ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 1, pp: (31-37), Month: January - March 2018, Available at: www.researchpublish.com

# Moringa *oleifer*a Leaf Extract Potential in Reducing the Incidence of Food Allergy in Experimental Rats

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***Abstract:*** **The incidence of food allergy outbreak worldwide is becoming a public health concern. Food allergy is growing at an epidemic proportion affecting all age groups of human population especially such occurrence in young children is worrisome. Management of food allergy without side effect is still a challenge to the medical community. The objective of this study was to investigate the effectiveness of Moringa Oleifera leaf extract in the treatment of food allergy without side effects in experimental rats. Sixty (60) Wistar male rats, (4-6) weeks old, weighing between (100-120) g were grouped into five (5) sections. They were grouped under: Positive Control group, Negative Control group, Epinephrine control group, Chemotherapeutic and Chemo-preventive groups. The animals were allowed to acclimatize for two weeks where the rats were given free food ration and pure water. After 15 days of consecutive administration of 1 ml crude peanut extract and 10µl cholera toxin as a potent adjuvant in rats, made food allergy established. Results from the study indicated that the means of rectal temperatures after 20$^{th}$ day of allergy induction were declined from (37.17-34.47)◦C, (37.0-34.67)◦C, and (37.52-3542)◦C in Positive control group, Chemo-preventive and Chemotherapeutic groups respectively. The reductions in rectal temperatures confirmed that food allergy was established in experimental rats. Also the study showed that Moringa oleifera leaf extract reduced serum Immunoglobin levels from (324.50-275.4)ng/ml, (181.20-170.50)ng/ml, (279.50-44.38) ng/ml respectively under Epinephrine treatment, Chemopreventive and Chemotherapeutic experimental group treatments .This suggested that Moringa oleifera leaf extract had potential to prevent and control food allergy.**

***Keywords:*** **Food Allergy, Immunoglobin E, Chemotherapeutic group, Chemopreventive groups, Moringa plant extract.**

## 1. INTRODUCTION

Allergy disorder and food intolerance are major public health problems, affecting all human age groups and can appear at any time in life (Jenna, et al., 2013). The remedy available is to avoid the risk factor in food (Fox, 2013). Allergy pathway involves responses to foreign novel protein allergens. The symptoms experienced by susceptible people can include: sudden increase in heart rate, shortness of breath, sneezing, skin rashes, diarrhea, scratching, puffiness around the eyes and mouth, teeth gnawing, wheezing of over labored respiration, tremor and convulsions. Allergy disorder can impose heavy social and economic burden on the sufferers and consequently affect national work force. According to Philpott, et al., (2009), more than eleven millions American have food allergies of various degree of severity among 2% of adult population and 8% of children that are below three years of age. Food allergy disorders are often become object of frustration especially when is experienced by people already suffering from chronic diseases like: diabetes, hypertension, heart diseases and in malnourished infants. The most common foods that can cause food allergy are: cow's milk, egg, nuts, kiwi fruit, seafood, soy and peanut. There are various kinds of allergies that may include: skin allergy, dust allergy, insect sting allergy, pet allergy, eye allergy sinusitis allergy and drug reaction allergy. Also allergy disorder may be as a result of environmental conditions that become suddenly polluted and contaminated, such risk factors may include the outdoor and indoor allergens, air pollution, polluted water, lifestyle, diet, and the exposure to cigarette smoke (De-Yun-Wang, 2005). Prevalence of allergy disorder in industrialized societies of the world is growing at alarming proportion in countries of United States, United Kingdom and Australia, known to have the highest rate of food allergies. Prevalence rates in the United State for instance have increased by at least 18% (Branum and Lukas, 2009). Data information on the prevalence of allergy disorders in developing societies is scanty and not consistent despite the fact that there is no known cure for allergic diseases. There should be an increase in the pharmaceutical intervention to find alternative cure for food related allergic disorders without any side effects. The objective of this study is to investigate into *Moringa oleifera* leaf extract as an alternative option in the management of allergic diseases. Moringa plant is used for treatment of different aliments in the indigenous system of medicine (Morton, 1999). The plant has the cure potential as anti-inflammation, antitumor, antidiuretic, cholesterol lowering property, antifungal, antibacterial and histamine reducing properties (Fahey, 2005) Moringa oleifera is rich in many bioactive and phytochemical components including: flavonoids, quercetin, indoles, carbinol resveratrol, biochanin, diadzein, genestein, keampferol and natural hormone and some powerful detoxifying enzymes that may provide strong anti-histamine properties.

## 2. MATERIALS AND METHODS

**The Study Area:**

The study was conducted in the Department of Nutrition and Dietetics of Babcock University as well as in the medical laboratory of Olabisi Onabanjo University Teaching Hospital, Shagamu in Ogun, State Nigeria.

**Animal Acclimatization:**

Forty (40) male Wistar rats (4-6) week old, weighing (100-400) g were purchased from the Animal Research Center in Babcock University. The rats were housed in colony cages of eight (8) rats per cage, at room temperature of 23◦C at relative humidity (30-70) percent under the light/dark cycle of 12 hr for at least two weeks of acclimatization. All animal rats had equal access to free food and free pure water.

## 3. METHODOLOGY

*Moringa Oleifera* leaves were harvested from the Botanical Garden of Nigerian National Development agency at about 6 a.m. The leaves were washed several times under tap running water. The leaves were then dried in a herb drier at 40◦C for 3 days. The leaves were then grinded manually into a coarse powder, to ease the extraction processes.

**Preparation of Moringa plant extract:**

Two (200 g) hundred, of Moringa powder was agitated in 10% methano organic solvent for 18 hr. A mercerated extract was filtered through (5μm) Whatman filter paper, and was concentrated to 10% of the original volume, at 40◦C in a rotatory evaporator in order to extract the organic solvent. The concentrates were dried at 37◦C to yield 35% of crude Moringa extract in accordance with Ugwu, et al., (2013) procedure. The extract was reconstituted into a sock solution of 250 mg/kg per rat body weight prior to extract administration.

**Antigen Preparation:**

Antigen was prepared following Farideh, et al., (2010) procedure, with a slight modification. Peanut proteins were used as the Antigen source and were extracted from fresh crude peanut. The peanut germs were grounded in a mill into a paste. The paste was defatted using n-Hexane solvent (1:10 v/v), which was extracted by shaking overnight at 4◦C. The suspension was centrifuged twice for clarification at 400 rpm for 50 min. The supernatant was filter-sterilized through (0.45μm) pore size in a sterile syringe filter. The extract was frozen at -20◦C and kept until use.

**Crude Protein Extract-Allergy Sensitization:**

Blood samples of experimental rats induced with allergy were tested to establish that Immunoglobin E allergen was effective. Forty (40) rats were subjected to allergic sensation five times in three days apart following Roy, (1999) procedure. Manifestation of signs and symptoms were evaluated after (30-40) min. when the allergic reaction was induced by feeding the rats with (1 mg of Crude Protein Extract plus Cholera toxin) according to the procedure of Li, (2001). Possible and expected signs under observation included: possible death, scratching and rubbing the head and snout, puffiness around the eyes and mouth, teeth gnawing, diarrhea, urine dis-coloration, Anorexia, wheezing, labored respiration, tremor and convulsion. After 15 days expiration, the experimental rats in affected group were sacrificed, followed by the collection of orbital plexus blood samples. Also organs of interest were harvested that included the liver, and the kidney.

**Grouping of Experimental rats:**

Grouping of rats according to experimental procedures were carried out by five sections that included:

1. **Positive Control Group**:

In this section experimental rats were fed with normal food ration for 6 weeks and the differences in weight were observed and recorded.

2. **Negative Control Group:**

A mixture of crude Peanut Extract and controlled dose of cholera toxin that was used to induce and establish allergy were fed to the experimental rats.

3. **Epinephrine Administration Group**:

The experimental rats received a controlled amount of epinephrine drug after the rats exhibited some allergic reaction. Epinephrine dose is the normal prescribed treatment drug for allergic reactions. The effectiveness of this treatment drug was to be compared to the Chemopreventive and Chemotherapeutic administration to allergic rats.

4. **Chemotherapeutic Group:**

In this section the experimental induced allergic rats were treated with Moringa plant extract to manage the allergic reactions.

5. **Chemopreventive Group:**

In this section the experimental rats were given Moringa plant extract before exposing them to allergy to prevent the allergic reactions in the rats.

**Statistical Analysis:**

Data collected were subjected to Analysis of Variance (ANOVA) using (SAS, 2004) and significantly ($P<0.05$) different means were compared using Duncan Multiple Range Test (DMRT) obtained in the same statistical package. Statistical values were reported as means of triplicates.

## 4. RESULTS AND DISCUSSION

Means of rectal temperature observed in allergy induced rat groups are presented in **Table 1.** The Table classified the data information under: Positive control group, Chemopreventive group and Chemotherapeutic group and within: Initial Temperature before allergy induction, also temperatures after $10^{th}$ and $20^{th}$ day of allergy induction respectively. Under Positive control group the means rectal temperature, 30 min after allergy induction was 37.17◦C, followed by 37.02◦C and 37.52◦C for Chemopreentive and Chemotherapeutic groups respectively. The means temperature after the $10^{th}$ day allergy induction was reduced from (37.17-to-36.30)◦C followed by Chemopreventive group means rectal temperature reduction from (37.0- to- 36.90)◦C, while that of Chemotherapeutic group experienced a reduction in mean rectal temperature from (37.52-to-36.92)◦C. The means temperatures after the $20^{th}$ day allergy induction fell from (37.17-to-34.47)◦C followed by Chemopreventive group means rectal temperature reduction from (37.0- to- 34.67)◦C, while that of Chemotherapeutic group experienced a reduction in mean rectal temperature from (37.52-to-35.42)◦C.

Farideh, et al., (2010), noticed that there was a reduction in rectal temperatures whenever allergy was established. The current data information also recorded reductions in the means rectal temperatures 30 min after the allergy induction at $10^{th}$ and $20^{th}$ day of allergy induction. These reductions confirmed the establishment of food allergy among the experimental rat animals.

**Table 1. Means of Rectal Temperature pattern in Allergy Induced experimental Rats**

| Groups | 1st Day Initial Temperatures at Allergy Induction | 10th Day Temperature After Allergy Induction | 20th Day Temperature After Allergy Induction |
|---|---|---|---|
| Positive Control rat Group | 37.17◦C | 36.30◦C | 34.47◦C |
| Chemo-preventive rat Group | 37.02◦C | 36.90◦C | 34.67◦C |
| Chemotherapeutic rat Group | 37.52◦C | 36.92◦C | 35.42 ◦C |

Values are Means ± SE of triplicate.

Means in a column are not significantly (P< 0.05) different.

The means of Serum Immunoglobin E activity levels in allergy induced rat groups are expressed in **Table 2.** The Table categorized the data information under: Positive control group, Negative control group, Epinephrine group, Chemopreventive group and Chemotherapeutic group. Also the serum immunoglobin levels were evaluated from the initial day of allergy induction and subsequent to those of 16th and 30th days after allergy inductions. The results were expressed as means of the evaluation. The means under the effects of Initial day of allergy induction were not significantly (P < 0.05) different in Positive Group, Negative Group, Epinephrine Group Chemoprevention and Chemotherapeutic Groups. The means after 16th day of allergy induction and Initial day of allergy induction under Positive control Group (50.00 – 34.79) µg/ml were not significantly different because this group was the experimental control. Under Negative control group the means of serum Immunoglobin levels after 6th day of allergy induction increased from (48.94 to 294.60) µg/ml. This high level of serum immunoglobin resulted in high level of oxidative stress caused by the effect of allergy disorder in the affected rats. Under the Epinephrine control group the means of serum immunoglobin level after 6th day of allergy induction increased from (47.40 to 324.50) µg/ml, indicating high level of oxidative stress caused by allergy disorder in the experimental rats. Under Chemopreventive group the means of serum immunoglobin level after 16th day of allergy induction increased from (46.09 to 181.20) µg/ml, resulting in an oxidative stress in the experimental rats. Under Chemotherapeutic group the means of serum immunoglobin level rose from (37.3 to 279.50) µg/ml indicating high level of oxidative stress in the experimental rats.

After 30th day of allergy induction the surviving rats received treatments using epinephrine (being the normal dug prescription for allergy disorder), Moringa oleifera leaf extract was used to prevent further complication of allergic reaction in rats under Chemopreentive group. Also Moringa oleifera leaf extract was used to treat allergy disorder in rats under Chemotherapeutic group. However, high level of oxidative stress killed majority of the rats under the Negative control group. After the epinephrine drug treatment, the mean of serum immunoglobin level declined from (324.50 to 275.40) µg/ml. This reduction indicated that epinephrine could be used to treat allergy disorder in experimental rats. Under the Chemopreventive group the mean of serum immunoglobin level reduced from (181.20 to 170.50) µg/ml. This reduction in the serum

**Table 2. Means of Serum Immunoglobin activity in Allergy Induced experimental Rats**

| Groups | 1st Day/Initial Allergy Induction | 16th Day after Allergy Induction | 30th Day after Allergy Induction |
|---|---|---|---|
| Positive Control Group | 34.79 ± 6.64 | 50.0 ± 0.100 | 34.79 ± 6.64 |
| Negative Control Group | 48.94 ± 6.23 | 294.60 ± 3.16 | Not available |
| Epinephrine Treated Group | 47.14 ±3.67 | 324.50 ± 5.25 | 275.4 ± 2.72 |
| Chemo-preventive Group | 46.09 ± 4.28 | 181.20 ± 6.11 | 170.50 ± 5.89 |
| Chemotherapeutic Group | 37.23 ± 8.35 | 279.50 ± 2.14 | 44.38 ± 1.96 |

Values are Means ± SE of triplicate.

Means in a column are not significantly (P< 0.05) different.

Immunoglobin level of activity indicated that *moringa oleifera* leaf extract had the potential to prevent and suppress allergy disorder in the experimental rats. Under the Chemothrapeutic group the mean of serum immunoglobin level declined from (279.50 to 44.38) µg/ml. This reduction in the serum immunoglobin level of activity suggested that *moringa oleifera* leaf extract had the potential to effectively treat allergy disorder in the experimental rats.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
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Group of Antioxidant hepatic enzymes involved in reducing and detoxifying oxidative stress caused by free radicals effects included: Superoxide Dismutase (SOD), Catalase and Glutathione detoxifying enzymes among others. The mean of antioxidant enzyme activity levels in detoxifying toxin caused by free radicals are presented in **Table 3.** The Table classified the Antioxidant enzyme activity against the free radicals under: Positive control group, Negative control group, and Epinephrine, Chemopreventive and Chemotherapeutic groups.

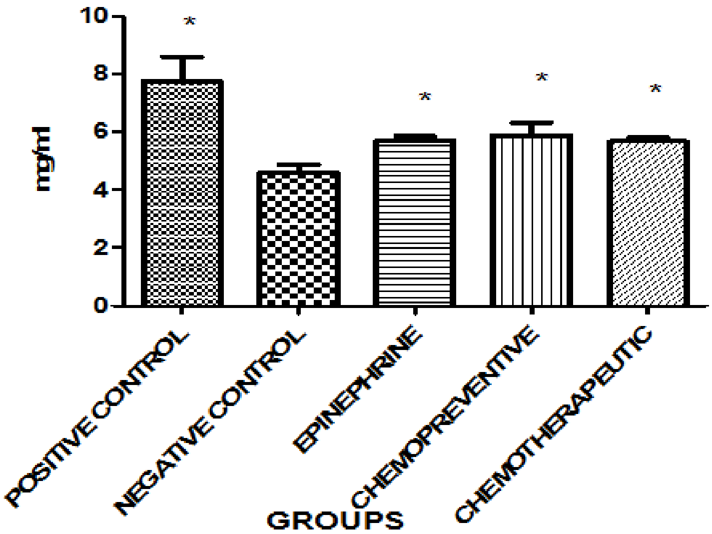


**Fig. 1. Means of Superoxide Dismutase enzyme activity against free radicals in allergic rats**

Under the Positive Control group the mean of hepatic SOD activity potential against free radical was (7.750) mg/ml, being the initial level of enzyme activity. Under the Negative group the means of (SOD) enzyme activity declined from (7.75 to 4.60) mg/ml indicating that allergy induction media (using the Cholera toxin and Crude Peanut extract) enable the free radical to suppress the SOD enzyme activity against free radical effects. However, under Epinephrine control group the mean of hepatic (SOD) enzyme activity increased from (4.60 to 5.72) mg/ml. This increase in antioxidant enzyme activity level, suggested that hepatic (SOD) and the epinephrine dug could be used to treat and control free radicals effects that contributed to oxidative stress in the experimental rats. The means of SOD enzyme activities increased from (4.60 to 5.89) mg/ml, and from (4.60 to 5.68) mg/ml in Chemopreventive and Chemptherapeuatic groups respectively. These increases indicated that hepatic SOD together with Moringa oleifera leaf extract could be used to control and suppress the activity of the free radicals, as well controlling the oxidative stress effects.

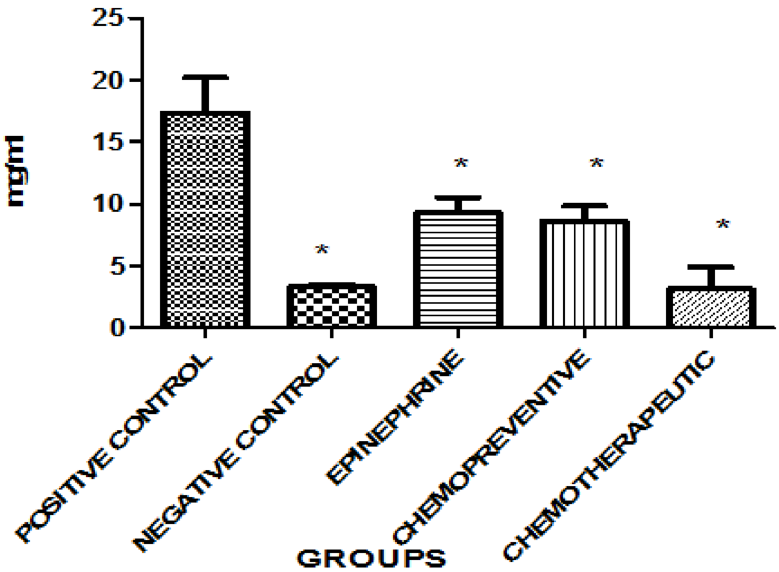


**Fig. 2. Means of Catalase enzyme activity against free radicals in allergic rats**

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Under the Positive Control group the mean of hepatic Catalase activity potential against free radical was (17.36) mg/ml, being the initial level of enzyme activity. Under the Negative group the means of Catalase enzyme activity declined from (17.36 to 3.30) mg/ml indicating that allergy induction media (using the Cholera toxin and Crude Peanut extract) were enable to suppressed the catalase enzyme activity against free radical toxin. However, under Epinephrine control group the mean of hepatic Catalase enzyme activity increased from (3.30 to 9.31) mg/ml. This increase in Antioxidant enzyme activity indicated that hepatic Catalase and epinephrine drug controlled and prevented free radicals contributing to the oxidative stress in experimental rats. The means of Catalase enzyme activities also increased from (3.30 to 8.60) mg/ml, and from (3.30 to 5.13) mg/ml in Chemopreventive and Chemptherapeuatic groups respectively. These increases in enzymes activity indicated that Catalase and Moringa oleifera leaf extract can be used to suppress and control the activity of the free radicals and oxidative stress in experimental rats.

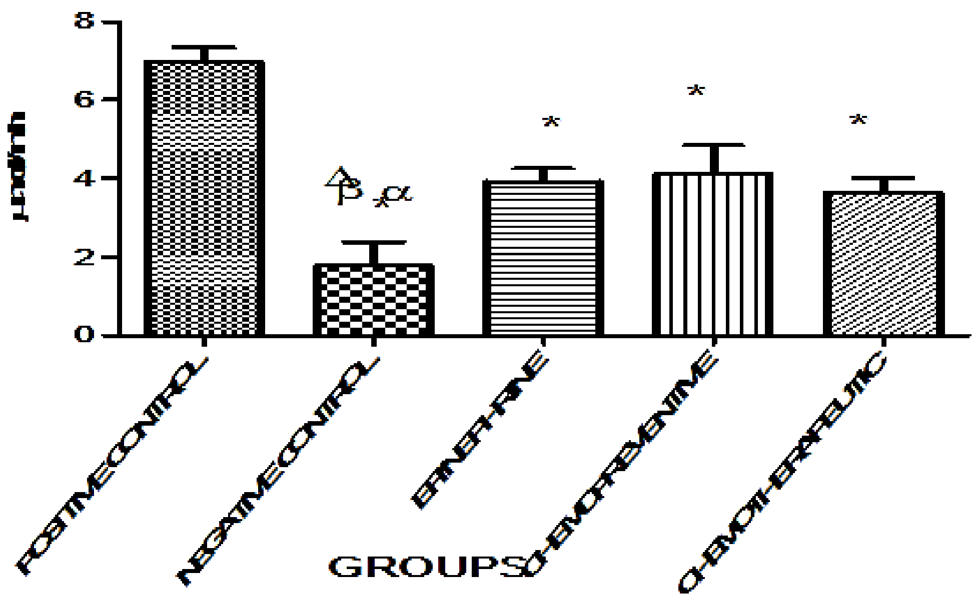


**Fig. 3. Means of Glutathione enzyme activity against free radicals in allergic rats**

Under the Positive Control group the mean of Glutathione activity against free radical was (6.99) mg/ml at initial enzyme activity. Under the Negative group the means of Glutathione enzyme activity declined from (6.99 to 1.77) mg/ml, indicating that allergy induction process (using the Cholera toxin and Peanut extract) enable free radical to suppressed the Glutathione enzyme activity against free radical effects. However, under Epinephrine control group the mean of Glutathione enzyme activity increased from (1.77 to 3.93) mg/ml. This increase in Antioxidant enzyme activity indicated that Glutathione and epinephrine drug could inhibit free radicals effects and the effects of oxidative stress in the experimental rats. The means of Glutathione enzyme activities increased from (1.77 to 4.14) mg/ml, also from (1.77 to 4.89) mg/ml in Chemopreventive and Chemptherapeuatic groups respectively. These increases indicated that

Glutathione in combination with Moringa oleifera leaf extract can be used to suppress free radical effects and that of oxidative stress in rats.

**Table 3. Effectiveness of Antioxidant enzymes against Free radical effects**

| Enzymes | Positive Control Group | Negative Control Group | Epinephrine treated Group | Chemo-preventive Group | Chemotherapeutic Group |
|---|---|---|---|---|---|
| SOD | 7.75 ± 0.83 | 4.60 ± 0.28 | 5.72 ± 0.14 | 5.89 ± 0.43 | 5.68 ± 0.13 |
| Catalase | 17.36 ± 2.82 | 3.30 ± 0.18 | 9.31 ± 1.18 | 8.60 ± 1.25 | 5.13 ± 0.33 |
| Glutathione | 6.99 ± 0.35 | 1.77 ± 0.61 | 3.93 ± 0.33 | 4.14 ± 0.71 | 4.89 ± 0.20 |

SOD = Superoxide Dismutase

Values are Means ± SE of triplicate.

Means in a column are not significantly (P< 0.05) different

## 5. CONCLUSION

The study indicated that cases of reduction in rectal temperatures 30 min after the allergy induction clearly suggested that food allergy was established. Application of crude peanut extract and cholera toxin were effective and active ingredients that promoted food allergy in experimental rats. However there were reported cases of patho-physiological manifestation in response to the severity of food allergy disorder. Some of the signs and manifestation were: labored respiration, rubbing of the snout and the head, wheezing, diarrhea and urine discolouration. These anaphylactic signs confirmed the presence of food allergy in the experimental rats.

The study further indicated that 250 mg/kg Moringa oleifera extract significantly reduced serum Immunoglobin levels, suggesting that food allergy disorder can be controlled and managed by suing Moringa oleifera leaf extract. Under Chemopreventive and Chemotherapeutic group treatments, the serum Immunoglobin levels were not significantly different at the point of allergy intervention using Moringa oleifera leaf extract. This suggested that Moringa oleifera leaf extract had the potential to prevent and control food allergy disorder to the minimum level of tolerance.

The study also revealed that Moringa oleifera leaf extract can improve the Antioxidant enzyme activities of hepatic (SOD), catalase and Glutathione effects against free radical effects. The synergy effects of Moringa oleifera leaf extract and the Antioxidant enzyme activity effectively prevented and controlled severity of oxidative stress effects in the experimental rats.

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