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Antioxidant and Anti-ulcer Activities of Fermented soybean (Natto) and Moringa oleifera leaves in Male Albino Rats

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ABSTRACT

Peptic ulcer disease is caused by spicy food, stress, alcohol, gastric surgery and *Helicobacter pylori*. Studies are being directed towards using natural products for developing ulcer drugs with minimal side effects. The antioxidant, total phenolic content (TPC), and anti-ulcer activity of the powders were investigated, in addition to the fortification of soup with them. TPC of *Moringa oleifera* (MO) leaves andfermented soybean (Natto) extracts was high for both soybean and MO leaves aqueous extracts. Gallic acid was discovered to be the main component of phenolic represent 1464.304µg/gm sample in soybean Natto, whereas chlorogenic acid was the main component of MO leaves (2218.493 µg/gm). According

to the antioxidant activity results, MO leaves had 2.6 times the antioxidant activity of fermented soybean Natto by 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Also, ferric reducing power activities results indicated that MO leaves gave high reducing power activities 64.4 times more than fermented soybean Natto. After pre-treated with Natto (25 , 50%) and aqueous extract of MO leaves (5, 10%)For 7 days, then all animalsadministration by asprineat a dose of 500 mg/kg bw suspended in water for the induction of acute gastric ulcer, ulcer index, protective index, volume of gastric juice and pH of gastric juice were evaluated. In biochemical parameters, lipid peroxidation, superoxide dismutase (SOD), gluthathione peroxidase (GPx), malondialdehyde (MDA) and Catalase (CAT) were evaluated. Ranitidine was used as a positive control. All the macroscopic and biochemical parameters showed significant anti-ulcer activity offermented soybeanNatto and aqueous extract of MO leaves. The anti-ulcer activity was almost comparable to the positive control. Ready to cook soup was an ideal product to be fortified with either soybean Natto or MO leaves and the addition of soybean natto was found to be highly acceptable comparing to the control sample in some cases because of its meat like flavor and taste which was more accepted for some panelists. While a gradient of admission was found in other samples.

Key words: Fermented soybean Natto; *M. oleifera* leaves; Antioxidant activities; total phenolic content; Aspirin; peptic ulcer; lipid peroxidation; Histopathology; and rats.

Introduction

Peptic ulcer is a disease characterized by mucosal damage that usually occurs in the stomach and proximal duodenum ***(Koffuor et al., 2013)***. It is a serious infection that occurs as a result of eating spicy food, stress, alcohol, stomach surgery, and *Helicobacter pylor i***(Bae et al., 2011).** Aspirin - acetylsalicylic acid- is an effective nonsteroidal anti-inflammatory drug (NSAID) which commonly used for inflammation, fever, and pain ***(Fornai et al., 2005).*** Aspirin is widely prescribed for the treatment of inflammatory diseases such as rheumatoid arthritis, and is commonly used for the prevention of cardiovascular thrombosis ***(Wang et al., 2011).*** Aspirin, one of the most widely used NSAIDs, damages gastrointestinal mucosa by irritant action, causing alterations in mucosal permeability and/or suppression of prostaglandin synthesis ***(Simmons et al., 2004).***

Currently, gastric ulcers are treated by chemical drugs that inhibit gastric acid secretion, antacid drugs that neutralise the acid, or cytoprotective drugs that prevent cellular

apoptosis***(Halabi et al., 2014).*** However, most of these drugs have side effects such as joint pain, altered heartbeat, haemopoeitic changes, gynaecomastia, impotence and systemic alkalosis ***(Handa et al., 2014).*** Nowadays, increasing studies are being directed towards the use of natural products for developing drugs with minimal side effects ***(Rasool et al., 2006).***

Moringa oleifera (MO) (family Moringaceae) is commonly known as “Drumstick”. It is a small or medium sized tree, about 10m height, found in the sub Himalayan tract ***(Trapti et al., 2009).*** Studies on MO has shown that the plant has antioxidant, antimicrobial, anti-inflammatory, antipyretic, ant diabetic, antiulcer, antitumor ant diarrheal and hypocholesteromic properties **(*Anitha et al., 2011).*** It is also an important food commodity that has received a great deal of attention as the natural nutrition of the tropics. The ancient Romans, Greeks, and Egyptians used these fast-growing trees. According to reports, Indians have been using MO as a regular component of traditional foods for nearly 5000 years. ***(Anwar and Bhanger, 2003).*** MO leaves has been reported to be a rich

source of β-carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidants ***(Mahmood et al., 2010).***

Its leaves have four times the calcium content of milk, seven times the vitamin C content of oranges, three times the potassium content of bananas, three times the iron content of spinach, four times the amount of vitamin A in carrots, and two times the protein content of milk. ***(Kamal, 2008).*** The use of these natural plant antioxidants has greatly improved preventive medicine. Plants contain numerous free radical scavenging molecules, including alkaloids, phenolic acids, amines, betalains, terpenoids, lignins, stilbenes, tannins, and vitamins, as well as secondary metabolites with high antioxidant activity***.(Manjula and Ammani, 2012).***

A long time ago, Soybean (Glycine max) was a perfect source of protein and oil, with sufficient amounts of minerals and vitamins ***(Cao et al., 2019).*** Soybeans are used to make a variety of foods, particularly fermented foods like Temph and Natto. Fermentation has been used to improve the bioavailability of vitamins, minerals, and isoflavones in soy, as

well as to change its flavour, increase its stability, and even create new food products. ***(Rekha and Vijayalakshmi, 2010; Chiang and Pan, 2011).*** Concentrations of some vitamins in soy foods including vitamin K_2 in Natto, vitamin B_{12} in Tempeh increase significantly after fermentation ***(Mo et al., 2013),*** due to the metabolic activity of starter cultures such as *Bacillus subtilis*natto used to produce Natto which is a traditional fermented food from soybeans, with a widespread popularity Japane. ***(Yanagisawa and Sumi, 2005).***

Also, as a result of soybean protein degradation by fermentation, bioactive peptidesare produced and it have been reported to play an important role in human health ***(Karami and Akbari-adergani, 2019)***.Fermentation, on the other hand, works to impart a distinct smell, taste, and flavour to the product while also altering its functional properties. Fermentation processes also result in the formation of some biologically active compounds. Furthermore, the microbes that perform the fermentation process or the enzymes produced as a result of fermentation may significantly contribute to the vital activity of the fermented products. However, research on

fermented products and their effects on human health is still limited***(Cao et al., 2019).***

It has been established that there is a link between chronic diseases and dietary habits. Malnutrition is the leading cause of immune deficiency-related health complications. Consumers do not have enough time to cook and have resorted to fast foods with low nutritional values such as high fat, sugar, and salt content. ***(Kaushik et al., 2011).*** This problem could be overcome by supplying easy-to-Cook nutrient-enriched foods ***(Farzana et al., 2017).*** Dry soups are much more preferable among foods because of its easy to make, reduce of weight, long shelf life and transportation. Most of popular market soups are low in nutritional values (high in carbohydrate and low in protein). For that, the aim of this study was to study the antioxidant and anti-ulcer activities of fermented soybean natto and MO leaves as sources of strong bioactive compounds in experimental rats and adding them to the soup product to turn it into a healthy nutritious soup that falls under the category of functional foods.

Materials and Methods

Soybean *(Glycine max)* and *M. oleifera* leaves were obtained from the Agriculture Research Center-Giza-Egypt, samples were obtained at 2019 season. While, Commercial kits used for determining superoxide dismutase (SOD), gluthathione peroxidase (GPx), malondialdehyde (MDA) and Catalase (CAT) were obtained from Biodiagnostic Co. Dokki, Egypt. Ranitidine and aspirin were obtained from El-Gomhoreya Co., Cairo, Egypt.

2.1. Preparation of fermented soybean (Natto)

The following Natto processing method was used, as described by ***Mahmoud et al., 2015:*** To avoid fermentative acidification, soybean was soaked in tap water at 1:3 w/v ratios for 24 hours. Autoclaves were used to cook the soaked beans at 121^{o}C for 3 minutes. Fifty g of cooked soybeans were cooled to 38^{o}C, inoculated with the Bacillus strain, and incubated for 24 hours at 38^{o}C. The natto product was obtained and stored until it could be analysed. Natto was dried in an oven dryer set to 50^{o}C.

2.2. Proximate composition of soybean and *M. oleifera* leaves

Proximate chemical composition (moisture, protein, ash, fat and crude fiber) of soybean and *M. oleifera* leaves were evaluated according to the methods described in the ***AOAC, (2000)*** carbohydrate was calculated by difference.

2.3. Preparation of extracts and Determination of total phenolic compounds

Extracts were performed according to ***Hayat et al., (2010)*** with some modifications. Dried Moringa leaves and soybean Natto powder (10 g) was extracted with 100 ml of either methanol 80% or water in an ultrasonic device (200 W, 59 kHz, Shanghai Kudos Sonication Machine Company Ltd., China) for 60 min at room temperature, the extracts were centrifuged for 15 min at 4000rpm. The Folin– Ciocalteu assay, adapted from ***Ramfulet al., (2011)*** was used for the determination of total phenolics present in the citrus fruit extracts.

2.3. Determination of polyphenols by HPLC

HPLC analysis was carried out using an Agilent 1260 series. Kromasil C18 column (4.6 mm x 250 mm i.d., 5 m) was used for separation. At a flow rate of 1 ml/min, the mobile phase was composed of water (A) and 0.05 percent trifluoroacetic acid in acetonitrile (B). The mobile phase was programmed in a linear gradient in the following order: 0 minutes (82 percent A); 0–5 minutes (80 percent A); 5-8 minutes (60 percent A); 8-12 minutes (60 percent A); 12-15 minutes (85 percent A); and 15-16 minutes (85 percent A) (82 percent A). At 280 nm, the multi-wavelength detector was monitored. For each of the sample solutions, the injection volume was 10 l. The column temperature was kept constant at 35 °C.

2.4. Antioxidant Activities

2.4.1. DPPH radical scavenging activity:

The effect of extracts on the free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was calculated using the procedure described by ***Aboelsoued et al., (2019).*** A spectrophotometer was used to measure the absorbance at 517nm. In place of the sample, ethanol was used as a control. The following equation was us

$$\text{Scavenging activity (\%)} = \frac{Ac - As}{Ac} \times 100$$

Where Ac and As are the absorbance's at 517nm of the control and sample, respectively.

2.4.2. Determination of Ferric reducing power (FRAP) assay

The FRAP assay is based on phenolics' ability to reduce Fe3+ to Fe2+. The FRAP reagent was made by combining 0.1 M acetate buffer (pH 3.6), 10 mM TPTZ, and 20 mM ferric chloride (10:01:01, v/v/v). To 150 l of reagent, 20 microlitres of previously diluted extract were added. A Microplate spectrophotometer was used to measure absorbance at 593 nm. The analysis was carried out in triplicate, with an aqueous Trolox solution serving as the standard, and the results were expressed as lmolesTrolox equivalents/100 g of sample***(Barros et al., 2012).***

2.5. Fortification of soup by *M. oleifera* leaves and fermented soybean natto

2.5.1. Formulation of a soup with powder

Three different formulations of soup mixtures were prepared by using either soy natto and/or MO leaves powder as given in Table (1).

2.5.2. Cooking procedures of soup powders

Twenty-five grams of the newly developed soup powder was added into 350 ml water and boiled for 5–6 min.

2.6. Sensory analysis

Sensory attributes such as flavor, taste, texture, after taste, colour, and overall acceptability using a nine-point hedonic-scale scorecard. by a trained 10-member panelists selected from the staff members of the Division of Food Science and Technology (DFST), National Research Centre (NRC), Egypt. Soup samples were evaluated using a 10-point hedonic scale (1-dislike extremely to 10=like extremely) according to ***Mahmoud et al., 2017***. The rating scale was used for all other parameters according to method described by ***Larmond (1980).***

2.7. Biological experiment

Forty two male Albino rats weighing about 150±5 g were obtained from Agricultural Research Center, Giza, Egypt. The animal groups were kept in an atmosphere of filtered, pathogen-free air, water, and a temperature of 20-25^{o}C for 8 weeks, with a 12 hour light/dark cycle and a light cycle (8-20 h) and a relative humidity of 50%. For one week, all rats were fed a basal diet. The basal diet was designed to contain 14% casein, 10% sucrose, 4% corn oil, 5% fibre (cellulose), 3.5 percent mineral mixture, 1% vitamin mixture, 0.25 percent choline chloride, 0.3 percent D-L methionine, and 61.95 percent corn starch ***(Reeves et al., 1993).***Before starting the experiment for acclimatization. All the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals. The experiment was conducted at Agricultural Research Center, Giza, Egypt.

2.7.1. Experimental design:

After one week for adaptation, the rats were divided into two groups. The first group (n= 6 rats) was fed only the basal diet as a negative control group (-). (Healthy rats). According to the protocol, all rats in the second main group (n= 36 rats) received a single oral dose of aspirin at a dose of 500 mg/kg bw

suspended in water for the induction of acute gastric ulcer **(*Mahmoud et al., 2019).*** Then, The second major group is divided into five groups, as follows: Group (2): The control positive (+) group was only fed a basal diet. For 7 days, Group (3) was fed a basal diet as well as an oral dose of *M. oleifera* leaves extract5 ml (5%). For 7 days, group (4) was fed a basal diet plus oral administration of *M. oleifera* leaves extract 5 ml (10%). For 7 days, group (5) was fed a basal diet plus oral Natto (25%) feeding. For 7 days, group (6) was fed a basal diet plus oral Natto (50%) feeding. For 7 days, group (7) was fed a basal diet plus oral ranitidine (50 mg/kg BW) as a standard drug. 1 hour before aspirin administration. The animals were sacrificed after 4 h of the administration of aspirin.

At the end of the experiment, animals from each group were sacrificed, and the blood was collected in a clean dry centrifuge tube, left at room temperature until the clot was formed, completely retracted, and then centrifuged to separate serum by centrifugation at 4000 R.P.M., for 10 minutes at room temperature, followed by storage in a plastic vial (well stoppered) until analysis.

2.7.2. Assessment of gastric mucosal damage (Ulcer Index, UI):

All rats were sacrificed and their stomachs were tied around both openings (cardiac & pyloric sphincters) and injected with 3ml distilled water. The gastric juice was then collected in sterilized tube. **Determination of ulcer index**: Ulcer index was determined using magnifying glass as described by ***Bandyopadhyay et al., (2004).*** The sum of the area of the lesions for each stomach was used to calculate the UA ***(Robert et al., 1984)*** and UI was calculated using the following formula:

UI (%) = [(UA of C–UA of T) /UA of C] × 100%

Where UI is the ulcer inhibition, T is the treatment, and C is the negative control.

2.7.3. Measurement the volume of gastric juice:

Each animal's gastric juice was centrifuged at 3000 rpm for 10 minutes to remove any solid debris. The volume of gastric juice was measured with a graduated cylinder and expressed in millilitres (ml). The pH of the supernatant was then determined. **(*Moore, 1968).***

2.7.4. Determination pH of gastric juice:

PH value was determined according to ***(Debnathet al., 1974).***

2.7.5. Biochemical investigation

Measuring the lipid per oxidation product as MDA according to ***Satoh (1978).*** Activity of CAT was assayed using the method of ***Aebi et al., (1984)***, SOD was assayed by ***Nishikimi et al., (1972),*** and GPx was assayed by ***Paglia and Valentine (1967).***

2.7.6. Histopathological studies:

All stomachs were quickly removed, opened along the greater curvature, and thoroughly rinsed with ice-cold saline. Following the recording of stomach ulcers, a longitudinal section of gastric tissue was taken from the anterior part of the stomach and fixed in a 10% formalin solution. According to Bancroft et al., after 24 hours of fixation followed by embedding in a paraffin block, it was cut into 5 micron sections onto a glass slide and stained with hematoxylin-eosin for histological assessment of the gastric mucosa according to ***Bancroft et al., (1996).***

2.8. Statistical Analysis

The data obtained from the present study was statistically subjected to analysis of variance (ANOVA) according to ***Snedecor and Cochran (1980)*** by the computerized program SPSS software, version “20” for Windows. The least significant difference (LSD) value was used to determine significant difference between means. Data was represented as Mean _ SD. Values were considered significant at $P < 0.05$, otherwise were considered non-significant.

Results and Discussion

3.1. Proximate composition of soybean and *M. oleifera* leaves

Table (2) represents the chemical composition of soybean and MO leaves. The results show that soy, since it is considered a seed, contained a higher percentage of protein and fat (45.14 and 20.08 %) than *M. oleifera* leaves (21.44 and 6.73%), while *M. oleifera* leaves were characterized by containing a high percentage of carbohydrates and ash (55.29 and 9.31%).

3.2. Total phenolic compounds

Phenolic compounds are a group of heterocyclic compounds vary in solubility in water and polar solvents depending on their chemical structure. **Table (3)** represent the total phenolic compound content of both of MO leaves and soybean Natto aqueous and methanolic extract. Results reflected that, both of soybean Natto and MO leaves were rich in phenolic compounds. There was a significant ($p \leq 0.05$) difference between soybean Natto and MO leaves in total phenolic content which was ranged from 17.972±.0314 mg gallic acid/g for soybean to 40.922±.0443mg gallic acid/g for Moringa aqueous extract, while it was 38.264±0.203and 22.766±.0212766mg gallic acid/g for soybean Natto and MO leaves, respectively. The results were agreed with ***Rocchetti et al., (2020)*** who found that the total phenolic content of MO was (31.84 mg/g). ***Shin et al., (2014***) found that fermentation with *Bacillus subtilis* increase the phenolic compounds of soybean to reach 16.92 mg GAE/g.

3.3. Characterization of phenolic compounds by HPLC

Fig. (1) Display the distribution of phenolic compounds of both of soybean Natto and MO leaves. There were 13 phenolic compounds identified by HPLC-DAD **(table 3).** Gallic acid was found to be the main component of phenolic represent 1464.304 µg/g in soybean Natto while, chlorogenic acid was the main component of moringa leaves (2218.493 µg/g). Catechin was the second one in soybean Natto followed by 4`.7-DihydroxyisoFlavone (598.2141 and 166.001µg/g). On the other hand, MO Leaves contained high amount of both Naringenin and Vanellin (1512.507 and 1490.551 µg/gm).

3.4. Antioxidant activities

A substance functions as an antioxidant if it can delay, retard or prevent the oxidation or free radical mediated oxidation of a substrate when present in low concentrations, leading to the formation of stable radicals after scavenging ***(Singh et al., 2016).***The total antioxidant capacity needs to reflect both lipophilic and hydrophilic capacity, and at least for physiological activity it needs to reflect and differentiate both hydrogen atom transfer (radical quenching) and electron transfer (radical reduction). Both of soybean Natto and MO leaves antioxidant activities were determined by two different

methods radical scavenging activities (DPPH) and ferric reducing power activities FRPA (TPTZ) as seen in **table (4).**

The results of screening of antioxidant activities showed that both of soybean Natto and MO leaves had high antioxidant activities and could be considered as good cheap source for natural antioxidants. There were significant differences ($p \leq 0.05$) between the antioxidant activities of soybean Natto and MO leaves, while,MO leaves had more radical scavenging activities than soybean Natto (90.31±0.10% and 83.59±0.23% for moringa leaves aqueous and methanolic extract,respectively.But, soybean natto gave radical scavenging activities 34.63±0.68% and 34.69±0.44% for aqueous and methanolic extract, respectively. The results indicated that MO leaves gave 2.6 times antioxidant activities than soybean Natto. On the other hand, ferric reducing power activities results indicated that MO leaves gave high reducing power activities 64.4 times more than soybean Natto and the aqueous extract of both soybean natto and MO leaves had more reducing power activities than methanolic extract (8.79±0.07 and 1.21±0.031 for soybean Natto aqueous and methanolic extract

and 579.58±04.29 and 369.23±1.92µg Troloxeq/g sample for MO leaves aqueous and methanolic extract, respectively).

Mwamatope et al., (2020) demonstrated the high antioxidant activities of moringa which ranged from 42.72 to 89.09% by DPPH. Also, ***Shin et al., (2014)*** discovered that fermenting soybean with Bacillus subtilis CSY191 increased its antioxidant activities by converting soybean isoflavones into active form (aglycon) and producing antioxidant peptides as a result of protein degradation***. (Marwa et al., 2014).***

This means that most of existing phenolic compounds in soybean Natto and MO leaves are seems to be water soluble and their antioxidant activity didn't not depend on the amount of phenol compounds, but it was depended on the varieties and chemical structure of these compounds. It could be summarized that soybean Natto and MO leaves are an excellent source of an antioxidant water soluble phenolic compounds, and could be used as a natural bioactive compound in our diet to prevent from many diseases.

3.5. Effect of soybean natto and/or *M. oleifera* leaves as bioactive compounds sources on sensory attributes of produced soup

Ready to cook soup was an ideal product to be fortified with either soybean Natto or Moringa leaves. The sensory evaluation mean scores of parameters were statistically analyzed and the results are shown in **Table (5)**. Results showed that sensory attributes which represented in color, taste, flavor and after taste showed significant differences ($p < 0.05$) between control and samples with additives. All samples had good accepted appearance with high score in T1 soybean natto additives. Sample T1 Soybean natto additive 10g to soup (T1) was ranked high scores comparing to T2 soybean natto + MO leaves and T3 MO leaves 10g. On the other hand, sample T3 10g MO leaves had an unusual look of soup as a result of its green color, and both of color and overall acceptability were less than other samples, while T2 which was a combination of both 5g soybean natto and 5g MO leaves gave more acceptable scores result.

Generally, the addition of soybean natto was found to be highly acceptable comparing to the control sample in some cases because of its meat like flavor and taste which were more accepted for some panellists than control. While a gradient of admission was found in other samples.

3.6. Biochemical Analysis:

Aspirin causes gastric ulcers by decreasing the hydrophobicity of the mucus gel layer, altering the action of surface-active phospholipids, and suppressing prostaglandin synthesis ***(Saeed et al., 2006).*** Prostaglandin plays an important role in the protection of gastric mucosal integrity by increasing local blood flow and promoting mucus and bicarbonate synthesis and secretion ***(Byron and Kenneth, 2014).*** While bicarbonate provides protection by lowering the acidity of the gastric lumen, the mucus layer acts as a barrier, protecting against the effects of pepsin and hydrochloric acid, which are the most common causes of ulcers. ***(Nurhidayah et al., 2014).*** The disruption of this cascade of body defense activities by a non steroidal anti-inflammatory drug (NSAID) such as aspirin is to blame for the development of ulcerations characterized by mucosal bleeding. ***(Suleiman et al., 2010).***

From **Table (6)**, aspirin administration caused a remarkably high ulcer index (8.71± 0.72) when compared to control group. Pretreatment with natto, aqueous extract of MO leaves and ranitidine offered significant (p≤ 0.05) protection against aspirin -induced gastric ulcer in the experimental rats. Natto (25 and 50 %) reduced ulcer index to 1.37 ± 0.43and 1.00 ± 0.18showing 84.2 and 88.5% prevention respectively. Also aqueous extract of MO leaves (5 and 10 %) reduced ulcer index to 1.72 ± 0.17and 1.05 ± 0.30showing 80.2 and 87.9 % prevention respectively. whereas RAN reduced ulcer index to 2.70 ± 0.18showing 69.0%prevention against gastric mucosal injury.

Dipicolinic acid, found in natto, has anti-bacterial activity against *E. coli* O157 and against *Helicobacter pylori*, the causative agent of stomach ulcers.**(*Sumi et al., 2006).** *M. oleifera* was discovered to have ulcer-protective properties. MO leaves also contain isothiocyanate, which has anti-inflammatory and immune-modulatory properties ***(Shaila et al., 2010).*** According to ***Biswas et al., (2012),*** the presence of flavonoids

in MO leaves extract reduces gut ulceration by improving microcirculation and increasing capillary resistance, making the cells more resistant to inflammatory factors.

Induction with aspirin could significantly ($p \leq 0.05$) increase volume of gastric juice and decreasing the pH of the gastric contents ulcer group (+) when compared with the normal animals (-) **(Table 7)**. High gastric secretion volume and low pH of the gastric juice cause severe gastric mucosal injury ***(Yi et al., 2015).***Pre-treated with (natto (50%),MOE (10%), natto (25%) and MOE (5%)) respectively has anti secretory activity, as observed by the decrease in volume of gastric juice and increasing the pH of the gastric contents when compared with the ulcer control group (+). also when compared with the reference control group **(**Ranitidine group).

Most bean products can protect the stomach by using their alkaline properties to neutralise the amount of hydrochloric acid produced by gastric damage and relieve stomach injuries. Soy isoflavones in soybean products, in addition to their alkalinity, may play an important role in preventing stomach damage. ***(Zhao, Qian and Li, 2014).***

Because of the increased content of small bioactive compounds, fermentation is an excellent processing method for improving the nutritional and functional properties of soybean. Enzymatic hydrolysis during fermentation breaks down the large protein, lipid, and carbohydrate molecules in raw soybean to small molecules such as peptides, amino acids, fatty acids, and sugars, which are responsible for the unique sensory and functional properties of the final products***(Beak et al., 2008)*** Bioactive oligopeptides from fermented soybeans are an emerging area of research with great promise peptides from the soybean is currently the subject of investigation into new drugs and functional food ingredients for gut health and modulating the intestinal absorption of nutrients ***(Shimizu and Son, 2007)*** *M. oleifera* aqueous extracts may have gastric protective effects due to direct action on mucus secretion or by increasing prostaglandins, thus protecting the stomach from acid alcohol injury. It has also been reported to alter antioxidant factors such as total tissue sulfhydryl group (glutathione), implying that *Moringa oleifera*'s antioxidant action is responsible for its ability

to prevent the development of gastric ulcers in rats. ***(Debnat et al., 2013).***

The gastro-protective and antioxidant effects of MO is as a results of it several active constituents such as alkaloids, sterols, glycosides, flavonoids, and terpenoids ***(Mahajan et al., 2008).*** Also, its leaves are rich in benzyl isothiocyanate which has anti-inflammatory activity **(*Lee et al., 2009).***

As shown in **Table (8),** gastric MDA levels were significantly ($p \leq 0.05$) elevated in ulcer control groups (+) (2.44 ± 0.10) compared with control group (-) (1.41 ± 0.31)this was in agreement with ***Kim et al., (2016)***who reported that gastric ulceration develops mainly through the production of oxygen free radicals and lipid peroxides. ***Cuevas et al., (2011)*** found that aspirin significantly increased MDA gastric concentration when compared to control animals. Whereas the activities of SOD, CAT, and GPx were significantly lower in comparison to the control group (-), ***Chattopadhyay et al., (2006)*** reported that NSAIDs induced reactive oxidative metabolites in animal models, which may contribute to mucosal injury. These free radicals also damage the cellular antioxidant enzymes such as CAT and SOD, acting as the first line of cellular defense

against oxidative injury and this might lead to aggravated tissue damage during gastric ulceration as mentioned by ***El-Missiry et al., (2001).***

Stomach of the rats pre-treated with (natto (50%),MOE (10%) , natto (25%) and MOE (5%)) respectively could significantly($p \leq 0.05$) increase those activities SOD, CAT and GPx when compared with the ulcer control group (+) .Gastric MDA significantly decreased and reverted to near normal levels whenpre-treated with (natto (50%) , MOE (10%) , natto (25%) and MOE (5%)) respectively when compared with the ulcer control group (+) , also when compared with the reference control group **(**Ranitidine group).

Soybean isoflavone is a flavonoid subclass with antioxidant properties. It has the ability to remove free radicals; a byproduct produced by cells during the normal metabolism process, and thus prevents the occurrence of cancer caused by free radical damage to DNA ***(Patel et al., 2001).*** Daidzein has the ability to increase catalase activity. ***(Liu et al., 2005)*** this was in agreement withour results**.** Natto Bacillus subtilis can

prevent diarrhoea, dysentery, enteritis, and constipation by inhibiting spoilage microorganisms that cause abnormal fermentation in the intestine. Overall, it is important in regulating the microecological balance of intestinal flora. When drinking alcohol, natto mucus covering the surface of the gastrointestinal mucosa can protect the gastrointestinal system and alleviate drunkenness. ***(Hong et al., 2017)***this is also supported our results**.**

Hessah, (2018) demonstrated that MO can restore the antioxidant activities of GST, GPx, and SOD and decrease the lipid peroxidation induced by oral administration of acid alcohol and with notable decrease in gastric lesions this was apparent in our results. This is also supported by ***Bhattacharya et al., (2000)*** who reported that antioxidant properties of MO leaf extracts exert its action via alteration in superoxide dismutase (SOD), gluthathioneperoxidase (GPx), and malondialdehyde (MDA) levels in rat gastric mucosathis was agree with our results. In the presence of a gastric ulcer, there is an increase in gastric mucosal SOD and lipid peroxidation activities, indicating that the production of reactive oxygen species (ROS) such as superoxide anion (O2 -), H_2O_2, and hydroxyl radical

(OH-) induces cell degeneration by increasing lipid peroxidation of cell membrane lipids and may be the cause of gastric peroxidase inactivation. As a result, the antioxidant effects of MO leaf extracts are most likely achieved by metabolising lipid peroxides and scavenging endogenous hydrogen peroxide (H_2O_2)***(Biswas et al., 2012).***

3.7. Histopathological Examination:

Stomach sections of rats in different experimental groups were examined and the photomicrographs are illustrated in **Fig. (3 to 4)**

The stomach is in a protected anatomical position in abdominal cavity and can move within some limits, so it is not easy to injure it with outside violence ***(Zhao et al., 2013).***Gastric mucosal damage is common and caused by a variety of factors, including chemical factors such as smoking, drinking strong tea or coffee, and drugs that stimulate the gastric mucosa such as aspirin and indomethacin, physical factors such as excessive cold or heat, eating rough food, bacteria or their toxins***.(Zhao et al., 2014).***

In the present study, gastric ulcer group(+) showed Congestion in the blood vessels of the lamina propria of the mucosa as well as the underlying submucosxa associated with oedema and inflammatory cells infiltration in the submucosa compared with control group (-) which showed no histopathological alteration and the normal histological structure of the mucosa with glandular structure , submucosa muscularis and serosa were recorded in (Fig.3).This is consistent with the findings of ***Zhang et al., (2014),*** who found that aspirin caused severe congestion and multiple haemorrhagic erosion in stomach tissue, particularly in mucous secreting cells, as evidenced by gastric pit damage and vacuolation of the glandular portion. The lamina propria showed mononuclear cellugrouolar infiltration, which was supported by ***Abdelgawad et al., (2013)***who stated that oral administration of a single dose of aspirin (200 mg/kg) to rats caused edoema in the lamina propria with mononuclear cellular infiltration. **.**

Pre-treated with MOE (5%) The submucosa showed diffuse oedema, while MOE (10%) showed no histopathological alteration as recorded in (Fig.4).The gastroprotection provided

by Moringa extract, as evidenced by histological plates, could be attributed to the presence of phytocomponents such as flavonoids, tannins, terpenoids, sterols, alkaloids, and phenols, which have been reported to be present in Moringa leaf extract. When tested for antiulcer and gastroprotective properties, these phytochemical agents yielded positive results. ***(Noemi et al., 2014). Kumar et al., (2013)*** reported the usefulness of flavonoids in wound healingpromotion, cellular regeneration and cytoprotection as key antiulcer dynamics. In view of the fact that ulcer is greatly linked with oxidative stress **(*Shokouhsadat et al., 2015),*** the antioxidant properties of flavonoids and phenols in Moringa leaf extract may have contributed to the antiulcer effect observed. Separate studies on isolated flavonoids revealed high levels of gastroprotection in association with flavonoids administration. **(*Zayachikiwaka et al., 2005).***

Pre-treated with natto (25%) Mild oedema was observed in the muscularis and serosa while the mucosa and submucosa were intact, but Pre-treated with natto (50%) There was no histopathological alteration as recorded in (Fig.4). Most bean products can protect the stomach by using their alkaline

properties to neutralise the amount of hydrochloric acid produced by gastric damage and relieve stomach injuries. Soy isoflavones in soybean products, in addition to their alkalinity, may play an important role in preventing stomach damage**.** ***(Zhao et al., 2014).*** Phenolic compounds, on the other hand, have been reported to have gastroprotective effects via a variety of mechanisms such as antisecretory activity, cytoprotection, and modulation of inflammatory mediators, antioxidative stress defence, and enhancement of antioxidant enzyme levels in the body***.(Shokouhsadat et al., 2015*****).*** also the reference control group (Ranitidine group)The submucosa showed congestion in the blood vessels as well as oedema with inflammatory cells infiltration while the mucosa was intact

Table 1: Composition of the different soup mixtures

Ingredients (g)	**control**	**T 1**	**T 2**	**T 3**
Corn starch	25	25	25	25
Onion	5	5	5	5
Garlic	3	3	3	3
Black and white paper	2	2	2	2
salt	10	10	10	10
Soy natto	_	10	5	-
M. oleifera leaves	_	_	5	10

T1: soup +10 soybean natto, T2: soup + 5g soybean natto + 5g MO leaves and T3: soup + 10g MO leaves

Table (2): Proximate composition of soybean and M. oleifera leaves %

Sample	Protein%	Fat%	Moisture%	Ash%	Carbohydrate%
Soybean	45.14	20.08	6.75	5.08	22.95
M.oleifea leaves	21.44	6.73	7.23	9.31	55.29

Table (3): Phenolic compounds of Natto and *M. oleifera* leaves

Phenolic acids	Natto Conc. (µg / g)	*M. oleifera* Conc.(µg/ g)
Gallic acid	1464.304	760.4625
Chlorogenic acid	104.9917	2218.493
Catechin	598.2141	0
Caffeine	72.82006	0
Coffeic acid	0	0
Syringic acid	0	0
Rutin	0	1470
Ellagic acid	0	126.0939
Coumaric acid	0	0
Vanillin	21.64667	1490.511
Ferulic acid	0	52.13495
Naringenin	151.4941	1512.507
Propyl Gallate	83.7824	0
4`.7-DihydroxyisoFlavone	166.0066	0
Querectin	29.40176	171.6399
Cinnamic acid	18.2218	11.61279

Tble (4): Total phenolic compounds and the antioxidant activities of soybean natto and M. oleifera leaves.

Sample	Total Phenols (mg/g)	DPPH%	TPTZ(µgTroloxeq/ gm sample)
Soybean Natto (Aqueous)	17.972±0.314[a]	34.63±0.68[a]	8.79±0.07926[b]
Soybean Natto (methanol)	22.766±0.212766[b]	34.69±0.44[a]	1.21±0.031[a]
M.oleifera leaves(Aqueous)	40.922±0.443[d]	90.31±0.10[c]	579.58±04.29[d]
M. oleifera leaves ((methanol))	29.381±0.241[c]	63.88±0.15[b]	391.44±2.56[c]

Data are presented as means ± SDM (*n=3*). a, b, c and d: Means with different letter among treatments in the same column are significantly different($P \leq 0.05$)

Table (5): Sensory evaluation mean scores of parameters of soybean natto and M. oleifera leaves.

Treatment	Colour	Taste	Flavour	After taste	Texture	Overall acceptability
Control	9.11[d]±1.17	9.50[d]±1.9	9.31cd±2.69	9.504[a]±0.9	9.71[d]±09	9.72[c]±1.62
T1	8.91[c]±1.8	9.41[c]±1.4	9.216[c]±1.9	8.91[b]±0.71	8.38[c]±17	9.68[c]±1.4
T2	7.018b±1.17	8.72[b]±0.8	8.72[b]±0.98	8.32c±1.08	8.02[b]±1.7	7.92[b]±0.64
T3	5.92[a]±2.98	8.53[a]±0.8	8.82[a]±0.8	7.52d±1.03	7.38[a]±17	7.22[a]±0.49

Data are presented as means ± SDM (*n=3*).a, b, c and d: Means with different letter among treatments in the same column are significantly different($P \leq 0.05$)T1: soup +10 soybean natto, T2: soup + 5g soybean natto + 5g MO+, T3: soup + 10g MO leaves

Table 6: Effect of Natto and aqueous extract of M. oleifera leaves and ranitidine on gastric lesion surface induced by aspirin

Groups	Gastric Mucosal Injury	
	Gastric Mucosal Injury Area (mm^2)	Protection (%)
Control (-)	0 ± 0.00^{a}	100
Control (+)	8.71 ± 0.72^{e}	0
MOE (5%)	1.72 ± 0.17^{c}	80.2
MOE (10%)	1.05 ± 0.30^{b}	87.9
Natto (25%)	1.37 ± 0.43^{bc}	84.2
Natto (50%)	1.00 ± 0.18^{b}	88.5
Ranitidin(50 mg/Kg)	2.70 ± 0.18^{d}	69.0

Data are presented as means ± SDM (*n=6*).a, b, c and d: Means with different letter among treatments in the same column are significantly different ($P \leq 0.05$)MOE: aqueous extract of M. oleifera leaves Natto: Fermented soy beans

Table 7: Effect of Natto, aqueous extract of *M. oleifera* leaves and ranitidine on Volume of gastric juice (mL) and pH of gastric juice induced by aspirin

Groups	Volume of gastric juice (mL)	pH of gastric juice
Control (-)	0.28±0.01^{a}	3.20±0.00^{e}
Control (+)	1.12±0.02^{e}	1.30±0.00^{a}
MOE (5%)	0.52±0.01^{cd}	2.94±0.36^{c}
MOE (10%)	0.46±0.03^{bc}	3.22±0.00^{e}
Natto (25%)	0.57±0.02^{d}	3.11±0.5^{d}
Natto (50%)	0.45±0.02^{bc}	3.31±0.57^{f}
Ranitidine (50 mg/Kg)	0.43±0.10^{b}	2.67±0.00^{b}

Data are presented as means ± SDM (*n*=*6*).a, b, c and d: Means with different letter among treatments in the same column aresignificantly different($P \leq 0.05$),MOE: aqueous extract of M. oleifera leaves Natto: Fermented soy beans

Table 8: Effect of Natto, aqueous extract of M. oleifera leaves and ranitidine on Lipid Peroxidation induced by aspirin

Groups	Lipid Peroxidation			
	SOD (IU/L)	CAT(IU/L)	MDA (µmol/L)	GPx (IU/L)
Control (-)	2.38 ±0.10^{b}	52.54 ±0.27^{d}	1.41±0.31^{ab}	49.86 ±0.09^{d}
Control (+)	1.28 ±0.05^{a}	45.8 ±0.26^{a}	2.44±0.10^{d}	42.75 ±0.14^{a}
MOE (5%)	1.82 ±0.35^{a}	48.34 ±0.52^{b}	1.33 ±0.35^{ab}	45.56 ±0.08^{b}
MOE (10%)	3.02 ±0.04^{c}	53.69 ±0.41^{e}	1.06 ±0.00^{a}	50.34±0.39^{e}
Natto (25%)	2.27 ±0.40^{b}	51.02 ±0.77^{c}	1.69±0.03^{bc}	45.54 ±0.52^{b}
Natto (50%)	3.22 ±0.56^{c}	54.11 ±0.79^{e}	1.39 ±0.25^{ab}	49.02±0.04^{c}
Ranitidine(50 mg/Kg)	1.92 ±0.40^{b}	46.39 ±0.29^{a}	1.86±0.02^{c}	45.9 ±0.08^{b}

Data are presented as means ± SDM (n=6).a, b, c and d: Means with different letter among treatments in the same column are significantly different($P \leq 0.05$)SOD: superoxide dismutase CAT: Catalase MDA: malondialdehyde GPx: gluthathione peroxidase , MOE: aqueous extract of M. oleifera leaves Natto: Fermented soy beans

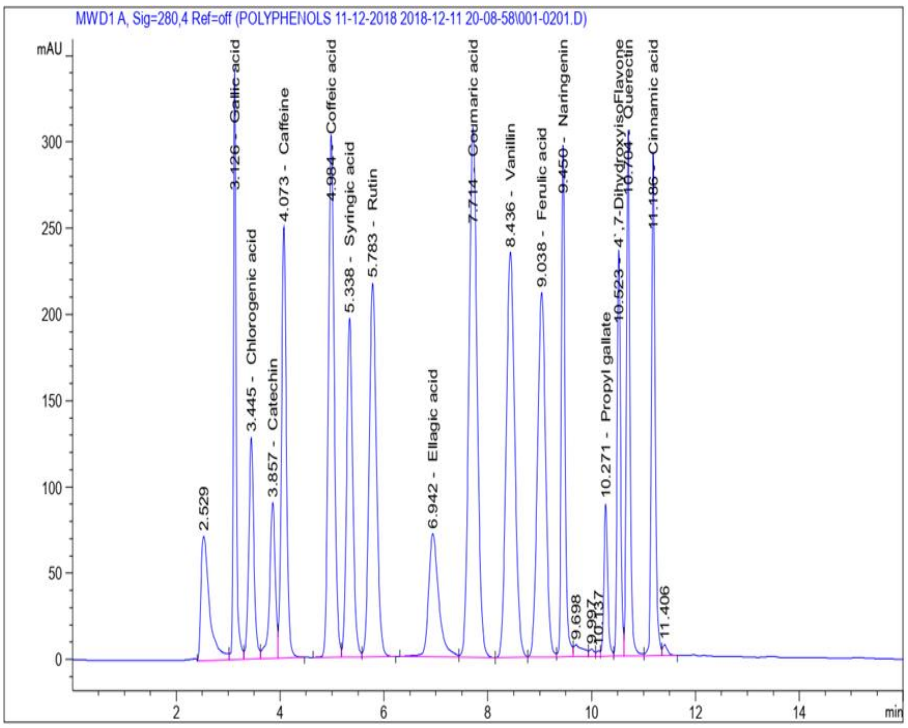

Fig. (1)

Distribution of phenolic compounds of both of soybean Natto and *M. oleifera* leaves

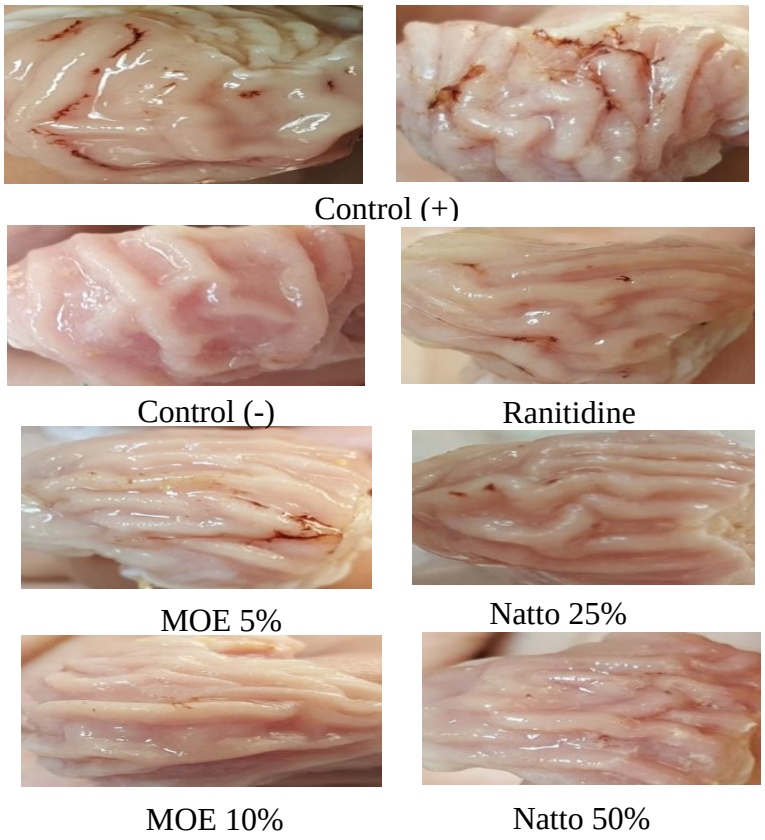

Fig. 2.
Photomicrographs showing mucosal surface of rat stomach; stomach from normal control (-), stomach from aspirin control (+), stomach from rat treated with Natto 25, 50% + aspirin. , stomach from rat treated with aqueous extract of *M. oleifera* leaves 5, 10%+ aspirin. And stomach from rat treated with ranitidine 50 mg/kg + aspirin.

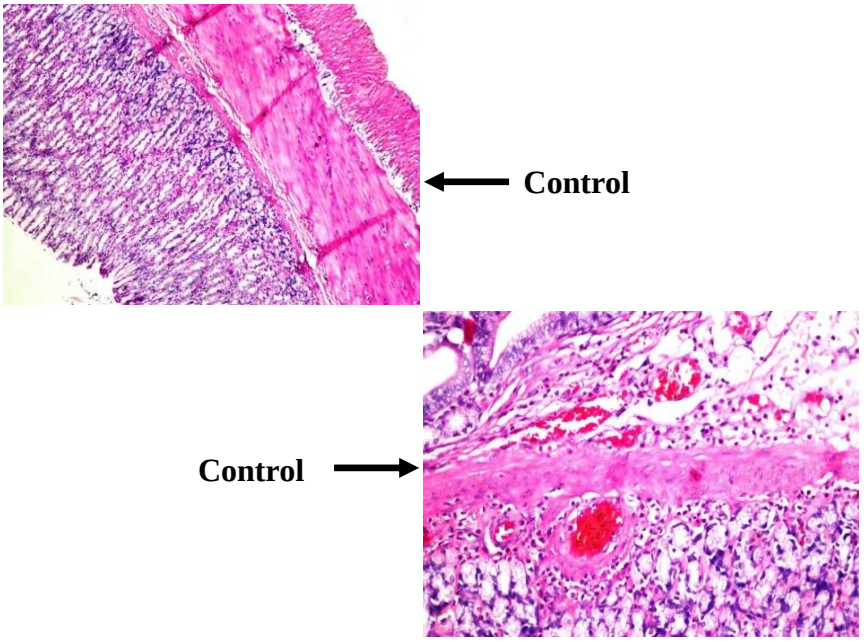

Fig (3):
Photomicrograph of Sections of stomach of control (-) group and control (+) group, stained with H & E, X 400.

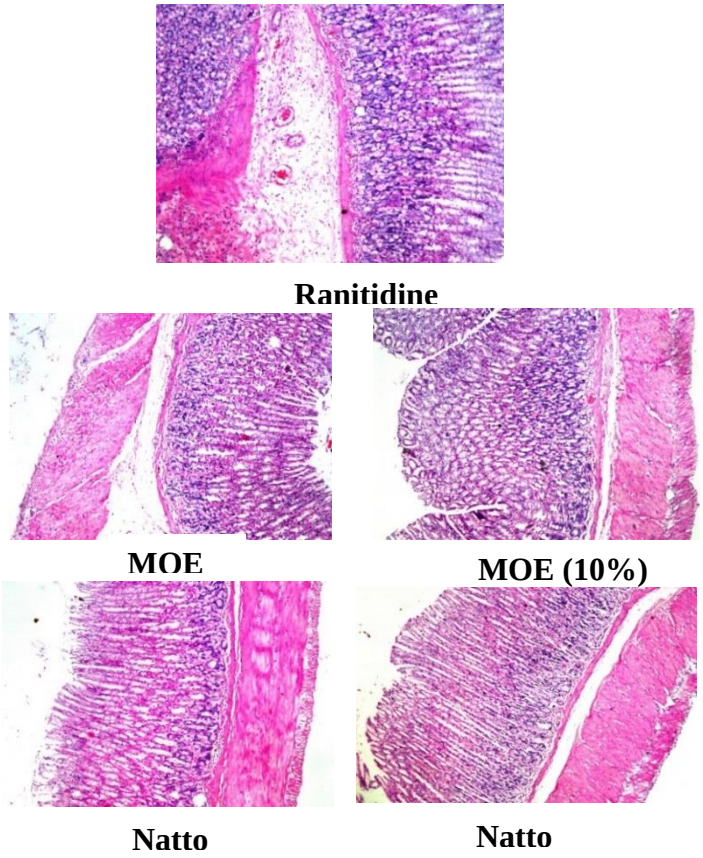

Fig (4):

Photomicrograph of Sections of liver of different rats groups Natto (25 and 50%), aqueous extract of M. oleifera leaves (5 and 10 %) and ranitidine stained with H & E, X 400**.**

Refrences

Abdelgawad, H. S., Manal A, Header E. A. (2013):

Influence of aqueous extract of Red Chillis pepper as curative for gastric ulcer in albino rats. J Nat Sci Res; 3(4):78-87.

Abdullah, N., Gopal, D. and Abdulla, M., (2012):

Effect of soya beans and soya beans fermented with Schizophyllum commune Fr. On ethanol-induced gastric ulcer in Sprague-Dawley rats. Acta Alimentaria. 41. 334-342. 10.1556/AAlim.41.2012.3.5.

Aboelsoued, D., Abo-Aziza, F. A. M., Mahmoud, M. H., Abdel Megeed, K. N., Abu El Ezz, N. M. T., & Abu-Salem, F. M. (2019):

Anti cryptosporidial effect of pomegranate peels water extract in experimentally infected mice with special reference to some biochemical parameters and antioxidant activity. Journal of Parasitic Diseases, 43(2), 215–228. Https://Doi.Org/10.1007/S12639-018-01078-Z.

Aebi, H. (1984):

Methods Enzymol 105, 121 – 126

Anitha, J.R., Velliyur, K.G., Sangilimutu, A.Y. and Sudarsanam D., (2011).

Antimicrobial Activity of *Moringa oleifera* (Lam) Root extract. J Pharm Res 4(5):1426–1427

Anwar, F. and Bhanger, M.I., (2003):

Analytical characterization of *Moringa oleifera* seed oil grown in temperate regions of Pakistan. J. Agric. Food Chem. 51, 6558-6563.

AOAC, (2000):

Official methods of analysis of the association of official analytical chemists, 17th edn. The Association of Official Analytical Chemists, Rockville.

Bae D., Park D., Lee S, Yang G., Yang Y., Kim T., (2011):

Different antiulcer activities of pantoprazole in stress, alcohol and pylorus ligation-induced ulcer models. Laboratory animal research; 27(1): 47-52.

Banchroft, J., Stevens, A. and Turner, D., (1996).

Theory and practice of histological techniques. Fourth Ed. Churchil Living stone, New York, London, San Francisco, Tokyo.

Bandyopadhyay D., Ghosh G., Bandyopadhyay A. and Reiter R.J., (2004):

Mela-tonin protects against piroxicam-induced gastric ulceration, J. Pineal.Res; 36: 195–203.

Barros, H.R.M., Ferreira, T.A.P.C. and Genovese, M.I., (2012):

Antioxidant capacity and mineral content of pulp and peel from commercial cultivars of citrus from Brazil. Food Chem 134:1892–1898.

Beak, L.M. L.Y. Park, K.S. Park, and S. H. Lee, (2008):

Effect of starter cultures on the fermentative characteristics of cheonggukjang," Korean J. Food Science Technology, vol. 40, pp.400-405.

Bhattacharya, S.K., Bhattacharya, A., Kumar, A., and Ghosal S., (2000):

Antioxidant activity of Bacopamonniera in rat frontal cortex, striatum and hippocampus.Phytother.Res.14, 1-6.

Biswas, S. K., Chowdhury A., Das J., Roy A. and Zahid Hosen S.M., (2012):

Pharmacological potential of MoringaOleifera LAM: A review. IJPSR. 3(2):305-310.

Byron, C. and Kenneth, W.M., (2014):

Gastrointestinal ulcers; role of aspirin and clinical outcomes in pathobiology, diagnosis and treatment. J Multidiscip Health c 7:137–146

Cao, Z. H., Green-Johnson, J. M., Buckley, N. D. and Lin, Q. Y. (2019):

Bioactivity of soy-based fermented foods: A review. *Biotechnology advances*, *37*(1), 223-238.

Chattopadhyay, L., Bandyopadhyay, U., Biswas, K., Maity, P., Banerjee, R.K., (2006):

Indomethacin inactivates gastric peroxidase to induce reactive- oxygen mediated gastric mucosal injury and curcumin protects it by preventing peroxidase inactivation and scavenging reactive oxygen. Free RadicBiol Med. 40(8):1397-408.

Chiang, S.S. and Pan, T.M., (2011):

Antiosteoporotic effects of Lactobacillus-fermented soy skim milk on bone mineral density and the microstructure of femoral bone in ovariectomized mice. J. Agric. Food Chem. 59, 7734–7742.

Cuevas, V., Calzado, Y., Guerra, Y., Year, A., Despaigne, S., Ferrerio, R., (2011):

Effects of grape seed extract, vitamin C and vitamin E on Ethanol and Aspirin induced ulcers. AdvPharmacol Sci. 740687.

Debnat, A., Ansari, M. A. and Kumar, B., (2013):

Anti ulcerogenic Effect of Gallic Acid in Rats and Its Effect on Oxidant and Antioxidant Parameters in Stomach Tissue." Indian Journal of Pharmaceutical Sciences 75(2), 149–155.

Debnath, P.K., Gode, K.D., Gobinda Das, D. and Sanyal, A. K., (1974):

Effect of propranolol on gastric secretion in albino rats. Br J Pharmacol 51:213-216.

El-Missiry, M.A., El-Sayed, I.H. and Othman, A.I., (2001:

Protection by metal complexes with SOD- mimetic activity against oxidative gastric injury induced by indomethacin and ethanol in rats. Ann ClinBiochem. 38:694-700.

Farzana, T., Mohajan, S., Saha, T., Hossain, M. N., &Haque, M. Z. (2017):

Formulation and nutritional evaluation of a healthy vegetable soup powder supplemented with soy flour, mushroom, and moringa leaf. Food science & nutrition, 5(4), 911-920.

Fornai, M. Natale, G. Colucci, R. Tuccori, M. Carazzina, G. Antonioli, L. Del-Tacca, M., (2005):
Mechanisms of protection by pantoprazole against NSAID-induced gastric mucosal damage, NaunynSchmiedebergs Arch. Pharmacol. 372: 79–87.

Halabi, M.F., Shakir, R.M., Bardi, D.A., Al-Wajeeh, N.S., Ablat, A., Hassandarvish, P., Hajrezaie, M., Norazit, A. and Abdulla, M.A., (2014):
Gastroprotective activity of ethyl-4-[(3, 5-di-tert-butyl-2-hydroxybenzylidene) amino] benzoate against ethanol-induced gastric mucosal ulcer in rats. Journal PLoS ONE EISSN: 1932-6203 .9 (5). E 95908 DOI:10.1371/journal.pone.0095908

Handa, O. Y. Naito, A. Fukui, T. Omatsu, T. Yoshikawa, (2014):
The impact of non-steroidal Anti-inflammatory drugs on the small intestinal epithelium, J. Clin. Biochem. Nutr.54: 2–6.

Hayat, K., Zhang, X., Farooq, U., Abbas, S., Xia, S., Jia, C., Zhong, F. and Zhang, J., (2010):
Effect of Microwave treatment on phenolic content and antioxidant activity of citrus mandarin pomace. Food Chem 123:423–429.

Hessah, M. A., (2018):

Effects of Moringa oleifera leaves extract on the oxidative and gastric mucosa ulcers induced by indomethacin in rats. Afr. J of biotec.17 (3), 2018, 51-56

Hong, C., Chen, Y., Li, L., Chen, S. and Wei, X. (2017):

Identification of a Key Gene Involved in Branched-Chain Short Fatty Acids Formation in Natto by Transcriptional Analysis and Enzymatic Characterization in Bacillus subtilis. Journal of Agricultural and Food Chemistry, 65, 1592-1597. https://doi.org/10.1021/acs.jafc.6b05518

Kamal, M., (2008):

Moringa oleifera Lam - The miracle tree A New Leucoanthocyanin from Moringa oleifera Gum. J. Ind. Chem. Soc., 74, 247-248.

Karami, Z., and Akbari-Adergani, B., (2019):

Bioactive food derived peptides: A review on correlation between structure of bioactive peptides and their functional properties. *Journal of food science and technology*, *56*(2), 535-547.

Kaushik, J. S., Narang, M. and Parakh, A. (2011):

Fast food consumption in children. *Indian pediatrics*, *48*(2), 97-101.

Kim, J.H., Jin, S., Kwon, H.J. and Kim, B.W., (2016):

Curcumin blocks naproxen-induced gastric antral ulcerations through inhibition of lipid peroxidation and activation of enzymatic scavengers in rats. J. Microbiol. Biotechnol. 26(8):1392-97.

Koffuor, G., Ainoonson, G., Amponsah, K., Addotey, J., Asiamah, E., Akuffo, S., (2013):

Anti- ulcerant Activity of an Aqueous Fruit Extract of Musa x para-disiaca on Acetic Acid- Induced Gastric Ulceration in ICR Mice. Journal of Medical and Biomedical Sciences . 2(2): 30-39.

Kumar, M., Manish, K.G., Anit, S. and Goel, R.J., (2013):

Healing effects of Musa sapientum var. Paradisiacal in diabetic rats with co-occuring gastric ulcer, cytokines and growth factor by PCR amplification. BML Complement Altern Med 13:305

Larmond, E. (1980):

Laboratory methods of sensory evaluation of food. Research branch Canada, Dept. Agric. Publications.

Lee, Y.M., Seon, M.R., Cho, H.J., Kin, J. and Park, H.J., (2009):

Benzyl isothiocyanate exhibits anti-inflammatory effects in murine macrophages and in mouse skin. J. Mol. Med. 87, 2009:1251-126.

Liu, J., Chang, S.K. and Wiesenborn, D. (2005):

Antioxidant Properties of Soybean Isoflavone Extract and Tofu in Vitro and in Vivo. Journal of Agricultural and Food Chemistry, 53, 2333-2340. https://doi.org/10.1021/jf048552e

Mahajan, S.G., Mehta, A.A., and Mali, R.G., (2008):

Protective effect of ethanolic extract of seeds of Moringa oleifera Lam. against inflammation associated with development of arthritis in rats. J. Immunotoxicol. 4(1), 39-47.

Mahmoud,M.H., Wahba, H. M., Marwa, H. Mahmoud, and Badawy, I. H (2017):

Newly Formulated Antioxidant Rich Dietary Supplement in Jelly Form for Alleviation of Liver Disease in Rats. *Journal of Biological Sciences*, 17 (7), 334-346.https://doi.org/10.3923/jbs.2017.334.346.

Mahmood, K.T., Mugal, T. and Haq, I.U., (2010):

Moringa oleifera: A Natural Gift -A Review. J. Phar. Sci. Res., 2, 775-781.

Mahmoud, M. H. and Abu-salem, a. F. M. (2015):

Synergistic effect of ascorbic acid and soybean bioactive compounds in extending shelf life of refrigerated burger.

Research journal of pharmaceutical biological and chemical sciences, 6(6), 17-26.

Mahmoud,M.H., Ferial M., Abu-Salem, El-Kalyoubi, M.H., Gibriel, A.Y. and Azza, A. Abou-Arab, (2014) :
Utilization of bioactive components produced from fermented soybean (Natto) in beef burger. International Journal of Biological, Veterinary, Agricultural and Food Engineering Vol: 8 (3): 309-316.

Mahmoud,Y.I., Eman A. and Abd El-Ghffar., (2019):
Spirulina ameliorates aspirin-induced gastric ulcer in albino mice by alleviating oxidative stress and inflammation, Biomedicine &Pharmacotherapy 109 (2019) 314–321

Manjula, C.H. and Ammani, K., (2012):
Phytochemical analysis and pharmacological importance of Sophorainterrupta leaves. Int. J. Res. Pharm. Biomed. Sci., 3(4), 1798-1804.

Mo, H., Kariluoto, S., Piironen, V., Zhu, Y., Sanders, M.G., Vincken, J., WolkersRooijackers, J., Nout, M.J.R., (2013):
Effect of soybean processing on content and bioaccessibility of folate, vitamin B12 and isoflavones in tofu and Tempe. Food Chem. 141, 2418–2425

Moore, E.W. (1968):

Determination of pH by the glass electrode: pH meter calibration for gastric analysis. Gastroenterology, 54(4):501-507.

Mwamatope, B., Tembo, D., Chikowe, I., Kampira, E. and Nyirenda, C., (2020):

Total phenolic contents and antioxidant activity of Sennasingueana, Meliaazedarach, Moringaoleifera and Lanneadiscolor herbal plants. *Scientific African*, *9*, p.e00481.

Nishikimi, M., Roa, N.A., and Yogi, K., (1972):

Biochem. Bioph. Res. Common., 46, 849 – 854

Noemi DP, Darryl HBC, Christine L (2014):

Chichioco-Hernandez, antiulcer activity of Leguminosae plants, Arq. Gastroenterol 51:1

Nurhidayah, A.B.R., Pouya, H., Shahram, G., Salmah, I., Saad, T., Mahmood, A.A., (2014):

Gastroprotective effect of ethanolic extract of Curcuma xanthorrhiza leaf against ethanol-induced gastric mucosal lesions in Sprague-Dawley rats 2014:1–10

Paglia D.E. and Valentine W. N. (1967):

J. Lab. Clin. Med. 70: 158 – 169.

Patel, R.P., Boersma, B.J., Crawford, J.H., Hogg, N., Kirk, M., Kalyanaraman, B., Parks, D.A., Barnes, S. and Darley-Usmar, V. (2001).

Antioxidant Mechanisms of Isoflavones in Lipid Systems: Paradoxical Effects of Peroxyl Radical Scavenging. Free Radical Biology and Medicine, 31, 1570-1581.

Ramful, D., Tarnus, E., Aruoma, Oi. Bourdon, E., Bahorun, T., (2011):

Polyphenol composition, vitamin c content and antioxidant capacity of mauritian citrus fruit pulps. Food Resinter 44:2088–2099.

Rasool, M. and Sabina, E.P., (2006):

Lavanya, B.Anti-inflammatory effect of *Spirulina fusiformis*on adjuvant-induced arthritis in mice, Biol. Pharm. Bull. 29; 2483–2487.

Reeves, P.G., Nielsen, F.H. and Fahey, G.C. (1993).

AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN- 76A rodent diet. J. Nutr., 123(11):1939-1951.

Rekha, C.R. and Vijayalakshmi, G., (2010):

Bioconversion of isoflavone glycosides to aglycones, mineral bioavailability and vitamin B complex in fermented soy milk by probiotic bacteria and yeast. J. Appl. Microbiol. 109, 1198–1208.

Robert, A., Böttcher, W., Golanska, E. and Kauffman, G.L., (1984):

Lack of correlation between mucus gel thickness and gastric cytoprotection in rats. Gastroenterology. 86: 670–674

Rocchetti, G., Pagnossa, J.P., Blasi, F., Cossignani, L., Piccoli, R.H., Zengin, G., Montesano, D., Cocconcelli, P.S. and Lucini, L., (2020):

Phenolic profiling and in vitro bioactivity of Moringaoleifera leaves as affected by different extraction solvents. *Food Research International*, *127*, 108712.

Saeed, S., Naim, A., Tariq, P., (2006):

In vitro antibacterial activity of peppermint. Paki J Botany 38(3):869–872

Satoh K., (1978):

ClinicaChimicaActa .90, 37.

Shaila, G., Mahjan, M. and Anita, A., (2010):

Immunosuppresive activity of ethanolic extract of seeds of Moringa oleifera Lam. in experimental immune inflammation. Ethnopharmacol. Commun. 130:183-186.

Shimizu, M. and D.O. Son, (2007):

Food-derived peptides and intestinal functions," Current Pharm Design, vol. 13, pp.885-895.

Shin, E.C., Lee, J.H., Hwang, C.E., Lee, B.W., Kim, H.T., Ko, J.M., Baek, I.Y., Shin, J.H., Nam, S.H., Seo, W.T. and Cho, K.M., (2014):

Enhancement of total phenolic and isoflavone-aglycone contents and antioxidant activities during Cheonggukjang fermentation of brown soybeans by the potential probiotic Bacillus subtilis CSY191. *Food Science and Biotechnology*, *23*(2), pp.531-538.

Shokouhsadat, H., Amir, A.A., Mohammed, H.F., (2015):

Gastroprotective effect of aqueous stem bark extract of Ziziphus jujube leaves against HCl/ethanol—induced gastric mucosal injury in rats. J Tradit Chin Med 35(6):665–670

Simmons, D.L., Botting, R.M., (2004):

HlaT.Cyclooxygenaseisoenzymes: the biology of prostaglandin synthesis and inhibition. Pharmacol Rev, 2004; 56(3): 387-437.

Singh, B., Singh, J.P., Kaur, A. and Singh, N., (2016):
Bioactive compounds in banana and their associated health benefits–A review. Food Chemistry, 206, pp.1-11

Snedecor, G.W., Cochran, W.G., (1980):
Statistical Methods, 7 th. IBIT Public. Co, Oxford.

Suleiman, H.I., Albayrak, A., Bilici, M., Cadirci, E., Halici, Z., (2010):
Different mechanisms in formation and prevention of indomethacin induced gastric ulcers. Inflammation 33(4):224–234

Sumi, H., Yatagai, C., Ikeda, S., Ohsugi, T., (2006):
Dipicolinic acid in Bacillus subtilisnatto and strong anti-H. Pylori activity. Clin Pharm Therapy 16:261-266

Trapti, R., Vijay B., Komal, M., Aswar, P.B., and Khadabadi, S.S. (2009):
Comparative Studies on Anthelmintic Activty of Moringa Oleifera and VitexNegundo. Asian J. Research Chem. vol. 2(2).

Wang, Z. Hasegawa, J. Wang, X. Matsuda, A. Tokuda, T. Miura, N. Watanabe, T., (2011):
Protective effects of ginger against aspirin-induced gastric ulcers in rats, YonagoActa Med. 54 11–19.

Yanagisawa, Y., Sumi, H., (2005):

Natto bacillus contains a large amount of water-soluble vitamin K (menaquinnone-7). J. Food Biochem. 29, 267–277.

Yi, R.K.; Wang, R.; Sun, P.; Zhao, X., (2015):

Antioxidant-mediated preventative effect of Dragon-pearl tea crude polyphenol extract on reserpine-induced gastric ulcers. Exp. Ther. Med. 10, 338–344. [CrossRef] [PubMed]

Zayachikiwaka, O.S., Konturek, S.I., Drozdawicz, D., Konturek, P.C., Birzozowski, I. and Ghegotsky, M.R., (2005).

Gastroprotective effects of flavonoids in plant extract. J Physiol Pharmacol 56(1):219–231

Zhang, J.Y., WU, Q.F., Wan, Y., Song, S.D., Xu, J. and Xu X.S., (2014):

Protective role of hydrogen rich water on aspirin induced gastric mucosal damage in rats. World J Gastroenterol. 20(6):1614-22.

Zhao, X., Qian, Y. and Li, G.J., (2014).

Fermentation period influences on gastric injury preventive effects of Shuidouchi. J. Chin. Cereals Oils Assoc 29, 14–17.

Zhao, X., Song, J.L., Jung, O.S., Lim, Y.I. and Park, K.Y., (2014):

Chemical properties and in vivo gastric protective effects of bamboo salt. Food Sci. Biotechnol. 23, 895–902. [CrossRef]

Zhao, X., Wang, Q. and Qian, Y., Ilex kudingcha, C.J. and Tseng. (2013):
(Kudingcha) prevents HCl/ethanol-induced gastric injury in Sprague-Dawley rats. Mol. Med. Rep. 7, 1613–1616. [PubMed]**.**

تأثير فول الصويا المتخمر (الناتو) واوراق المورينجا أوليفيرا كمضاد للاكسدة وقرحة المعدة علي ذكور فئران الالبينو

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الملخص العربى

ينتج مرض قرحة المعدة عن الأطعمة الحاره ، والإجهاد ، والكحول ، وجراحة المعدة ، وبكتيريا *Helicobacter pylori* . ويتم توجيه الدراسات نحو استخدام المنتجات الطبيعية لتطوير أدوية القرحة مع الحد الأدنى من الآثار الجانبية. تم تقدير مضادات الأكسدة والمحتوى الفينولي الكلي (TPC) والنشاط المضاد للقرحة في مسحوق أوراق المورينجا ومسحوق فول الصويا المتخمر ، بالإضافة إلى تدعيم الحساء بهما. كان المحتوى الفينولي الكلي لأوراق المورينجا ومستخلص فول الصويا المتخمر (الناتو) مرتفعًا لكل من المستخلصات المائية لفول الصويا المتخمر (الناتو) وأوراق المورينجا. وجد ان حمض الجاليك المكون الرئيسي للفينول ويمثل ١٤٦٤,٣٠٤ ميكروجرام / جم لعينة فول الصويا المتخمر (ناتو) ، بينما كان حمض الكلوروجينيك المكون الرئيسي لأوراق المورينجا٢٢١٨,٤٩٣ ميكروجرام / جم) . وأظهرت نتائج نشاط مضادات الأكسدة بواسطة DPPH ، أن أوراق المورينجا لديها ٢,٦ ضعف نشاط مضادات الأكسدة لفول الصويا المتخمر (الناتو). كما أشارت نتائج أنشطة الطاقة المختزلة الحديديك إلى أن أوراق المورينجا اكثر ٦٤,٤ مرة من فول الصويا المتخمر ناتو. تم تغذية الفئران أولا علي (٢٥ ،

٥٠ %) من فول الصويا المتخمر والمستخلص المائي لأوراق المورينجا (٥ ، ١٠ %) لمدة ٧ أيام ، ثم تم إعطاء جميع الفئران الأسبرين بجرعة ٥٠٠ مجم / كجم من وزن الجسم معلقة في الماء للإصابة بقرحة المعدة الحادة، وتم تقدير مؤشر القرحة ، وحجم عصير المعدة ودرجة الحموضة لعصير المعدة. وكذلك في التحاليل البيوكيميائية (superoxide dismutase (SOD), gluthathione peroxidase (GPx), malondialdehyde (MDA) and Catalase) وتم استخدام رانيتيدين كدواء معالج إيجابي. أظهرت جميع النتائج الباثولوجية والكيميائية لفول الصويا المتخمر (الناتو) ومستخلص أوراق المورينجا لهما نشاطًا مضادًا للقرحة مشابها الرانيتيدين كدواء معالج إيجابي. واظهر التقييم الحسي لمنتج الحساء المدعم بمستخلص أوراق المورينجا وفول الصويا المتخمر (الناتو) فوجد أن إضافة فول الصويا المتخمر مقبول للغاية مقارنة بالمعاملة الضابطة حيث انها كانت ذات نكهة ومذاق شبيه اللحم و كان أكثر قبولًا اثناء التحكيم وكان في تدرج في درجات القبول لبقية المعاملات .

الكلمات المفتاحية: فول الصويا المتخمر ناتو ؛ أوراق المورينجا اوليفيرا. النشاط المضادات للأكسدة. محتوي الفينولات الكلية ؛ الأسبرين؛ القرحة الهضمية؛ بيروكسيد الدهون ؛علم التشريح؛فئران التجارب.