

Moringa leaf extract potential in the incidence of 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 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 distilled 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 20th day of allergy induction were declined from (37.17-34.47) fC, (37.0-34.67) fC, and (37.52-35.42) fC 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 leaf extract reduced serum Immunoglobulin E 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 leaf extract had potential to prevent and control food allergy.

Keywords: Food Allergy, Immunoglobulin E, Chemotherapeutic group, Chemopreventive groups, moringa plant extract

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 have often become object of frustration especially when was experienced by people already suffering from chronic diseases like: diabetes, hypertension, heart diseases and in malnourished infants. The most common

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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). 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 was to investigate into moringa leaf extract s an alternative option in the management of allergies.

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 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 distilled water.

Methodology

Moringa 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 crushed manually into a coarse powder to ease the extraction processes.

Preparation of moringa plant extract

Two hundred grams of moringa powder was agitated in 10% methano organic solvent for 18 hr. A macerated 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 stock 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 ground 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. 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 Immunoglobulin 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:

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.

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.

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.

Chemotherapeutic group

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

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.

Oxidative stress tests

Animal liver sample preparation

4.5ml phosphate buffer was added to 0.5g sample of rat liver. The liver sample mixture was then homogenized and centrifuged at 3,000rpm for 10min and the supernatant was decanted.

Superoxide dismutase enzyme activity

Ability of SOD enzyme to inhibit auto-oxidation of epinephrine to adrenochrome at pH 10.2 buffer solution make this reaction an Assay for SOD enzyme activity according to Valerino and Mc-Cornack (1971), procedure.

Determination of SOD activity unit

0.9ml of distilled water was added to 0.1ml to supernatant liver sample preparation. An aliquot of 0.2ml of diluted SOD enzyme preparation was added to 2.5ml of 0.05M carbonate buffer at pH 10.2. The reaction started when 0.3ml of 0.3mM of adrenaline was added to the mixture. The complex was rapidly mixed together by inversion of the test tube. A reference solution containing 2.5ml of 0.005M carbonate buffer plus 0.3ml adrenaline substrate and 0.2ml distilled water were all contained in a cuvette. The initial absorbance was set at 480nm on spectrophotometer. The increase in absorbance was observed every 30secs for a span of 150secs. A unit of SOD enzyme

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activity was given as the amount of SOD needed to cause 50% inhibitors of oxidation of adrenaline to adrenochrome in 1 min.

Determination of Glutathione (GSH) enzyme activity

Serial dilution of GSH stock was prepared before the experiment commenced according to Sedlack and Lindsay (1965) procedure.

1.5ml of phosphate buffer was added to each test tube containing dilution of GSH stock, followed by addition of another 0.5ml of phosphate buffer. Slowly, 0.5ml of Ellman's reagent of 5, 5, di-thio-bis-2-nitrobenzoic acid was added. The absorbance of a yellow colored complex formed on addition of Ellman's reagent was read within 5 min. at 4.12nm on spectrophotometer.

Determination of Catalase enzyme activity

Catalase activity was determined according to Sinha (1971) procedure. Catalase enzyme sample as added to split H_2O_2 in a reaction that terminates when dichromate-acetic acid was added. The remaining H_2O_2 was determined by measuring chromic acetate using colorimeter after heating the reactant mixture. Quantity of 20-160moles of H_2O_2 was measured into small test tubes. 0.2ml of dichromate acetic acid was added to each test tube producing an unstable blue precipitate of per-chromic acid. Subsequent heating in a warm water bath for 10min. changes the color to stable green chromic acetate. Cooling to a room temperature 3.0ml of distilled water was added. Colorimeter was set at 570nm to measure the absorbance of the green chromic acetate.

Statistical analysis

Data 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.

Results and discussions

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 10th and 20th 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 chemopreventive and chemotherapeutic groups respectively. The means temperature after the 10th 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 20th 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 10th and 20th day of allergy induction. These reductions confirmed the establishment of food allergy among the experimental rat animals.

Table 1: Rectal temperature pattern in allergy induced experimental rats

Groups	1 st day initial temperatures at allergy induction	10 th day temperature after allergy induction	20 th day temperature after allergy induction
Positive Control rat Group	---	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 Immunoglobulin 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 immunoglobulin E 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 Immunoglobulin E levels after 6th day of allergy induction increased from (48.94 to 294.60) µg/ml. This high level of serum immunoglobulin E 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 immunoglobulin E 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 immunoglobulin E 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 immunoglobulin E 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 drug prescription for allergy disorder), moringa leaf extract was used to prevent further complication of allergic reaction in rats under chemopreventive group. Also moringa 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 immunoglobulin E 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 immunoglobulin E level reduced from (181.20 to 170.50) µg/ml. This reduction in the serum

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Table 2: Serum immunoglobulin activity in allergy induced experimental rats

Groups	1 st Day/Initial Allergy Induction	16 th Day after Allergy Induction	30 th 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.

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

Groups 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.

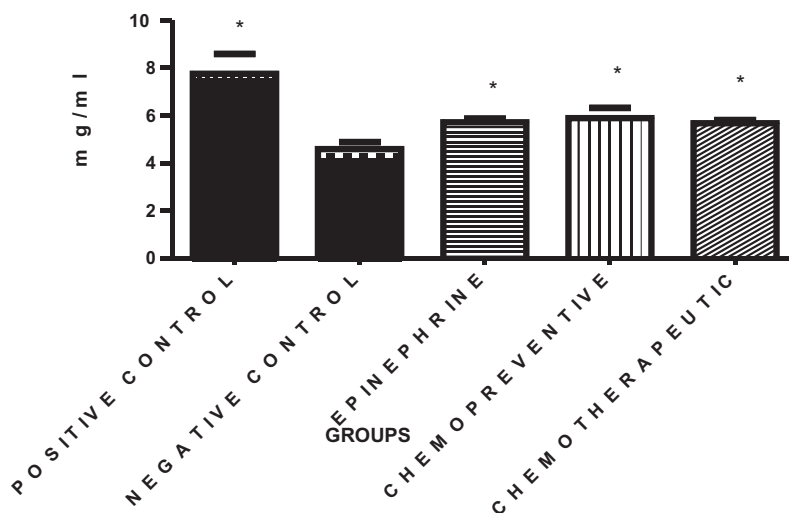


Figure 1: Superoxide Dismutase enzyme activity against free radicals in allergic rats

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 chemotherapeutic groups respectively. These increases indicated that hepatic SOD together with Moringa leaf extract could be used to control and suppress the activity of the free radicals, as well controlling the oxidative stress effects.

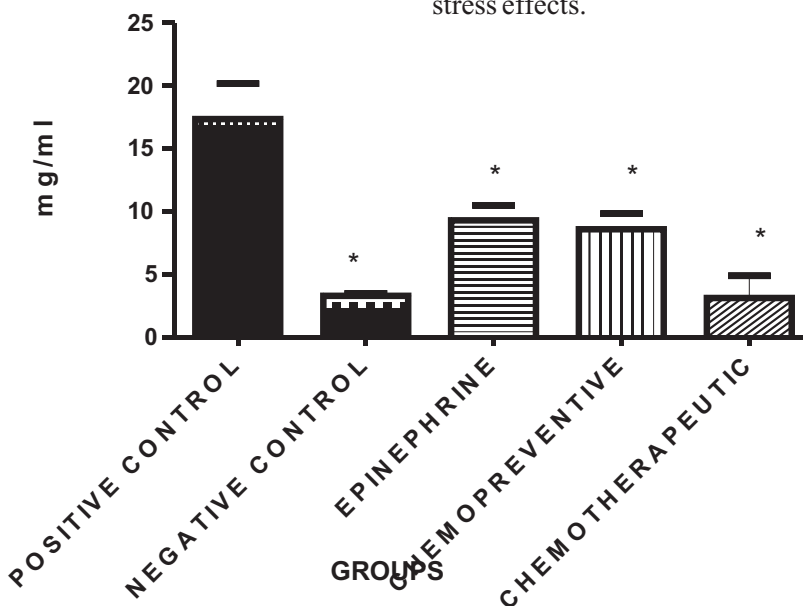


Figure 2: Catalase enzyme activity against free radicals in allergic rats

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 unable to suppress 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

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chemopreventive and chemotherapeutic groups respectively. These increases in enzymes activity indicated that Catalase

and moringa leaf extract can be used to suppress and control the activity of the free radicals and oxidative stress in experimental rats.

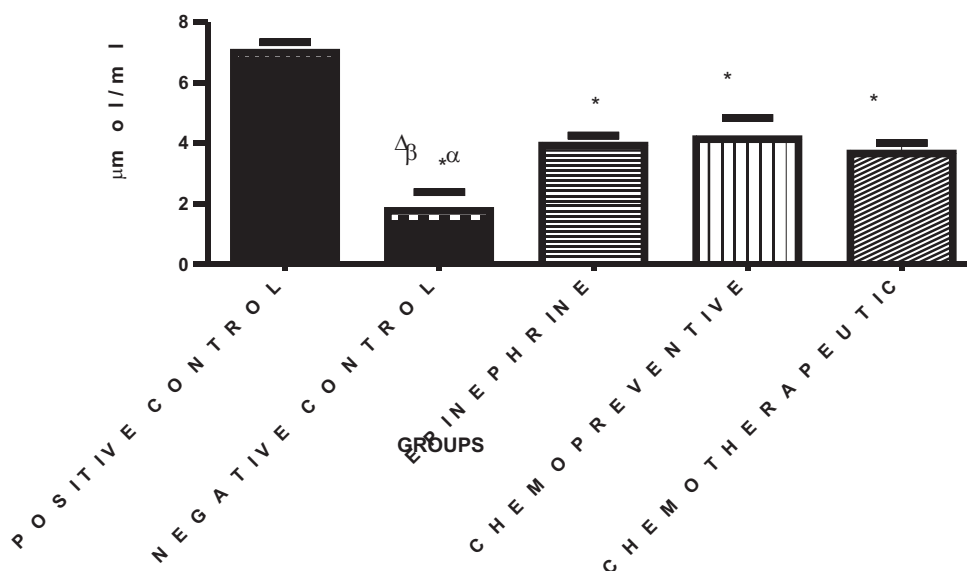
Table 3: Effectiveness of antioxidant enzymes against free radical effects

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



Under the positive control group the means 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 activities increased from (1.77 to 4.14) mg/ml, also from (1.77 to 4.89) mg/ml in chemopreventive and chemotherapeutic groups respectively. These increases

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

Conclusions

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 extract significantly reduced serum Immunoglobulin E levels, suggesting that food allergy disorder can be controlled and managed by using moringa leaf extract. Under Chemopreventive and Chemotherapeutic group treatments, the serum Immunoglobulin E levels were not significantly different at the point of allergy intervention using moringa 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 leaf extract can improve the antioxidant enzyme activities of hepatic (SOD), catalase and glutathione effects against free radical effects. The synergy effects of moringa leaf extract and the antioxidant enzyme activity effectively prevented and controlled severity of oxidative stress effects in the

experimental rats.

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