International Journal of Biochemistry Research & Review
3(3): 248-258, 2013

SCIENCEDOMAIN *international*
www.sciencedomain.org

Evaluation of Anti-diabetic and Liver Enzymes Activity of Aqueous Extracts of *Moringa oleifera* and *Bridelia ferruginea* Leaves in Alloxan Induced Diabetic Albino Rats

P. M. Aja[1*], E. J. Nwafor[1], A. U. Ibiam[1], O. U. Orji[1], N. Ezeani[1] and B. U. Nwali[1]

[1]Department of Biochemistry, Faculty of Biological Sciences, Ebonyi State University, Abakaliki, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author PMA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors EJN and OUO managed the analyses of the study. Authors NE and BUN managed the literature searches. Author AUI read through the original manuscript and made some vital contributions. All authors read and approve the final manuscript.

Research Article

Received 19th March 2013
Accepted 9th May 2013
Published 28th June 2013

ABSTRACT

Aims: To evaluate anti-diabetic and liver enzymes activities of aqueous extracts of *Moringa oleifera* and *Bridelia ferruginea* leaves in alloxan induced diabetic albino rats.
Study design: Diabetes was induced in three groups of rats, one group was not treated while two groups were treated orally with *M. oleifera* and *B. ferruginea* extracts at 200, 400 and 800 mg/kg body weight of rats twice for 1 week respectively. One group was not induced and received distilled water only. The anti-diabetic and liver enzymes activities were determined from blood glucose and transaminases activities of the rats.
Place and Duration of Study: Department of Biochemistry Ebonyi State University, Abakaliki, Nigeria, September, 2012.
Methodology: Twenty-four albino rats were grouped into A, B, C and D group. Group C and D were further subdivided into C_1, C_2, C_3, D_1, D_2 and D_3, respectively. Diabetes was induced in all the groups, except group A (positive control). Group B (negative control) was

**Corresponding author: Email: ovumte@yahoo.com;*

not treated while group C and D were treated with aqueous extracts of *M. oleifera* and *B. ferruginea* leaves, which were administered orally to the animals twice daily for 1 week at varying concentrations of 200, 400 and 800 mg/kg body weights. The glucose and liver enzymes levels were determined using glucometeric and spectrophotometric methods.
Results: The results revealed that there was significant reductions ($P < 0.05$) in glucose level in rats treated with aqueous extract of *B. ferruginea* than *M. oleifera* treated rats. There were no significant reduction ($P > 0.05$) in Alkaline phosphatase level between the controls and the treated groups, except at 200 mg/kg dose in which Alkaline phosphatase level was high in rats treated with *M.* oleifera extract. There were significant reduction ($P < 0.05$) in Alanine aminotransaminase level in rats treated with both *M. oleifera* and *B. ferruginea in* comparison to the negative control while there was significant increase ($P < 0.05$) when compared to the positive control except at 200 mg/kg dose where there was decrease in Alanine aminotransaminase level in both plant extracts. Also there was a significant decrease ($P < 0.05$) in AST level in rats treated with *M. oleifera* when compared to controls while there was significant increase ($P < 0.05$) in Aspartate aminotransaminase level in rats treated with *B. ferruginea* except at 200 mg/kg where there was decrease when compared to the controls.
Conclusion: These suggest that both extracts can be used in ethno-medicine for the management of diabetes mellitus.

Keywords: Anti-diabetics; hepato-protective; Moringa oleifera; Bridelia ferruginea, liver enzymes.

1. INTRODUCTION

Diabetes mellitus is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar [1]. Diabetes is a disease condition distinguished by an inability to control blood glucose level due to the insufficient production of or heightened resistance to the hormone insulin [2]. Defective insulin secretion is the major cause for chronic hyperglycemia resulting in impaired function or serious damage to many of the body's systems, like eyes, kidneys, nerves, heart and blood vessels [3].

Since diabetes mellitus is a multi-factorial disease, the treatment is aimed to not only controlling blood sugar level to normal limit, but also at correcting the associated metabolic defects [4]. Early diabetes symptoms can be very mild and often even unnoticeable. Diabetes is characterized by chronic hyperglycemia, which causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidney, nerves and arteries. Along with hyperglycemia and abnormalities in serum lipid, diabetes is associated with micro and macro vascular complications the major causes of morbidity and death [5].

The cells of the liver, which are known as hepatocytes carry out many biochemical activities. Some of these biochemical activities carried include excretion of bile, carbohydrate metabolism, protein metabolism, synthesis of blood clotting factors, storage of iron and some vitamins, detoxification and lipid metabolism [6]. It is therefore very obvious that any disease condition or adverse physiological conditions, which affect the hepatocytes, will cause concerted and tremendous metabolic derangement. In such conditions also, there will be an increase in the serum activities of the mitochondrial-bound liver enzymes since hepatocytic damage causes their release into the serum. It is therefore very pertinent to ascertain the effect of any ingestible food or drug on the serum activities of the liver enzymes so as to

ensure the hepato-protectiveness of such food or drug. This can be achieved through liver function tests, which include estimation of plasma protein, aspartate aminotransferase(AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and bilirubin [6].

The World Health Organisation has estimated that perhaps 80% of earths 6 billion inhabitance relies upon traditional medicine for their primary health care needs, and a major part of this therapy involves the use of plant extracts or their active principles. Traditionally, some plants species are known to have anti-diabetic and hepato-protective properties, with the folkloric use of some of these plants species in the treatment of diabetes has been proven experimentally [7]. *Moringa oleifera* and *Bridelia ferruginea* are one of such plants species used for the management of diabetes [8, 9].

Moringa oleifera belongs to the family of Moringacaea, a fast growing drought-resistant tree native of Sub-Hamalayan tracts of Northern India but now distributed worldwide in the tropics and sub tropics [10]. The tree's leaf and seed pod are widely consumed as food. The bark, leaf and root have ethno-medicinal properties [11]. *Moringa oleifera* is a common vegetable in Nigeria especially in the Eastern Nigeria. However, apart from ethno-medicinal and nutritional uses, there are several reports on biological activities of *Moringa oleifera* in literature. These include hypotensive activities [12], hypocholesterolemic effects [13,14], anti- inflammatory [15] and anti-helmic, analgesic, management of heart diseases, dyspepsia and ulcers [12].

Bridelia ferruginea belongs to the family Euphorbiaceae that is commonly found in savannah regions [16]. *Bridelia ferruginea* has diverse uses. The leaves have been used to treat diabetes. The species is furthermore used as a purgative and vermifuge [17]. The bark extract is being used for milk coagulation and also in limejuice for the formulation of traditional gargle "Ogunefu" [18].

2. MATERIALS AND METHODS

2.1 Plants Collection

Fresh leaves of fully grown *Moringa oleifera* and *Bridelia ferruginea* were collected from Kpiripkiri in Ebonyi local government area, Ebonyi State and identified by Prof. S. S. Onyekwelu of the department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria.

2.2 Preparation of Extract

The leaves of *Moringa oleifera* and *Bridelia ferruginea* were rinsed in clean water and air-dried under room temperature. The dried leaves were pulverized to fine granules using electric blender. The powder was soaked in distilled water at room temperature for 24 hours. It was then filtered using sieve cloth and the filtrate evaporated to dryness using rotator evaporator. The extracts were subsequently reconstituted in distilled water at appropriate concentrations for the experiment.

2.3 Experimental Animals

Twenty-four male albino rats weighing between 90 –140g (4-6-weeks old) were obtained from the animal house of the faculty of Veterinary Medicine University of Nigeria Nsukka. 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. 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.

2.4 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 C_1, C_2, C_3, D_1, D_2 and D_3 corresponding to 200, 400 and 800 mg/kg doses of the extracts. Group C_1 to C_3 was treated with *M. oleifera* extract while group D_1 to D_3 was treated with *B. ferruginea* **twice daily** for seven days.

2.5 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 intraperitoneally using diabetic syringe [19]. The animals with sugar level more than 180mg/dl were considered as experimental diabetic [20].

2.6 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.

2.7 Determination of Blood Glucose Level

Glucose level was determined using glucose oxidase method (Glucometer).

2.8 Determination of Liver Enzymes

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were determined by method described by Reitman and Frankle, [21], which was reviewed by Bhutia et al*., [*22].

2.9 Statistical Analysis

Data obtained were .subjected to a one way analysis of variance ANOVA using the General Liner Model procedure of SAS (version 6.04) (SAS Institute, 1994). Comparison of significant treatment means was by least significance differences (LSD) as outlined by Obi [23].

3. RESULTS AND DISCUSSION

There was significant reductions (*P<0.05*) in the mean body weight of rats in diabetic control compared to positive group while rats in treated groups showed significant increase (*P<0.05*) in their mean body weight compared to diabetic control group (Fig. 1).

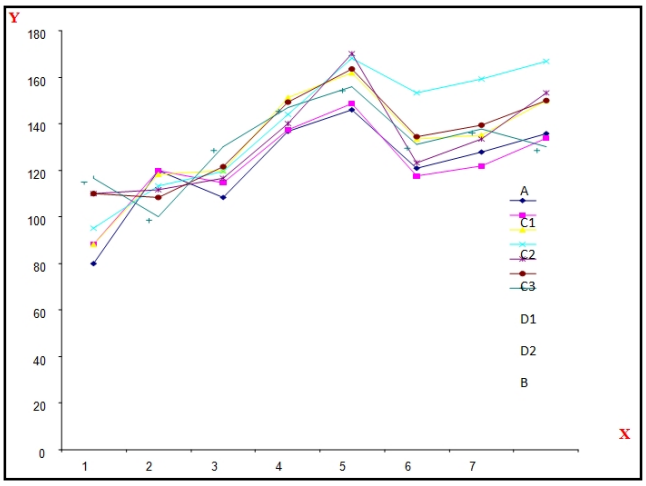

Fig. 1. Effect of aqueous extracts formulated from *M. oleifera* and *B. ferruginea* leaves on the body weight of albino rats. The data are shown as mean ± S.D (n=3). Means values in bars with (⁎) have significance differences (*P<0.05*)

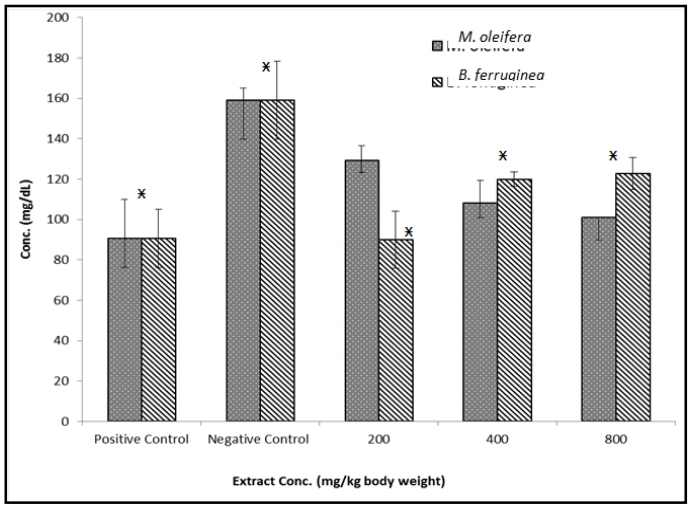

Fig. 2. Comparison of the effects of aqueous extracts of *M. oleifera* and *B. ferruginea* leaves on serum glucose levels in diabetic albino rats.

The data are shown as mean ± S.D (n=3). Means values in bars with (⁎) have significance differences (P<0.05)

The effects of aqueous extract of *Moringa oleifera* and *Bridelia ferruginea* leaves on fasting blood glucose level is measured on 1st and 7th day of post induction. The diabetic control group was compared with normal control and then treated groups were compared with

diabetic control group. The values are shown in Fig. 2. Alloxan induced rats showed significant increase (*P<0.05*) in fasting blood glucose level compared to normal rats. Oral administration of the two extracts at the dose of 800 mg/kg body weight showed a significant decrease (*P<0.05*) in blood glucose level on 7th day of treatment.

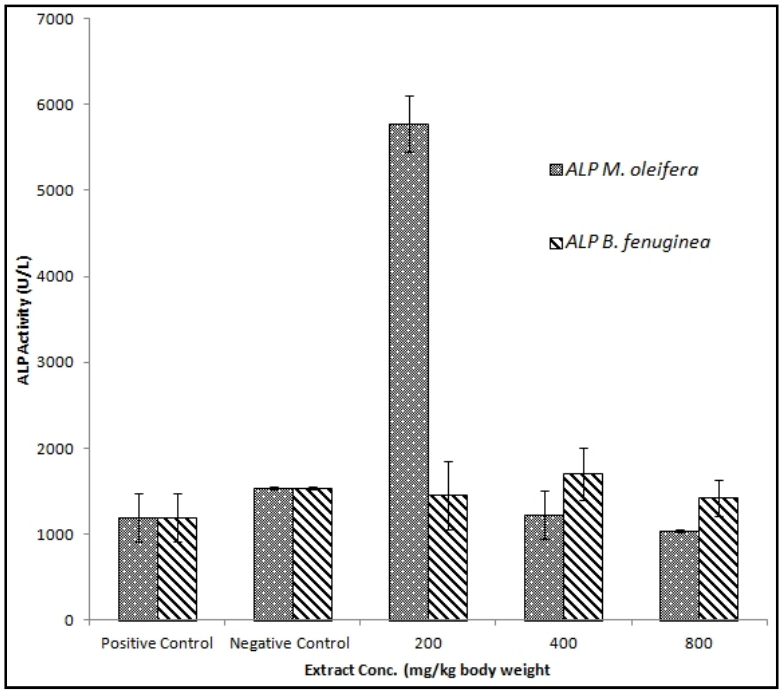

Fig. 3. Comparison of the effects of aqueous extracts of *Moringa oleifera* and *Bridelia fenuginea* leaves on the levels of alkaline phosphatase activity in diabetic albino rats

There were no significant increase (*P>0.05*) in the levels of ALP in diabetic rats compared to rats in normal control group (Fig. 3) while rats in treated groups showed no significant reductions (*P>0.05*) compared to rats in diabetic groups except the rats in treated group administered with 200 mg/kg body weight of *M. oleifera* which showed significant increase (*P<0.05*) in the level of ALP.

There was significant reduction (*P<0.05*) in the ALT levels in rats in diabetic group compared to the normal positive group (Fig. 4) while rats in the groups treated with 400 and 800 mg/kg body weight of rats showed significant increase (*P<0.05*) in ALT levels compared to rats in diabetic group.

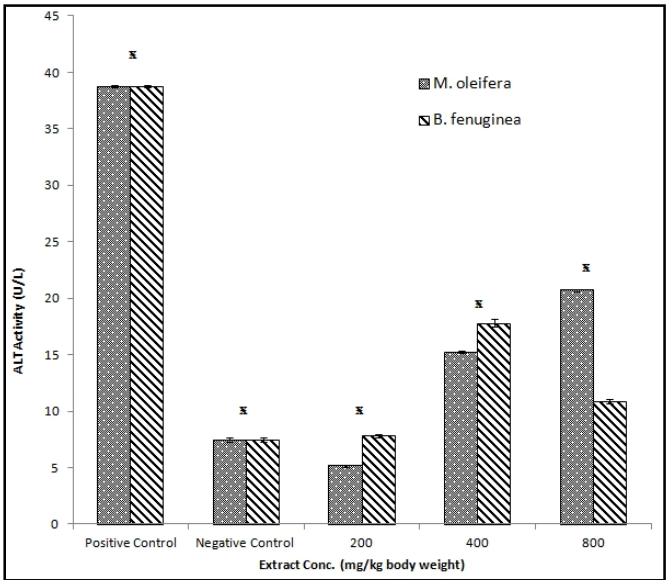

Fig. 4. Comparison of the effects of aqueous extracts of *Moringa oleifera* and *Bridelia fenuginea* leaves on the levels of Alanine aminotransferase activity in diabetic albino rats

The data are shown as mean ± S.D (n=3). Means values in bars with (⁎) have significance differences (P<0.05)

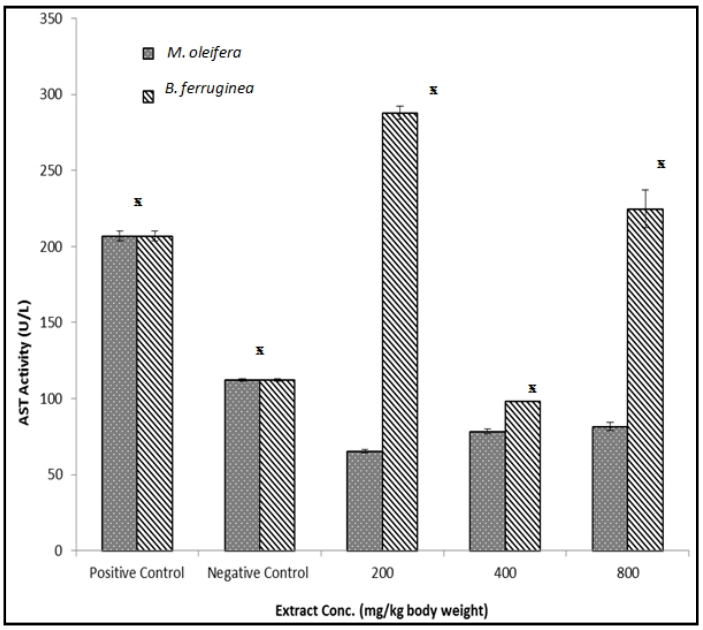

Fig. 5. Comparison of the effects of aqueous extracts of *M. oleifera* and *B. ferruginea* leaves on the levels of aspartate aminotransferase activity in diabetic albino rats. The data are shown as mean ± S.D (n=3). Means values in bars with (⁎) have significance differences (*P<0.05*)

There were significant decrease (*P<0.05*) in AST level in rats in diabetic group compared to rats in normal positive control while rats in the treated groups showed no significant reductions (*P>0.05*) in AST level compared to rats in diabetic group except at 400 and 800 mg/kg body weight of rats where *B. ferruginea* extract showed significant increase (*P<0.05*) in AST level in rats in treated group compared to rats in diabetic control group (Fig. 5).

Aqueous extraction of *Moringa oleifera* and *Bridelia ferruginea* yielded 26.3 and 19.1 % extracts. In the antidiabetic activity, the effects of aqueous extracts of *Moringa oleifera* and *Bridelia ferruginea* leaves on body weight is measured on 1st and 7th day of post induction. The diabetic control group was compared with normal control and then treated groups were compared with diabetic control group. There was significant reductions (*P<0.05*) in the mean body weight of rats in diabetic control compared to positive group while rats in treated groups showed significant increase (*P<0.05*) in their mean body weight compared to diabetic control group (Fig. 1**)**.This shows that as the glucose level decreases, the body weight improves also. This is in line with the work done by Grover *et al*, [24].

The effects of aqueous extract of *Moringa oleifera* and *Bridelia ferruginea* leaves on fasting blood glucose level is measured on 1st and 7th day of post induction. The diabetic control group was compared with normal control and then treated groups were compared with diabetic control group. The values are shown in Fig. 2. Alloxan induced rats showed significant increase (*P<0.05*) in fasting blood glucose level compared to normal rats. Oral administration of the two extracts at the dose of 800 mg/kg body weight showed a significant decrease (*P<0.05*) in blood glucose level on 7th day of treatment. The blood glucose level was seen to be lowered in the treated animals than that of the untreated. The results of this experiment validate the anti-diabetic effects of *Moringa oleifera* and *Bridelia ferruginea* as earlier asserted [7, 25]. The reduction of blood glucose level by *M .oleifera* leaves extract may be due to its numerous bioactive compounds such as flavonoids, alkaloid, coumarins, caroteniods and phenols [26]. Some of these bioactive compounds may exert their hypoglycemic effects by, reducing insulin resistance, increasing release and decreasing glucagon's secretion, slowing the digestion and absorption of carbohydrates or by decreasing hepatic glucose production [27]. *Bridelia ferruginea* also contains flavonoid, terpenoids, glycosides and alkaloids [9], as its bioactive compounds, which elicit their anti-diabetic effect by causing an increase in insulin output or by inhibition of the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. It could also be achieved through increasing glucose transport (decreasing glycolysis and glucose oxidation in adipose tissue] [28].

The effects of aqueous extract of *Moringa oleifera* and *Bridelia ferruginea* leaves on the level of liver enzymes are measured on 1st and 7th day of post induction. The diabetic control group was compared with normal control and then treated groups were compared with diabetic control group. There were no significant increase (*P>0.05*) in the levels of ALP in diabetic rats compared to rats in normal control group (Fig. 3) while rats in treated groups showed no significant reductions (*P>0.05*) compared to rats in diabetic groups except the rats in treated group administered with 200 mg/kg body weight of *M. oleifera* which showed significant increase (*P<0.05)* in the level of ALP. There was significant reduction (*P<0.05*) in the ALT levels in rats in diabetic group compared to the normal positive group (Fig. 4) while rats in the groups treated with 400 and 800 mg/kg body weight of rats showed significant increase (*P<0.05*) in ALT levels compared to rats in diabetic group. There were significant decrease (*P<0.05*) in AST level in rats in diabetic group compared to rats in normal positive control while rats in the treated groups showed no significant reductions (*P>0.05*) in AST level compared to rats in diabetic group except at 400 and 800 mg/kg body weight of rats

where *B. ferruginea* extract showed significant increase (*P<0.05*) in AST level in rats in treated group compared to rats in diabetic control group (Fig. 5). The significant increase in the enzymes activities between the positive control and treated groups at the stated concentrations may be due to cholastasis in which conjugated bilirubin is equally retained [29]. Though flavonoids and flavonoid-containing compounds also have been shown to cause hepatic disorders [30], and may be the result of the significant increase ($p < 0.05$) in these enzymes activities against the stated hepato-protectiveness actions of these leaves extract [31]. It could also be attributed to age variation and physical activity as most biochemical parameters are high in infancy followed by a fall towards childhood after which there is increase in level between puberty and adulthood [32]. According to Steffen and Menzel, [33], significant increase (*P>0.05)* in the serum activities of liver enzymes is an indication of hepatic injury, but since there was no significant increase in the serum activities of the enzymes except AST activity in *B. ferruginea* extract where there was reduction and elevation respectively which shows that *M. oleifera* is more safe to be used as anti-diabetic drug than *B. ferruginea which* may be as a result of the above reasons.

4. CONCLUSION

It therefore, means that *M. oleifera* and *B. ferruginea* leaves have hepato-protective activity and can be considered as alternative strategy to current pharmacotherapy of diabetes mellitus due to their hypoglycemic effects, less side effects of medicinal plants as compared to synthetic drugs, enormous cost of modern drug as well poor availability of the advanced therapies for many rural population in developing countries. The findings suggest that *M. oleifera* and *B. ferruginea* leaf extracts have anti-diabetic effect and relatively safe because of their effect on liver enzymes.

ACKNOWLEDGEMENT

Authors are grateful to Prof. Dr Johnny Ogunji (Director of research, innovation and commercialization (DRIC), EBSU) and TET Fund for financial support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Susheela T, Padma B, Jane T, Narender R and Reddy P. Evaluation of hypoglycemic and anti-diabetic effect of *Melia dubia* fruit in mice. Curr. S.c, 2008;94(9): 1191-1195.
2. Ali L, Kahn AK, Mosihuzzaman M. Studies on the hypoglycemic effects of fruit pulp, seed and whole plant of *Momordica cheranta* on normal and diabetic rat model. Planta Medica. 1993;56:408-412.
3. Arun BV, Govindarao M, Ravi CS, Reddy D, Harsh B, Vishwanath J Amarnath RG. Anti-diabetic Activity of Htdroalcoholic Extract of *Annas comosus* L. Leaves in Streptozotocin Induced Diabetic Rats. Int. J .of Pharma. 2012a;2(1):142-147.
4. Hattersley A. Multiple facts of diabetes in young people. Curt Sc. 2002;82:273- 276
5. Ramachandra A, Snehalata C, Vishwanathan V. Burden of Type 2 diabetes and its complications-the Indian Scenario. Curr Sc. 2002;83:1471-1477.
6. Ochei J, Kolhatkar A. Medical Laboratory Science: Theory and Practice, 6th edition, Tata McGraw Hill, New Delhi. 2007;106-158.

7. Onunkwo GC, Akah PA, Udeala OK. Studies on *Bridelia ferruginea* leaves. Stability and hypoglycemic actions of the leaf extract tablets. Phytother Res. 1996;10(5):420-423.
8. Dieye AM, Sarr A, Diop SN, Ndiaye M, Sy GY, Diarra M, et al. Medicinal plants and the treatment of diabetes in Senegal: survey with patients. Fundam. Clin. Pharmacol. 2008;22:211–216.
9. Iwu MM. Handbook of African Medicinal Plants. Chemical Rubber Company Press Incorporation, Boca Raton, U.S.A. 1993;133-135.
10. Fuglie LJ. The miracle tree: *Moringa oleifera* natural nutrition for the tropics. Church world service, Dakar. 1999;68-70.
11. Joseph C. *Moringa oleifera*: a review of the medicinal evidence for its nutritional, therapeutic and prophylatic properties. Trees of Life J. 2011;1:15-17.
12. Nikkon F, Haque ME, Aragianis K, Mosaddik MA. Isolation of Aglycone of Deoxy- Niazimicin from, *Moringa oleifera* and its cytotoxicity. Rev. Latinoamur aunim. 2003; 31(1):5-9
13. Dangi SY, Jolly CI and Narayanan S. Anti – Hypertensive activity of the total alkaloids from the leaves of *Moringa oleifera.* J. Pharmaceutical Biol. 2002;2:144–148.
14. Ghosi S, Nwobodo E, Ofili JO. Hypocholesterolemic effect of crude extract of leaf of *Moringa oleifera* in high fat diet fed wistar rats. J of Ethno pharmacol. 2000;69(1):21 – 25.
15. Rao KS, Mishra H. Anti – inflammatory and antihepatotoxic activities of fruits and the roots of Moringa pterygosperma Gaertn, Indian J of pharmaceutical Sc. 1998;60:12.
16. Ekanem JT, Kolawole OM, Abbah OC. Trypanocidal potential of methanolic extract of *Bridelia ferruginea* benth bark in *Rattus novergicus.* African J. of Biochem Res. 2008;*2*(2):45-50.
17. Cimanga K, DeBrugne T, Apers S, Dietersh TJ, Kambu K, Tona L, Bakana P, et al. Complement-inhibiting constituent of *Bridelia ferruginea* stem bark. Planta Medica. 1999;65(3):213-217.
18. Orafidiya LO, Lamikanra A, Adediji JA. Phytotherapy Res. (United Kingdom). 1990;4(5):189-194.
19. Lenzen S. The mechanisms of Alloxan and Streptozotocin induced diabetes. Diabetogica. 2008;51:216-226.
20. Stanley M P and Venugopal MP. Anti-oxidant action of *Tinospora cordiflia* root extract in Alloxan-induced diabetic rats. Phytother Res. 2001;15:213-218.
21. Reitman S, Frankel SA. Calorimetric method of determination of serum glutamic oxaloacetic and glutamic pyruvate transaminase. American J. of Clin. Path. 1957;28:56-67.
22. Bhutia DR, Upadhyay B and Meneesh M. Association of Plasma level of Thiobarbituric Acid Reactive Substances with Extent of Hepato-cellular injury in Preterin infants with Cholestatic Jaundice. Indian J. of Clin. Biochem. 2006;721(2):39-41.
23. Obi IU. Statistical Methods of Detecting Differences between treatment Means and Research Methodology issue in laboratory and field experiments. A.P Company Ltd., Nigeria; 2002.
24. Grover JK, Yadav S, Vats V. Medicinal plants of India with ant-diabetic Potential. Journal of Ethno-pharmacology. 2002;81:81-100.
25. Ndong M, Uehara M, Katsumata S, Suzuki K. Effects of oral administration of *Moringa oleifera* Lam on glucose tolerance in gotokakizaki and wistar rats. J. of Clin Biochem and Nutri. 2007;40:229-233.
26. Anwar F, Lotof S, Ashara M, Gilani AH. *Moringa oleifera*: a food plant with multiple medicinal uses. J of Phytother Res. 2007;21:17-25.

27. Katzung BG, Masters SB, Trevor AJ. Basic and Clinical Pharmacology, 11th edition, Large Medical Publications, Singapore. 2009;727-736.
28. Clark FJ, Burgess RC, Chapin JW, Lipscomb WT. Role of intramuscular receptors in the awareness of limb position. J of Neurophysiol. 1985;54:1529-1540.
29. Crook MA. Clinical Chemistry and Metabolic Medicine, 7th edition, Edward Road Publishers, London. 2006;250-268.
30. Gandolfo GM, Girelli G, Conti LM, Perrone P, Arista MC, Damico C. Haemolytic anaemia and thrombocytopenia induced by Cyanidanol. Acta Haematologia, 1992;88:96-99.
31. Fakurazi S, Wnthini U, Hairuszah I. Hepatoprotective and antioxidant action *Moringa oleifera*. Int J of Pharmacol. 2003;4:270-275.
32. Owiredu WKBA, Ephraim RKD, Amidu N, Eghan BA, Laing EF. Oxidative Stress among Ghanaian Patients presenting with Chronic Kidney Disease, J. of Medical and Biomedical Sc. 2012;1(1):28-37.
33. Steffen C, Menzel J, Development and current status of Non-specific Enzyme therapy, 8th edition, McGrew Hill companies Inc, New York. 2009;10-14.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=231&id=3&aid=1592