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Research paper

Comparative Evaluation of Anti-diabetic and Hepatoprotective Effects of *Moringa oleifera* and *Bridelia ferruginea* Leaves Aqueous Extracts on Alloxan Induced Diabetic Albino Rats.

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Abstract

This study was designed to evaluate comparative antidiabetic and hepatoprotective activities of aqueous extracts of *Moringa oleifera* and *Bridelia ferruginea* leaves in alloxan induced diabetic albino rats. Twenty-four ~~(24)~~ 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. The glucose and liver enzymes levels were determined using glucometeric and spectrophotometric methods. Diabetes was induced in all the groups, except group A (positive control). Group B (negative control) was 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 800mg/kg body weights. 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 ALP level between the controls and the treated groups, except at 200mg/kg dose in which ALP level was high in rats treated with *M.* oleifera extract. There were significant reduction ($p < 0.05$) in ALT 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 200mg/kg dose where there was decrease in ALT 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 AST level in rats treated with *B. ferruginea* except at 200mg/kg where there was decrease when compared to the controls. These suggest that both extracts can be used therapeutically for the management of diabetes mellitus.

Key Words: **Anti-diabetics, hepato-protective, *Moringa oleifera, Bridelia ferruginea***

Introduction

Diabetes mellitus is one of the most common endocrine diseases in domestic animals, especially man (Anaga, 2003). 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 (Susheela *et al.*, 2008). 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 (Ali *et al.,* 1993). It could also be described as a disorder that prevents the body using food properly (Chen *et al.*, 1995).

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Diabetes mellitus is a major problem worldwide; approximately 5% of the world population suffers from diabetes (WHO, 1998). 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 (Hattersley, 2002). 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 ~~due to diabetes~~ (Ramachandra *et al.,* 2002).

~~Liver is the largest internal organ. It is related by its domed upper surface to the diaphragm, which separates it from pleura, lungs, pericardium and heart (Harold, 2002).~~ The cells of the liver, which are known as hepatocytes, ~~house a lot of enzymes~~ and carries out many biochemical activities. Some of these biochemical activities carried ~~out by the hepatocytes~~ include excretion of bile, carbohydrate metabolism, protein metabolism, synthesis of blood clotting factors, storage of iron and some vitamins, detoxification, lipid metabolism, etc (Ochei and Kolhatkar, 2007). 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 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), bilirubin, etc (Ochei and Kolhatkar, 2007).

Traditionally, some plants are known to have anti-diabetic and hepato-protective properties, the folkloric use of some of these plants in the treatment of diabetes has been proven experimentally (Onukwo *et al.,* 1996). *Moringa oleifera* and *Bridelia ferruginea* are one of such plants used for the management of diabetes (Dieye *et al*., 2008; Iwu, 1993).

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 (Fuglie, 1999). ~~*Moringa oleifera* has Horseradish tree, Drumstick tree and Ben oil tree, as its English names. While the local names in Nigeria include: "Gawara" (Fulani), "Zogall" (Hausa), "Ikwe oyibo" (Igbo) and "Ewe ile" (Yoruba). The Moringa tree grows mainly in semi-arid, tropical and subtropical areas. Currently it is widely grown in Africa, Central and South America, Sri-lanka, India, Mexico, Malaysia and the Philippines.~~ It has diverse uses. The tree's leaf and seedpod are widely consumed as

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food. The bark, leaf and root have medicinal properties ~~and have been used for centuries in folk medicine for a variety of health promoting purposes~~ (Joseph, 2011).

Bridelia ferruginea belongs to the family Euphorbiaceae that is commonly found in savannah regions (Ekanem *et al*., 2008). It is an indigenous medicinal plant in Nigeria. ~~Its common names in Nigeria include "kirni" (Hausa), "Maren" (Fulani), "Iralodan" (Yoruba) and "Ola" (Igbo). Its habitat is the savannah, especially in the moisture regions from Guinea to Zaire and Angola.~~ *Bridelia ferruginea* has diverse uses. The leaves have been used to treat diabetes. The plant is used as a purgative and vermifuge (Cimanga *et al*., 1999). The bark extract is being used for milk coagulation and also in limejuice for the formulation of traditional gargle "Ogunefu" (Orafidiya *et al.,* 1990).

Materials and Methods

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.

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.

Experimental Animals

Twenty-four ~~(24)~~ albino rats of either sex 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 ~~(7)~~ 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.

Experimental Design

At the end of the 7 days acclimatization period, the animals were randomly assigned into eight ~~(8)~~ different groups of three (3) rats each, designated as group A-D. Group A received water and feed only

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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 800mg/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 7 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 intraperitoneally using diabetic syringe (Lenzen, 2008). The animals with sugar level more than 180mg/dl were considered as experimental diabetic (Stanley and Venugopal, 2001).

Collection of Blood from Animals:

After 7 days of treating the animals with the plants extract. The animals were starved for ~~twenty-four (~~24~~)~~ hours. 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 0th 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 (Glucometer).

Determination of Liver Enzymes: Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were determined by method described by Reitman and Frankle, (1957), which was reviewed by Bhutia *et al., (*2006).

Statistical Analysis: Results were expressed as mean ± standard deviation. The significance of difference between the controls and treated groups were determined, using two-way analysis of variance (ANOVA) SAS software version.

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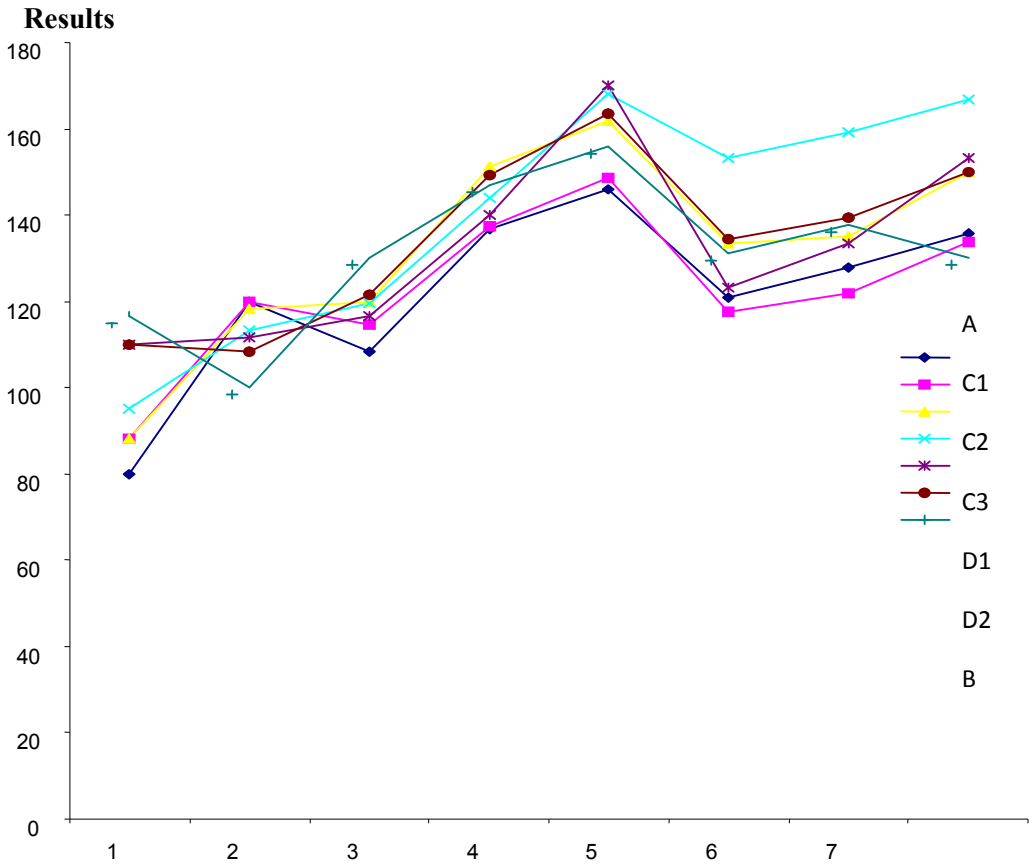

Figure 1: Effect of aqueous extracts formulated from *M. oleifera* and *B. ferruginea* leaves on the body weight of albino rats.

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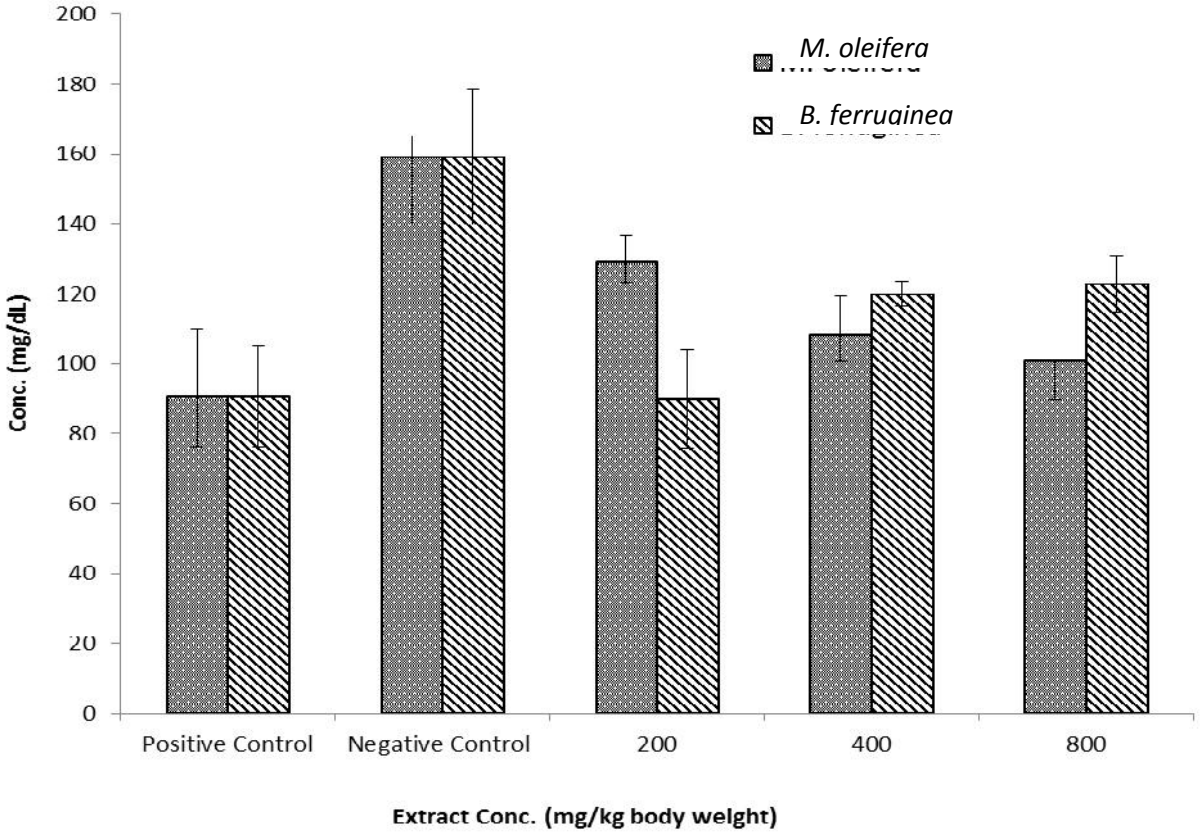

Figure 2: Comparison of the effects of aqueous extracts of *M. oleifera* and *B. ferruginea* leaves on serum glucose levels in diabetic albino rats.

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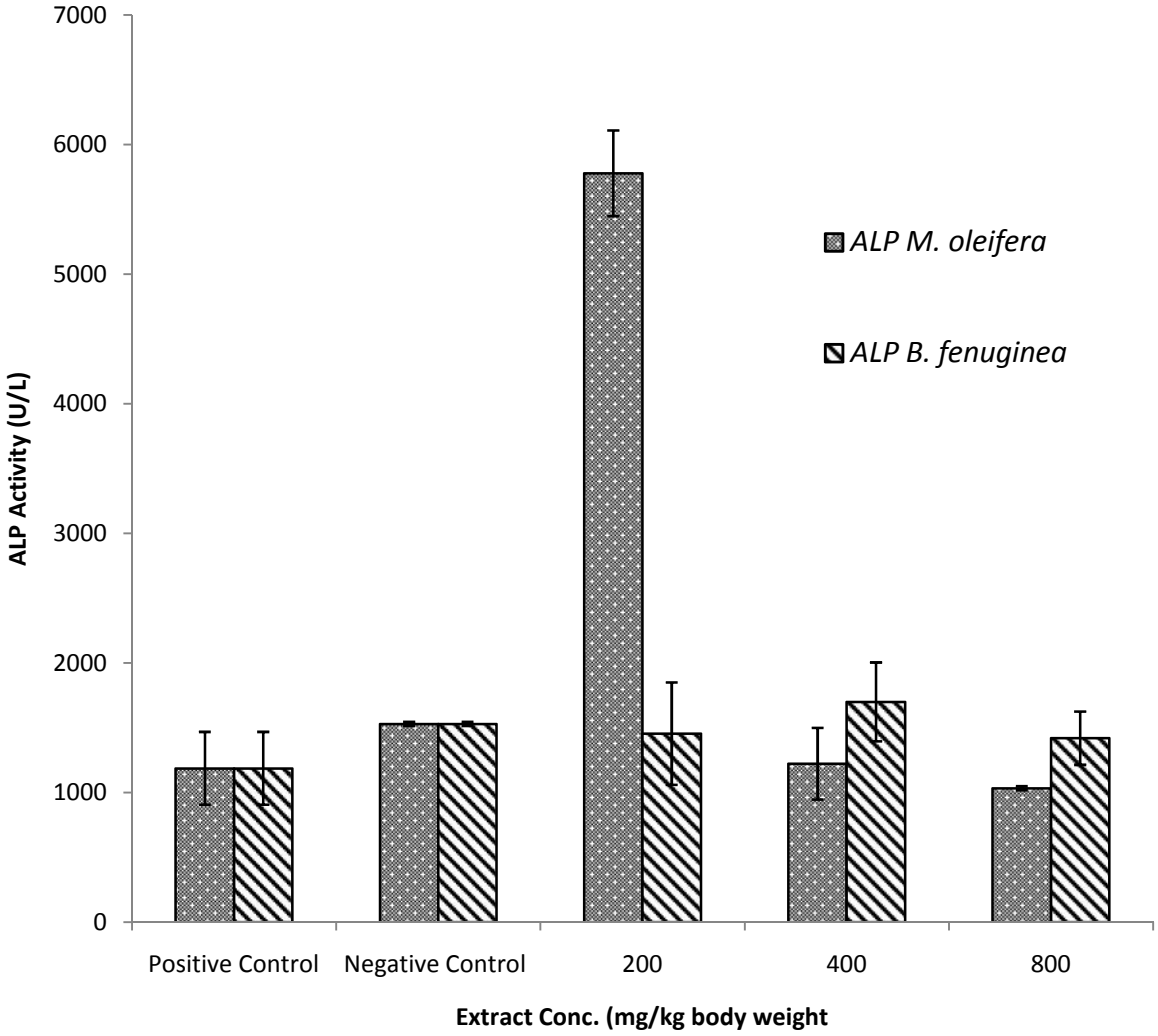

Figure 3: Comparison of the effects of aqueous extracts of *Moringa oleifera* and *Bridelia fenuginea* leaves on the levels of Alkaline phosphatase activity in diabetic albino rats

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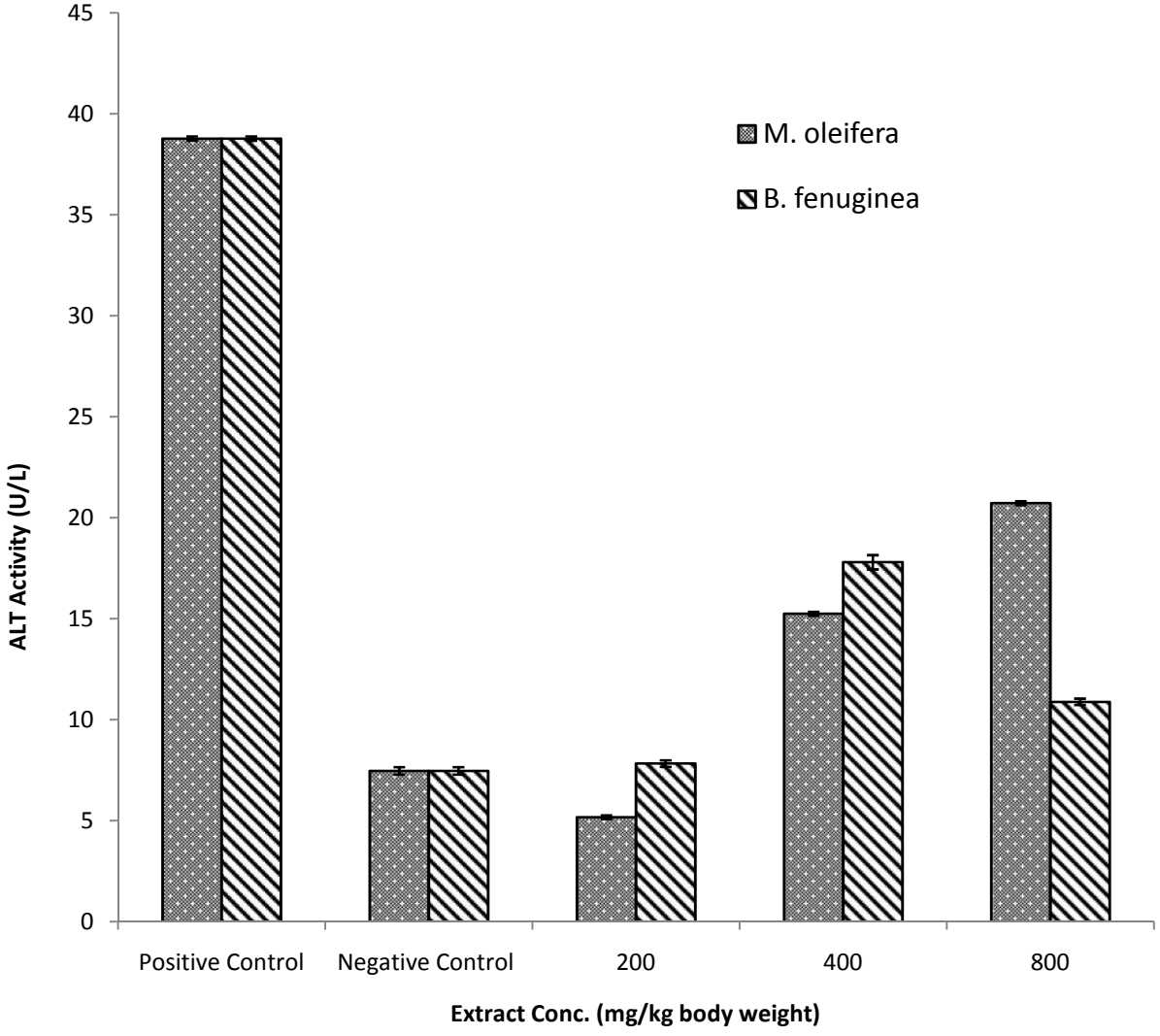

Figure 4: Comparison of the effects of aqueous extracts of *Moringa oleifera* and *Bridelia fenuginea* leaves on the levels of Alanine aminotransferase activity in diabetic albino rats

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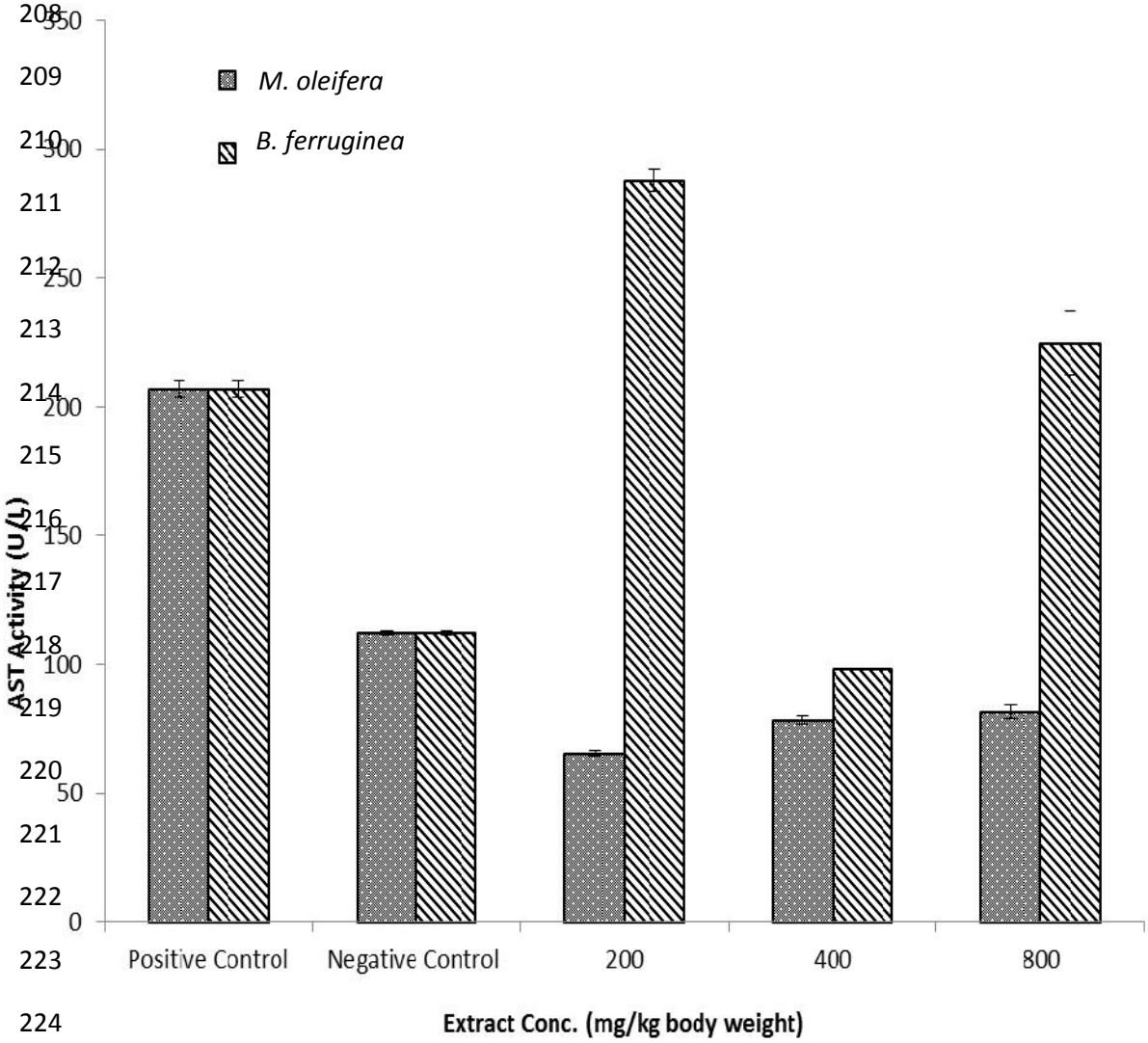

Figure 5: Comparison of the effects of aqueous extracts of *M. oleifera* and *B. ferruginea* leaves on the levels of aspartate aminotransferase activity in diabetic albino rats.

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Discussion

~~Figure 1 show variations on effect of aqueous extracts formulated from *M. oleifera* and *B. ferruginea* leaves on the body weight of albino rats during 7 days of administration.~~ The treated animals and the positive control shows gradual increase in the body weight while negative control show decrease. 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*, 2002.

~~Figure 2 shows that the glucose levels of all the animals were raised above normal after the administration of alloxan. It also demonstrated the anti-diabetics effects of the extracts on blood glucose level.~~ 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 (Onunkwo *et al.,* 1996; Ndong *et al.,* 2007). The extracts showed significant reduction ($p < 0.05$) in the level of blood glucose in all the treated groups in dose dependent manner. 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, phenols, ~~etc~~ (Anwar *et al.,* 2007; Ferriera *et al.,* 2008). 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 (Kakzung *et al.,* 2009). *B. ferruginea* also contains flavonoid, terpenoids, glycosides, alkaloids, ~~etc~~ (Iwu, 1993), 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) (Clark *et al.,* 1985).

It can be deduced from the figure 3 that there were no significant increases ($p>0.05$) in the levels of ALP at the end of the extracts administration except at 200mg/kg of *M. oleifera.* ~~Figure 4 shows that~~ there were significant reductions ($p<0.05$) in the ALT levels in the treated animals when compared to the negative control. ~~The figure 5 shows that~~ there were significant decreases ($p<0.05$) in the AST level in animals treated with *M. oleifera* extract compared to the controls ~~while~~ there were increases in animals treated with *B. ferruginea* except at 400mg/kg dose where there was a decrease indicating that 400mg/kg is the therapeutic dose of *B .ferruginea.*

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The results of hepatotoxicity markers showed there were no significant reduction ($p > 0.05$) in ALP level between the controls and the treated groups, except at 200mg/kg dose in which ALP level was high in rats treated with *M. oleifera* extract. There were significant reduction ($p < 0.05$) in ALT 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 200mg/kg dose in both plant extracts. There were significant reduction ($p < 0.05$) in ALT level in rats treated with both *M. oleifera* and *B. ferruginea in* comparison to the negative control while there was significant increase ($p < 0.05$) in ALT level when compared to the positive control except at 200mg/kg dose where there was decrease in ALT level in both plant extracts. Also there was a significant decrease in AST level in rats treated with *M. oleifera* compared to controls while there was significant increase ($p < 0.05$) in AST level in rats treated with *B. ferruginea* except at 200mg/kg where there was decrease when compared to the controls. 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 (Crook, 2006). Though flavonoids and flavonoid-containing compounds also have been shown to cause hepatic disorders (Gandolfo *et al.,* 1992), 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 (Fakurazi *et al.,* 2008; Owiredu *et al.,* 2001). 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 (Owiredu *et al.,* 2011). According to Steffen and Menzel, 2009, significant increase 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.

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.

Conclusion

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In conclusion, these findings suggest that *M. oleifera* and *B. ferruginea* leaf extracts have anti-diabetic effect and relatively safe because of their hepato-protective properties.

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