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Research Article

Phytoconstituents and *In-vivo* Antioxidant Activity of Ethanol Leaf Extract of *Moringa oleifera* Lam (Moringacea) in Mice

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Abstract

Antioxidants also known as free radical scavengers interact and neutralize free radicals thereby preventing cellular damage in the biological system. This work evaluated the bioactive components and antioxidant activity of *Moringa oleifera* ethanol leaf extract in mice. Ethanol leaf extract was prepared from a freshly collected leaves of *Moringa oleifera* using cold maceration method and preliminary phytochemical screening of the extract was conducted using standard method of Prashant. Antioxidant activity of the extract at doses of 100, 200 and 400 mg/kg was evaluated using serum superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) and glutathione peroxidase (GPX) activity tests. Alkaloids, glycosides, flavonoids, tannins, and phenolic compounds were detected from the extract. The extract produced a significant ($p < 0.01$) increase in the serum level of superoxide dismutase (SOD) activity at 200 and 100 mg/kg when compared to the normal saline treated group. The extract also produced a significant ($p < 0.05$) increase in the serum level of catalase (CAT) activity at all the tested doses. The extract produced a significant ($p < 0.001$) and dose dependent increase in the malondialdehyde (MDA) levels in the serum when compared to control group, however, there was no significant statistical reduction in the serum level of glutathione peroxidase (GPX) enzyme activity as compared to control. Ethanol extract of possess phytochemical constituents that have antioxidant activity.

Keywords: Antioxidant; Phytochemistry; Superoxide Dismutase; Catalase; Malondialdehyde Glutathione Peroxidase

Introduction

Diseases such as coronary atherosclerosis, ischemia, diabetes, cancer, immunosuppression and many neurodegenerative disorders are risk factors for oxidative damage caused by reactive oxygen species (ROS) and free radicals which can be prevented by the action of the endogenous antioxidant enzymes such as catalase (CAT), glutathione peroxidase and superoxide dismutase [1]. Reactive oxygen species (ROS) are not only produced naturally during metabolic process but also due to cases of radiation, smoking, psychological or even emotional stresses [2]. Oxidation is necessary in many living organisms for their daily biological activities and thus the role of the reactive oxygen species (ROS) for oxidation reaction is needed [1]. Inadequate resources and decreased safety profile of the clinically available antioxidants have eventually led to the search for new herbs with antioxidant potentials [3].

Moringa oleifera belonging to the family Moringaceae remain an important medicinal plant that is widely cultivated all over the world and it is highly valued for its known and vast medicinal properties [4]. The importance of this medicinal plant globally can never be overemphasized as almost every part of this tree possesses product useful to man [5]. *Moringa oleifer*a has naturalized in West Africa, especially in Nigeria where it is cultivated for its nutritional and medicinal values [4]. Common names includes "Drumstick tree", "Horseradish tree" because of the taste of the roots, "Ben oil tree" from the oil which is obtained from the seeds, "Ben aile" in French (Ramachandran,1980) and in Nigeria is popularly known as "Adagba malero" in Yoruba, "Zogale ganji" in Hausa, Igbos called it "Odudu oyibo" and "Konamarede" in Fulfulde [6].

Plant material collection and identification

Fresh leaves of the plant (*Moringa oleifera*) were collected from a garden in Misau Local Government Area of Bauchi State- Nigeria in January, 2017. The plant was identified and authenticated by a botanist (Mr Baha'uddeen) at the herbarium Unit, Department of Plant Biology, Bayero University Kano (BUK). The sample was compared with an already deposited specimen and voucher number (BUKHAN0011) was given for reference.

Preparation of plant extract

Fresh leaf of *Moringa oleifera* was shade dried at room temperature to a constant weight. The dried leaf was blended into fine powder using mortar and pestle and sieved until a fine powder was obtained. About 600g of the powdered plant material was then macerated with five (5) litres of 70% V/V ethanol in a container for three (3) days with occasional agitations and stirring which was then filtered using whatmaan filter paper No:1 to get residue and filtrate. The resultant filtrate was concentrated and evaporated to dryness in an oven at a temperature of 50°C and air dried. The yield was weighed and stored in an air-tight container. The percentage yield was then calculated as follows:

$$\text{Percentage yield of the extract} = \frac{\text{(Weight of the extract (g))}}{\text{(Weight of the powdered leaf (g))}} \text{ X } 100$$

Preliminary phytochemical analysis of the extract

Phytochemical analysis was conducted based on the method described by Prashant., *et al*. [7].

Detection of alkaloids

Little portion of the extract was dissolved in dilute hydrochloric acid (HCl) and filtered. Filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

Detection of glycosides

500 mg of the crude extract was dissolved individually in dilute Hydrochloric acid and filtered.

Modified Borntrager's Test: Filtrates (2 ml) were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Rose-pink colored substance appeared in the ammonical layer and that indicates the presence of anthranol glycosides.

Detection of flavonoids

Alkaline Reagent Test: Extract (2 ml) was treated with few drops of sodium hydroxide solution. Intense yellow colored precipitate was formed, which becomes colorless on addition of dilute acid, indicating the presence of flavonoids.

Detection of tannins

Gelatin Test: To the extract (2 ml), 1% gelatin solution containing sodium chloride was added. White precipitate formed which indicates the presence of tannins.

Detection of phenols

Ferric Chloride Test: 50 mg of extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

This method was similar to that of Onoja., *et al.* [8]. Twenty four (24) rats were randomly selected and divided into four (4) groups of six animals each. Daily oral administration for the following was carried out; Group-I received normal saline 1 ml/kg; Group-II, III and IV received 100, 200 and 400 mg/kg of the ethanol leaf extract of *Moringa oleifera* respectively. On the 28th day, all animals were fasted overnight and blood was collected aseptically via retro-orbital puncture which was allowed to stand for 30 minutes before centrifuged at 1,500 rpm for 10 minutes [9] and the serum was harvested and used to assay for superoxide dismutase (SOD), catalase (CAT), Malondialdehyde (MDA) and glutathione peroxidase (GPX).

Estimation of Superoxide Dismutase (SOD)

Method described by Sun., *et al.* [10] was employed to assay the Superoxide dismutase activity. In this method, xanthine-xanthine oxidase system was used to generate a superoxide flux, and nitroblue tetrazolium (NBT) was used as an indicator of superoxide production. The activity of superoxide dismutase was then measured by the degree of inhibition of the reaction unit of enzyme taking 50% inhibition of NBT reduction. Values were expressed as U/mL. Values of all the test groups (Extract) were compared with normal saline treated group. An increased in the superoxide dismutase activity indicates antioxidant like action.

Estimation of catalase activity

The method described by Atawodi [11] was adopted for the determination of catalase activity in serum: Serum (10 µL) was added to test tube containing 2.80 mL of 50 mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1 mL of fresh 30 mM hydrogen peroxide and the decomposition rate of hydrogen

peroxide was measured at 240 nm for 5minutes using spectrophotometer. Catalase activity was calculated using molar extinction coefficient of 0.041 mM/cm. An increased in the catalase activity showcase the antioxidant like effect.

Estimation of malondealdehyde activity

This was carried out following the method described by Spirlandeli., *et al*. [12] 100 µl of the plasma obtained from the blood of the individual rat of the respective groups was added to 700 µl of one percent phosphoric acid and 200 µl of 42 mmol/L of thiobarbituric acid (TBA). The mixture was then heated at 100°C over thirty minutes and then ice cooled for about two (2) minutes. A 250 µl of the aliquot was separated and added to 250 µl of 1 M sodium hydroxide in methanol at the ratio of 1:6. It was then centrifuged and filtered and 20 µl of the supernatant was subjected to high performance liquid chromatographic (HPLC) machine, maintaining the flow rate of 1 ml/min, column temperature and pressure at 26°C and 2 200 psi, respectively. The absorbance was read at 532 nm. The higher the absorbance, the better malondealdehyde activity. Values of all extract treated groups were compared with control group.

Results

Percentage Yield of the Ethanol Leaf Extract of *Moringa oleifera* (ELEMO)

Ethanol leaf extract of Moringa oleifera was found to be dark green substance with the percentage yield of 16.2 w/w.

Phytochemical Constituents of Ethanol Leaf Extract of *Moringa oleifera*

The Phytochemical screening of ethanol leaf extract of *Moringa oleifera* revealed the presence of alkaloids, glycosides, flavonoids, tannins and phenolic compounds (Table 1).

Phytochemical constituent	Inference
Alkaloids	+
Glycosides	+
Flavonoids	+
Tannins	+
Phenols	+

Table 1: Phytochemical Constituents of Ethanol Leaf Extract of *Moringa oleifera* (ELEMO)

(+) = Present, (-) = Absent

In-vivo Antioxidant Effects of Ethanol Leaf Extract of *Moringa oleifera* (ELEMO) in Wistar Rats after 28 days Treatment

The extract produced a significant ($p \leq 0.01$) increase in the serum level of superoxide dismutase (SOD) activity at 200 and 100 mg/kg when compared to the normal saline treated group. The extract also produced a significant ($p \leq 0.05$) increase in the serum level of catalase (CAT) activity at all the tested doses. The extract produced significant ($p \leq 0.001$) and dose dependent increase in the malondealdehyde (MDA) levels in the serum when compared to control. However, there was no significant ($p \leq 0.001$) statistical reduction in the serum level of glutathione peroxidase (GPX) enzyme activity as compared to control (Table 2).

Discussion

The results of our phytochemical screening have shown that the ethanol leaf extract of *Moringa oleifera* contains alkaloids, glycosides, flavonoids, tannins and phenolic compounds and this conforms to the findings of Santos., *et al.* [13] who found similar phytoconstituents in the leaves extract of this valuable medicinal plant.

Antioxidants also known as free radical scavengers interact and neutralize free radicals making them devoid of causing cellular damage in the biological system [14].

In this study, the results of the *in-vivo* antioxidant activity of the ethanol leaf extract of *Moringa oleifera* have shown that the extract increased the activity of serum superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) and decreased the serum level of glutathione peroxidase (GPX). Superoxide dismutase is an enzyme catalyzing dismutation of superoxide to hydrogen peroxide and oxygen, thereby reducing the likelihood of superoxide anion reacting with nitric oxide to form reactive peroxynitrite [15], thus, increased activity of this enzyme by ELEMO amounts to antioxidant activity. Catalase is a ubiquitous enzyme that catalyzes the decomposition of hydrogen peroxide, a reactive oxygen species, which is a toxic product of both normal aerobic metabolism and pathogenic reactive oxygen species production [15,16]. In this study, ELEMO was able to significantly increase the level of catalase in the extract treated groups suggesting the potential antioxidant activity of the plant. The increased serum activities of catalase and SOD as observed in this study suggest that the extract has potential *in-vivo* antioxidant activity and is capable of ameliorating the effect of reactive oxygen species in biologic system [17,18]. Antioxidant activity observed with this extract might be due to the presence of one or more phytochemical constituents of the plant. Numerous

studies have shown that flavonoids possess potent antioxidant activities capable of scavenging hydroxyl radicals, superoxide anions, and lipid peroxy radicals [19]. Phenolic compounds and flavonoids have been implicated in literature to be responsible for the antioxidant activity [20,21] thus the observed antioxidant activity with this extract might be due to the flavonoids or phenols contents of the plant. The results of this study agrees with the findings of Torres-Castillo., *et al.* [22] and Aa., *et al.* [23] who independently reported the antioxidant activity of the leaf extract of *Moringa oleifera* possibly due to same bioactive components such as flavonoids and/or phenols.

Conclusion

Moringa oleifera leaf extract possesses secondary metabolites that have antioxidant activity, suggesting the potential use of this medicinal plant as antioxidant.

Conflict of Interest

Authors declare no conflict of interest.

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