Effect of Seeds and Sprouts of Red Radish and Soybeans on Oxidative Stress Caused by Paracetamol on Rats

Hoda M. Hanafi
Nutrition and Food Science, Home Economic Dept., Faculty of Specific Education, Fayoum Univ., Egypt

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البريد الإلكتروني للمجلة E-mail JSROSE@foe.zu.edu.eg

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Abstract

Background: Oxidative stress resulting from increased free radicals and decreased antioxidants can be controlled by consuming natural antioxidants from safe sources such as red radish and soybean seeds and sprouts.

Methods The chemical composition, antinutritional factors, total polyphenols, total flavonoids and antioxidant activity by DPPH were estimated. Two main groups contain 36 rats split into the first group (ve-) 6 rats, and the second group 30 rats which took 2 g/kg BW from Paracetamol for 7 days. Then divided into 5 groups, (ve+), (ve-), 10% red radish seeds and 10% red radish sprouts, 20% soybean seeds and 20% soybean sprouts for 6 weeks. MDA, GHS and CAT were evaluated.

Results: significant differences in nutritional composition, polyphenols, flavonoids and antioxidant activity (DPPH). Sprouting was found to have significant nutritional value in both red radish and soybean sprouts. A Supplementation diet with 20%sprouted of soybean and 10% red radish sprouts demonstrated strong antioxidant activity across all macroscopic and biochemical parameters tested.

Keywords: antioxidant enzymes, polyphenols, flavonoids, sprouting

تأثير بذور وبراعم الفجل الأحمر وفول الصويا على الإجهاد التأكسدي الناتج عن الباراسيتامول لدى الفئران

المستخلص

الخلفية: يمكن السيطرة على الإجهاد التأكسدي الناتج عن زيادة الجذور الحرة وانخفاض مضادات الأكسدة عن طريق استهلاك مضادات الأكسدة الطبيعية من مصادر آمنة مثل الفجل الأحمر وبذور فول الصويا وبراعمها.

الطرق: تم تقدير التركيب الكيميائي، والعوامل المضادة للتغذية، ومجموع البوليفينولات، ومجموع الفلافونويدات، وكذلك تقدير النشاط المضاد للأكسدة وتم التحكم في التأثيرات البيولوجية داخل الجسم من خلال دفاعات إنزيمات مضادات الأكسدة. قُسِّمت الفئران (٣٦ فارا) إلى مجموعتين رئيسيتين: المجموعة الأولى (فئران سليمة) ضمت ٦ فئران، بينما تلقت جميع فئران المجموعة الثانية الرئيسية (عددها ٣٠ فارا) جرعة فموية من الباراسيتامول بجرعة ٢ جم/كجم من وزن الجسم لمدة ٧ أيام. ثم قُسِّمت إلى ٥ مجموعات: مجموعة ضابطة سالبة ومجموعة ضابطة موجبة ومجموعتان ١٠% بذور فجل أحمر و١٠% براعم فجل أحمر، ومجموعتان ٢٠% بذور فول الصويا و٢٠% براعم فول الصويا.

النتائج: ظهرت فروقٌ كبيرة في التركيب الكيميائي، والبوليفينولات الكلية، والفلافونويدات، ونشاط مضادات الأكسدة. ووُجد أن الإنبات له قيمة غذائية كبيرة في كلٍّ من بذور الفجل الأحمر وبذور فول الصويا. ويمكن لنظام غذائي مُكمّل يحتوي على براعم فول الصويا وبراعم الفجل الأحمر أن يُحسّن بشكلٍ ملحوظ من نشاط إنزيمات مضادات الأكسدة الرئيسية في الكبد والخصيتين من خلال تحسين آثار الإجهاد التأكسدي.

الكلمات المفتاحية: إنزيمات مضادة للأكسدة، البوليفينول، الفلافونويد، الإنبات

INTRODUCTION

Oxidative stress occurs when pro-oxidative activities overwhelm cellular antioxidant defenses, altering redox signaling and responses **(Ji and Yeo, 2021)**. Smoking, a variable lifestyle choice, significantly alters the equilibrium between ROS generation and antioxidant defenses **(Agarwal et al., 2008 and Durairajanayagam 2018)**. Oxidative stress (OS) causes by the redox equilibrium, which impairs liver function, affects inflammatory pathways, and leads to sickness **(Asadi et al., 2017).**

OS reduces male fertility indices by causing oxidative damage to reproductive cells and intracellular components **(Dutta et al.,2021).** Antioxidants work in cells, either enzymatically or non-enzymatically. Flavonoids, glutathione, vitamins E and, C are examples of non-enzymatic antioxidants which play an important and vital role in the direct reaction with free radicals **(Sies et al., 2022).** The concern about the effects of oxidative stress on people has become a serious topic**.** The WHO reports that bioactive chemicals originating from plants account for more than 80% of conventional pharmaceuticals **(Krishnaiah et al., 2011).** Antioxidant

chemicals found naturally in plants can be utilized therapeutically. This is considered to be dietary supplements which can reduce oxidative stress and the need for further medicine **(Goyeneche et al., 2015 and Rezk et al., 2019).** Seed sprouts are becoming increasingly popular for consumption, either alone or as a raw material for other goods, due to their higher nutritional value and associated with a healthy lifestyle **(Demir and Bilgiçli, 2020).**

Red radish (*Raphanus sativus* L.) belongs to the Brassicaceae family. The Pharaohs and Greeks utilized red radish, which is one of the earliest known meals. It is a vital vegetable that has a variety of therapeutic properties. Radish root, seeds, and leaves are all used medicinally **(Gutiérrez and Perez 2004**). It includes numerous vital micronutrients, as well as fibre and antioxidants (**Capus et al., 2016 and Jaafar et al., 2020).** Radish seeds perform some important functions such as improving bone density and strengthening it, stimulating enzymes, treating sexual weakness, and reducing high blood pressure and cholesterol **(El-Beltagi et al., 2010 and Jing et al., 2014).**

Furthermore, it includes a large amount of anthocyanins, which are commonly used as natural food colorings due to their high durability and comparable features to artificial food Red No. 40 **(Chen et al., 2017).** Anthocyanin prevents ROS from developing when exposed to stressful conditions during development so it considered to be an important antioxidant source (**Jing et al. 2014).** The accumulation of primary and secondary metabolites is due to sprouting process. Because of these alterations, sprouts contain more health-promoting phytochemicals than other vegetables. Sprouts are high in vitamins, protein, and amino acids, all of which are beneficial to health **(Ghani et al., 2016).** The germination process maximizes the nutritional and functional value of nutrients. Many enzymes are active during germination, which increases protein digestibility and mineral bioavailability **(Choi et al., 2000).** Moisture, protein, and total dietary fiber content rose in germinated samples, but fat levels declined.

Red radish sprouts (RRS) contain more health-promoting compounds than seeds. Gutiérrez and Perez, et al. (2004). Raphanus sativus L. seeds contain active pharmacological substances such as alkaloids, flavonoids, glycosides, phenols, sterols, and tannins **(Ahmad et al., 2012 and Umamaheswari et al., 2012).**

Soybean (*Glycine max* (L.) Merr., Fabaceae) is a significant crop for both human and animal use (**Malenčić et al., 2008).** Soybean seeds contain several antinutritional compounds, including lectins and enzyme inhibitors. Germination has been discovered as an affordable and effective approach to improve the nutritional content of soybeans **(Bau et al., 1997).** Consumption of soybean sprouts has several health advantages, including reducing the risk of cancer as well as cardiovascular diseases **(Prakash et al. 2007).**

Furthermore, another benefit of germination is eliminating antinutrients like tanins and phytic acids **(Shi et al. 2010).** Thus, this research aimed at studying the effect of Seeds and Sprouts of Red Radish and Soybeans as a strong and natural antioxidant on rats with oxidative stress**.**

MATERIALS AND METHODS

Materials

During the 2024 season, samples of red radish seeds (*Raphanus sativus*) and soybeans (Glycine max) were purchased from Al- Fajr private company of agricultural seeds, spices, and medicinal plants, -October City, Egypt. and identified and authenticated at Cairo University Research Park (CURP). Paracetamol was purchased from Memphis Company for Pharmaceutical and Chemical Industries in Cairo, Egypt. The kits utilized were purchased from Bio-diagnostic Co. Dokki in Egypt.

Methods

Experimental procedures

Preparation of sprouted red radish and soybean seeds.

The seeds were washed of any contaminants and steeped for 16 hours, then wrap with a wet cotton tissue. The seeds started to germinate in the dark at a temperature of (25-30° C), and the sprouts were taken 3-4 days later and used in the study, according to **Lotfy and Abd-Elrahman (2021)**. Rinse the soybeans in cold water and refrigerate overnight. Place the cleaned soybeans in a planter with small holes on the bottom, and add extra water every 5 hours to keep them moist. The water will drain through the planter's little pores at the bottom. A black fabric is also used to cover the planter in order to block out light for three days. Rinse and drain the sprouts several times before placing them in the refrigerator **(Zinia et al.,2022).**

Analytical methods:

Proximate chemical composition of non-sprouted and sprouted red radish and soybean seeds

Moisture, ash, fat, fiber and protein were measured using the **AOAC method (2015),** and the carbohydrate content was computed by difference. Total carbs = 100 - (Moisture+ ash+ fat+ fiber + protein). All determinations were performed in triplicate.

Determination of antinutritional factors: phytate, tannin content

Phytate Onwuka's (2005) method for determining phytate content was used. Red Radish, soybean seeds and sprouts extraction by using 0.2 N hydrochloric acid. Then a test tube with a ground glass stopper was used to transfer the extract (0.5 6669 mL). 1 ml of Ferric solution was added to the tube, heated in a boiling water bath for half an hour after covering it. Use the centrifuge (3000 xg) for half hour after cooling. 1.5 ml of 2,2-bipyridine solution was added to 1 ml supernatant in a new test tube. Test conducted against distilled water at 519 nm absorbance. calibration curve was used for determining the concentration.

Tannins The quantitative measurement of tannins was carried out according to Pulipati et al. (2014) as follows: 1 gram of each sample was combined with 10 ml of HCl in 1% methanol (v/v) in a dark container and agitated for 20 minutes at room temperature before filtering. In a test tube, 1 ml of the supernatant was combined with 5 ml of (vanillin / HCl) combination (made by combining equal amounts of 2% vanillin in methanol and 8%HCl in methanol) and left at room temperature for 20 minutes. The color was measured at 500 nm with a spectrophotometer (Jenway 6300 VIS, Laborned Inc., USA). Catechin was employed to prepare the standard curve. Tannins were determined as mg of catechin equivalent (CE) per 100 g dry weight basis.

Determination of Total Phenolic and flavonoid content

The technique of Folin-Ciocalteu was used for determining **total phenolic content** as described by **Elsayed et al., (2022),** with 1 ml of each sample (extracts before evaporation) 1 ml of Folin reagent added after deposited in a test tube. After three minutes, one milliliter of sodium carbonate (7.5%) was added. The combination was kept in the dark for one hour before the absorbance at 740 nm was measured. A standard curve of gallic acid concentration used for determining the total phenolic content.

and reported as mg of gallic acid per milligram of sample.

The flavonoid content was determined using the aluminum trichloride technique, with catichin serving as a reference component. Colorimetric technique was used for determining total flavonoid concentration

Catichin standard solutions were generated by dissolving catechin in water at concentrations ranging from 10 to 50 µg/mL. In brief, 1 ml of adequately diluted aqueous catichin standard solutions or red radish and soybean seed samples was mixed with 4 ml of distilled water. At time 0, 0.3 ml of 5% (w/v) nano2 was introduced. 0.3 mL of 10% (w/v) alcl3 was added 5 minutes later. At 6 minutes, 2 ml of 1 mol l-1 naoh was added, and the solution was diluted to 10 ml with distilled water and mixed.

Using a spectrophotometer, the spectrum was scanned against a blank between 850 and 290nm. calibration curve was used for determining the total flavonoid concentration and catechin equivalents was used as mg per ml of sample.

Antioxidant Activities by DPPH:

1, 1-diphenyl-2-picrylhydrazyl (DPPH) was used for determining Radical scavenging activity of extracts according to **Aboelsoued et al. (2019).**

Ethanol used as a control. Using spectrophotometer at 517nm

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

Ac: absorbance at 517nm A: sample

Biological experiment

Experimental rats:

A total of 36 male Albino rats weighing around 180±5 g were procured from the Agricultural Research Center in Giza, Egypt. Rats groups were housed for 6 weeks. Filtered environment, pathogen-free air and tap water were used.

The temperature was about 20-25^{o}C, a 12-hour light/dark cycle, a light cycle (8-20 h), and a relative humidity of 50%. For one week, all rats were given a baseline diet according to **(Reeves et al., 1993).**

Before commencing the acclimatization experiment. All experimental protocols followed international criteria for the care and management of laboratory animals.

Experimental design:

Seven days as an adaptation phase, rats divided into two major groups.

The first group (n= 6 rats) was fed only the baseline diet as (ve-). According to the protocol, the 30 rats received an oral dose2 g/kg BW of Paracetamol at for 7 days. suspended in water for the induction of oxidative stress according to **Abd el Latif *et al*., (2021)**. Then, the 30 rats divided into five groups, as follows:

Group (1): The positive group(ve+) fed only on a basal diet.

Group (2): fed on the basal diet plus red radish seed (10%) / diet / day.

Group (3): fed on the basal diet plus fresh sprouted red radish seed 10%) / diet / day.

Group (4): fed on the basal diet plus soybean (20%)/ diet / day.

Group (5): fed on the basal diet plus sprouted soybean (20%) / diet / day.

All groups weighed every week until the end of the experimental period (٦ weeks) finally, the rats were fasted overnight, anesthetized with ether and sacrificed for the collection of liver and testis samples for further analysis to determine.

Biological Determination:

Body weight gain% (BWG %) and organs weight / body weight% were determined according to **Chapman *et al*., (1959).**

Biochemical analysis

By using capillary glass tubes, blood samples were taken from the orbital plexus veins, placed in centrifuge tubes without anticoagulant and allowed to clot. Following the serum generated by centrifugation (3000 rpm for 15 minutes)

Determination of serum Urea Nitrogen according to the method described by **Fawcett and Soctt, (1960)** using spectrophotometer (model DU 4700) adjusted nm 550 nm.

serum uric acid was determined by **Barham and Trinder, (1972)** using spectrophotometer (model DU 4700) adjusted at 510 nm.

Creatinine was determined by **Larsen, (1972)** using spectrophotometer (model DU 4700) adjusted at 510 nm.

ALT and AST were determined calorimetrically using spectrophotometer (model DU 4700) at 505 nm according to the method of **Reitman and Frankel, (1957**).

serum total protein was determined by colorimetric method (biuret reagent) described by **Tietz, (1990)**

Oxidative stress evaluation:

Reduced glutathione (GSH) in tissues was evaluated using **Ellman's (1959)** technique using a spectrophotometer (model DU 4700) set to 412 nm. Catalase activity in tissues was measured using **the Aebi (1984) technique** with a spectrophotometer (model DU 4700) set to 240 nm. **Albro et al.'s (1986)** technique was used to measure the hepatic malondialdehyde (MDA) level (µmol/g tissue).

Histopathological Investigation:

The liver and testis tissues were fixed in 10% neutral formalin for 24 hours after dissection, then dehydrated with increasing concentrations of alcohol, washed in xyline, and embedded in paraffin wax. Tissue sections were 3 micron thick and stained with hematoxylin and fosin **(Banchroft et al., 1996).** All tissues were examined under a light microscope to detect any histological changes.

Statistical analysis:

The current study's data were statistically analyzed using the computerized program SPSS software, version "20" for Windows, following Snedecor and Cochran's (1980) ANOVA. 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 $P < 0.05$, else not significant.

RESULTS AND DISCUSSION

Table (1): Proximate chemical composition of non-sprouted and sprouted red radish and soybean seeds (g/100g) on the dry weight basis.

Constituents	Red radish seeds(RRS)		soybean seeds(SBS)	
	Non-sprouted	Sprouted	Non-sprouted	Sprouted
Moisture	4.23^{d}±0.11	8.92^{a}±0.05	7.40^{c}± 0.10	8.31^{b} ± 0.20
Protein	23.46^{d}±0.56	26.76^{c}±0.33	32.29^{b}±0.20	35.71^{a}±0.10
Crude fat	23.79^{a}±0.05	16.55^{d}±0.49	21.4^{b} ± 0.2	10.97^{c} ±0.10
Ash	4.40^{c}±0.08	8.37^{a}±0.28	4.00^{d}± 0.10	5.90^{b} ± 0.30
Crude fiber	13.47^{c}±0.10	18.79^{a}±0.20	9.67^{d} ± 0.10	17.4^{b} ± 0.1
Carbohydrates	30.65^{a}±0.70	20.61^{d}±0.59	25.24^{b} ± 0.2	21.71^{c}± 0.2

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

Table (1) showed significant differences in the content of nutrients in non-sprouted and sprouted seeds. Moisture, Ash, fiber, and Protein, increased after sprouted in each of red radish seeds and soybean seeds. On the contrary, the content of carbohydrates and fats decreased after sprouted.

The moisture content varied between (4.23±0.11 and 8.92±0.05) in non-sprouted and sprouted red radish seeds) and (7.40% and 8.31%) in non-sprouted and sprouted soy bean. This finding is similar to the results reported by (**Khatoon and Prakash 2006 and Warle et al.,2015).** This is related to the germination mechanism, which happens in 3 steps depending on the seed's water intake capacities and the seed's microstructure **(Agbo et al., 1987)**. The first step is the imbibition stage, which is distinguished by fast absorption of water; in the second step, water uptake is reduced; and in the third step, water uptake rises once again, accompanied by the protrusion of the radicle from the seed coat **(Bewley et al., 2013).**

Protein and fiber also showed increasing in their content in sprouts than seeds. These results confirmed the finding **of Maldonado-Alvarado et al., (2023)** which indicated that germination increased the fibre and protein content. Protease enzymes break down seed proteins into peptides and amino acids which given to the developing embryo**(Joshi, 2018 and Dweh, and Choudhury 2023). Azulay (1997)** also reported that during germination, minerals, proteins, vitamins, and enzymes increase by 25 to 4,000 percent**. Joshi (2018)** indicated the reason of decreasing carbohydrate content in sprouts is due to amylase which is responsible for solubilizing spare food resource in the form of starch, appropriately in seed, and delivering energy and other vital food materials to the sprouting embryo **.**

Fat decline in sprouts is due to the use of the lipid as energy source during germination **(Mostafa and Rahma 1987)** which confirmed finding fat decreasing during germination in red radish and soybean seeds and their sprouts.

Table (2): Antinutritional factors: phytate, tannin content of non-sprouted or sprouted red radish and soybean seeds (g/100g) on the dry weight basis.

Antinutritional factors	Red radish seeds		soybean seeds	
	Non-sprouted	Sprouted	Non-sprouted	Sprouted
Phytate	0.64^{c}±0.05	0.27^{d}±0.11	6.87^{a}±0.10	2.21^{b}±0.07
Tannin	0.89^{b}± 0.02	0.28^{d}±0.33	1.70^{a}±0.04	0.69^{c}±0.02

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

Seeds contain antinutritional substances that impair the digestion and nutritive value of plant-based proteins. Phytic acid and tannins are examples of antinutritional factors (**Sá et al., 2020).**

Results in **table (2)** indicate that phytate and tannin had significant decrease content in both of sprouted Red radish seed and soy bean. Phytate in legumes is more than seeds as shown with soybean compared to red radish seeds. Phytic acid, a powerful chelating agent, forms complexes with minerals and proteins making nutrients inaccessible for absorption and utilization **(Kumar et al., 2010 and Ojo 2021).** Phytic acid content decreased and phytase activity in all varieties increased due to germination **(Maldonado-Alvarado et al., 2023).**

For tannin content, non-sprouted soy bean had higher content of tannin compared to non-sprouted red radish seeds. That is due to the high content of tannins in legumes than seeds. Reduced tannins improves protein degestabilty. **Sá et al., (2019**) reported that animal proteins are known to be more digestible than plant proteins due to the presence of antinutritional agents. Thus, inactivation of antinutritional agents during food processing may improve plant protein quality.

Enzymatic changes during germination is the reason behind decreased in tannin and phytic acid content **(Rusydi and Azrina 2012).** Germination can produce functional foods that promote human health **(San gronis and Machado, 2007).**

Table (3): Total flavonoids, total phenolic contents and antioxidant activities by DPPH in non-sprouted and sprouted red radish seeds.

Constituents	Red radish seeds		soybean seeds	
	Non-sprouted	Sprouted	Non-sprouted	Sprouted
Total flavonoids (mg- catechin)	123.17^{d}±1.26	207.67^{c}±0.58	339.8 ^{b}± 0.10	548.3^{a}± 0.20
Total phenolic (mg-^{1} Gallic acid)	281.27^{b}±1.10	819^{a}±1.00	210.28^{d}±0.81	249.40^{c}±0.83
DPPH%	71.98^{c}±0.98	81.9^{b}± 0.20	61.06^{d}±0.42	88.87^{a}±0.97

Results in **table (3)** indicated that content of flavonoids in sprouted seeds is more than total flavonoids in non-sprouted seeds. Soy bean sprouts had a high significant content of flavonoids (548.3±0.20) compared to Red radish sprouts (207.67±0.58). Legumes flavonoids play an important function, since the plant excretes them in reaction to bacteria-produced nodulation factors (**Velazquez** et al.**, 2010**). Isoflavones, a kind of flavonoid present in soybean seeds, contain significant secondary products with diverse chemical properties. They act as antioxidant and anticancer (**Kim et**

al.,2005). Total phenolic showed high content in sprouted red radish seeds (819±1.00) compared to soy bean sprouts (249.40±0.83). Red radish (*Raphanus sativus*) is part of the Brassicaceae family. Cruciferous vegetables are rich in natural antioxidants **(Soengas et al., 2011).** Various studies have explored the polyphenol content and antioxidant activity of red radish **(Eugenio et al., 2017).** Phenolic compounds
and flavonoids can interact with ROS/RNS, halting chain reactions before cell viability is significantly compromised **(Kumar and Pandey, 2013).**
The antioxidant activity of sprouted soybean and red radish sprouts differed significantly ($p \leq 0.05$). Sprouted soybeans had higher levels of DPPH 88.87±0.97 than sprouted red radish seeds. This result is explained by the high content of total flavonoids in both soybean seeds and sprouts (339.8 ± 0.10 and 548.3± 0.20) respectively than in red radish seeds and sprouts (123.17±1.26 and 207.67±0.58) respectively. On the contrary red radish seeds had higher levels of DPPH (1.98±0.98) than soybean seeds. This may be due to the higher content of total phenols in red radish seeds (281.27±1.10 and 210.28±0.81 respectively).
Glucosinolates are sulfur and nitrogen-containing glucosides that are virtually entirely present in cruciferous plants like red radish **(Bowen-Forbes et al.,2023).** Glucoraphenin in radish was the prevalent in raw seeds, and, although their quantity fell during germination, they were retained in relatively substantial amounts in sprouts **(Martinez-Villaluenga et al., 2010)**

Table (4): Mean body weights (g) and body weight gain% of experimental rats which induced oxidative stress by paracetamol and treated with non-sprouted and sprouted red radish and soybean seeds

Body weight (g)	Control (ve-)	Control (ve+)	Groups			
			Red radish seed		Soybean seeds	
			Non-sprouted	sprouted	Non-sprouted	sprouted
IBW	180.8a±2.33	184.16a±2.02	181.50b±3.01	180.00b±3.81	182.83b±2.27	180.66b±2.29
7	199.66a±3.55	173.50e±2.09	184.66d±2.66	189.83c±3.48	189.83c±1.64	194.50b±2.99
14	213.33a±3.90	183.33f±4.11	197.00e±4.90	204.66d±3.73	207.83c±2.66	209.66b±3.38
21	230.83a±2.72	186.00f±37.65	219.33d±6.80	217.50e±3.94	221.50c±4.02	226.83b±3.53

FBW	266.5a±17.90	200.16e±1.75	232.16d±4.06	234.50c±4.36	238.50b±3.23	239.66b±2.18
BWG/w %	**47.28a±5.57**	8.71e±0.72	28.10d±3.16	30.45c±4.71	30.53c±2.34	32.72b±1.50

Data are presented as means ± SDM(n=6). Data in a row with different superscript letters are statistically different (P ≤ 0.05) IBW= Initial body weight; FBW= Final body weight; BWG= Body Weight gain; W= Week

To ensure being well in rats' life, there is a sensitive indicator and its body weight since it incorporates multiple factors, including food consumption. The increased weight growth compared to the control group might be due to a nutritive balanced diet in the animal feed.

Data in table (4) There is a significant difference (P≤0.05) in the control group (+) (200.16±1.75) compared to the remaining treatment groups in the final weight of the rats. The soybean sprouts group is expected to achieve the best results. The data also indicated that weekly body weight gain (BWG/wk) was highest (47.28±5.57 g) in the healthy group (-), while in the remaining treatment groups ranged from 30.45±4.71, 30.53±2.34, 32.72±1.50, and 28.10±3.16 g, from same table, it was noticed that the positive control group recorded the lowest weekly body weight gain (8.71±0.72 g), while the highest weekly body weight gain (32.72±1.50 g) was recorded in the soybean seed sprouts group, followed by the non-sprouted soybean seed group (30.53±2.34 g).

Table (5): Mean organ weight/body weight (%) of experimental rats which induced Oxidative stress by paracetamol and treated with non-sprouted and sprouted red radish and soybean seeds

Organs	**Control (ve-)**	**Control (ve+)**	**Groups**			
			Red radish seed		**Soybean seeds**	
			Non-sprouted	**sprouted**	**Non-sprouted**	**sprouted**
Liver	2.98^{d}±0.4	4.04^{a}±0.56	3.68^{b}±0.85	3.33^{c}±0.34	3.37^{c}±0.87	3.34^{c}±0.4
Testis	1.6^{b}±0.13	1.98^{a}±0.22	1.92^{a}±0.21	1.38^{c}±0.29	1.41^{c}±0.19	1.61^{b}±0.08

Organs weight / body weight % of experimental rats which induced Oxidative stress by paracetamol and treated with non-sprouted and sprouted red radish and soybean seeds

Data are presented as means ± SDM(n=6). Data in a row with different superscript letters are statistically different (P ≤ 0.05)

Results in **Table (5)** indicated that the weight of liver and testis in the positive group were higher than the treatment groups with non-sprouted and sprouted red radish seeds and soybean seeds. Results indicated that liver and testis weight in non- sprouted group are higher than in sprouted groups. This weight gain in these groups may be due to the increased

carbohydrates and fat in the non-sprouted seeds groups compared to the sprouted seeds groups. Oxidative stress and inflammation are the leading causes of liver disease, according to several studies **(Reyes-Gordillo et al., 2017). Simona et al., (2020)** illustrated that Common liver disorders such as steatosis, fibrosis, and cirrhosis can alter organ weight and size, although the exact relationship is unknown.

Biochemical analysis

Table (6): Liver enzymes(U/l) of experimental rats which induced Oxidative stress by paracetamol and treated with non-sprouted and sprouted red radish and soybean seeds

Parameters (U/I)	Control (ve-)	Control (ve+)	Groups			
			Red radish seed		Soybean seeds	
			Non-sprouted	sprouted	Non-sprouted	sprouted
AST	60.5^{f}±1.53	149.33^{a}±0.99	114.00 ^{b}±2.08	٦٥.33^{d}±1.5	٧8.33^{c}±1.52	64.00 ^{e} ±1.15
ALT	٢٢.33^{e}±1.52	٦٥.66^{a}±0.77	٥٩.66^{b}±1.51	٢5.66^{d}±1.15	27.01c±1.56	21.66f±2.3
T. protein	6.4^{a}±٠.36	4.03^{d}±0.25	4.43^{d}±0.51	٦.7^{a}±٠.36	٥.16^{c}±1.01	٥.9٦^{b}±٠.15

Data are presented as means ± SDM(n=6). Data in a row with different superscript letters are statistically different (P ≤ 0.05). AST: aspartate amino transferase; ALT: alanine amino transferase

Table (6) recorded that administrated with paracetamol caused acute liver damage. Total protein had a significant decrease (P≤0.05). On contrary, liver enzymes had a significant increase (P≤0.05) in in rats administered with paracetamol (control +) compared with (control -) group.

Lancaster et al., (2015) confirmed that the most prevalent cause of acute liver failure (ALF) is paracetamol hepatotoxicity, which remains a public health problem and often leads to urgent liver transplantation.

The best result of AST in groups treatment with sprouted soybean (64.00 ±1.15) followed by sprouted red radish seeds (٦٥.33±1.5). The best result of ALT was in the sprouted soybean group (21.66±2.3).

Table (7): Kidney function (mg/dl) of experimental rats which induced Oxidative stress by paracetamol and treated with non-sprouted and sprouted red radish and soybean seeds

Parameters (mg/dl)	Control (ve-)	Control (ve+)	Groups			
			Red radish seed		Soybean seeds	
			Non-sprouted	sprouted	Non-sprouted	sprouted
Creatinine	$0.41^{d}\pm0.05$	$0.٩1^{a}\pm0.06$	$0.٨3^{b}\pm0.07$	$0.٤٣^{d}\pm0.03$	$0.56^{c}\pm0.06$	$0.6^{c}\pm0.01$
Urea	$36.43^{e}\pm4.93$	$66.8^{a}\pm3.3$	$٦٤.16^{b}\pm8.93$	$٣٩.5^{d}\pm4.32$	$49.9^{c}\pm6.33$	$٤7.06^{d}\pm2.33$
Uric acid	$3.57^{d}\pm0.44$	$5.94^{a}\pm0.64$	$٥.٠9^{ab}\pm0.67$	$3.61^{d}\pm0.59$	$4.47^{b}\pm0.15$	$٤.٣1^{c}\pm0.29$

Data are presented as means ± SDM(n=6). Data in a row with different superscript letters are statistically different ($P \leq 0.05$)

Results showed an increase in the mean values of uric acid, urea nitrogen and creatinine with increasing the level of protein in the diet. **Frey (2007)** reported that There are two cases result in rising serum urea nitrogen level: using large amounts of protein or there is a problem in kidney function.

Results in **table (7) also** showed the mean values of urea and creatinine decreased ($p<0.05$) in all tested rat groups that fed on sprouted red radish seeds followed by non-sprouted soybean seeds and sprouted soybean seeds. A substantial reduction ($P < 0.05$) was found in sprouted red radish seeds. As compared to the control (+) group.

(Yokozawa et al 2003) indicated that there are two ways for identifying if there is Kidney disorder either by urea concentration level analysis or by Blood urea nitrogen concentration due to disease severity

Table (8): MDA, GSH and CAT levels on liver and testis tissues of experimental rats

Parameters	Control (ve-)	Control (ve+)	Groups			
			Red radish seed		Soybean seeds	
			Non-sprouted	sprouted	Non-sprouted	sprouted
Liver						
MDA (µ mol/g. tissue)	٩٥.14f±1.22	١٤٢.24^{a}±1.11	112.72^{b}±0.9	96.27e±0.47	102.68^{c}±1.24	99.61d±0.47
GSH (µ mol/g. tissue)	٣٥٣.53^{a}±1.40	١٧٠.44f±3.26	301.32e±2.69	349.16^{b}±1.62	320.9d±0.86	332.73c±1.62

CAT $s^{-1}g^{-1}$	173.99[a]±1.69	28.45f ±0.54	112.99e±0.96	161.67b±1.6	141.63d±1.6	154.08c±1.92
Testis						
MDA (µ mol/g. tissue)	114.4f±3.92	353.98[a]±1.1	134.48[b]±1.75	116.28e±9.36	120.16d±0.85	129.9[c]±2.4
GSH (µ mol/g. tissue)	٤6.14[a]±3.06	٢١.45e±2.11	34.77d±0.31	43.46b±2.06	39.82c±3.88	41.29c±1.17
CAT $s^{-1}g^{-1}$	3.84[a]±0.05	2.24e±0.05	2.70d±0.05	3.53b±0.29	2.89c±0.41	3.49b±0.16

Data are presented as means ± SDM(n=6). Data in a row with different superscript letters are statistically different ($P \leq 0.05$). MDA: malondialdehyde; GSH: Reduced glutathione; CAT: Catalase

Lipid peroxidation (MDA, GSH and CAT) levels on liver and testis tissues in **table (8**) indicated that MDA is a biomarker for oxidative stress. The highest value of MDA found in the positive control group (1٤٢.24±1.11) in liver and (353.98±1.1) in Testis compared to the all treatment groups. Testis is higher than liver in MDA content and thus may be due to the antioxidant defense systems are insufficient to counteract the negative consequences of excess reactive oxygen species (ROS) (**Dutta et al., 2020).** On contrary GSH and CAT in liver were found to be higher than GSH and CAT in testis.

Allameh (2023) illustrated the reasons that liver cells have extensive antioxidant defense systems that include both enzymatic and non-enzymatic components, allowing them to keep ROS levels below physiological limits. **Casas-Grajales and Muriel, (2015)** also reported that antioxidant enzymes include Catalase (CAT), Glutathione (GSH) and dietary antioxidants, such as the role of flavonoids in protection against oxidative stress.

Histopathological examination:

Liver:

The sections of liver showed normal histological architecture of hepatic lob by using Light microscopic examination in negative control group. In adverse the result showed, liver sections from control (Ve+) group showed steatosis of hepatocytes (black arrow) and congestion of hepatic sinusoids (red arrow), hyperplasia of biliary epithelium (black arrow) and the infiltration of portal with inflammatory cells (red arrow) as seen in (Fig.1). Oxidative stress and activating ROS-sensitive cells have

been shown to dramatically impair normal cellular function and induce inflammation **(Roehlen et al., 2020 and**, **Ivanov et al., 2017).** Because of mitochondrial dysfunction, oxidative stress induces by the HBx protein of HBV **(Lee et al., 2004).** It enhances hepato carcinogenesis, even without liver fibrosis **(Yang et al., 2021 and Zhang et al., 2021).**

Meanwhile, the liver of rats from 10% red radish seed group described slight hydropic or vacuolar degeneration of hepatocytes, necrosis of sporadic hepatocytes (black arrow) and activation of Kupffer cells (red arrow) in (Fig.1). On the other hand, the liver of rats from 10% of sprouted red radish seed group exhibited no histopathological damage (Fig.1). Furthermore, rats liver from 20% soybean seeds group revealed slight vacuolar degeneration of hepatocytes (black arrow) (Fig.1). Moreover, liver of rats from 20% sprouted soybean seeds group exhibited no histopathological damage as seen in (Fig.1).

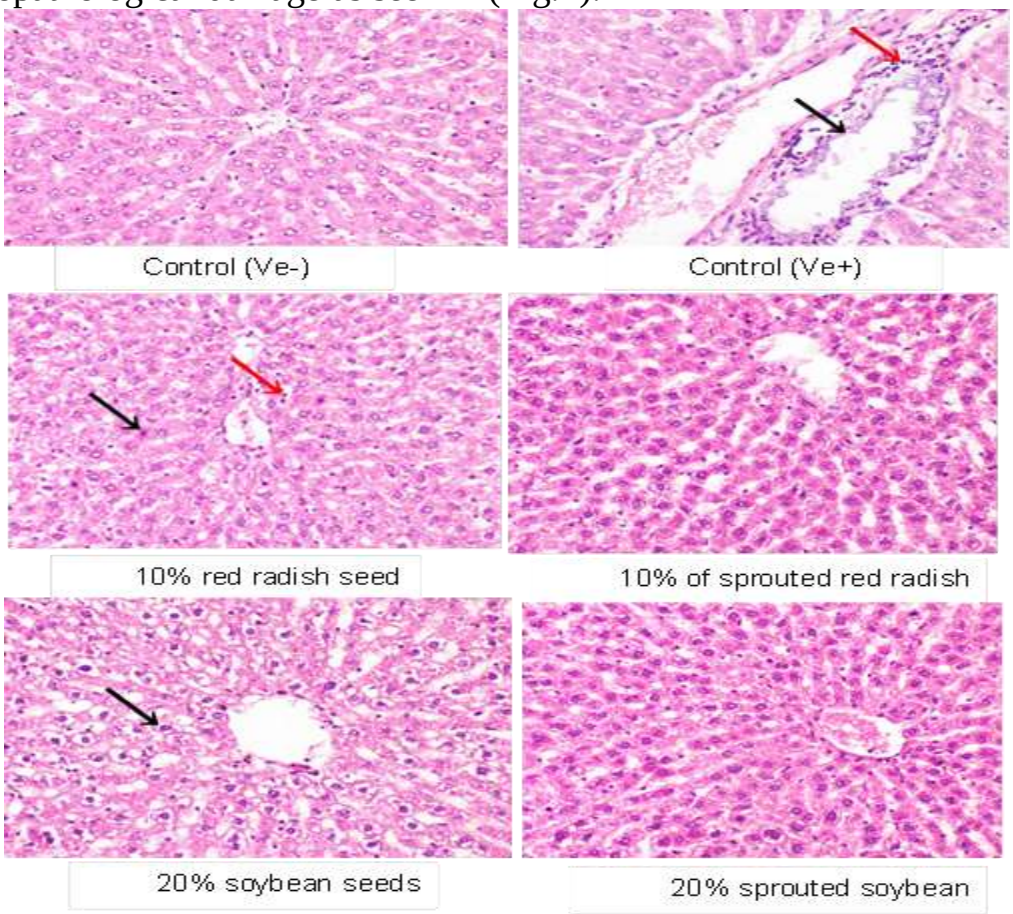

Fig. (1): Photomicrograph of Sections of liver for different rats' groups stained with H & E, X 400.

Testis:

Testis was taken because, it is one of the most organs affected by oxidative stress. **Nematollah Asadi et al., (2017**) indicated that oxidative stress connected to sperm abnormalities and male infertility. Because of the rapid rate of cell division and mitochondrial oxygen consumption in testicular tissue, which also has greater quantities of unsaturated fatty acids compared to other tissues. Also **Sengupta et al., (2024)** reported that elevated ROS levels significantly impact sperm quality. Spermatozoa are vulnerable to ROS because they lack DNA repair mechanisms and have large levels of polyunsaturated fatty acids in their membranes.

The histological structure of the seminiferous tubule in the testis of rats from the control (Ve-) group was normal, with normal spermatogoneal cells and complete spermatogenesis, as observed under a microscope. In contrast, the testis of the control (Ve+) group exhibited degeneration of spermatogoneal cells that line seminiferous tubules (black arrow), as illustrated in Figure 2.

Maintaining redox equilibrium is critical for healthy sperm function. An disparity in the formation and removal of reactive oxygen species (ROS) can lead to oxidative injury and worse sperm quality **(Kothari et al., 2010).**

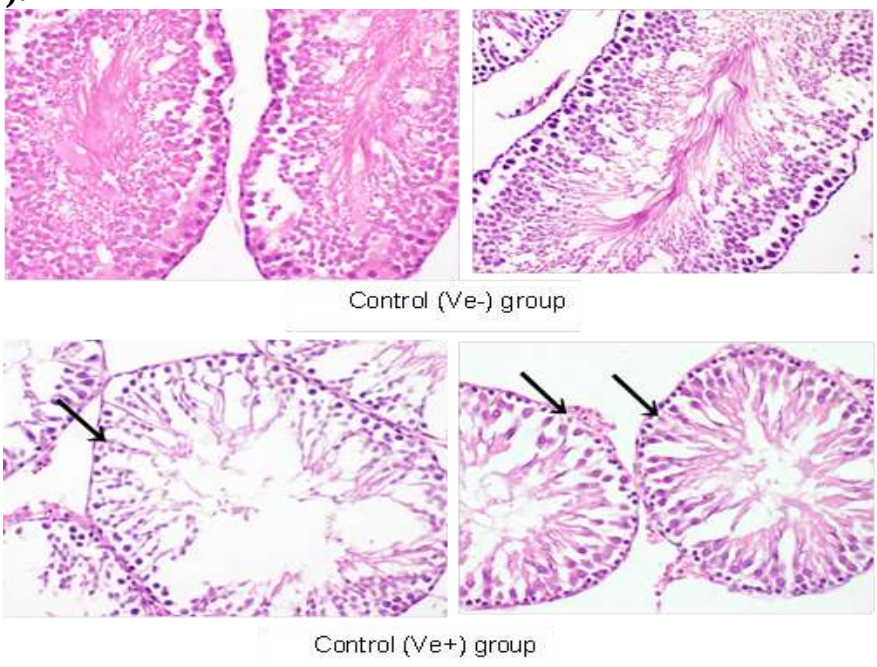

Fig. (2): Photomicrograph of Sections of Testis for control (ve-) group and control (ve+) group, stained with H & E, X 400

Meanwhile, some examined sections from 10% red radish seed group showed slight degeneration of spermatogoneal cells lining some seminiferous tubules (black arrow), whereas other sections from group 10% sprouted red radish seed group as well as sections from 20% soybean seeds group and 20% sprouted soybean seeds group exhibited normal seminiferous tubules with no histopathological alterations (Figs. 3).

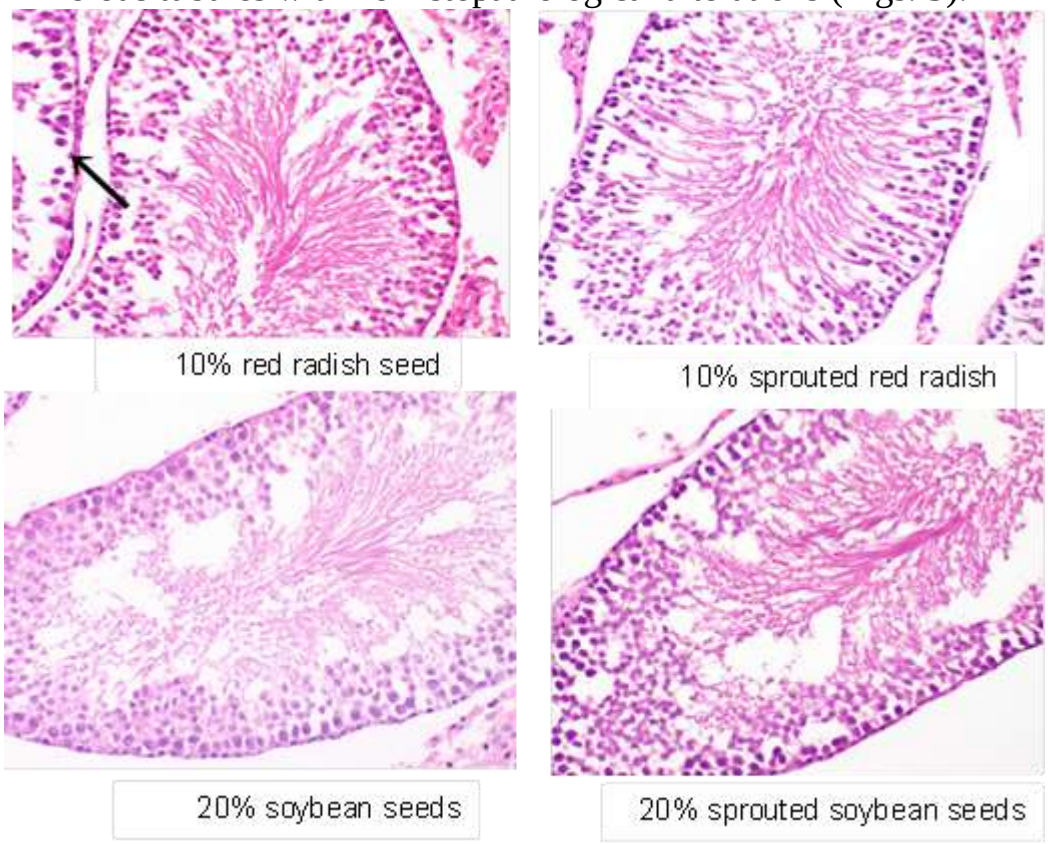

Fig. (3): Photomicrograph of Sections of Testis for different rats' groups stained with H & E, X 400.

Conclusion

An evaluation of sprouted and non-sprouted red radish and soybean seeds demonstrates a considerable increase in antioxidant capacity after germination. Sprouting induces metabolic changes that increase the synthesis and bioavailability of polyphenolic compounds, particularly flavonoids, which are known for their powerful free radical scavenging properties. These phytochemicals serve to minimize the oxidative stress. Experimental results show that soybean sprouts and seeds, followed by red radish sprouts, have high antioxidant activity, as seen by elevated levels of total phenolic, total flavonoid, and superior performance in antioxidant

tests like DPPH. As a consequence, incorporating sprouted seeds into your diet may operate as a natural, functional way to battle oxidative stress and related ailments, making them an essential source of dietary antioxidants.

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