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Tel: 02/26844594

Web Site :

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Email :

egyjournal@sedu.asu.edu.eg

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سعادة أ. د. رئيس تحرير المجلة المصرية للدراسات المتخصصة المحترم
جامعة عين شمس، كلية التربية النوعية، القاهرة، مصر
تحية طيبة وبعد،،،

يسر معاميل التأثير والاستشهادات المرجعية للمجلات العلمية العربية (أرسياف - ARCIF)، أحد مبادرات قاعدة بيانات "معرفة" للإنتاج والمحتوى العلمي، إعلامكم بأنه قد أطلق التقرير السنوي التاسع للمجلات للعام 2024.

ويسرنا تهنئكم وإعلامكم بأن المجلة المصرية للدراسات المتخصصة الصادرة عن جامعة عين شمس، كلية التربية النوعية، القاهرة، مصر، قد نجحت في تحقيق معايير اعتماد معاميل "أرسياف Arcif" المتوافقة مع المعايير العالمية، والتي يبلغ عددها (32) معياراً، وللاطلاع على هذه المعايير يمكنكم الدخول إلى الرابط التالي: <http://e-marefa.net/arcif/criteria>

وكان معاميل "أرسياف Arcif" العام لمجلتكم لسنة 2024 (0.4167).

كما صنفت مجلتكم في تخصص العلوم التربوية من إجمالي عدد المجلات (127) على المستوى العربي ضمن الفئة (Q3) وهي الفئة الوسطى، مع العلم أن متوسط معاميل "أرسياف" لهذا التخصص كان (0.649).

وبإمكانكم الإعلان عن هذه النتيجة سواء على موقعكم الإلكتروني، أو على مواقع التواصل الاجتماعي، وكذلك الإشارة في النسخة الورقية لمجلتكم إلى معاميل "أرسياف Arcif" الخاص بمجلتكم.

ختاماً، نرجو في حال رغبتكم الحصول على شهادة رسمية إلكترونية خاصة بنجاحكم في معاميل "أرسياف"، التواصل معنا مشكورين.

وتفضلوا بقبول فائق الاحترام والتقدير

أ.د. سامي الخزندار
رئيس مبادرة معاميل التأثير
"أرسياف Arcif"



+962 6 5548228 -9
+962 6 55 19 10 7

info@e-marefa.net
www.e-marefa.net

Amman - Jordan
2351 Amman, 11953 Jordan

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- Effect of Sprouted Red Radish Seeds (Raphanus Sativus) Against Gentamicin - Induced Nephrotoxicity in Rats

Prof. Zenab Mostafa Mosa
Prof. Eman Mohamed El-metwally
A. Prof. Safaa Talaat Gohari Mohamed
Hadeer Magdy Ahmed Abdullah

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Prof. Zenab Mostafa Mosa ^(١)

Prof. Eman Mohamed El-etwally ^(٢)

A.Prof. Safaa Talaat Gohari ^(٣)

Hadeer Magdy Ahmed Abdullah ^(٤)

-
- (1) Prof. of Food Sci.& Nutrition, Home Economic Dept. Fac. Of Specific Edu., Ain Shames University
(2) Prof. of Food Sci.& Nutrition, Home Economic Dept. Fac. Of girl's college., Ain Shames University
(3) Assistant Prof. of Food Sci.& Nutrition, Home Economic Dept. Fac. Of Specific Edu., Ain Shames University
(4) Research of Home Economic Dept. Fac. Of Specific Edu., Ain Shames University

Effect of Sprouted Red Radish Seeds (Raphanus Sativus) Against Gentamicin- Induced Nephrotoxicity in Rats

Prof. Zenab Mostafa Mosa

Prof. Eman Mohamed El-metwally

A. Prof. Safaa Talaat Gohari Mohamed

Hadeer Magdy Ahmed Abdullah

Abstract

The present study aimed to evaluate the effect of germinated radish seeds against gentamicin- induced nephrotoxicity in rats. Results showed that the germinated seeds content high amount of carbohydrates, protein, fiber and ash and a decrease in their content of fats compared to non-germinated seeds. Germinated seeds are rich in total flavonoids, total phenolics and antioxidants. Also, sprouted red radish seeds mitigate the GM-inflicted liver and kidney damage.

Keywords: Sprouted Red Radish, Raphanus Sativus, Gentamicin- Induced Nephrotoxicity

ملخص:

العنوان : تأثير بذور الفجل الأحمر المنبتة ضد السمية الكلوية الناتجة عن الجنتاميسين في اناث الفئران

المؤلفون : زينب مصطفى موسى ، إيمان محمد المتولي ، صفاء طلعت جوهري ، هدير مجدي أحمد عبد الله

هدفت الدراسة الحالية إلى تقييم تأثير بذور الفجل المنبتة ضد السمية الكلوية المحدثة بالجنتاميسين في الجرذان. أظهرت النتائج أن البذور المنبتة تحتوي على كمية عالية من الكربوهيدرات والبروتين والألياف والرماد وانخفاض في محتواها من الدهون مقارنة بالبذور غير المنبتة. البذور المنبتة غنية بالفلافونويدات والفينولات ومضادات الأكسدة. كما أن بذور الفجل الأحمر المنبتة تخفف من تلف الكبد والكلية الناتج عن الجنتاميسين.

الكلمات الدالة : بذور الفجل الأحمر ، السمية الكلوية ، الجنتاميسين.

Introduction

Nephrotoxicity is the most common kidney problems and occurs when body is exposed to a drug or toxin. When kidney damage occurs, body unable to get rid of excess urine and wastes from the body and blood electrolytes (such as potassium and magnesium) will all become elevated (**Konam and Yalamuri, 2014**). Gentamicin (GM) is a commonly utilized aminoglycoside antibiotic as a therapy for severe gram-negative bacterial infections and possesses a higher potency compared to other aminoglycosides (**Nafiu et al., 2019**). Despite its wide therapeutic uses, it is well renowned for giving rise marked cytotoxicity, including renal and hepatic toxicity (**Al-khamas et al., 2020**). The liver and kidney are highly susceptible to drug toxicities due to its contribution in metabolic pathways, detoxification, and excretion of drugs and their metabolites (**Yarijani et al., 2019**).

The pathogenesis of GM-induced injury is multi-factorial; however, the overgeneration of harmful free radicals with subsequent oxidative damage plays a major role in GM mediated-renal and hepatic tissue injuries (**Arjinajarn et al., 2017**). The generated of reactive oxygen species ROS by GM can directly attack the cellular components resulting in lipid peroxidation (LPO), protein denaturation, and DNA damage. These events are known to be accompanied by exhaustion of endogenous enzymatic and non-enzymatic antioxidant defense mechanisms. These mechanisms dedicate the potential use of antioxidant supplementations to counteract the GM-induced oxidative damage (**Ahmed et al., 2021**).

Radish seeds (*Raphanus sativus*) belong to cruciferous family. Radish seeds has been cultivated for thousands of years in both the Mediterranean region and China. In general radish seeds comprises carbohydrates, ether extract, dietary fiber and crude protein. (**Aly, 2015**). Radish contains very potent phytochemicals. Radish contains many mineral elements important to human health such as calcium Ca, potassium K, iodine I, sulfur S and magnesium Mg, and active secondary compounds with multiple

biological effects such as alkaloids, glycosides, glucosinolates, Phenolic compounds, tannins and sulforhane. Radish plant is rich in vitamin C and vitamin A. So, radishes are plants of high nutritional value as well as being appetizing and digesting. Radish plants (roots, leaves and seeds) have many medical uses, including treatment of tuberculosis, whooping cough, asthma attacks, laxative to treat constipation, Fracture of kidney and bladder stones, thinner, cholesterol-lowering, antiradical and analgesic pain ((**Richard *et al.*, 2010** and **Magda *et al.*, 2021**).

Germination resulted in a significant increase in the phytonutrient and micronutrient content of all the selected seeds, which proves that there is a noticeable increase in the nutritional value of the seeds on sprouting. This ultimately indicates that sprouts should be considered a vital component of the diet and can be combined to improve agricultural productivity and their use easily by low-income families (**Wagner *et al.*, 2013**).

Germinated radish seeds found to contain unique biologically active compounds that have health benefits for humans (**Aly, 2015**). Cruciferous sprouts are distinctive functional foods because of their rich in composition bioactive compounds compared to other plants. Germinated seeds may contain more than twice as various phytochemicals depending on varieties, species, and environmental conditions (**Baenasa *et al.*, 2017**). **Hanlon and Barnes (2011)** showed that, compared with the mature radish taproot, sprouts contained significantly higher concentrations of glucosinolates (3.8-fold), isothiocyanates (8.2-fold), and phenolics (6.9-fold). Sprouts are source of nutrients with antioxidant properties, which is of great importance to human health (**Andreas, 2022**). Therefore, the current investigation has been conducted to investigate the effect of sprouted red radish seeds against gentamicin- induced nephrotoxicity in rats.

Materials and Methods

Materials

Red radish seeds (*Raphanus sativus*) were obtained from Food Technology Research Institute, Agriculture Research Center, Egypt, during the 2022 season. Samples were identified and authenticated at Cairo University Research Park (CURP). The kits used for analysis were obtained from Bio-diagnostic Co. Dokki, Egypt. Gentamicin (GM) was obtained from Memphis Company for Pharmaceutical and Chemical industries, Cairo, Egypt. Casein (85% protein), cellulose, dextrose, choline chloride, DL-methionine, vitamins and salt mixture were obtained from Cairo Company Chemical Trading, Cairo, Egypt. Corn oil was obtained from the local market. Corn starch was obtained from Starch and Glucose Co. Helwan, Cairo, Egypt. Thirty-six female Albino rats (Sprague Dawley strain), was obtained from Agricultural Research Center, Giza, Egypt.

Methods

Preparation of sprouted red radish seeds.

The seeds were cleaned from all impurities and soaked for 16 h in water then the seeds were placed on a wet cotton tissue and covered the seeds by another wet tissue. The seeds were left to sprout in the dark at room temperature (25-30° C) and then sprouts were harvested after 3-4 days from seed sowing and applied for use in the study according to **Lotfy and Abd-Elrahman, (2021)**.

Chemical analysis

Proximate composition of non-sprouted and sprouted red radish seeds.

Moisture, protein, fat, crude fiber and ash were determined according to the method of **AOAC, (2015)**, and then the carbohydrate content was calculated by difference (**Fernandes et al., 2015**). All determinations were made in triplicate.

Total carbohydrates = 100 – (moisture + protein + fat + fiber + ash)

Estimation of total flavonoid content (TFC)

Total flavonoid content (TFC) was calorimetrically estimated by aluminum chloride assay as described by **(Han and May 2012)**. The TFC was expressed as mg rutin⁻¹ dry weight (mg rutin g⁻¹ DW) by the calibration curve of rutin. All samples were measured in triplicates.

Analyzing the total phenolic (TPC)

The total phenolic content (TPC) in the extracts of non-sprouted and sprouted red radish seeds was determined by the Folin–Ciocalteu reagent. Gallic acid was used as a standard and the total phenolic was expressed as µg/mg gallic acid equivalent to (GAE). All determination was performed in triplicate. The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue color upon reaction. This blue color was measured spectrophotometrically **(Chun *et al.*, 2013)**.

Antioxidant Activities:

Radical scavenging activity (RSA) by DPPH:

The effect of extracts on the free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was calculated using the procedure described by **Moure *et al.*, (2001)**.

Diet Preparation and Experimental Design

Thirty-six male albino rats weighing about 1[∕]0±20 g was kept in an atmosphere of filtered, pathogen-free air, water, and a temperature of 20-25°C, 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% fiber (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**). All the experimental procedures were carried out in accordance with international guidelines for the care and use of laboratory animals. The experiment was conducted at Agricultural Research Center, Giza, Egypt. The rats were weighed weekly through the experimental period.

After an acclimation period of one week, the animals were randomly divided into two main groups. The first group of rats control (ve-) (6 rats) was fed on standard diet, while the second group (30 rats) was fed on standard diet and received gentamicin (GM) at a dose rate of 80 mg / kg body weight of rat by intraperitoneal injection for 8 consecutive days to induce nephrotoxic (**Reddy *et al.*, 2011**), then rats were divided into five groups six rats each, and treated orally by different concentrations of non-sprouted and sprouted red radish seeds for 6 weeks. as follows: Group (2) the Positive control group (ve+) received only standard diet. Group (3) treated with 5% red radish seeds of diet / day. Group (4) treated with (10 % red radish seeds of diet / day). Group (5) treated with (5% sprouted red radish seeds of diet / day). While Group (6) treated with (10 % sprouted red radish seeds of diet / day).

At the end of the experiment, the animals were sacrificed for the collection of Kidney and liver samples for further analysis and the blood was collected in a clean dry centrifuge tube, left at room temperature until the clot was formed and then centrifuged to separate serum by centrifugation, followed by storage in a plastic vial (well stoppered) until analysis.

Biological evaluation

Feed intake was recorded every day and body weight was recorded every week. Biological evaluation of the different diets was carried out by calculating of body weight gain% (BWG %), feed efficiency ratio (FER) and organs weight as a percent of total body weight according to **Chapman *et al.*, (1959)** using the following equations: -

Feed intake (FI) = Initial weight of diet (g) – Left over diet weight (g)

BWG% = [(Final weight g - Initial weight g) / (Initial weight g)] X 100

FER= Gain in body weight / feed intake (g)

Relative organs weight ROW % = (Organ weight / Final weight) X 100

Biochemical analysis

Blood samples were withdrawn from orbital plexus venous by using fine capillary glass tubes, placed in centrifuge tubes without anticoagulant and allowed to clot. After the serum prepared by centrifugation (3000 rpm for 15 min), serum samples were analyzed by bio diagnostic kits:

A. Determination of some liver functions:

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined calorimetrically using spectrophotometer (model DU 4700) at 505 nm according to the method of **Reitman and Frankel, (1957)**. Total protein was determined at 550 nm according to the method described **Gornal et al., (1949)**. Total bilirubin was determined at 535 nm according to the method described **Walter and Gerade, (1970)**.

B. Determination of the kidney functions:

Serum uric acid was determined by **Barham and Trinder, (1972)** using spectrophotometer (model DU 4700) adjusted at 510 nm. Serum urea nitrogen was determined according to the method described by **Batton and Crouch, (1977)** using spectrophotometer (model DU 4700) adjusted nm 550 nm. Serum creatinine was determined by **Tietz, (1986)** using spectrophotometer (model DU 4700) adjusted at 510 nm.

C. Determination of the oxidative stress:

Serum Catalase (CAT) activity, Superoxide Dismutase (SOD) activity and malondialdehyde (MDA) were determined according to (Beutler *et al.*, 1963; Kakkar *et al.*, 1984 and Draper and Hadly, 1990) respectively.

Histopathological Examination:

The tissues sample from kidney were fixed immediately after dissection in 10% neutral formalin for 24 h, then collected and dehydration was done on concentration of alcohol, cleaned in oxyline and embedded in paraffin wax. Tissues were sectioned at a thickness of 3 micron and stained with hematoxylin and eosin stains (Banchroft *et al.*, 1996) and examined by the light microscope for detection of any histopathological alteration.

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 $\pm$ SD. Values were considered significant at $P \leq 0.05$, otherwise were considered non-significant.

Results and Discussion

Proximate chemical composition of non-sprouted and sprouted red radish seeds on the dry weight basis:

The data tabulated in Table (1) showed the Proximate composition (g/100g) of non-sprouted and sprouted red radish seeds. It can be noticed that non-sprouted red radish seeds contained moisture, protein, fat, ash, fiber, and carbohydrates with values (4.27, 23.46, 33.79, 4.40, 14.47 and 19.61, respectively). While the values of sprouted red radish seeds were (8.64, 28.96, 2.65, 10.31, 18.79 and 31.65, respectively). These

results are like **Amira and Wesam, (2021)** that reported the chemical composition of red radish sprouts (g/100g) on the dry weight basis as follows (moisture 4.15, protein 31.30, fat 1.08, ash 3.57, fiber 14.10 and carbohydrates 59.85).

In our results the fat content was 33.79% of non-sprouted red radish seeds, like those reported by **Mohamed *et al.*, (2022)** who found that radish seeds contained 36.80 %, **Bingjun *et al.*, (2017)** found that radish seeds contained 37.00-43.00 % (w/w) oil whereas **Uluata and Özdemir (2012)** reported that the seeds contained $43.12 \pm 0.34\%$ fat. On the other hand, other studies have reported that germination reduces fat content (**Jan *et al.*, 2017; Wanasundara *et al.*, 1999**) due to hydrolysis and utilization of fats as an energy source for biochemical reactions during germination (**Chinma *et al.*, 2009; Jan *et al.*, 2017; Moongngarm and Saetung, 2010**).

From obtained results, it can be noticed that germination increased moisture, protein, ash, fiber and carbohydrates while decrease fat content. These results are in agreement with **Aly *et al.*, (2018) and Elshahat, (2019)** that indicated the content of germinated radish for crude protein and ash, increases markedly in comparison to non-germinated seeds on dry weight basis, the increase crude protein content in germinated seeds might be due to reduction of seed nitrates such as lipids into plant protein (metabolic enzymes), and also loss in dry weight through respiration during germination. The results showed a decrease in the fat content in radishes sprouts compared to non-germinated seeds. Also, may be due to the using of lipid to produce energy which necessitated for protein biosynthesis process (**Abdallah, 2008**). The content of germinated seeds of total carbohydrates and fibers was increased compared to non-germinated seeds. The results agree with (**Islam *et al.*, 2019**) reported that chemical composition of radish seeds content 22.40 % protein, 3.66 % ash, 38.69 % ether extract, 18.68 % crude fiber and 30.15 % total carbohydrates While, germinated radish seeds 35.80 % protein,

9.38 % ash, 8.69 % total lipid 14.68 % crude fiber and 45.26 % total carbohydrate.

Also, our data agree with **Amira and Wesam, (2021)** that demonstrate the crude protein and carbohydrate content in red radish sprouts RRS recorded the highest percent. Data also showed a higher content of fibers in RRS indicating that the sprouts can be consumed as dietary fiber (DF) supplements. Where many researchers showed that germination can enhance protein and DF contents, decrease the tannin and phytic acid contents, and increase the vitamins concentration, bioavailability of minerals, and trace elements (**Ghavidel and Prakash (2007), Khattak et al., (2007), Kaushik et al., (2010) and Khandelwal et al., (2010).**

Compared to the seeds it was established that the sprouts possess higher nutritional value due to its transformed protein content, which is of higher biological value, the higher polyunsaturated fatty acid content, higher vitamins and minerals content. During the germination the polysaccharides degrade into oligo- and monosaccharides, the fats into free fatty acids, whereas the proteins into oligopeptides and free amino acids, which processes support the biochemical mechanisms in our organism. They improve the efficiency of both the protein-decomposing and the carbohydrate and fatty acid decomposing enzymes therefore germination can be considered as one kind of predigesting that helps to break down the high-molecular complex materials into their building blocks (**Marton et al., 2010).**

Abdallah, (2008) reported that, development of food products from germinated Egyptian radish may be promising in Egyptian human foods for their nutritional advantage due to low content of fats and high protein utilization. Moreover **Salama et al., (2014)** reported that radish sprout at cotyledon stage grown in clay medium are good source of protein, fiber and vitamin C with low content of lipid and sugars.

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

Constituents (%)	Red radish seeds	
	Non-sprouted	Sprouted
Moisture Content	4.27±0.11 ^b	8.64±0.05 ^a
Crude Protein Content	23.46±0.56 ^b	28.96±0.33 ^a
Crude fat Content	33.79±0.05 ^a	2.65±0.49 ^b
Ash	4.40±0.08 ^b	10.31±0.28 ^a
Crude fiber	14.47±0.10 ^b	18.79±0.20 ^a
Carbohydrates	19.61±0.59 ^b	31.65±0.70 ^a

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 ($P \leq 0.05$).

Total flavonoids, total phenolic acid contents and antioxidant activities by DPPH in non-sprouted and sprouted red radish seeds:

Data presented in Table (2) showed that the highest content was of total phenolic acids followed total flavonoids then DPPH% of both non-sprouted and sprouted red radish seeds. The values were (181.27, 232.11, 123.17, 207.67, 71.98 and 88.87, respectively). These results are like **Amira and Wesam, (2021)** that found total phenolic 185±0.30, total flavonoids 126±0.30 for Red Radish Seeds while total phenolic 234±0.20, total flavonoids 209±0.10 for Red Radish sprouts.

It can be noticed that the germination increased the values of both total flavonoids, total phenolic acid contents and antioxidant activities by DPPH. These results agree with **Paulina et al., (2014)** that reported germination increased the total phenolic and flavonoid levels, as well as the antioxidant activity of the seeds, and influenced the profile of free and bound phenolic compounds.

The current results accord with the results of **Devaraj et al., (2011)** who noticed that the ethanolic extract of radish leaves and its fractions showed the existence of carbohydrates, proteins, tannins, alkaloids, saponins, flavonoids, and glycosides in the phytochemical assessment. As well, according to **Mute et al., (2011)**, the aqueous extract of radish root found to contain

triterpenes, flavonoids, alkaloids, saponins, and coumarin. Glucoraphasatin, a glucosinolate, was mainly observed in red radish roots and sprouts as reported by **Montaut *et al.*, (2010)**. Many authors also stated that RRS has a high concentration of the main glucosinolates (GLSs) (i.e. glucoraphasatin (4-methyl thio-3-butenyl) and glucoraphenin). Glucoraphenin is hydrolyzed to the isothiocyanates (ITCs) sulphoraphene (SFE), a bioactive compound that assists to decrease the oxidative stress of cell and provide santimutagenic activity antimany malignant cell types (**Bhandari *et al.*, 2015, Baenasa *et al.*, 2017 and Abellán *et al.*, 2019**). Moreover **Salama *et al.*, (2014)** reported that radish sprouts are good source of total phenols, total flavonoids and vitamin C.

Germination increases total phenolics, flavonoid, and condensed tannin contents (**Zhang *et al.*, 2015**). Phenylalanine ammonia-lyase PAL, the enzyme that catalyzes the pathways responsible for biosynthesis of the different phytochemicals, is responsible for limiting the rate of biosynthesis of phenolic acids and flavonoids. PAL activity is enhanced during the process of germination. Another possible explanation is that there could be hydrolysis of bound phenolic compounds as well as de novo biosynthesis of phenols in the embryonic axis of the sprouts. The increased content of phytochemicals results into increased antioxidant activity of germinated cereals and legumes (**Onyango *et al.*, 2013; Zilic *et al.*, 2015 and Smith *et al.*, 2018**).

The results agree with (**Paulina *et al.*, 2014**) indicated that germinated radish seeds had higher total phenolic (TP), total flavonoid (TF) and antioxidant activity content in compared to non-germinated radish seeds. Therefore, germinated edible seeds are a very valuable source of natural antioxidants and sprouts could be used as ingredients in functional foods. **Pasko *et al.*, (2009)** also reported that higher total phenolic content in germinated seeds compared with non-germinated seeds, suggesting that synthesis of phenolic antioxidants during germination may occur, it is thought that seeds mainly act as a

reservoir for the development of the sprouts (Elshahat, 2019). Moreover, **Tork, et al. (2019)** stated that germination significantly increased the micronutrients and phytonutrient contents of radish seeds, hence verifying an obvious increase in the nutritive value of the sprouting seeds.

Table (2) Total flavonoids, total phenolic acid contents and antioxidant activities by DPPH in non-sprouted and sprouted red radish seeds.

Constituents	Red radish seeds	
	Non-sprouted	Sprouted
Total flavonoids (mg-1rutin)	123.17±1.26 b	207.67±0.58 a
Total phenolic acids (mg-1 Gallic acid)	181.27±1.10 b	232.11±1.00 a
DPPH%	71.98±0.98 b	88.87±0.97 a

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 ($P \leq 0.05$).

Biological evaluation of non-sprouted and sprouted red radish seeds on experimental rats induced nephrotoxicity by gentamicin.

The mean values of initial weight, final weight, body weight gain % of experimental rats treated with non-sprouted and sprouted red radish seeds tabulated in table (3) showed that, the initial weight of all groups had similar values to that of the control (ve-) group. The weights ranged from 176.02 to 180.83 g., where there was none statistically significant difference among groups.

The positive control group had significant ($P \leq 0.05$) decrease in final body weight, body weight gain (g), body weight gain %, feed intake and FER compared with control negative group, while all treatment groups that treated with non-sprouted and sprouted red radish seeds showed significant ($P \leq 0.05$) increase in these parameters compared to positive control group. These data agree with **Ali et al., (1992)** that found gentamicin reduced the body weights of rats in a dose-dependent manner. The weight reductions were most marked on days 4, 5 and 6 of the treatment. Another study by **Venkatesha and Veeru (2019)** recorded that reducing body weight and food consumption

was noticed on Day 7 in rats treated with 100 mg/kg of gentamicin, the reduction in body weight is related to nephrotoxicity as demonstrated in histological changes.

Table (3): Mean body weight gain (g), body weight gain %, feed intake and feed efficiency ratio (FER) of experimental rats which treated with non-sprouted and sprouted red radish seeds.

Groups	Parameters					
	Initial weight (g)	Final weight (g)	Weight gain (g)	Weight gain (%)	Feed intake(g)	FER
Control -ve	176.02±4.34 a	236.33±8.38b	60.31±5.3 b	34.26±2.25 b	17.99±1.5 a	0.060±0.006 c
Control +ve	178.17±6.18 a	197.29±5.98 c	19.12±1.26 c	10.73±1.32 c	14.44±1.19 c	0.024±0.002 d
5 % RRS	178.67±7.42 a	247.19±9.82 a	68.52±4.3 a	38.35±3.04 a	15.95±1.35 b	0.077±0.007 ab
10 % RRS	180.83±8.28 a	239.94±8.42 b	59.11±3.2 b	32.69±2.33 b	15.98±1.41b	0.066±0.005 c
5 % Sprouted RRS	179.83±7.94 a	249.51±9.36 a	69.68±4.4 a	38.75±2.94 a	16.34±1.52 b	0.076±0.004 ab
10 % Sprouted RRS	178.51±8.24 a	251.17±9.18 a	72.66±5.7 a	40.70±3.09 a	16.31±1.42 b	0.080±0.006 a

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 ($P \leq 0.05$). RRS: Red radish seeds IBW= Initial body weight; FBW= Final body weight; BWG= Body Weight gain

Relative organs weight of experimental rats treated with non-sprouted and sprouted red radish seeds.

Data of mean organ weight/body weight (%) of experimental rats which treated with non-sprouted and sprouted red radish seeds represented in table (4) recorded that, the positive control group showed significant increase in relative liver and kidney weight organs (3.90 ± 0.72 and 0.94 ± 0.10 , respectively) compared to negative control group (2.63 ± 0.19 and 0.68 ± 0.03 , respectively).

These results are agreed with **Venkatesha and Veeru (2019)** that recorded kidneys were enlarged and perirenal edema in rats treated with gentamicin. These findings were correlated with organ weight increase.

Treatment with non-sprouted and sprouted red radish seeds significantly decreased the liver weight and kidney weight % when compared to the positive control group. Interestingly, the best result was group 10% sprouted RRS where, there was no significant difference of liver and kidney organs weight % compared to negative control group. The results are agreed with

Elshahat (2019) that showed decrease in the kidney and liver weight in the groups feeding on germinated radish seeds compared to the control group.

Table (4): Relative organ weight of experimental rats treated with non-sprouted and sprouted red radish seeds.

Groups	Organ's weight (%)	
	liver	kidney
Control (ve-)	2.63±0.19 c	0.68±0.03 c
Control (ve+)	3.90±0.72 a	0.94±0.10 a
5 % RRS	3.11±0.98 b	0.79±0.08 b
10 % RRS	2.88 ±0.48 b	0.74±0.02 b
5 % Sprouted RRS	3.06±0.37 b	0.77±0.09 b
10 % Sprouted RRS	2.71±0.27 bc	0.70±0.06 bc

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 ($P \leq 0.05$). RRS: Red radish seeds

Biochemical analysis

Effect of non-sprouted and sprouted red radish seeds on some liver functions of rats induced nephrotoxic by gentamicin.

Data presented in Table (5) showed the effect of treatment with non-sprouted and sprouted red radish seeds. The data revealed that aspartate transaminase (AST) and alanine transaminase (ALT) activities and total bilirubin were elevated significantly by gentamicin administration, while, there was significant ($P \leq 0.05$) decrease in serum total proteins the values were (123.6±9.94, 63.4±6.91, 0.66±0.08 and 3.20±0.69, respectively) compared to negative control (89.01±6.44, 42.60±4.45, 0.32±0.09 and 6.75±0.29, respectively). In agreement with our results, **Ahmed *et al.*, (2021)** found that GM evoked significant alterations in liver function biomarkers.

Significant ($P \leq 0.05$) reduction was observed in activities of AST, ALT enzymes and total bilirubin for all treatment groups treated with non-sprouted and sprouted red radish seeds compared to positive control group. Whereas there was significant increasing of serum total protein for all treatment groups

compared to positive control group. Also, our results showed an improvement in all the treated groups compared to negative control groups for liver functions. The best result was in 10 % sprouted RRS group compared to negative control group.

These results accorded with previous findings of **Kalantari et al., (2009)** who studied the protective effect of radish seeds in liver toxicity motivated by carbon tetrachloride in mice and observed that crude extract of radish seeds in a dose of 600 and 800 mg/kg⁻¹ may be sufficient to protect liver damage. In addition, **Sadeek, (2011)** noticed that the pretreatment with radish juice significantly restored the liver enzyme activities; AST, ALT, and ALP, to the normal level and counter CCl₄- caused hepatotoxicity and oxidative stress in rats. **Aly et al., (2015)** showed that the treatment with radish sprout improved the liver and kidney functions. **Elshahat, (2019)** showed that rats fed germinated radish seeds powder reduced AST, ALT, ALP and bilirubin levels compared to positive and negative control, which shows that radish sprouts have an effective role in improving liver function. In addition, **Amira and Wesam, (2021)** reported that there was a highly significant reduction in liver enzyme; AST and ALT by red radish sprouts treating.

Non-sprouted and sprouted red radish seeds could mitigate the GM-inflicted liver damage, in a dose-response pattern, due to its high content of phenolics and flavonoids. Numerous studies have proven that herbs and plant products have an effective role in protecting the liver, due to their antioxidant properties as a source of ascorbic acid and a sulfur-containing compound, radish seeds can compete with free radicals in cell components and protect the liver from infection (**Naghibi et al., 2003**). Research stated that methanol extract from germinated radish seeds had higher activity than L-ascorbic acid in protecting the liver because it was rich in antioxidants like flavonoids and sinapinic acid esters and was responsible for this activity (**Takaya et al., 2003**).

Table (5): Liver functions of all experimental rats which treated with non-sprouted and sprouted red radish seeds.

Groups	Parameters			
	AST(U/L)	ALT(U/L)	Total Bilirubin (g/dl)	Total Protein (g/dl)
Control (ve-)	89.01±6.44 c	42.60±4.45 c	0.32±0.09 c	6.75±0.29 a
Control (ve+)	123.60±9.94 a	63.40±6.91a	0.66±0.08 a	3.20±0.69 b
5 % RRS	104.61±8.13 b	53.64±5.32 b	0.45±0.04 b	6.10±0.52 a
10 % RRS	100.22±7.56 b	50.41±4.72 b	0.42±0.07 b	6.76±0.44 a
5 % Sprouted RRS	96.01±6.86 b	49.03 ±3.74 b	0.42±0.09 b	7.06±0.24 a
10 % Sprouted RRS	89.81 ±6.49 c	43.62±4.99 c	0.30±0.02 c	7.11±0.56 a

Data are presented as means $\pm$ SDM ($n=6$). a, b, c and d: Means with different letter among treatments in the same column are significantly different ($P \leq 0.05$). RRS: Red radish seeds; AST: aspartate amino transferase; ALT: alanine amino Transfers; ALP: alkaline phosphatase.

Effect of non-sprouted and sprouted red radish seeds on kidney functions of rats induced nephrotoxic by gentamicin.

Data presented in Table (6) showed the effect of treatment with non-sprouted and sprouted red radish seeds. The data revealed that serum urea, uric acid and creatinine were elevated significantly by gentamicin administration, the values were (81.34 ± 9.17 , 6.10 ± 0.33 and 2.96 ± 0.18 , respectively) compared to negative control (38.61 ± 4.16 , 3.19 ± 0.16 and 0.54 ± 0.09 , respectively).

Significant ($P \leq 0.05$) reduction was observed in serum urea, uric acid and creatinine for all treatment groups treated with non-sprouted and sprouted red radish seeds compared to positive control group. Also, the results showed an improvement in all the treated groups compared to negative control groups for kidney functions. The best result was in 10 % Sprouted RRS group compared to negative control group.

These results accorded with previous findings by **Elshahat, (2019)** who showed that acute intoxication of rats induced significant increases in serum levels of urea, uric acid and creatinine. Meanwhile, a decrease in total protein and albumin. The results showed that decrease levels of urea, uric acid and

creatinine and increases in total protein and albumin in the groups feeding on germinated radish seeds compared to the positive and negative control. **Ahmed et al., (2021)** found that GM evoked significant alterations in kidney function biomarkers (uric acid, creatinine, and blood urea). However, in our results non-sprouted and sprouted red radish seeds could mitigate the GM-inflicted kidney damage especially, 10% sprouted RRS, due to its high content of phenolics and flavonoids. These results agree with **Aly et al., (2015)** explained that the treatment with sprouts improved the kidney functions.

Table (6): Kidney functions of experimental rats which treated with non-sprouted and sprouted red radish seeds.

Groups	Parameters(mg/dl)		
	Urea	Uric Acid	Creatinine
Control (ve-)	38.61±4.16 c	3.19±0.16 c	0.54±0.09 c
Control (ve+)	81.34±9.17 a	6.10±0.33 a	2.96±0.18 a
5 % RRS	55.16±9.10 b	4.74±0.17 b	0.99±0.44 b
10 % RRS	48.02±4.42 b	4.63±0.15 b	0.79±0.26 bc
5 % Sprouted RRS	50.63±7.18 b	4.37±0.16 b	0.91±0.08 b
10 % Sprouted RRS	40.20 ±3.11 c	3.64±0.22 c	0.55±0.07 c

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 ($P \leq 0.05$); RRS: Red radish seeds.

Effect of non-sprouted and sprouted red radish seeds on oxidative stress of rats induced nephrotoxicity by Gentamicin.

As seen in Table (7) serum catalase CAT and superoxide dismutase SOD activities were decreased significantly by gentamicin GM administration, while there was significant elevated in serum malondialdehyde MDA compared to negative control group. Oxidative stress was the main modulatory mechanism in GM-induced hepato-renal toxicity. Reactive oxygen species (ROS) such as superoxide anion, $O_2^{\bullet-}$; hydrogen peroxide, H_2O_2 ; and hydroxyl radical, $OH^{\bullet}$, have been confirmed to be generated by GM (**Ahmed et al., 2021**). Lipid peroxidation LPO and oxidative stress after GM treatment expounded by elevation of MDA level together with considerable suppression in the CAT activity. Intriguingly, CAT is an antioxidant enzyme

required for the breakdown of H₂O₂ into H₂O and O₂ (**Rashid and Khan, (2017)**). When CAT enzyme was exhausted by GM-generated ROS, Fenton's reaction will be promoted; thereby, abundant quantities from OH• are formed. OH• is as the most prejudicial radical disrupting the lipid membrane causing LPO detected by the increased MDA level (**Abdeen *et al.*, 2019**).

The results showed enhancement for all treatment groups treated with non-sprouted and sprouted red radish seeds, back to significant increase in serum catalase CAT and superoxide dismutase SOD activities and significant decrease in serum malondialdehyde MDA compared to positive control group. The best results showed of 10% sprouted red radish seeds group compared to negative control group. Numerous studies have proven that radish seeds can compete with free radicals in cell components and protect from infection (**Naghibi *et al.*, 2003**). Research stated that germinated radish seeds had higher activity because it was rich in antioxidants like flavonoids and sinapinic acid esters (**Takaya *et al.*, 2003**).

Therefore, treatment by non-sprouted and sprouted red radish seeds could mitigate the GM-inflicted liver and kidney damage, in a dose-response pattern, due to its high content of phenolics and flavonoids. Numerous phenolic and nonphenolic compounds in sprouts have been identified to have antioxidant activities. The activity of ascorbic acid in sprouts has been described (**Hamilton and Vander, 1979**) and (**Simon *et al.*, 2021**).

Table (7): Serum catalase, superoxide dismutase and malondialdehyde of rats induced nephrotoxicity by Gentamicin which treated with non-sprouted and sprouted red radish seeds.

Groups	Parameters		
	CAT (μ /L)	SOD (μ /dl)	MDA (ng/ml)
Control (ve-)	98.69 $\pm$ 2.18a	91.54 $\pm$ 3.71 a	11.33 $\pm$ 0.31 c
Control (ve+)	39.74 $\pm$ 1.25c	41.13 $\pm$ 1.85c	56. 95 $\pm$ 2.71 a
5 % RRS	81.22 $\pm$ 4.47 b	79.98 $\pm$ 3.16 b	23.58 $\pm$ 1.64 b
10% RRS	80.12 $\pm$ 3.16 b	80.13 $\pm$ 2.91 b	24.09 $\pm$ 1.12 b

5 % Sprouted RRS	85.12 ±3.11b	81.25±3.45 b	19.23±1.02 b
10 % Sprouted RRS	93.22±3.78a	89.23±3.78 a	13.02±1.22 c

Data are presented as means ± SDM ($n=6$). a, b, c and d: Means with different letter in the same column are significantly different ($P \leq 0.05$). CAT: catalase SOD: Superoxide dismutase MDA: malondialdehyde

Histopathological examination of Kidney:

Microscopically, kidney sections of rats from control (ve-) group exhibited normal histological structure of renal parenchyma. In contrariwise, kidneys of rats from control (ve+) group showed histopathological lesions characterized by cytoplasmic vacuolization of epithelial lining renal tubules, congestion of renal blood vessel and necrobiosis of epithelial lining renal tubules (photo 1). These results agree with **Ahmed *et al.*, (2021)** who found that in gentamicin administered rats, incidence of LPO in the renal epithelial membrane clarified by disintegrated cortical tubular brush border in renal histology in tandem with a significant increase in serum levels of renal biomarkers. GM is excreted unchanged through the glomerular filtrate, but a portion is reabsorbed back into the renal tubules and accumulates mainly in epithelial cells of proximal. Furthermore, mitochondria are abundant in the proximal tubules; wherein, high energy is required for active transport. That mitochondrial abundance makes the renal cell vulnerable to more oxidative damage. The histopathological findings demonstrated more damage located in the cortical region than other parts of the kidney in response to GM insult. Also, **Abdeen *et al.*, (2014)** demonstrating that the proximal tubule is a primary target to GM-induced renal injury, attributing to impaired tubular reabsorption and consequently increased serum creatinine and urea levels. The current study strongly assumed that oxidative stress is the modulatory mechanism linked with GM toxicity. Besides, our and others earlier studies suggested a positive correlation between inflammatory reactions and oxidative stress in response to various insults (**2019 & 2020 and Salzano *et al.*, 2014**).

Meanwhile, kidneys of rats from 5% red radish seeds group revealed no histopathological changes except cytoplasmic

vacuolization of epithelial lining some renal tubules. On the other hand, kidneys of rats from 10% red radish seeds group showed eosinophilic proteinaceous casts in the lumen of some renal tubules and pyknosis of the nuclei of epithelial lining some renal tubules. Otherwise, kidneys of rats from 5% sprouted red radish seeds group described cytoplasmic vacuolization of epithelial lining some renal tubules and pyknosis of the nuclei of epithelial lining some renal tubules. Furthermore, kidneys of rats from 10% sprouted red radish seeds group exhibited no histopathological lesions (photo 2).

Cruciferous sprouts contain non-nutrient health-promoting compounds, like diverse types of glucosinolates and phenolic compounds (**Baenasa *et al.*, 2017**). The biological activity developed by these compounds is mainly due to their antioxidant capacity, which could lower the deleterious consequences of excessively high levels of Reactive oxygen species (ROS) in cells. These compounds have direct effects on several cellular processes triggered by ROS, which are related to inflammation and oxidative reactions on DNA, proteins, and cell lipids (**Gagné, 2014**). In addition, to lower oxidative stress, many bioactive phytochemicals present in edible sprouts display biological functions that are crucial for the prevention of carcinogenesis processes and other chronic diseases (**Gan *et al.*, 2017**).

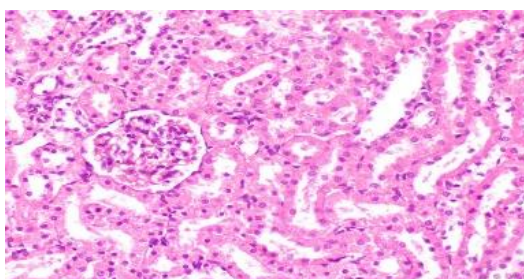
Red radish seeds especially after germinated revealed the existence and elevation of total phenolics and flavonoids among other bioactive components. Notably, these compounds exhibit various therapeutic potential mainly antioxidant properties and anti-inflammatory (**Kumaradevan *et al.*, 2015**). Various reports have shown a respectable positive association between the phenolic compound constitutes and the antioxidant capacity of the plants (**Minatel *et al.*, 2017**).

Interestingly, in the current experiment, treatment with seeds especially 10% high dosage of sprouts red radish seeds caused significant improvement of hepato-renal function in GM-intoxicated rats. Such improvements were seen by retrieving the

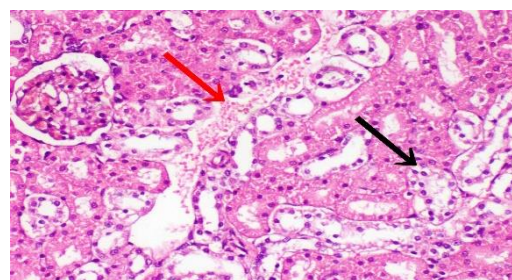
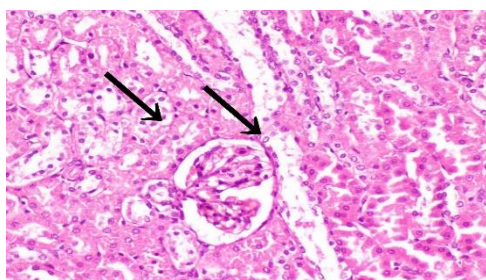
histopathology of kidney that close to normal. Our data found that RRS treatment could confer a sturdy antioxidant activity against GM-stimulated oxidative stress indicated by the recovery of the MDA level, CAT and SOD activities in the GM intoxicated rats. The present work has also postulated an anti-inflammatory activity of sprouts RRS in the GM-intoxicated rats which was observed by a remarkable reduction in the lymphocytic infiltration in kidney. That improvement might be directly attributed to the anti-inflammatory content. It is also assumed that the scavenging activity of the antioxidant constituents may be another reason behind the mechanisms by which 10% sprouts RRS could suppress the ROS-induced inflammatory reaction.

Conclusion

This study revealed that red radish sprouts have a higher amount of phenolic, flavonoid compounds and antioxidants activity than the seeds. The results indicated that these sprouts that rich in antioxidants influenced improving biological parameters, liver and kidney functions and reducing oxidative stress. Therefore, this study encouraged the consumption of red radish sprouts as vital sources of biomolecules with beneficial effects on health. However, more studies are required to study the effect of sprouted red radish on different diseases.

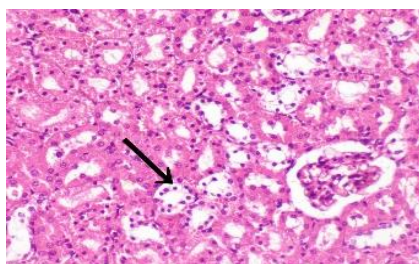


Control (-ve)

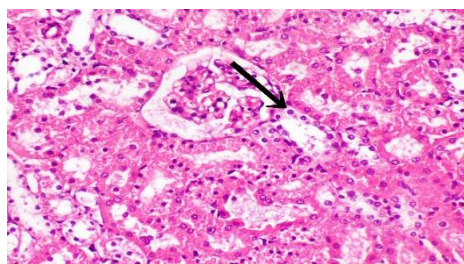


Control (+ve)

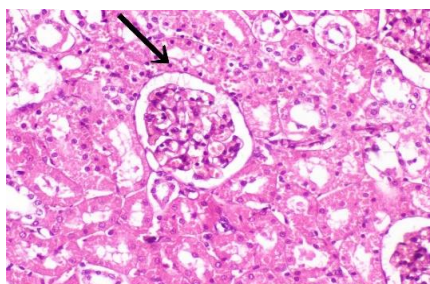
Photo (1): Photomicrograph of kidney in control (-ve) and control (+ve) groups (H&E X 400)



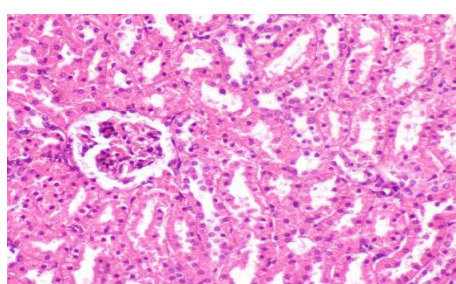
5% Red radish seeds



10% Red radish seeds



5% Sprouted red radish seeds



10% Sprouted red radish

Photo (2): photomicrographs of kidney in all treated experimental groups (H&E X 400)

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Instructor at the Ontario institute for studies in
education (OISE) at the university of Toronto
and consultant to UNESCO

Prof. Nicos Souleles (Greece)

Multimedia and graphic arts, faculty member,
Cyprus, university technology



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البريد الإلكتروني:

egyjournal@sedu.asu.edu.eg

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الترقيم الدولي الموحد الإلكتروني : 4353 - 2682

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