ($P<0.05$) reduction in total bilirubin in dose dependent manner in alloxan induced diabetic albino rats treated with *Cajanus cajan* except the group treated with 400 mg/kg which revealed no significant ($p<0.05$) decrease in total bilirubin level. A significant ($P<0.05$) increase in total bilirubin level in rats treated with 400 mg/kg of *Moringa oleifera* ethanol leaf extract except for the treated with 800 mg/kg of *Moringa oleifera* ethanol leaf extract which showed no significant ($p<0.05$) increase in total bilirubin level. At 200 mg/kg of *Moringa oleifera* ethanol leaf extract no significant ($P<0.05$) reduction in total bilirubin level was observed. The result equally showed significant ($P<0.05$) reduction in conjugated bilirubin level in albino rats treated with *Cajanus cajan* except for the group treated 400 mg/kg of *Cajanus cajan* which showed significant ($P<0.05$) increase in conjugated bilirubin level. A significant ($P<0.05$) reduction in dose dependent manner was observed in conjugated bilirubin level rats treated with *Moringa oleifera* ethanol leaf extract.

Key words: *Moringa oleifera* · *Cajanus cajan* · Liver Function Indices · Glucose Level · Anti-Diabetic

INTRODUCTION

In the recent times, there has been growing interest in exploiting the biological activities of different ayurvedic medicinal herbs, due to their natural origin, cost effectiveness and lesser side effects [1]. Plants produce vast array of secondary metabolites as defense against environmental stress or other factors like pest attacks, wounds and injuries [2]. The complex secondary metabolites produced by plants have found various

Corresponding Author: P.M. Aja, Department of Biochemistry, Faculty of Science,
Ebonyi State University P.M.B 053 Abakaliki, Nigeria.

therapeutic uses in medicine from time immemorial [2, 3]. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness [3]. Among these are *Moringa oleifera* and *Cajanus cajan*. *Moringa oleifera* is a multipurpose tree used as vegetable, spice, a source of cooking and cosmetic oil and a medicinal plant [4, 5]. It is known as drumstick in English, "Zogallagand" in Hausa, "Zogali" in Nupe, "Okweoyibo" in Igbo, "Ewe- igbala" in Yoruba [6]. All part of *Moringa* tree are edible and have been consumed by humans, a plethora of its curative power and scientific validation of these claims are available in literature [4]. *Cajanus cajan* is extensively eaten as a dal (Preparation of beans). It is known as pigeon pea in English and "Fiofio" in Igbo [7]. It is an important grain legume crop of rain-field agriculture in the tropic and subtropics. It is known to be useful in diverse ways [8, 9].

The leaves are used for the treatment of wound, bedsores, malaria as well as diet induced hypercholesterolemia [10, 11]. Protective effects of extracts from pigeon leaf against hypoxic-ischemic brain damage and alcohol induced liver damage have also been reported [12].

Diabetes mellitus (DM) is a metabolic disease characterized by a persistent elevation of fasting blood glucose level (FBGL) above 200 mg/dl, due to insufficient or complete cessation of insulin synthesis or secretion and/or peripheral resistance to insulin action [13]. The latest World Health Organization (WHO) publication (Global Burden of Disease) estimate the presence of diabetes in adults to be around 173 million [14]. In diabetes, lipid abnormalities, anemia, alteration of liver and kidney functional indices has been implemented as major risk factors to the progression of both microvascular and macrovascular diabetes complications [15, 16]. Several anti-diabetic drugs such as biguanid and sulphonyureas along with insulin have been employed for the treatment of this disease. Still none of these drugs were able to cure the disease without adverse reaction [17]. There is a growing interest in the use of medicinal plants of low side effect for the management of DM especially in countries where access to conventional treatment of diabetes mellitus is inadequate [17, 18].

The liver is a vital organ present in vertebrates and some other animals. It has a wide range of functions, including detoxification, protein synthesis and production of biochemical necessary for digestion [19]. Despite the considerable progress in the treatment of diabetes using oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. In recent times, there has been renewed interest in plant remedies for which WHO has recommended attention [20]. This study therefore evaluate the anti-diabetic effect and liver function indices of ethanol extracts of *Moringa oleifera* and *Cajanus cajan* leaves in alloxan-induced diabetic albino rats.

MATERIALS AND METHODS

Materials: The chemicals and reagents used are of analytical quality. Fresh leaves of fully grown *Moringa oleifera* and *Cajanus cajan* were collected from a local farm at Aghara Oza village, Izzi Local Government area, Ebonyi State and identified by Prof. J.C Okafor, a plant taxonomist in the Department of Applied Biology, University of Nigeria Nsukka, Enugu State, Nigeria.

Preparation of Extract: The leaves of *Moringa oleifera* and *Cajanus Cajan* were air-dried under room temperature. The dried leaves were pulverized to fine granules using manual grinder. The powder was soaked in 98% ethanol at room temperature for 24 hours. It was then filtered using sieve cloth and the filtrate evaporated to dryness using rotator evaporator.

Experimental Animals: Twenty-four male albino rats weighing between 125 –200g (4-6-weeks old) were obtained from the animal house of the faculty of Veterinary Medicine Nnamdi Azikiwe University (UNIZIK), Awka Anambra State and transported to the animal house of Biochemistry Department Ebonyi State University, Abakaliki.

They were acclimatized for seven days in stainless steel cages under good laboratory conditions. They were fed with commercial poultry growers mash feed (Vital feed ®, Jos, Nigeria). Clean water was provided daily and access was free. Proper sanitation was maintained in the animal house to ensure healthy and clean environment. The animals were weighed using triple beam weighing balance. Handling, management and use of animals for the experiment were as such that allowed minimal stress. Ebonyi State University Animal Ethical Committee approved the animal studies.

Experimental Design: At the end of the seven days acclimatization period, the animals were randomly assigned into eight different groups of three rats each, designated as group A-D. Group A received water and feed only and served as positive control. Diabetes were induced in group B, C and D. Group B was not treated and

served as negative control while group C and D were further subdivided into C1, C2, C3, D1, D2 and D3 corresponding to 200, 400 and 800 mg/kg doses of the extracts. Group C1 to C3 was treated with *M. oleifera* extract while group D1 to D3 was treated with *C. cajan* once daily for seven days.

Induction of Diabetes: About 2g of alloxan were dissolved in 20ml of distilled water and were administered to group B, C and D, based on the body weight of the rats and on a dosage of 100mg/kg. The administration was done intra-peritoneally using diabetic syringe [21]. The animals with sugar level more than 180mg/dl were considered as experimental diabetic [22].

Collection of Blood from Animals: After seven days of administering the animals with the plants extracts. The animals were starved. The blood samples were collected from the tail vein puncture for the measurement of blood glucose. The blood glucose levels were measured in the fasting animals on 1st and 7th days. Blood samples used for liver enzyme assay was collected through ocular puncture using capillary tubes from the eyes of the animal.

Determination of Blood Glucose Level: Glucose level was determined using glucose oxidase method (GOD) (Glucometer).

Determination of Liver Enzymes: Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined by method described by Reitman and Frankel [23].

Determination of albumin (ALB) was carried out by the method described by Tietz [24] and Grant [25].

Determination of total bilirubin and conjugated bilirubin were carried out by the method described by Jendrassik and Grof [26].

Determination of Unconjugated Bilirubin: This was determined by subtracting the conjugated bilirubin level from the total bilirubin level.

Data Analysis: Results were expressed as mean ± standard deviation. The significance of difference between the controls and treated groups were determined using one-way analysis of variance (ANOVA).

RESULTS

Percentage Yield of Extracts: The percentage yield of ethanol extract of *Moringa oleifera* and *Cajanus cajan leaves are* 25% and 6.25% as shown in the table. This means that ethanol is a good solvent for extraction of some of the active ingredients in *Moringa oleifera* leaves when compared with *Cajanus cajan* which has a percentage yield of 6.25%.

Result of the Weight of Animals Based on Varying Extract Doses During the 7 Days of Treatment: The result showed that there was an increase in the body weight of rats in the positive control group and those treated with 400mg/kg body weight of *Cajanus cajan* and *Moringa oleifera* leaves. The result also revealed a decrease in the body weight of rats in the negative control.

Result of Blood Glucose Level in Alloxan Induced Diabetic Albino Rats after 7 Days of Treatment: The administration of ethanol extracts of *Moringa oleifera* and *Cajanus cajan* leaves in alloxan induced diabetic albino rats at various doses of 200, 400 and 800mg/kg body weight significantly ($P<0.05$) reduced the blood glucose level in the treated rats (Figure 2). The result also showed that there was significant ($P<0.05$) reduction in the blood glucose level in rats treated with *Moringa oleifera* ethanol leaf extract in a dose dependent manner than the *Cajanus cajan* ethanol leaf extract (Figure 2).

Result of Levels of Liver Enzymes in Alloxan Induced Diabetic Albino Rats Treated with Ethanol Extracts of *Moringa oleifera* and *Cajanus cajan* Leaves: The result showed significant ($P<0.05$) increase in ALT, AST and ALP levels in alloxan induced diabetic albino rats treated with *Cajanus cajan* ethanol leaf extract when compared

Table 1: Percentage yield of Extracts of Dried *Moringa oleifera and Cajanus cajan* leaves using Ethanol

Solvent used	Plant	Plant Part	Mass of leaf before extraction (g)	Mass of leaf after extraction (g)	Volume of solvent used (ml)	Mass of extract (g)	Percentage yield (%)
Ethanol	*Moringa oleifera*	Leaves	200	150	600	50	25
Ethanol	*Cajanus cajan*	Leaves	200	187.5	1600	12.5	6.25

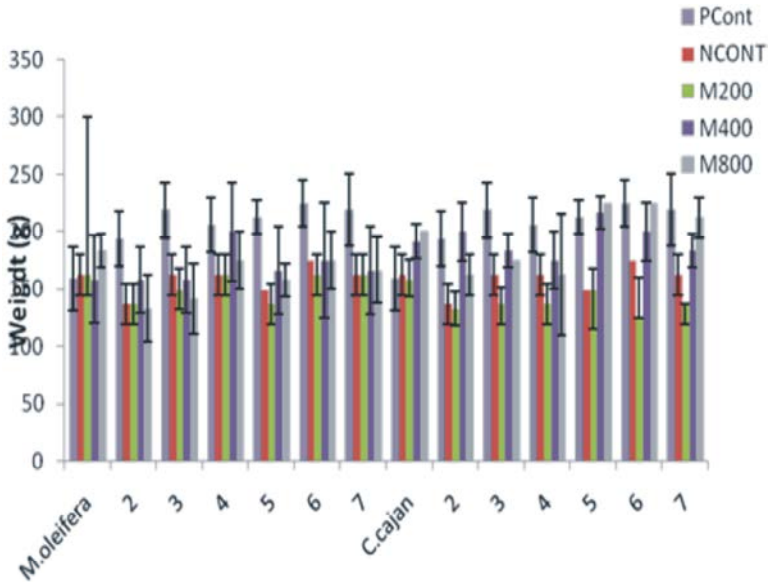

Fig. 1: Weight of Albino Rats during the Seven (7) days Treatment with Ethanol Extract of *Moringa oleifera* and *Cajanus cajan* Leaves.
Data are shown as mean ± S.D (n=4) and significant difference at P<0.05 in comparison with the controls.

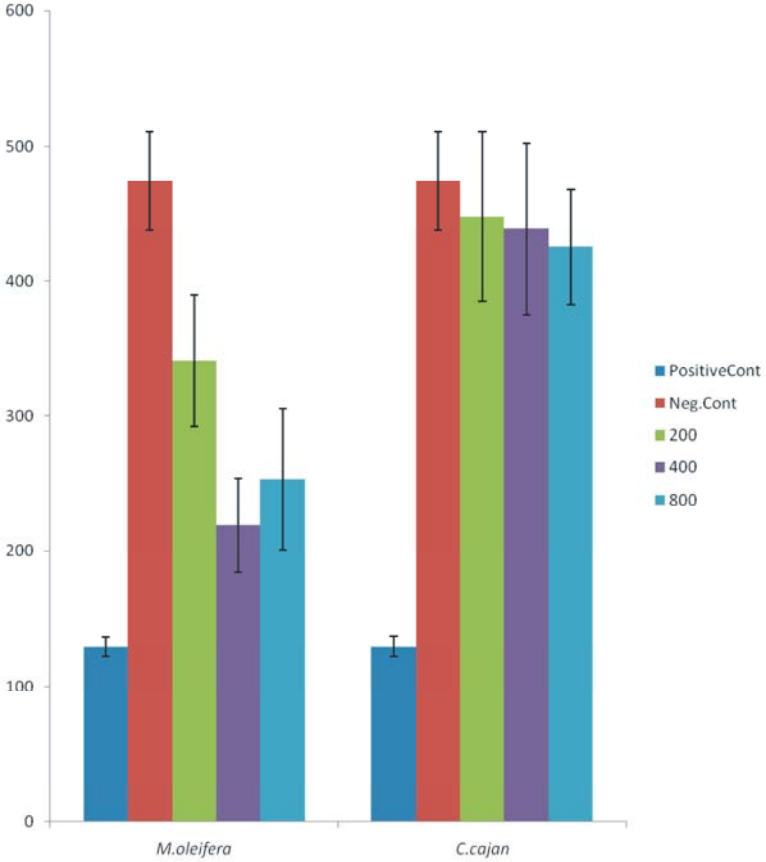

Fig. 2: Blood glucose level in Alloxan Induced Diabetic Albino rats Treated with Ethanol Extract of *Moringa oleifera* and *Cajanus cajan* leaves
Data are shown as mean ± S.D; (n = 3), significant differences at (p<0.05).

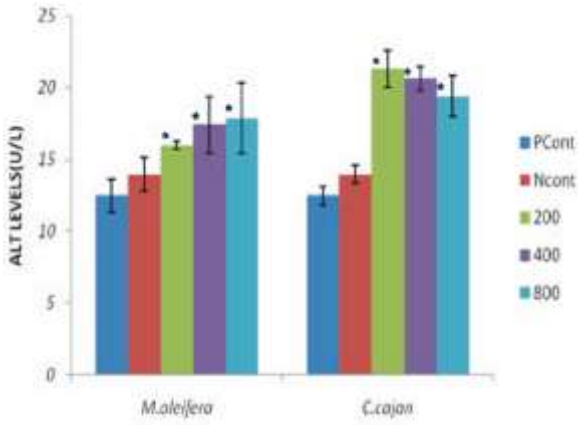

Fig. 3: ALT level in Alloxan Induced Diabetics Albino rats Treated with Ethanol Extract of *Moringa oleifera* and *Cajanus cajan* leaves.
Data are shown as mean ± S.D; (n = 3), mean values with (*) have significant differences (P<0.05) when compared with the negative and the positive control.

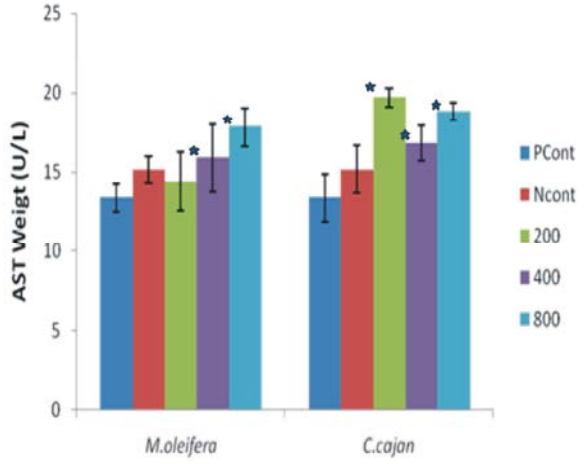

Fig. 4: AST level in Alloxan Induced Diabetics Albino rats Treated with Ethanol Extract of *Moringa oleifera* and *Cajanus cajan* leaves.
Data are shown as mean ± S.D; (n = 3), mean values with (*) have significant differences (P<0.05) when compared with the negative and the positive control.

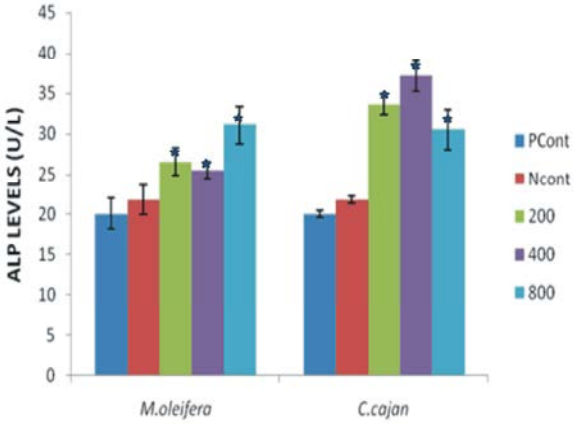

Fig. 5: ALP level in Alloxan Induced Diabetics Albino rats Treated with Ethanol Extract of *Moringa oleifera* and *Cajanus cajan* leaves.
Data are shown as mean ± S.D; (n = 3), mean values with (*) have significant differences (P=0.05) when compared with the negative and the positive control.

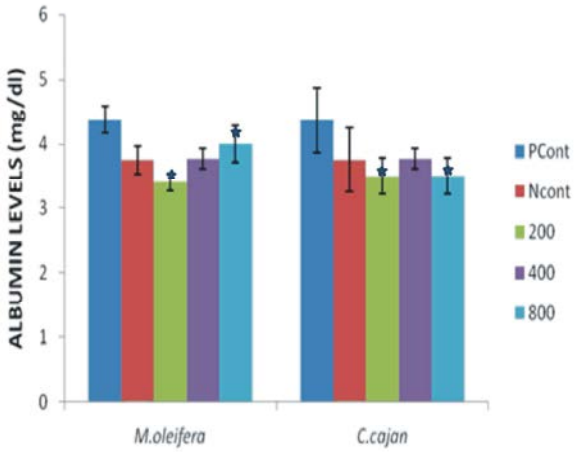

Fig. 6: Albumin level in Alloxan Induced Diabetic Albino rats Treated with Ethanol Extract of *Moringa oleifera* and *Cajanus cajan* Leaves.
Data are shown as mean ± S.D; (n = 3), mean values with (*) showed significant differences at P<0.05.

with those treated with *Moringa oleifera* ethanol leaf extract which also showed a significant (P<0.05) increase in dose dependent manner (Figure 3-5).

Result of level of Albumin, Total and Conjugated Bilirubin in Alloxan Induced Diabetic Albino Rats Treated with Ethanol Extract of *Moringa oleifera and Cjanus cajan* Leaves: The result showed significant (P<0.05) reduction in albumin level in alloxan induced diabetic albino rats treated with *Cajanus cajan* and *Moringa oleifera* ethanol leaf extracts except for 400mg/kg of both extract which showed no significant (p<0.05) decrease in albumin level (Figure 6). Also a significant (p<0.05) increase in albumin level in alloxan induced diabetic albino rats treated with 200 mg/kg of *Moringa oleifera* ethanol leaf extract was observed (Figure 6). The result also showed a significant (P<0.05)

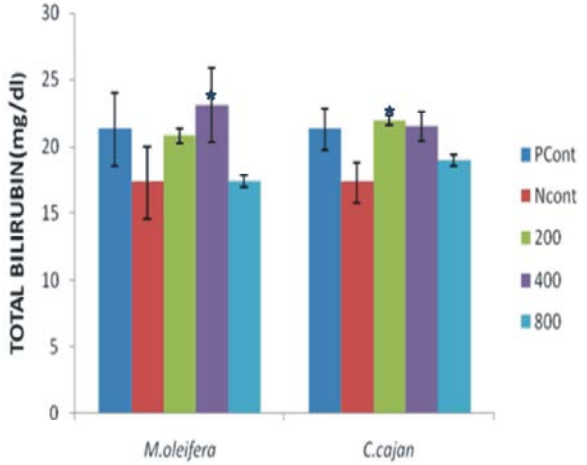

Fig. 7: Total bilirubin level in Alloxan Induced Diabetic Albino rats Treated with Ethanol Extract of *Moringa oleifera* and *Cajanus cajan* Leaves. Data are shown as mean ± S.D; (n = 3), mean values with (*) showed significant differences at P<0.05.

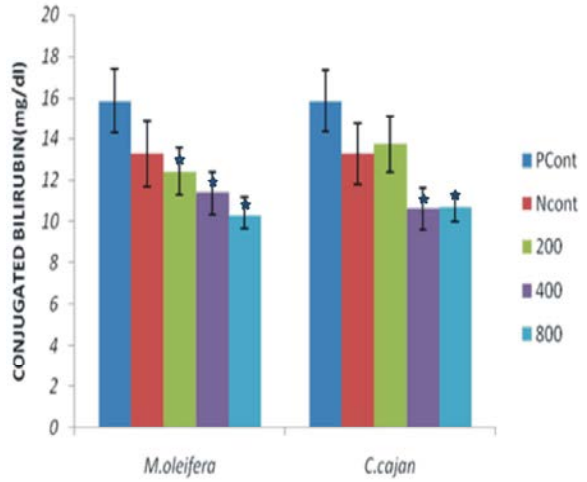

Fig. 8: Conjugatedl bilirubin level in Alloxan Induced Diabetic Albino rats Treated with Ethanol Extract of *Moringa oleifera* and *Cajanus cajan* Leaves. Data are shown as mean ± S.D; (n = 3), mean values with (*) showed significant differences at P<0.05.

reduction in total bilirubin in dose dependent manner in alloxan induced diabetic albino rats treated with *Cajanus cajan* except the group treated with 400 mg/kg which revealed no significant (p<0.05) decrease in total bilirubin level (Figure 7). A significant (P<0.05) increase in total bilirubin level in alloxan induced diabetic albino rats treated with 400 mg/kg of *Moringa oleifera* ethanol leaf extract except for the treated with 800 mg/kg of *Moringa oleifera* ethanol leaf extract which showed no significant (p<0.05) increase in total bilirubin level (Figure 7). At 200 mg/kg of *Moringa oleifera* ethanol leaf extract no significant (P<0.05) reduction in total bilirubin level was observed (Figure 7). The result equally showed significant (P<0.05) reduction in conjugated bilirubin level in alloxan induced diabetic albino rats treated with *Cajanus cajan* except for the group treated 400 mg/kg of *Cajanus cajan* which showed significant (P<0.05) increase in conjugated bilirubin level(Figure 8). A significant (P<0.05) reduction in dose dependent manner was observed in conjugated bilirubin level rats treated with *Moringa oleifera* ethanol leaf extract (Figure 8).

DISCUSSION AND CONCLUSION

A general significant (p<0.05) decrease in the body weights in alloxan induced diabetic albino rats was observed in the study (Figure 1). This is widely associated with diabetes patients due to loss of appetite which was also confirmed in this study. The result in Figure 2 showed an increase in the body weight of treated animals this is an indication that diabetes reduces the weight of animals. Aja *et al.* [27], reported a significant (P<0.05) reductions in the mean body weight of rats in diabetic control compared to positive group while rats in treated groups showed significant (P<0.05) increase in their mean body weight compared to diabetic control group in work done earlier on anti-diabetic effect of aqueous extract of *Moringa oleifera* and *Bridelia ferruginea* leaves in alloxan-induced diabetic albino rats. Grover *et al.* [28] had also reported that aqueous extract of *Aeglemarmelose* leaves was equally effective in comparison to insulin in restoring blood glucose and body weight to normal levels. Uraku *et al.* [29] had earlier reported a significant (p<0.05) increase in serum glucose of the untreated diabetic group compare to the control group.

The administration of ethanol extract of *Moringa oleifera* and *Cajanus cajan* leaves to the alloxan induced diabetic rats showed a significant (P<0.05) reduction in the blood glucose level of the rats (Figure 2). The result of this study correlates with Aja *et al.* [27] which reported a significant (p<0.05) decrease in glucose level in alloxan induced diabetic albino rats treated with aqueous extracts of *Moringa oleifera* and *Bridelia ferruginea* leaves. Some of the medicinal plants such as *Moringa oleifera* and *Cajanus cajan* are among the numerous plant adjuvant for the treatment of diabetes mellitus. WHO has pointed out that prevention of diabetes and its complications is not

only a major challenge for the future, but essential if health for all is to be an attainable target [30]. The WHO study groups in its report had emphasized strongly the need of optimum and rational uses of traditional and natural indigenous systems of medicine in health care of general public of any specific country [30]. There is still insufficient evidence to draw definite conclusions about the efficacy of individual herbs and supplement for diabetes; however, they appear to be generally safe [31]. Some of these bioactive compounds found in medicinal plants may exert their hypoglycemic effects by reducing insulin resistance, increasing release and decreasing glucagon secretion, slowing the digestion and absorption of carbohydrates or by decreasing hepatic glucose production [32]. *Moringa oleifera* and *Cajanus cajan* also contains flavonoids, stilbeans, terpenoids, glycoside and alkaloids [33] as its bioactive compounds, which elicit their anti-diabetic effect by causing an increase in insulin output or by inhibition of the intestinal absorption glucose or to the facilitation of metabolites in insulin dependent processes.

The result of the study showed that ethanol extract of *Moringa oleifera* and *Cajanus cajan* leaves were able to raised liver function indices in alloxan induced diabetics albino rats (Figure 3-8) Also Aja *et al.* [27] had reported a significant ($p<0.05$) decrease in liver enzymes level in alloxan induced diabetic albino rats treated with aqueous extracts of *Moringa oleifera* and *Bridelia ferruginea* leaves. Administration of aqueous-ethanolic extracts of crude extracts of *A. esculentus* caused a marked ($p<0.05$) increased in the albumin levels in the diabetic rats [29]. There was a significant ($P<0.05$) increase (82%) in total bilirubin levels in diabetic control group over the normal control. Administration of aqueous-ethanolic extracts caused a marked significant ($p<0.05$) decrease in the bilirubin levels in the diabetic rats [29].

Whereas liver protective herbal drugs contain a variety of chemical constituents like coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavonoids, organic acids, lipids, alkaloids, xanthenes, terpenoids, reducing sugars, resins saponins and stilbeans [33]. These are present in the *Moringa oleifera* and *Cajanus cajan* and so may be responsible for this effect. It can deduce that the leaves extract of *Moringa oleifera* and *Cajanus cajan* have appreciable ability to prevent damage to the liver. The significant decrease in the level of these parameters especially with the 400mg/kg dose may be an indication that this may be the safest dose to use when administering this extract for medicinal purpose. They are normally present at low levels in the blood so if the liver cells are damaged, it will be that some of the enzymes leak into the blood and increase in levels.

CONCLUSION

The intra-peritoneal injection of alloxan in rats induces type 2 diabetes with instability of some biochemical parameters like liver function indices. The data obtained from the study specified that oral administration of ethanol extract of *Moringa oleifera* and *Cajanus cajan* leaves have anti-diabetic properties. It is therefore concluded from the result that *Moringa oleifera* and *Cajanus cajan* ethanol leaf extracts possesses anti-diabetic activity and it may be useful in the management of diabetes.

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