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ABSTRACT

Background Sprouted seeds are rich in bioactive substances including unsaturated fatty acids, polyphenols and flavonoids, which have a good influence on stomach health due to their anti-inflammatory and antioxidant properties.

Methods The chemical composition, fatty acids, polyphenols and antioxidant activity were estimated. Thirty-five rat were divided into the main group (5 rat), which was the control (ve-), and five subgroups. Four groups were fed the basal diet including (5, 10%) flax sprouts and (5, 10%) broccoli sprouts as a pre-treatment for 7 days. Then, all groups were given ibuprofen (400 mg/kg, orally) twice a day after fasting for 24 hours, the final group was given ranitidine. Ulcer index, gastric juice volume, gastric juice pH, and protection index were measured. Biochemical indicators such as lipid peroxidation, superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) were evaluated.

Results: The results showed significant differences in nutritional composition, fatty acids, polyphenols, and antioxidant activity (DPPH and FRAP). Sprouting was found to have significant nutritional value in both flaxseed and broccoli seeds. Fatty acids varied between increased palmitic and oleic acid contents after germination and decreased stearic acid content in both flax and broccoli sprouts. Broccoli sprouts exhibited higher potency reducing activities compared to flax sprouts. Antiulcer activity was almost identical to that of the positive control. Sprouted broccoli seeds (10%) demonstrated strong antiulcer activity across all macroscopic and biochemical parameters tested.

Keywords: Antioxidant activity, lipid peroxidation, ranitidine, anti-inflammatory properties, polyphenols

INTRODUTION

A peptic ulcer, also known as a benign gastric ulcer, is a round or semi-round/oval image that extends beyond 5 mm into the stomach submucosa due to a rupture in the gastric mucosa's continuity (**Nigam and Paarakh, 2011**). Peptic ulcers(PU) are caused by gastric acid secretion and affect the mucosa of the stomach and/or duodenum. They often spread beyond the muscularis mucosa to the muscle layer **(Goli 2017).** An imbalance between protective gastric mucosal integrity and forceful stomach acid production leads to this condition, which affects a large section of the global population (**Ateufack et al.,2015).** Symptoms of peptic ulcer disease include epigastric discomfort that can be alleviated with food or antacids **(Ramakrishnan et al.,2007).** The most common risk factors for developing peptic ulcer disease are nonsteroidal anti-inflammatory are Helicobacter pylori infection medications. **(Monjur,2019).** H. pylori infection in peptic ulcer disease can be diagnosed by both invasive (endoscopic) and noninvasive (serologic or breath test) tests. The most extensively researched treatment is two weeks of triple antibiotic therapy **(Susan, 1998).**

Nonsteroidal anti-inflammatory medicines (NSAIDs) are among the most commonly recommended medications for rheumatoid arthritis and other pain-related conditions. Ibuprofen's most common side effects are vomiting, nausea, stomach discomfort, gastric ulcers, and gastrointestinal bleeding **(Xiao-Ling et al., 2016).** It has been demonstrated that the effectiveness and toxicity of NSAIDs are mediated by their inhibition of COX, which has two structurally and functionally different isoforms, COX-1 and COX-2. The COX-1 isoform is found throughout the body and is essential for gastrointestinal protection and platelet aggregation. COX-2 is an inducible COX that contributes to the inflammatory response. Explicit data reveals that COX-2 inhibitors produce significantly less gastrointestinal ill effects than nonselective NSAIDs **(Brown et al., 2006 and Yang et al., 2015).** Ibuprofen is a nonselective NSAID, hence it can easily induce gastrointestinal issues.

Plant-based solutions are gaining appeal as an alternative to commercially accessible synthetic medications for the treatment of peptic ulcers. This is attributed to H. pylori's rising antibiotic resistance, low cost, availability, and the occurrence of few or no negative side effects **(Hamedi et al., 2015).**

In the twentieth century, we relied on a diet rich in milk and milk cream, supplemented with antacids, to heal gastrointestinal ulcers, on the theory that milk would give stomach alkalinisation while also relieving pain. Milk is no longer advised due to its buffering action and considerable stomach acid output **(Reis, 2003).** The relevance of bioactive components in foods raises the prospect of improving public health through nutrition **(Jeffery et al., 2003).** The use of natural sources to improve nutritional quality for human health is gaining traction.

Sprouts include a high concentration of enzymes, which aid in health maintenance **(Bhardwaj and Hamama, 2012).** Around 3000 B.C., Ancient Egyptians began sprouting seeds **(Abdallah 2008).** Phytic acid, trypsin inhibitor, tannins, pentosans, and cyanides are some of the anti-nutritional chemicals that decrease during germination. On the contrary, taste, nutritional bioavailability, and the concentration of health-related phytochemicals (glucosinolates and natural antioxidants) improve (**Vidal-Valverde et al., 2002 and Márton et al., 2010).**

Sprouts are germinated crop seeds, such as legumes, cereals, pseudo-cereals, oilseeds, vegetables, and herbs **(Voinea et al., 2019 and Verlinden, 2020**). The sprouting process activates hydrolytic enzymes, which release minerals from their phytate chelates, making them more accessible; moreover, vitamins are synthesised and accumulate **(Lemmens et al., 2019).** Numerous researchers have discovered that sprouts are abundant in proteins, polyphenols, isothiocyanates, glucosynolates, vitamins and minerals **(Moreno et al., 2006 and Mir et al., 2021).**

Flaxseed (*Linum usitatissimum* L.) belongs to the Linaceae plant family **(Bhardwaj et al., 2012).** The oil of flax seeds contains 55% alpha linolenic acid, which benefits human cardiovascular health **(Prasad, 2009 and Herchi et al., 2011).** Flaxseeds oil include three types of chemicals, each with its own biological activity and functional properties: ALA (omega 3), linoleic acid (omega 6), and oleic acid (omega 9) (**Yu et al., 2018 and Parikh et al., 2019).** Flax has the ability to supplement the human diet as ground seeds **(Bhardwaj et al., 2012)** and critical elements in sprouts **(Narina et al., 2012).**

Flax sprouts had much higher amount of linoleic acid (C18:2) than seeds. Seeds contained a higher amount of linolenic acid (18:3) than sprouts**. (Satya et al., 2013).** It also included bioactive components such as phenolic acids, lignans, tocopherols, flavonoids, phytosterols, and cyclolinopeptides **(Shim et al., 2014).** Phenolic chemicals and tocopherols were identified as the primary components reflecting flaxseed and flaxseed oil antioxidant capabilities **(Deng et al., 2018).**

Broccoli (*Brassica oleracea L. var. italica*) seeds are high in polyphenolic chemicals, which have powerful antioxidant effects. Broccoli may also help to prevent oxidative stress-related ailments like as cardiovascular and neurological problems **(Khedr et al., 2020).** Broccoli sprouts are popular brassica vegetables that have anti-inflammatory, anticancer, anti-obesity and antimicrobial properties **(Baenas et al., 2017 and L´opez-Chill´on et al., 2019).** Germination process seeds takes 7-9 days of age, result in very rich in glucoraphanin and indol-glucosinolates (e.g., glucobrassicin) sprouts, also rich in phenolic compounds and other relevant nutrients include minerals (Fe, S, Ketc.), vitamins (E, A, C), and carotenoids (e.g., lutein) **(P´erez-Balibrea et al., 2008 and Abellan et al., 2019).** The sprouts of cruciferous had more than ten times the amount of glucosinolates as mature inflorescences (broccoli heads) due to their immature physiological condition following seed germination **(Baenas et al., 2016).**

The purpose of this study was to investigate the effect of germinated flaxseeds and broccoli seeds on gastric ulcer as antioxidant and anti-ulcer

MATERIALS and METHODS

Materials

Flaxseeds and Broccoli seeds were delivered from Al Fajr company of agricultural seeds, spices, and medicinal plants, -October City, Egypt. Ranitidine and Ibuprofen from pharmacy, Fayoum. Casein, sucrose, maize oil, mineral and vitamin mixtures, choline chloride, D-L methionine were purchased from El Gomhoreya Company, Cairo, Egypt. The rats were taken from the Giza Agricultural Research Centre.

Methods

Sprouts preparing

-The germination of flaxseed was carried out using the technique described by **Wang et al. (2015),** with minor modifications. Flaxseed was soaked using water of the tap and acidic electrolyzed water for half an hour minutes, distributed uniformly into sprouting plates and kept in a constant temperature and humidity incubator (25 ± 2°C; 75 ± 5%) for 0-5 days. Flaxseed spouts were sprayed with tap water and acidic electrolyzed water every 8-10 hours, collected daily, freeze-dried, and kept at -20°C for future examination.

- Broccoli seedlings were germinated using the methods described by **Perez-Balibrea et al., (2011)** with minor changes. For each germination stage, fifteen grams of seed were submerged in a sodium hypochlorite solution of 5gL1 for 2 hours.

These seeds were emptied, washed three times with distilled water, and then steeped for one hour. In a germination tray, the imbibed seeds were put on top of damp pressed cotton. The tray was put in a seed germinator and the seeds were watered continually via capillarity. Germination conditions were kept at 22 - 2 °C, with a light-dark cycle of 16 hours and 8 hours, with relative humidity (RH) levels ranging from 80% to 90%. Sprouted seeds were collected after three, five, eight, and eleven days. A portion of sprouting seeds was freeze-dried and kept at 20 degrees Celsius for further investigation

Proximate composition

Moisture, crude protein, fat, ash, and crude fiber were assessed using **AOAC's** standard technique of analysis **(2010)**. The crude protein was determined with the conversion factor of N × 6.25.

Fatty Acids Composition

Flaxseeds, broccoli seeds and their sprouts fatty acids were quantified using gas chromatography and methyl esters, following the method of **Sanchez-Machado et al. (2004)** with minor modifications. Duplicate samples were analyzed. A sample (0.5 g) was weighed in a tube with a screw cap and treated with 3 mL of HCl methanolic 5% and 2 mL of toluene. The mixture was mixed and left in a water bath at 70°C for 2 hours. In a room temperature, samples were cooled and Na2SO4 anhydride was used for drying the organic phase. The organic phase was filtered through a 0.45 lm membrane. A 3-liter sample of this solution was placed in a gas chromatograph.

Polyphenol contents

Gutfinger's (1981) approach was used to extract and measure total phenolic acid and flavonoid levels.

The antioxidant activity measured by (DPPH radical scavenging activity and FRAP test)

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical test was performed in accordance with the technique of **Boly et al., (2016).** To start, 100 µL of newly produced DPPH reagent (0.1% in methanol) was combined with 100 µL of sample on a 96-well plate (n=5). The reaction was then kept at room temperature for half an hour in the dark. At the end of the incubation time, the decrease in DPPH colour intensity was evaluated at 540 nm. The data is stated using the following equation: Percentage inhibition = ((average absorbance of blank - average absorbance of test)/(average absorbance of blank) * 100

FRAP test was performed using the technique of **Benzie and Strain., (1996**) with slight adjustments to be performed in microplates. A newly made TPTZ reagent (300 mM acetate buffer (PH=3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl3, in a 10:1:1 v/v/v ratio, respectively). 190 uL of newly made TPTZ reagent was combined with 10 uL of sample in a 96-well plate (n=3), and the reaction was incubated at room temperature. For half an hour in darkness. At the conclusion of the incubation time, the ensuing blue color was detected at 593nm.

Experimental rats:

The study used 35 female albino rats weighing 180 ± 10g b.wt from the Animal House of Agricultural Research Centre in Giza, Egypt.

Experimental Design:

Thirty-five albino rats were housed in a clean laboratory and fed a basic diet following AIN-93 recommendations **(Reeves et al., 1993)** for seven days as an adaptation phase. Then, separate into two major groupings. Each group consisted of five rats. The first group was given the basal diet as a negative control group (ve-) **(Mahmoud et al., 2019).** Additionally, five groups were separated under the second major group for seven days, as indicated below: Group 2: The positive control (ve+) group was just given a baseline diet. Group (3) ate a standard diet with 5% sprouted flaxseeds. Group (4) was provided a basic meal with 10% sprouted flaxseeds. Group (5) had a standard meal supplemented with 5% sprouted broccoli seeds. Group (6) followed a baseline diet supplemented with 10% sprouted broccoli seeds. Group (7) received a baseline diet and oral ranitidine (50 mg/kg BW) as a standard medicine. One hour before ibuprofen delivery. The animals were murdered four hours after being given ibuprofen.

Ulcer induction in the major second group occurred on the last day of the trial. The ulcer was produced by administering ibuprofen (400mg/kg was administrated orally) twice a day at a 12-hour interval. Ibuprofen was administered orally via a baby feeding tube. Rats were starved for 24 hours before ulcer induction **(Santhosh et al., 2007).**

After completing the feeding for 7 days, the mice from each group were slaughtered and the blood was collected in a clean, dry centrifuge tube and left at room temperature until a clot formed, then it was completely withdrawn. Using centrifugation at 4000 rpm for 10 minutes at room temperature to separate the serum then stored in a plastic bottle (well sealed) until analysis.

Evaluating the gastric mucosal degeneration (Ulcer Index)

Rats were collected in each group, slaughtered, and then their abdomens were wrapped around the two openings (pyloric sphincter and cardiac sphincter) and injected with 3 ml of distilled water. The stomach liquid was then collected into a sterile tube. **Bandyopadhyay et al. (2004)** reported on the use of a magnifying lens to compute the ulcer index. The UA was calculated by summing the area of each stomach lesion **(Robert et al., 1984),** and the UI was obtained using this Equation:

$$UI\ (\%) = \frac{UA\ of\ C - UA\ of\ T}{UA\ of\ C} \times 100.$$

UI refer to ulcer inhibition, T for treatment, and C refer to (ve-)

The volume of gastric juice

Centrifuge samples at 3000 rpm for 10 minutes then use graduated cylinder for measuring and expressing in milliliters **(Moore, 1968).**

Determination gastric juice pH:

pH of gastric juice was measured using **Debnath et al.'s (1974)** method.

Analysis of lipid peroxidation:

Nishikimi et al. (1972) measured the lipid peroxidation product as SOD, while **Satoh (1978)** determined MDA. The activity of catalase was measured using the **Aebi et al. (1984)** technique.

Histopathological Investigation:

The stomachs of rats from all experimental groups were collected at the end of the period, fixed in 10% neutral buffered formalin (pH=7.0), dehydrated in ethyl alcohol, cleared in xylol, and embedded in paraffin. Sections were prepared and stained with heamtoxylin and eosin to examine both fore and glandular parts of the stomach **(Carleton, 1976).**

Statistical Analysis

The current study's data were statistically analysed using the computerised program SPSS software, version "20" for Windows, in accordance with Snedecor and Cochran's (1980) ANOVA method. The least significant difference (LSD) value was used to calculate the difference between means. Data were presented as mean ± SD. Values were considered significant if $P < 0.05$, and otherwise non-significant.

Results and Discussion

Chemical Composition

Table (1) shows of flaxseeds and broccoli seeds and sprouts chemical composition. The results show significant differences in the nutritional composition in flaxseeds and broccoli seeds and their sprouts. The percentage of moisture, protein and ash increased after the germination. Moisture increased (from 4.7±0.1 and 6.9±0.13) in flaxseeds and broccoli seeds to be (24.4±0.36 and 8.67±0.37) respectively in sprouts. Protein increased from (22.29±0.29 and 25.87±0.19) to be (23.47±0.45 and 39.73±0.25) respectively. **Shutov and Vaintraub, (1987)** reported that seed germination requires the digestion of seed protein, a multi-step process aided by proteolytic enzymes. According to **Wu et al., (2019),** flaxseed is a preferred protein source because it contains significant amounts of sulfur-based amino acids, and essential amino acids. **(Joshi, 2018)** reported that, germination lead to produces amino acids, from protein which are then employed in the production of enzymes, hormones, and proteins The higher percentage of protein in broccoli sprouts than in flaxseed sprouts may be due to the higher carbohydrate content of broccoli seeds than flaxseed. Where **(Araga˜o et al., 2015)** explained that, sucrose is the most abundant soluble sugar in mature seeds, but decreased dramatically during germination. Glucose and fructose are only found in seedlings, indicating that sucrose breakdown to monosaccharides is crucial for seedling development.

Ash was (2.97±0.07 and 5.24±0.25) to be (6.23±0.2 and 7.25±0.25) respectively. on the contrary fat, fiber and carbohydrates were decreased in sprouts compared to seeds. This is consistent with finding of **(Eshraq et al., 2016)** Sprouting breaks down seed reserves, leading to higher protein and ash content. Fiber increased from (13.28±0.22 and 6.52±0.35) in seeds respectively to be (19.08±0.21 and 17.49±0.44) after germination. This is consistent with the results of **(Benítez et al., 2013)** who reported that total dietary fiber contents increased during germination and improved insoluble/soluble dietary fiber ratio.

Fat in flax seeds and broccoli seeds decreased from (34.13±0.41 and 8.61±0.22) to be in flax sprouts and broccoli sprouts (26.94±0.13 and 6.21±0.23) respectively. During germination, **(Quettier and Eastmond, 2009)** explained that triacylglycerol's are hydrolysed to provide energy that is required for the production of carbohydrates and amino acids Which leads to a decrease in its percentage over time.

Carbohydrates recorded (16.83±1.00 and 35.89±0.21) in seeds and (5.68±0.74 and 31.61±0.49) respectively. This is what **(Benítez et al., 2013)** confirmed, germination resulted in a considerable decrease in resistant starch and a rise in accessible starch percentage.

Fatty Acid Composition

Table (2): shows that, after germination, changes occur in all saturated and unsaturated fatty acids. Overall, the results showed that total saturated fatty acids reduced in both flax and broccoli sprouts (11.09 - 10.25) and (9.45 - 8.02) during germination. While total unsaturated and total polyunsaturated fatty acids have grown in both flax and broccoli sprouts (26.87-27.04) and (8.23-14.32), (58.16-59.9) and (28.00-54.04) respectively. Palmitic acid is somewhat deficient in flax seeds and sprouts (5.78-5.5) and broccoli seeds and sprouts (6.95-5.63). Stearic acid is also somewhat deficient in flax (5.31-4.74) and broccoli (2.5-2.39). The bulk of the oil shed by the seed was used as energy for the production of sprouts (**Orhan et al., 2007 and Bhardwaj and Hamama, 2009).**

Linolenic (C18:3) was found to be the most unsaturated fatty acid in flax seeds and sprouts (44.03 and 45.34, respectively). This results matching with the finding of **Choo et al., (2007) and Kanmaz and Ova, (2015)** who noticed that flaxseed oil has significant quantities of α-linolenic acid, followed by linoleic and oleic acid**, and** present in flaxseeds and sprouts with high content**.** In flax and broccoli sprouts, saturated fatty acids (palmitic and stearic) reduced (5.51 and 5.63) and (4.74 and 2.39), respectively.

Antioxidant Activity

Table (3) shows the total polyphenols, flavonoids concentration, FRAP (ferric reducing power), and DPPH radical scavenging ability activities. There was a substantial increase in polyphenol content ($P < 0.05$) between flax and broccoli sprouts. Flax sprouts had lower content, whereas broccoli sprouts had the greatest concentration (14.96 ± 0.51 and 40.54 ± 1.41, respectively).

Broccoli sprouts had higher total flavonoid content (4.96 ± 0.32) than flax sprouts (3.25 ± 0.34). The total flavonoid content of broccoli sprouts increased significantly during germination **(Lopez-Cervantes et al., 2013).**

The antioxidant activity of sprouted broccoli and flax seeds differed significantly ($p \leq 0.05$). Sprouted broccoli had higher levels of FRAP and DPPH (160.23±0.25) and (77.8 ± 0.98) than sprouted flax seeds (95.64±0.35) and (72.43±0.22), respectively. Broccoli sprouts are more antioxidant-rich than flax sprouts because they contain more secondary metabolites like phenolic compounds, flavonoids, and sulforaphane. Glucosinolate concentrations vary significantly between mature broccoli and broccoli seedlings. Sprouts found to have more than 20 times more glucosinolates. Small amounts of cruciferous sprouts produce a high concentration of inducible enzymes which protect against carcinogenesis in the diet. The sprouts of cruciferous have Stimulate activity that is 10-100 times more than mature plants **(Fahey et al., 1997).**

Biochemical Analysis:

Data in **Table (4)** shows Gastric Mucosal Injury Area and the protection percentage in rat's groups induced by Ibuprofen. Which inhibits cyclooxygenase (COX), an enzyme in the prostaglandin synthesis pathway, hence lowering fever, discomfort, and inflammation. The most likely cause of toxicity is impaired stomach cytoprotection due to prostaglandins **(Argentieri et al., 2012).** According to Table (5), ibuprofen treatment resulted in a significantly higher ulcer index (9.12 ±0.6) than (ve+). Pretreatment with SF, SB, and ranitidine significantly protected rats from ibuprofen-induced stomach ulcers ($p < 0.05$). SF at 5% and 10% decreased ulcer index to 2.47±0.4 and 0.65±0.52, resulting in 73.1 and 92.9% prevention, respectively.SB (5% and 10%) decreased ulcer index to (0.86±0.19 and 0.63 ±0.69), resulting in 90.7 and 93.1% prevention, respectively. Ranitidine (50 mg/kg BW) decreased ulcer index to 0.80 ± 0.10, indicating 6.73 ± 0.21 protection against stomach mucosal damage.

Broccoli sprouts are very effective free radical scavengers. This family of compounds includes flavonoids such as kampeferol, quercetin, and myricetin. Broccoli sprouts were discovered to have significant levels of flavonoids and phenolic acid. Sulforaphane, which is found in broccoli, is thought to be a strong inducer of detoxification of potentially carcinogenic substances by activating phase II enzymes (glutathione transferases, quinone reductases) **(Zhang and Callaway, 2002**) and can protect against electrophiles such as carcinogens, oxidative stress, and inflammation **(Ahn et al., 2010).** It also has antibacterial action against Helicobarcer pylori in vitro, providing further health benefits **(Moon et al., 2010).**

Flaxseed sprouts have high levels of omega-3 fatty acids, specifically α-linolenic acid **(Kanmaz and Ova, 2015).** Flax seeds are emerging as a major source of phytochemicals. Furthermore, it is one of the highest sources of lignans, high-quality protein, and soluble fibre **(Kraievska et al., 2017).**

Table (4) also shows that the control (+) group has a higher ulcer index (9.12 ±0.6) than the healthy group (-). Pretreatment with 5%SF, 5%SB, and Ranitidine provided considerable ($p \leq 0.05$) protection against ibuprofen-induced stomach ulcers in rats. 10% SF and 10% SB lowered ulcer index to (0.65 ±0.52 and 0.63 ±0.69), resulting in 92.9 and 93.1 percent prevention, respectively. Pretreatment with 5%SF and 5%SB decreased ulcer index to (2.47±0.4 and 0.86±0.19), resulting in 73.1 and 90.7% prevention of stomach mucosal damage, respectively.

Additionally, results show that broccoli sprouts had the best results, as they contain sulforaphane, which lowers colonisation and alleviates gastritis in H. pylori-infected mice and people **(Yanaka et al., 2009).** Sulforaphane is also a potent inducer of phase 2 detoxification enzymes, such as glutathione S-transferase, and has antioxidant, anticancer and anti-inflammatory activities **(Fahey et al., 1997 and McColl, 2010).**

Table (5) shows Ibuprofen induction substantially increased gastric juice volume and decreased pH in the ulcer group (+) (1.80 ±0.26 ml) and (4.33 ±0.15) compared to normal animals (-) (0.40±0.10ml) and (5.37±0.21). Gastric acid contributes significantly to the etiology of gastric mucosal sores **(Nishino et al., 2010).** Results show decreasing in the volume of gastric juice in pre treaded groups. The pretreated group 10%SF and 10%SB has the less volume of gastric juice (0.50 ±0.10 and 0.53 ±0.15 mL) compared to the ranitidine treatment group (0.80±0.10 mL). Flaxseed is the richest source of phytoestrogens (lignans) **(Kajla et al., 2014).** Lignans have antioxidative actions, such as activating antioxidant enzymes and scavenging free radicals, as well as phytoestrogenic characteristics **(Barakat et al., 2018).** It has anti-inflammatory qualities by blocking the nuclear factor kappa-light-chain-enhancer of activated B cells, a protein complex that regulates DNA transcription. It lowers the levels of pro-inflammatory cytokines **(Pellegrini et al., 2010).** Flavonoids have been observed to reduce stomach acidity in peptic ulcers **(Bigoniya and Singh, 2014).**

Table (6) indicates that in gastric ulcers, oxidative stress occurs which leading to increase lipid peroxidation in the gastric mucosa. MDA in control(Ve+) has a high level (52.97 ±0.04) compared to MDA control (Ve-) (47.74 ±0.05) that's ensure the high oxidative stress occurs in ulcer group. This is matching with finding of (**Ighodaro and Akinloye, 2018)** who explained that lipid peroxidation increased in ibuprofen-induced stomach ulcers, and MDA levels were significantly higher ($p < 0.05$). Increased in ibuprofen-induced compared with control ($p < 0.05$). In the pretreatment groups (10%SF and 10%SB) are the lowest value of MDA compared to the control groups and the Ranitidine administrated group (**43.10 ± 0.09** and **43.23 ± 0.12**) respectively.CAT and SOD were significantly lower in control group (+) compared to the control group (-) (10.69 ± 0.48 and 14.71 ±1.50) in catalase and (0.90 ±0.52 and 1.13 ± 0.82) in superoxide dismutase respectively. This results matching with **(El-Missiry et al., 2001 and Tandon et al.,2004).** Pretreatment groups (10%SF and 10%SB) have the highest value (14.10 ± 0.43 and 15.35 ± 0.74) in CAT and (1.34 ± 0.92 and 1.09 ±1.01) in SOD respectively. These results may be due to the increased flavonoid content in both broccoli and flaxseeds. **Reuter et al., (2010**) ensured these results when explained that flavonoids not only directly scavenge ROS but also protect and activate antioxidant enzymes, hence protecting against oxidative damage in peptic ulcers. (SOD and CAT) are antioxidant enzymes that fight free radicals and reduce oxidative damage.

Table (1): Proximate composition of flaxseeds and broccoli seeds and their sprouts:

Sample g/100g dry matter	Constituents					
	Moisture	Crude Protein	Crude fat	Ash	Crude fiber	Carbohydrate
Raw material						
flaxseed	4.7±0.1d	22.29±0.29d	34.13±0.41a	2.97±0.07d	13.28±0.22c	16.83±1.00c
Broccoli seeds	6.9±0.13c	25.87±0.19b	8.61±0.22c	5.24±0.25c	6.52±0.35d	35.89±0.21a
Sprouted Material						
Flaxseeds sprouts	24.4±0.36a	23.47±0.45c	26.94±0.13b	6.23±0.2b	19.08±0.21a	5.68±0.74d
Broccoli seeds sprouts	8.67±0.37b	39.73±0.25a	6.21±0.23d	7.25±0.25a	17.49±0.44b	31.61±0.49b

*The data is reported as mean ± SDM (n=3). A, B, C, and D: Means with different letters in the same column show significant differences (P < 0.05).

Table (2): Fatty acids of flax and broccoli (seeds and sprouts):

Fatty Acids	Flax		Broccoli	
	Seeds	Sprouts	Seeds	Sprouts
TSFA	11.09	10.25	9.45	8.02
TUFA	26.87	27.04	8.23	14.32
TPUFA	58.16	59.9	28.00	54.04
C16:0 (Palmitic),	5.78	5.51	6.95	5.63
C18:0 (Stearic),	5.31	4.74	2.5	2.39
C18:1 (Oleic),	26.87	27.04	8.23	14.32
C18:2 (Linoleic),	14.13	14.56	16.75	27.83
C18:3 (Linolenic).	44.03	45.34	11.25	26.21

TSA: Total Saturated Fatty Acids * TUFA: Total Unsaturated Fatty Acids
* TPUFA: Total Poly Unsaturated Fatty Acids

Table (3): Total Phenolic and Total flavonoids of sprouted flax seeds and sprouted Broccoli seeds and their effects on antioxidant activities

	Sprouted flaxseeds	Sprouted broccoli seeds
Total Phenolic	14.96 ± 0.51^{b}	40.54 ± 1.41^{a}
Total flavonoids	3.25 ± 0.34^{b}	4.96 ± 0.32^{a}
Antioxidant Capacity		
FRAP $^{+}$	95.64 ± 0.35^{b}	160.23 ± 0.25^{a}
DPPH%	72.43 ± 0.22^{b}	77.8 ± 0.98^{a}

The data is reported as mean ± SDM (n=3). A, B, C, and D: Means with different letters in the same row show significant differences (P < 0.05).

Table (4): Effect of sprouted flax seeds, sprouted broccoli seeds, and ranitidine on the gastric lesion surface produced by ibuprofen in rats

Groups	**Gastric Mucosal Injury**	
	Gastric Mucosal Injury Area (mm^2)	**Protection (%)**
Control (Ve-)	$00±00^{f}$	100
Control (Ve+)	$9.12 ±0.6^{a}$	0
5%SF	$2.47±0.4^{b}$	73.1
10%SF	$0.65 ±0.52^{e}$	92.9
5%SB	$0.86±0.19^{d}$	90.7
10%SB	$0.63 ±0.69^{e}$	93.1
Ranitidine (50 mg/kg BW)	$2.3 ±0.41^{c}$	75.0

columns are significantly different ($P \leq 0.05$). SF: Sprouted flaxseeds; SB: Sprouted Broccoli seeds

Table (5): Effect of (flax seeds, Broccoli seeds) sprouts and ranitidine on volume of gastric juice and gastric juice pH

Groups	**Volume of gastric juice (mL)**	**pH of gastric juice**
Control (Ve-)	$0.40±0.10^{f}$	$5.37±0.21^{c}$
Control (Ve+)	$1.80 ±0.26^{a}$	$4.33 ±0.15^{d}$
5%SF	$0.67±0.15^{d}$	$5.33 ±0.15^{c}$
10%SF	$0.50 ±0.10^{e}$	$6.27±0.21^{b}$
5%SB	$0.73 ±0.15^{c}$	$5.40±0.20^{c}$
10%SB	$0.53 ±0.15^{e}$	$6.73 ±0.21^{a}$
Ranitidine (50 mg/kg BW)	$0.80±0.10^{b}$	$6.73 ±0.21^{a}$

The data is provided as mean ± SDM (n=5). A, B, C, and D: Means with different letters across groups in the same column show significant differences ($P < 0.05$). SF: sprouted flaxseeds, SB: sprouted broccoli seeds.

Table (6): Effect of sprouted flax seeds and sprouted Broccoli seeds and ranitidine on the levels of MDA, SOD, and CAT in rats induced gastric ulcer by ibuprofen

Lipid Peroxidation			
Groups	**CAT(IU/L)**	**SOD(IU/L)**	**MDA (µmol/L)**
Control (Ve-)	$14.71 ±1.50^{ab}$	$1.13 ± 0.82^{b}$	$47.74 ±0.05^{d}$
Control (Ve+)	$10.69 ± 0.48^{f}$	$0.90 ±0.52^{a}$	$52.97 ±0.04^{a}$
5%SF	$11.55 ±1.19^{d}$	$1.07 ±0.81^{b}$	$48.71 ±0.0^{c}$
10%SF	$14.10 ± 0.43^{b}$	$1.34 ± 0.92^{a}$	$43.10 ± 0.09^{f}$
5%SB	$13.80 ±0.37^{c}$	$0.94 ±1.73^{c}$	$46.67 ± 0.12^{e}$
10%SB	$15.35 ± 0.74^{a}$	$1.09 ±1.01^{b}$	$43.23 ± 0.12^{f}$
Ranitidine (50 mg/kg BW)	$10.93 ±0.25^{e}$	$0.97 ±1.45^{c}$	$49.34 ±0.08^{bc}$

The data is presented as mean ± SDM (n=5). A, B, C, and D: Means with different letters among groups in the same column show significant differences ($P < 0.05$). Sprouted flaxseeds (SF), sprouted broccoli seeds (SB), catalase (CAT), and malondialdehyde (MDA)

Histopathological examination

Fig (1) shows examination of liver sections of normal rats group, ulcer rats group and administrated rats with Randitine. Healthy rats showed normal histological architecture of hepatic lobule. Meanwhile a positive control demonstrating tissue damage such as epithelial breakdown and inflammatory infiltration (black and red arrows) compared to the healthy group.

The group of ulcer rats showed Kupffer cells proliferation, hepatocellular vacuolar degeneration, biliary epithelial hyperplasia and portal invasion by inflammatory cells**. Bessone (2010)** reported that NSAIDs have been associated with liver damage.

On the other hand, rats' liver from group pretreatment with 5%SF exhibited proliferation of Kupffer cells and infiltration of the portal area with few inflammatory cells. Meanwhile, liver sections from group pretreatment with10%SF showed proliferation of Kupffer cells, slight congestion of central vein, slight hepatocellular vacuolization of some hepatocytes and oval cells proliferation. Furthermore, liver of rats from group pretreatment with 5%SB showed proliferation of Kupffer cells. Likewise, some liver of rats from group pretreatment with 10% SB described no histopathological alterations. **Kikuchi et al., (2015)** reported that, the treatment with broccoli seeds extract significantly improve liver function by lowering oxidative stress levels. Sulforaphane found in broccoli **(Zhang et al., 1992)** is a unique phytochemical that inhibits the NF-κB pathway (**Baird and Dinkova-Kostova 2011 and Gerhauser, 2013).** Nrf2 may protect the liver from both hepatotoxic substances and lifestyle variables like high-energy food consumption **(Shimozono et al., 2013 and Kikuchi et al., 2015).**

Moreover, liver of rats from group administrated with randitine showing proliferation of Kupffer cells. **Roberts et al., (2007**) explained that Understanding the involvement of Kupffer cells in these various responses is critical for understanding the processes of liver damage. Idiosyncratic drug-induced liver disease causes morbidity and death, which has a significant influence on the development of novel pharmaceutical drugs.

Fig (2) illustrated rats' stomach sections in the control (-), control (+) and pre-treatment groups. Gastric ulcers are caused by a combination of factors, including acid, Helicobacter pylori, ethanol, bile acids, pepsin, and non-steroidal anti-inflammatory drugs (NSAIDs), which disrupt protective mechanisms like epithelial cell tight junctions, microvascular blood circulation, nitric oxide levels, bicarbonate secretion, and prostaglandin **(Kolgazi et al., 2017).** The figure shows that the normal group (-) revealed the normal histoarchitecture of stomach layers compared to (ve+) group which showed histopathological damage characterized by gastric mucosal necrosis is related with inflammatory cell infiltration.

This is what (**Laine et al., 2008)** explained about ibuprofen is a nonselective NSAID, which means it can readily cause gastrointestinal damage. Some research showed that ibuprofen might cause stomach ulcers. NSAID-related gastritis had a higher prevalence of focal hyperplasia, oedema, and vascular ectasia compared to H. pylori gastritis **(Graham, 2000).** Meanwhile, stomach of rats from group pretreated with (5%SF) described submucosal edema, congestion of submucosal blood vessel and few inflammatory cells infiltration. On the other hand, some sections from group pretreated with 10%SF exhibited apparent histologically normal gastric mucosa. These results are in a line with **(Ibrahim et al., 2016)** who found that using flax seeds oil before administration inducing gastric ulcer got better results by decreasing the damage of gastric mucosa. Also **(El Senosi et al., 2018)** explained that omega-3 oral therapy significantly protects against ethanol-induced stomach ulcers, as evidenced by antioxidant and anti-inflammatory properties. Omega-3 delivery may be an alternate therapy for stomach ulcers due to its cytoprotective and anti-inflammatory properties. Furthermore, some sections from pretreatment group(5%SB) revealed apparent histologically normal gastric mucosa. Otherwise, stomach of rats from groups 10%SB and randitine exhibited no histopathological alterations. Concentrations of glucosinolates might increase by up to 20 times. Consuming cruciferous sprouts in tiny amounts can provide significant nutrition. Cruciferous sprouts have 10-100 times higher inducing activity than mature vegetables **(Fahey et al., 1997). Alazzouni et al., (2020)** reported that ranitidine oral treatment significantly reduced NSAID-induced ulcers.

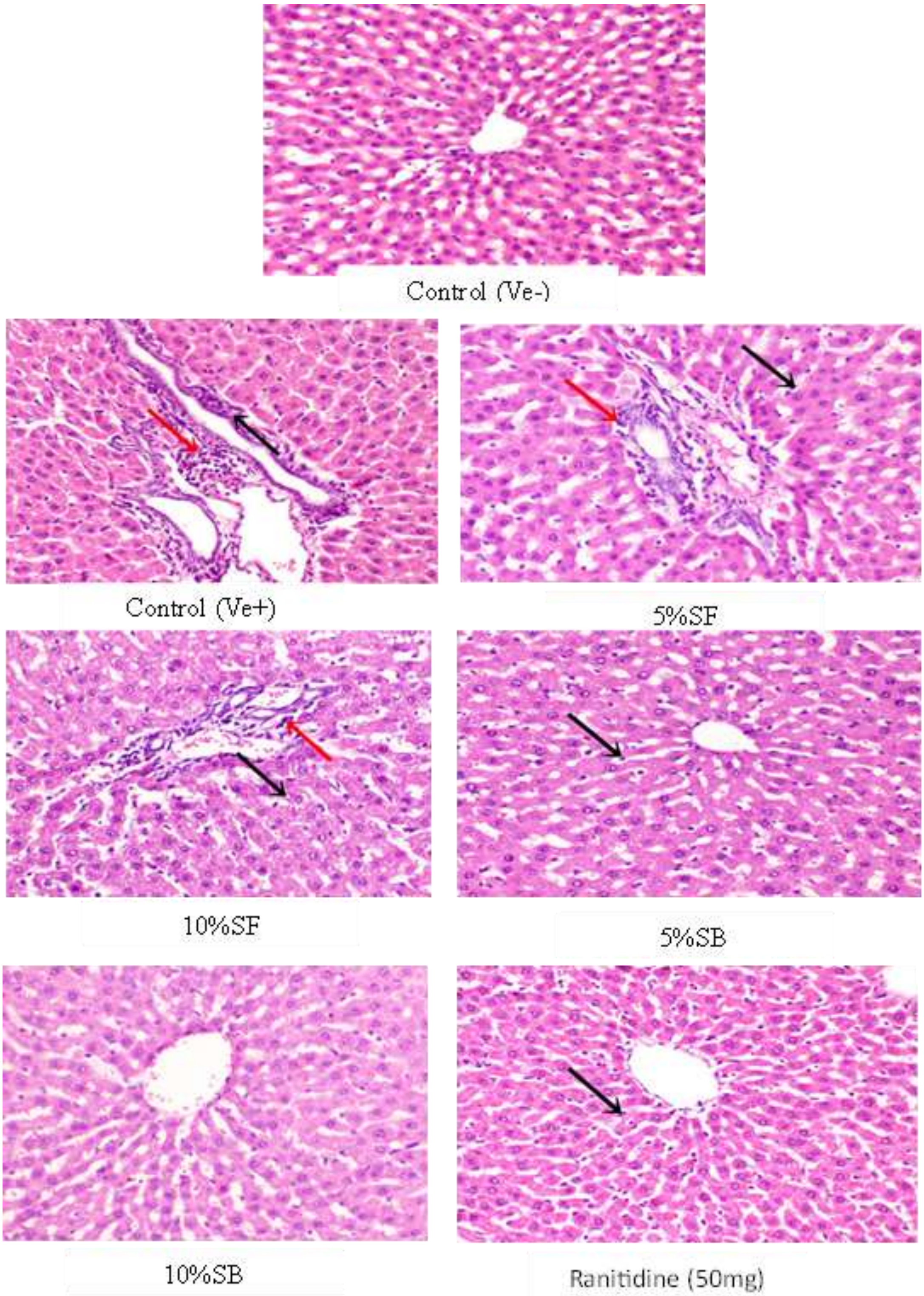

Fig. (1): Photomicrograph of Sections of liver for different rats' groups stained with H & E, X 400.

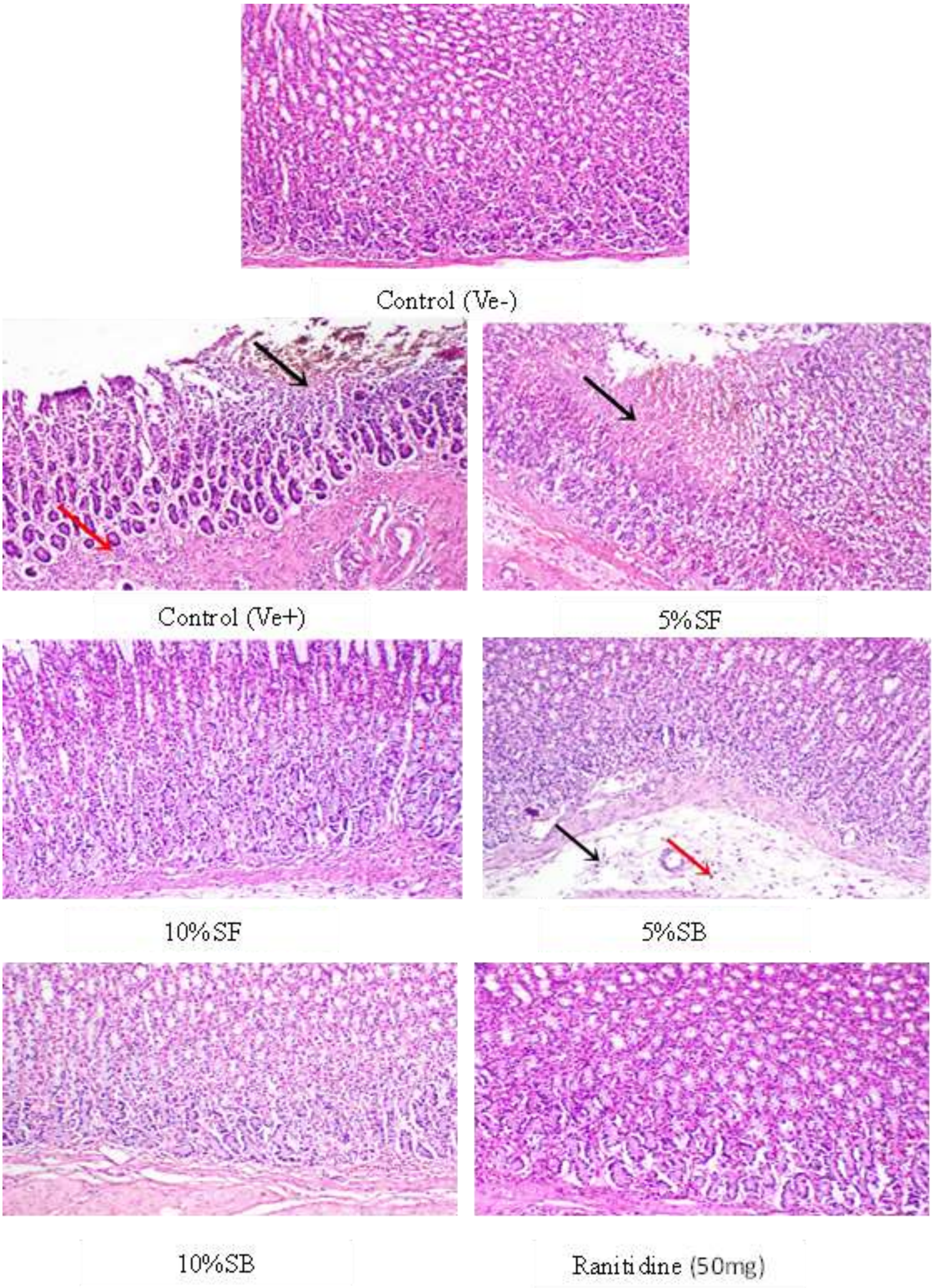

Fig. (2): Photomicrograph of Sections of stomach for different rats' groups stained with H & E, X 400.

Conclusion

Increased antioxidants, protein, ash content, fatty acids, and bioavailability of dietary components in germinated flaxseed and broccoli seeds provided anti-oxidant and anti-inflammatory health advantages to ulcer-treated rats. Significance and novelty Evidence suggests that germinated flaxseeds and broccoli seeds contain more nutrients than ungerminated seeds due to the activation of endogenous enzymes that break down antinutritional chemicals and give antioxidant qualities. Consuming broccoli and flaxseed sprouts is an inexpensive and simple method to maintain a balanced diet. The two sprouts evaluated in this study were high in protein and low in fat; their lipids included a substantial quantity of linolenic and linoleic acids, particularly flaxseeds. They also include high concentrations of flavonoids, polyphenols, and DPPH radical scavenging properties. Broccoli sprouts have a significant amount of sulforaphane, which has been related to health benefits.

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تأثير بذور الكتان وبذور البروكلي المنبتة كمضاد للأكسدة ضد القرحة الناتجة عن الإيبوبروفين في الفئران

المستخلص

الخلفية: تتميز البذور المُنبتة بغناها بالمواد النشطة بيولوجيًا، بما في ذلك الأحماض الدهنية غير المشبعة، والبوليفينولات، والفلافونويدات، والتي لها تأثير جيد على صحة المعدة بفضل خصائصها المضادة للأكسدة والالتهابات.

المنهجية: تم تقدير التركيب الكيماوي والاحماض الدهنية والبوليفينولات والنشاط المضاد للأكسدة كما تم استخدام ٣٥ فارا مقسمين إلى المجموعة الرئيسية (٥ فار) وهي الكنترول (الغير مصابة) و ٥ مجموعات فرعية تناولت أربع مجموعات منهم الغذاء الأساسي بالإضافة إلى (٥، ١٠ %) براعم الكتان و(٥، ١٠%) براعم البروكلي كمعالجة تمهيدية لمدة ٧ أيام، ثم أُعطيت جميع المجموعات إيبوبروفين (٤٠٠ ملجم/كجم، عن طريق الفم) مرتين بعد الصيام لمدة ٢٤ ساعة وأعطيت المجموعة الأخيرة الرانيتيدين كعنصر تحكم إيجابي. تم قياس مؤشر القرحة، وحجم العصارة المعدية، ودرجة حموضة العصارة المعدية، ومؤشر الحماية أثناء تعليقها في الماء لتوليد قرحة معدية حادة. قُيّمت المؤشرات الكيميائية الحيوية مثل بيروكسيد الدهون، وسوبر أكسيد ديسميتيز (SOD، والكتاليز ((CAT، والمالونديالدهيد (MDA) .

النتائج: أظهرت النتائج اختلافات كبيرة في التركيب الغذائي، والأحماض الدهنية، والبوليفينولات، ونشاط مضادات الأكسدة (DPPH) و(FRAP) .وجد أن الإنبات له قيمة غذائية كبيرة في كل من بذور الكتان وبذور البروكلي. تنوعت الأحماض الدهنية بين زيادة محتوى حمض البالمتيك والأوليك بعد الإنبات وانخفاض حمض الستياريك في كلا من براعم الكتان وبراعم البروكلي. وأعطت براعم البروكلي أنشطة عالية القوة المختزلة مقارنة ببراعم الكتان. كان النشاط المضاد للقرحة مطابقًا تقريبًا لنشاط الضبط الإيجابي. وقد أظهرت بذور البروكلي المُنبتة (١٠%) فعالية قوية في مكافحة القرحة عبر جميع المقاييس العيانية والكيميائية الحيوية المُختبرة.

الكلمات المفتاحية: النشاط المضاد للأكسدة، بيروكسيد الدهون، رانيتيدين، خصائص مضادة للالتهابات، البوليفينولات