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Inhibitory Activity of Aqueous extracts of Horseradiash *Moringa oleifera* (Lam) and Nutmeg *Myristica fragrans* (Houtt) on Oxidative Stress in Alloxan Induced Diabetic Male Wistar Albino Rats

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ABSTRACT

Plants contain many important and potential therapeutic agents for treating several diseases of man. In this study, we investigated and compared the hypoglycemic abilities and antioxidant enzymes activities of aqueous extracts of horseradish (*Moringa oleifera*) leaf and nutmeg (*Myristica fragrans*) seed on Alloxan-induced diabetic albino rats with the aim of alleviating oxidative stress. Hyperglycemia was induced in rats using alloxan intra-peritoneally (ip). Two groups of fifteen wistar albino rats each were fed with water and rat pellets for one week to serve as control stage 1 (Non-diabetic rats). Five rats from each group were separately weighed and sacrificed. The remaining animals from each group were injected with 0.5 mL each of alloxan (35 mg kg⁻¹ b.wt.) which was observed for another week with normal feeding, this served as stage 2 (Diabetic rats). Five animals from each group were also weighed and sacrificed. After the second stage, the remaining animals from each group were treated with 0.5 mL each of 30% aqueous extracts of *Moringa oleifera* leaf and *Myristica fragrans* seed, respectively for another week which served as stage 3 (Treated rats). The last sets of rats from each group were finally weighed and sacrificed. Blood samples were collected from each stage and group for fasting blood glucose determination in whole blood. While glutathione, glutathione peroxidase, superoxide dismutase, catalase and Malonaldehyde (MDA) were determined from plasma. The results of this study revealed that all the extracts caused hypoglycemia; increased weight, GSH, GPx, catalase and percentage inhibition of SOD but reduced Malonaldehyde (MDA) concentrations after treatments.

Key words: Aqueous extract, therapeutic agent, antioxidant enzymes, anti-diabetic activity, scavenging ability

INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder, has now become an epidemic, with a worldwide incidence of 5% in the general population. The fundamental defect in diabetes mellitus is an absolute or relative lack of biologically active insulin, which results in the impairment of uptake and storage of glucose, reduced glucose utilization for energy purpose (Chaurasia *et al.*, 2010). According to WHO projection, the prevalence of diabetes is likely to increase by 35%. Currently there are over 150 million diabetics world-wide and this is likely to increase to 300 million or more by the year 2025. Statistical projection about India suggests that the number of diabetic will rise

from 15 million in 1995 to 57 million in the year 2025 making it the country with the highest number of diabetics in the world (King *et al.*, 1998). *Moringa oleifera* is well known for its pharmacological actions and is used for the traditional treatment of diabetes mellitus (Babu and Chaudhuri, 2005; Bhishagratna, 1991). With such great medicinal value being suggested by traditional medicine, further clinical testing is very much needed. Most of the plants prescribed for Diabetes Mellitus are not edible (Atta-Ur-Rahman and Zaman, 1989) and therefore, the studies on edible plants which have a hypoglycemic effect would be of great value in the dietary management of the disease. *Moringa oleifera* is the most widely cultivated species of a monogeneric family, the Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. This rapidly-growing tree (also known as the horseradish tree, drumstick tree, benzolive tree, kelor, marango, or ben oil tree), was utilized by the ancient Romans, Greeks and Egyptians; it is now widely cultivated and has become naturalized in many locations in the tropics (Fahey, 2005). The leaves and pods of *Moringa oleifera* remove all kinds of pain, good vesicant, expectorant, stimulant and abortifacient. The decoction of the leaf is used as a stimulant, analgesic and diuretic (Rakesh and Singh, 2010). Leaves of *Moringa oleifera* are lopped for fodder (Sastri, 1962) and have been used as antiulcer, diuretic, anti-inflammatory and for wound healing (Caceres *et al.*, 1992; Udupa *et al.*, 1994; Pal *et al.*, 1995). Ethanolic extract of leaves have shown antifungal activity against a number of dermatophytes, (Chuang *et al.*, 2007) whereas methanol extract has a potent CNS depressant action (Pal *et al.*, 1996). The aqueous extract of the leaves has been found to possess antifertility activity (Shukla *et al.*, 1987). The pods are edible, seeds are useful as purgative, antipyretic, cures eye diseases, head complaints and are used in venereal affections (Chandrashekar *et al.*, 2010). There are very few studies of hypoglycemic and antihyperglycemic effect of *Moringa oleifera* leaves in rabbits. *Myristica fragrans* Houtt. (nutmeg) of the family Myristiceae is a spice seed from the fruit of an evergreen tree called *M. fragrans* Houtt. tree (Van Gils and Cox, 1994). Both *M. fragrans* (nutmeg) and mace (its sister spice) are native to tropical Asia and Australia. Nutmeg is the actual seed of the tree, while mace is the dried "lacy" reddish covering on the seed. It is the species used for culinary and medicinal purposes and grew naturally only on a small group of island called the Bandas. Nutmeg and mace taste similar though nutmeg is sweeter in flavour and mace more delicate. Many countries use nutmeg as a seasoning. In India, it is used in sweet dishes. In the Middle East, nutmeg spices savoury dishes. Europeans use it in most dishes to season potatoes, eggs, meats and even spinach with it along soups, sauces and baked goods. Nutmeg had been reported to have aphrodisiac (Tajuddin *et al.*, 2005), stomachic, carminative (Green, 1959; Khory and Katrak, 1985), tonic (Burkill, 1935), nervous stimulant (Ainslie, 1979), aromatic, narcotic, astringent, hypolipidemic, antithrombotic, antifungal, antidysentric and anti inflammatory (Tajuddin *et al.*, 2005) properties. Nutmeg is used by Arabs of Israel and people of its Jewish communities, especially Yemennities, as a drug of their folk medicine, as well as a spice and as an important ingredient in love-portions. It is used against vomiting and to regulate the movements of the bowels; it is good for liver and for the spleen. It is used in the treatment of tuberculosis, against colds, fever and in general for respiratory ailments. It is said to be antihelminthic and also used against skin diseases like eczema and scabies (Zaitschek, 1964).

In view of wide spread biological uses of nutmeg and *Moringa oleifera* and little information about the hypoglycemic and antioxidant enzymes evaluations, hence, this study was undergone to determine and compare the hypoglycemic and antioxidant enzymes evaluations of nutmeg and horseradish on alloxan-induced diabetic wistar albino rats.

MATERIALS AND METHODS

Sample collection and preparation: Fresh samples of the *Moringa oleifera* leaf was collected from Afao-Ekiti, while Nutmeg was purchased locally from Oja-Bisi market in Ado-Ekiti, Ekiti State, Nigeria. The identification and authentication of both plants were carried out at the Department of Plant Science, Ekiti State University, Ado-Ekiti. The samples were air dried and powdered for aqueous extract used in this study. All chemicals used were of analytical grade, while all-glass distilled water was used in the analysis. Alloxan used was obtained from Sigma/Aldrich.

Selection of animals and their care: Thirty matured male wistar albino rats weighing between 74.59 and 87.29 g were used for this experiment (15 rats per each group of *Moringa* and Nutmeg). The animals were acclimatized for a period of two weeks to the laboratory conditions prior to the experiment at the Animal house of Department of Biochemistry, Ekiti-State University. Rats were housed in cage at room temperature with 12 h light and dark cycle with free access to drinking water and rat feeds.

Experimental procedure: Rats in both the *Moringa* and Nutmeg groups were categorized into three as follows: Control stage1 (Non-diabetic rats). All the animals received normal rat feeds and water. After feeding them for one week, five animals were selected to determine body weights, fasting blood sugar and total protein levels in the blood. The animals were subsequently sacrificed to determine other parameters which included reduced Glutathione (GSH), Glutathione Peroxidase (GPx), Malonaldehyde (MDA), catalase and Superoxide Dismutase (SOD) activities from the plasma.

Induction of diabetes: After one week of control, rats were fasted for 24 h before injected intra-peritoneally with freshly prepared solution of alloxan at a dosage of 35 mg kg⁻¹ b.wt. The animals were considered to be diabetic as the fasting blood sugar level was observed greater than 115 mg dL⁻¹ from control 40 to 55 mg dL⁻¹. This dose of alloxan produced type 1 diabetes. Five animals were selected to determine body weights, fasting blood sugar and total protein levels in the blood. The animals were subsequently sacrificed to determine other parameters which included Glutathione (GSH), Glutathione Peroxidase (GPx), Malonaldehyde (MDA), catalase and Superoxide Dismutase (SOD) activities from the plasma which forms the stage 2 (diabetic rats).

At the expiration of one week of induction of diabetics, the animals were forced fed with aqueous extracts of *Moringa oleifera* leaf and *Myristica fragrans* seed for each group, respectively for a period of one week. The last five animals in each group, were selected to determine body weights, fasting blood sugar and total protein levels in the blood. The animals were subsequently sacrificed to determine other parameters which included Glutathione (GSH), glutathione peroxidase (GPx), Malonaldehyde (MDA), catalase and Superoxide dismutase (SOD) activities from the plasma which forms the stage 3 (Treated rats).

Determination of animal's weight: Individual animal weight was measured using Ohaus top-loading balance.

Determination of whole blood glucose: Glucose concentration in mg dL⁻¹ was measured in the animal with the aid of ON-CALL PLUS Glucometer using compatible glucose test strips according to prescribed instructions by pricking the tail and dropping blood on the glucometer.

Determination of plasma malonaldehyde (MDA): Lipid peroxidation was determined by measuring the formation of Thiobarbituric Acid Reactive Substances (TBARS) according to the method of Varshney and Kale (1990). An aliquot of 0.4 mL of the plasma was mixed with 1.6 mL of Tris-KCl buffer to which 0.5 mL of 30% Trichloroacetic Acid (TCA) was added. Then 0.5 mL of 0.75% TBA was added and placed in a water bath for 45 min at 80°C. This was then cooled on ice and centrifuged at 3000 g. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532 nm. The MDA level was calculated according to the method of Adam-Vizi and Seregi (1982). Lipid peroxidation in U mg⁻¹ protein or gram tissue was computed with a molar extinction coefficient of 1.56×10⁵ M⁻¹cm⁻¹.

Calculation:

$$\text{MDA (U mg}^{-1} \text{ protein)} = \frac{\text{Absorbance} \times \text{volume of mixture}}{E_{532\text{nm}} \times \text{volume of sample} \times \text{mg protein}}$$

Determination of reduced glutathione (GSH): The method of Jollow *et al.* (1974) was followed in estimating the level of reduced glutathione (GSH). The 0.2 mL of sample was added to 1.8 mL of distilled water and 3 mL of the precipitating solution was mixed with sample. The mixture was then allowed to stand for approximately 10 min and then centrifuged at 3000 g for 5 min. A 0.5 mL of the supernatant was added to 4 mL of 0.1 M phosphate buffer. Finally, 0.5 mL of the Ellman's reagent was added. The absorbance of the reaction mixture was read within 30 min of colour development at 412 nm against a reagent blank.

Determination of catalase activity: This experiment was carried out using the method described by Sinha (1972). A 0.2 mL of sample was mixed with 0.8 mL distilled H₂O to give 1 in 5 dilution of the sample. The assay mixture contained 2 mL of solution (800 μmol) and 2.5 mL of phosphate buffer in a 10 mL flat bottom flask 0.5 mL of properly diluted enzyme preparation was rapidly mixed with the reaction mixture by a gentle swirling motion. The reaction was run at room temperature. A 1 mL portion of the reaction mixture was withdrawn and blown into 1 mL dichromate/acetic acid reagent at 60 sec intervals. The hydrogen peroxide content of the withdrawn sample was determined by the method described above. The mononuclear velocity constant, K, for the decomposition of H₂O₂ by catalase was determined by using the equation for a first-order reaction:

$$K = 1/t \log S_0/S$$

where, S₀ is the initial concentration of H₂O₂ and S is the concentration of the peroxide at t min. The values of the K are plotted against time in minutes and the velocity constant of catalase K₍₀₎ at 0 min determined by extrapolation. The catalase contents of the enzyme preparation were expressed in terms of Katalase feiahigkeit or 'Kat. f' according to von Euler and Josephson (1927):

$$\text{Kat. f} = K_{(0)} \text{ mg protein mL}^{-1}$$

Determination of glutathione peroxidase (GPx): GPx activity was measured using Paglia and Valentine's method (Paglia and Valentine, 1967). The reaction mixture contained 2.6 mL of 100 mmol L⁻¹ phosphate buffer (pH 7.0) with 3 mmol L⁻¹ EDTA, 0.05 mL of 10 mg mL⁻¹ GSH

solution, 0.1 mL glutathione reductase (10 mg mL⁻¹), 0.05 mL of NADPH-Na salt (10 mg mL⁻¹), 0.1 mL 90 mmol L⁻¹ hydrogen peroxide solution and 0.1 mL of sample. The GPx activity was monitored by the decrease in absorbance at 340 nm due to the consumption of NADPH.

Determination of superoxide dismutase (SOD): The level of SOD activity was determined by the method of Misra and Fridovich (1972). One milliliter of sample was diluted in 9 mL of distilled water to make a 1 in 10 dilution. An aliquot of the diluted sample was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3 mL of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 mL buffer, 0.3 mL of substrate (adrenaline) and 0.2 mL of water. The increase in absorbance at 480 nm was monitored every 30 sec for 150 sec.

Calculation:

$$\text{Increase in absorbance per minute} = \frac{A_3 - A_0}{2.5}$$

where, A₀ is absorbance at 0 sec, A₃, absorbance at 150 sec.

$$\text{Inhibition (\%)} = \frac{\text{Increase in absorbance for substrate}}{\text{Increase in absorbance of blank}} \times 100$$

1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during 1 min.

RESULTS AND DISCUSSION

Figure 1 showed the effects of horseradish (*Moringa oleifera*) leaf and nutmeg (*Myristica fragrans*) seed aqueous extracts treatments on the weights of alloxan-induced diabetic rats. It was observed in this study that alloxan induced diabetic rats had a marked loss in body weight. This would be expected as one of the effects of diabetes is body weight loss, an observation

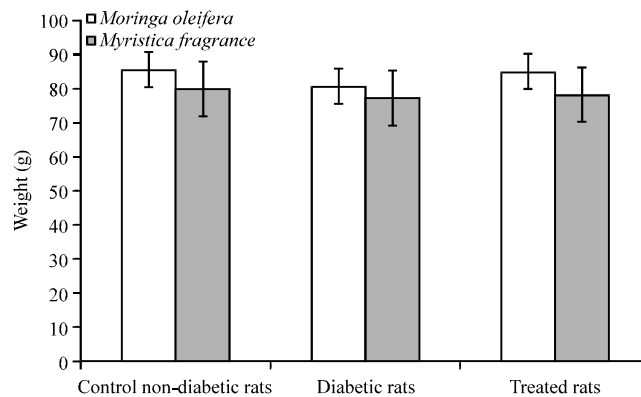


Fig. 1: Effects of aqueous extracts of horseradish (*Moringa oleifera*) leaf and nutmeg (*Myristica fragrans*) seed treatments on the weights of alloxan-induced diabetic rats, values are Mean±SEM

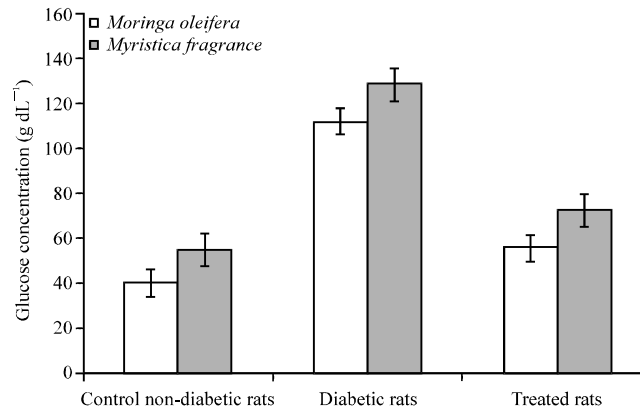


Fig. 2: Effects of aqueous extracts of horseradish (*Moringa oleifera*) leaf and nutmeg (*Myristica fragrans*) seed treatments on the glucose concentration of alloxan-induced diabetic rats, values are Mean \pm SEM

which was also seen in Eleazu *et al.* (2010) that reported weight loss in diabetic rat before the administration of unripe plantain. With the destruction of the pancreatic cells by alloxan, there is deficiency of insulin leading to increased synthesis of ketone bodies which are excreted in urine. The increased synthesis of ketone bodies coupled with increased lipolysis leads to a severe body weight loss. However, the diabetic rats treated differently with *Moringa oleifera* leaf and *Myristica fragrans* aqueous extracts had a remarkable gain in body weight.

Figure 2 revealed the effects of aqueous extracts of horseradish (*Moringa oleifera*) leaf and nutmeg (*Myristica fragrans*) seed treatments on the blood glucose concentration of alloxan-induced diabetic rats. The concentration of fasting blood glucose was increase in the alloxan induced diabetic rats in this study, which was in correlation with Edoga *et al.* (2013) who also reported increased glucose concentration at diabetics. Alloxan is known to destroy the cells of the pancreas that function in the regulation of insulin secretion and thus leads to an increase in the concentration of blood glucose.

However, the glucose concentration significantly reduced in the diabetic rats placed on *Moringa oleifera* leaf and *Myristica fragrans* seed aqueous extracts. This is in agreement with earlier studies done by (Edoga *et al.*, 2013; Jaiswal *et al.*, 2009; Kareem *et al.*, 2009) who earlier reported hypoglycemic actions in their various studies when treated diabetic animals with some plants extracts. Somani and Singhai (2008) had also reported hypoglycaemic activity of *Myristica fragrans* extract in alloxan induced diabetic rats. They all noted that the fasting blood sugar status to near the control level gradually after supplementation of *Moringa oleifera* leave in diets and *Myristica fragrans* extract in separate ways.

Figure 3 showed the effects of aqueous extracts of horseradish (*Moringa oleifera*) leaf and nutmeg (*Myristica fragrans*) seed treatments on the malonaldehyde concentration of alloxan-induced. The concentration of plasma Malonaldehyde (MDA) was shown to be significantly increased in diabetic rats when compared with the control but grossly reduced on treatments with the extracts, respectively this is in accordance with Erukainure *et al.* (2012), who reported decreased MDA in brain tissues of diabetic rats by certain plant fiber. It has also been reported by Ceriello *et al.* (1998) that diabetic patients show during the postprandial period, an increased plasma malonaldehyde levels.

However, the diabetic rats, when placed on *Moringa oleifera* and *Myristica fragrans* aqueous extracts had a remarkable decrease in their plasma malonaldehyde levels when compared

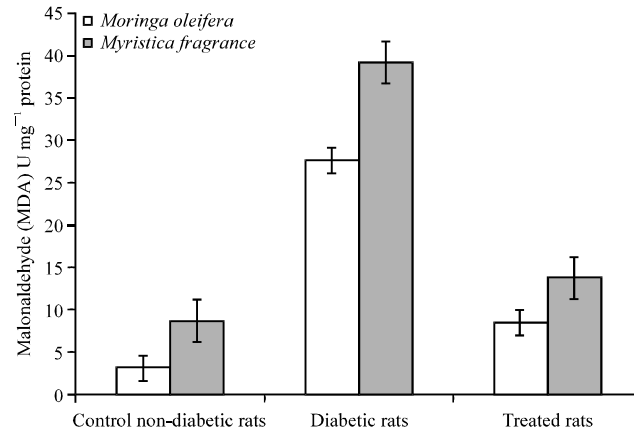


Fig. 3: Effects of aqueous extracts of horseradish (*Moringa oleifera*) leaf and nutmeg (*Myristica fragrans*) seed treatments on the malonaldehyde concentration of alloxan induced diabetic rat, values are Mean±SEM

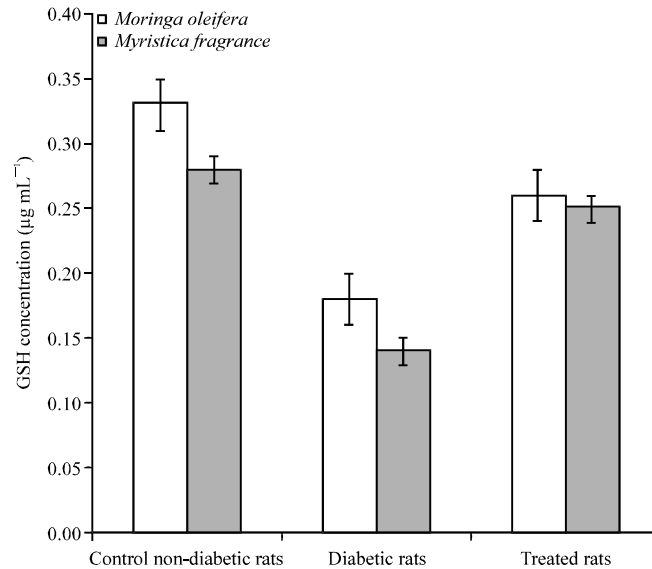


Fig. 4: Effects of aqueous extracts of horseradish (*Moringa oleifera*) leaf and nutmeg (*Myristica fragrans*) seed treatments on the Glutathione (GSH) concentration of alloxan-induced diabetic rats, values are Mean±SEM

with the control, thus indicating the free radical scavenging activity of both *Moringa oleifera* leaf and *Myristica fragrans* seed aqueous extracts on oxidative stress in diabetics.

The effects of aqueous extracts of horseradish (*Moringa oleifera*) leaf and nutmeg (*Myristica fragrans*) seed treatments on the reduced Glutathione (GSH) concentration of alloxan-induced diabetic rat showed a significant reduction in the reduced glutathione levels of the diabetic rats in both the *Moringa oleifera* and *Myristica fragrans* groups when compared with the control as seen in Fig. 4.

This depletion in blood glutathione is attributable primarily to the alloxan injected in the rats, a xenobiotic and an inducer of diabetes. Both xenobiotics and normal metabolism are known to

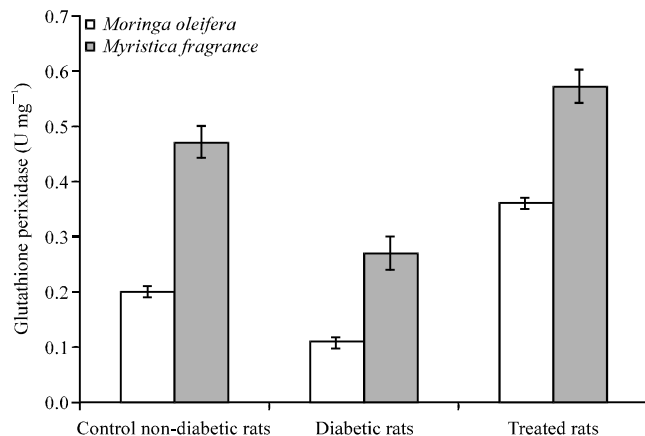


Fig. 5: Effects of aqueous extracts of horseradish (*Moringa oleifera*) leaf and nutmeg (*Myristica fragrans*) seed treatments on the Glutathione peroxidase (GPx) activity of alloxan-induced diabetic rat, values are Mean \pm SEM

deplete antioxidants as they are consumed in the course of scavenging reactive species generated. The reduction in glutathione to the level that was observed, could lead to a devastating decrease in the total antioxidant status of the animals because glutathione helps in recycling cellular antioxidants, inhibits free radical damage and plays a key role in the detoxification of harmful compounds (Robert *et al.*, 2000). This agrees with earlier works carried out by (Unt *et al.*, 2008; Eleazu *et al.*, 2010; Dominquez *et al.*, 1998; Polidori *et al.*, 2000) who reported reduced total plasma antioxidant capacity in uncontrolled diabetes. However, both plant extracts intake by the diabetic rats increased their glutathione status to near the control level and this is remarkable as this implies that both plants diet could have an ameliorating effect on the altered antioxidant status of a diabetic.

Figure 5 explained the effects of aqueous extracts of horseradish (*Moringa oleifera*) leaf and nutmeg (*Myristica fragrans*) seed treatments on the Glutathione peroxidase (GPx) activity of alloxan-induced diabetic rat. Glutathione peroxidase at the diabetics rats reduce when compare with the control in *Moringa oleifera* and *Myristica fragrans* leaf aqueous extract treated rats.

However, both plants intake by the diabetic rats increased their glutathione peroxidase (GPx) activity status to higher the control level and this is remarkable as this implies that both plants diet could have an ameliorating effect on the altered antioxidant status of a diabetic.

Figure 6 revealed the effects of aqueous extracts of horseradish (*Moringa oleifera*) leaf and nutmeg (*Myristica fragrans*) seed treatments on the catalase activity of alloxan-induced diabetic rat.

The reduction in catalase activity after injection of alloxan is another significant finding in this study. The decreased concentration of plasma catalase is attributable in part to the reduced synthesis of this antioxidant enzyme (which functions in the detoxification of hydrogen peroxide) whose concentrations would have fallen with the alloxan that was injected into the animals.

Though some studies have reported no alterations in the activity of red cell catalase in diabetics (Dohi *et al.*, 1988). However, this study is in agreement with earlier reports by (Erukainure *et al.*, 2012; Udoh *et al.*, 2007; Tagami *et al.*, 1992) who reported a decreased red blood cell catalase activity in diabetic. While treatment with *Moringa oleifera* leaf and *Myristica fragrans* seed aqueous extracts caused slight increase in catalase activity in their respective groups.

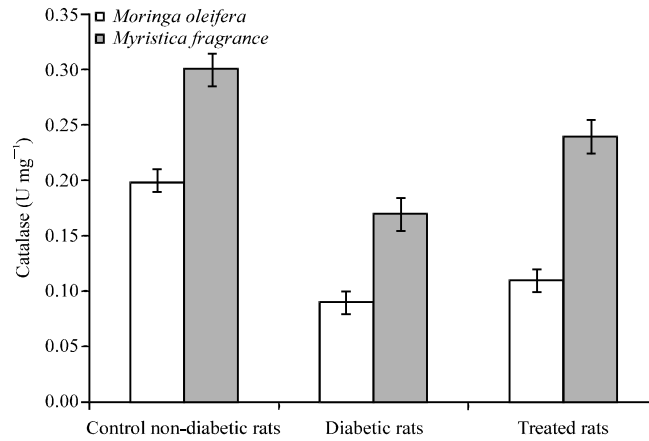


Fig. 6: Effects of aqueous extracts of horseradish (*Moringa oleifera*) leaf and nutmeg (*Myristica fragrans*) seed treatments on the catalase activity of alloxan-induced diabetic rat, values are Mean±SEM

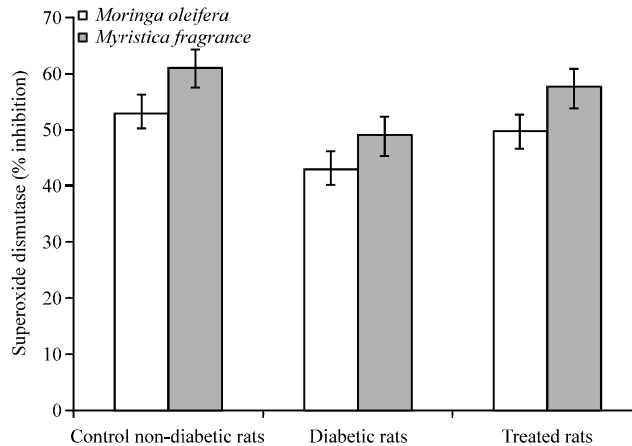


Fig. 7: Effects of aqueous extracts of horseradish (*Moringa oleifera*) leaf and nutmeg (*Myristica fragrans*) seed treatments on the superoxide dismutase (SOD) percentage inhibition in alloxan-induced diabetic rats, values are Mean±SEM

Figure 7 emphasized the effects of aqueous extracts of horseradish (*Moringa oleifera*) leaf and nutmeg (*Myristica fragrans*) seed treatments on the Superoxide dismutase (SOD) percentage inhibition in alloxan-induced diabetic rats. Similarly as observed for catalase, there was slight reduction in the alloxan-induced diabetic rats compared to control stage but treatment with the extracts caused an increase in the superoxide dismutase percentage inhibition activity. The ability of any plant to inhibit the formation of superoxide radicals which are toxic species can be viewed as a good index for measuring antioxidant activities. The observation in this study however agrees with other scientists Salawu *et al.* (2006) in their study on chemical composition and *in vitro* antioxidant activities of some Nigerian vegetables where it was evidenced that vegetables played a key role in inhibiting superoxide radicals.

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