

# The Activity of *Spinacia oleracea* Extract on Testicular Tissue, Antioxidant Enzymes, and Malondialdehyde in Male Albino Rats

Nabaa Qasim Al-Salamy, Nada Saad Naji AL-Tae

Department of Medical Biotechnology, College of Biotechnology, University of Al-Qasim Green, Babylon, Iraq

## Abstract

**Background:** In a new world, we are constantly exposed to environmental and natural stimuli that can lead to oxidative stress. Antioxidant enzymes and molecules help the human body eliminate reactive oxygen species and prevent oxidative damage. Green leafy vegetables such as *Spinacia oleracea* are a rich and well-studied source of antioxidant nutritional molecules. **Objective:** The research aims to study the effect of the medium effective dose (MED<sub>50</sub>: 58 mg/kg body weight) of the leaf extract on testicular tissue, antioxidant enzymes, and malondialdehyde (MDA) in male albino rats. **Materials and Methods:** About 20 healthy male rats were separated into two groups, each containing ten male adult rats. The first group, the control group, was treated orally with distilled water. The second group was treated orally with 58 mg/kg body weight of phenolic extract of spinach leaves for 60 days. **Results:** The testicular sections from the study's animals revealed that the control groups (left and right) displayed normal histology. In contrast, the treated groups (left and right) exhibited mature sperm cells within the seminiferous tubule lumens. A significant increase at *P* value < 0.05 in the concentration of antioxidant enzymes (superoxide dismutase, glutathione peroxidase, and catalase), also a significant increase in MDA concentration when compared to the control group. **Conclusion:** The findings of our study show that spinach extract could be a source of natural antioxidants due to its clear effect on testicular tissue and oxidation enzymes.

**Keywords:** Enzymes, male rats, MDA, *Spinacia*, testicular tissue

## INTRODUCTION

Infertility affects 30%–80% of males, with oxidative stress being a contributing factor.<sup>[1]</sup> The formation of ice crystals, reactive oxygen species (ROS), and their detrimental effects on the sperm membrane lipids can lead to two undesirable outcomes: the induction of oxidative stress (OS) during cryopreservation and oxidative stress itself. To keep men fertile, antioxidants have recently been the focus of research, due to the strong correlation between OS stimulation and cryopreservation.<sup>[2]</sup> Leafy green vegetables play a crucial role in maintaining a healthy diet by enhancing the overall well-being of the human body. Apart from providing fiber, protein, and other macronutrients, they also serve as a rich source of vital amino acids, organic acids, vitamins, minerals, and phytonutrients. These nutrients have been scientifically proven to play significant roles in promoting human health.<sup>[3]</sup> A well-established source of vital nutrients,

spinach, also contains ascorbic acid, various minerals, and beta-carotene, a precursor to vitamin A. Despite the fact that many researches have been done on spinach's antioxidant properties,<sup>[4-6]</sup> it has the ability to safeguard against free radicals that cause damage to cells within the human body. Spinach is commonly acknowledged as a functional food owing to its extensive range of nutrients, such as chlorophyll, carotenoids, dehydroascorbate reduced glutathione, beta-carotene, oxidized glutathione, lutein, and a variety of flavonoids that also serve as antioxidants.<sup>[7,8]</sup> Antioxidant enzymes found in spinach include peroxidase, superoxide dismutase (SOD), catalase

**Address for correspondence:** Dr. Nabaa Qasim Al-Salamy, Department of Medical Biotechnology, College of Biotechnology, University of Al-Qasim Green, Babylon 51013, Iraq. E-mail: nabaabiologist5@gmail.com

**Submission:** 13-Dec-2023 **Accepted:** 10-May-2024 **Published:** 30-Apr-2026

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 License (CC BY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

**For reprints contact:** WKHLRPMedknow\_reprints@wolterskluwer.com

**How to cite this article:** Al-Salamy NQ, Al-Tae NSN. The activity of *Spinacia oleracea* extract on testicular tissue, antioxidant enzymes, and malondialdehyde in male albino rats. *Med J Babylon* 2026;23:677-82.

### Access this article online

#### Quick Response Code:



**Website:**  
<https://journals.lww.com/mjby>

**DOI:**  
10.4103/MJBL.MJBL\_1796\_23

(CAT), glutathione peroxidase (GPx), and glutathione reductase.<sup>[9]</sup> Spinach possesses a wide array of proteins and molecules, rendering it a promising candidate for harnessing antioxidants that aid in regulating the levels of ROS within the human body. An array of proteins known as antioxidant enzymes is found in the cellular milieu and serves to both ease the electron-donating mechanism of antioxidants and recycle the oxidized antioxidants back into their reduced form as in a reverse reaction. Antioxidant enzymes are involved in the scavenging of free radicals by antioxidants.<sup>[10]</sup> The biological system contains a restricted number of antioxidant enzymes, of which CAT, GPx, and SOD are essential for scavenging free radicals.<sup>[11]</sup> Damage to DNA, proteins, and lipids can arise from OS, which is a result of an imbalance between the production of ROS/free radicals (such as superoxide radical, hydroxyl radical, and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)) and antioxidant defenses (such as manganese SOD and copper–zinc SOD).<sup>[12]</sup> It has been demonstrated that consuming foods and drinks high in polyphenols increases human plasma antioxidant capacity<sup>[13]</sup> and reduces OS in human placenta and human placental trophoblasts both *in vivo* and *in vitro*.<sup>[14]</sup> The aim of this research is to improve antioxidant enzymes and their effect on testicular tissue in animals treated with MED<sub>50</sub> of spinach leaf extract.

## MATERIALS AND METHODS

### Plants extraction

Spinach leaves were collected from Babylon in January. They were washed and dried, then blended with an electric grinder. The leaves were extracted according to Susanti *et al.*<sup>[15]</sup>

A total of 20 male albino rats (weighing 150–190 g) were 2 months old and held in plastic cages with 12/12 h light/dark cycle at 21 ± 2°C. The rats were divided randomly into two groups as follows:

1. Group 1: The Control group was receiving distilled water (DW).
2. Group 2: The treated group received 58 mg/kg of body weight daily of extract by oral administering for 60 days.

### Histopathological study

At the end of the experiment, both experimental rats and control were sacrificed after 60 days of treatment, and left and right tissue were taken. The testes were preserved in Bouin's solution to maintain their structure and molecular composition.<sup>[16]</sup> Subsequently, additional histological preparations were conducted, and the samples were stained with hematoxylin–eosin. These stained samples were then examined under a microscope at a magnification of 40×.<sup>[17]</sup>

## Antioxidant enzymes

### Superoxide dismutase

The enzyme's capacity to inhibit pyrogallol oxidation at pH 8.2 was used as the basis for a quick and easy way to calculate the measured activity<sup>[18]</sup>:

$$\begin{aligned} & \% \text{ Inhibition of pyrogallol autoxidation} \\ &= \frac{\Delta A_{\text{control}} - \Delta A_{\text{test}}}{\Delta A_{\text{control}}} \times 100\% \end{aligned}$$

$$\text{(Cu-Zn) SOD Activity (U/ml)}$$

$$= \frac{\% \text{ inhibition of pyrogallol autoxidation}}{50\%}$$

### Glutathione peroxidase

The measured activity of this enzyme was determined by method<sup>[19]</sup> with the equation:

$$\text{Glutathione peroxidase activity (U/L)}$$

$$= \left( \frac{\text{Conc. of GSH in STD} - \text{Conc. of residual GSH in test tube}}{\text{time (10min)}} \right) \times \left( \frac{\text{Total volume (ml)}}{\text{Volume of the sample (ml)}} \right) \times \text{D.f.}$$

### Catalase

In order to find the CAT activity, the following equation was used, as described in reference:<sup>[20]</sup>

$$\text{Catalase Activity of test kU} = \frac{2.303}{t} * \left[ \log \frac{S^0}{S-M} \right] * \frac{Vt}{Vs}$$

### Statistical analysis

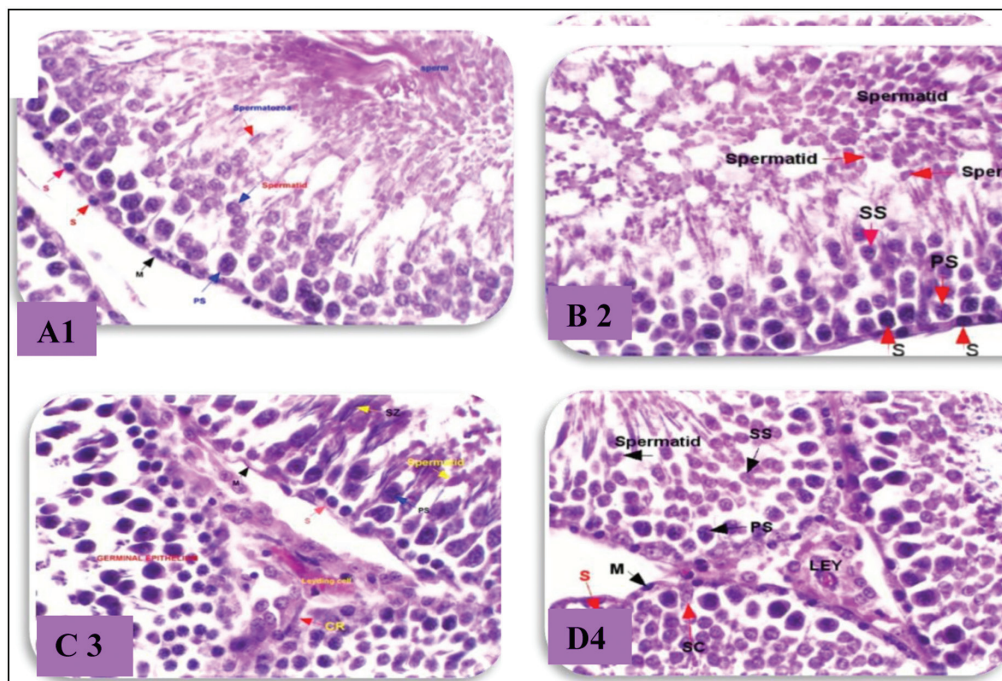
The data obtained were analyzed using the program Statistical Package for the Social Sciences 2010 (IBM Corp., Chicago, IL, USA). *t* Test and mean ± standard error (SE) were calculated. The level of statistical significance was set at *P* < 0.05.

### Approval ethical

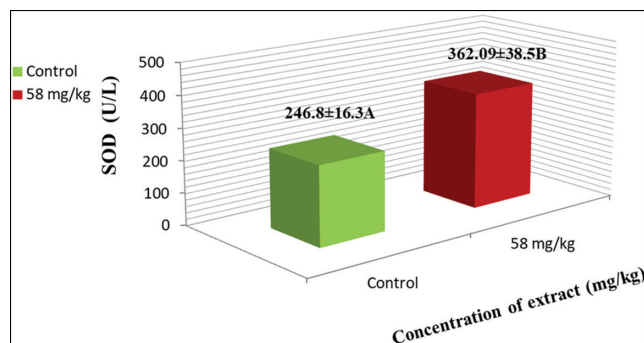
On a more serious note, the research ethics committee at Al-Qasim Green University's College of Biotechnology and Department of Medical Biotechnology gave their stamp of approval to this study.

## RESULTS

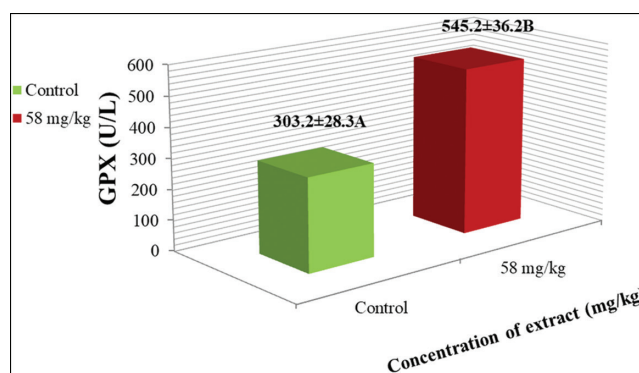
In Figure 1A and C show the normal seminiferous tubules with different spermatogenesis stages. At the base of the germinal epithelium are myoid cells, spermatogonia, primary spermatocytes (PS), and spermatids. The Leydig cells and crystals of Reinke (CR) were also evident; Figure 1B shows some stages of spermatogenesis starting from



**Figure 1:** Histological sections of the testis rat stained with hematoxylin and eosin (40×). (A) (C) Control group showing normal histology; (B) Right testis treatment with a distinctive thing is that the lumen is filled with different cells of spermatogenesis cells, most likely spermatids. (D) Left testis treatment, at the base of the germinal epithelium are myoid cells (M), spermatogonia (S), primary spermatocytes (PS), secondary spermatocytes (SS), spermatids, spermatozoa, and sperms fill all the lumen space. Also, Leydig (Ley) cells were evident



**Figure 2:** The effect of MED<sub>50</sub> of the extract on SOD level of the male albino rat. The outside columns represent the means ± standard error (SE). Number of animals = 10 in each group. The different letters denote the significance at ( $P < 0.05$ ) with  $T = 2.75$ ,  $P = 0.01317$



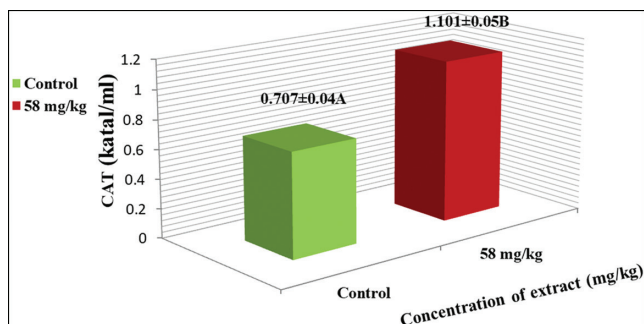
**Figure 3:** The effect of MED<sub>50</sub> of the extract on GPX level of the male albino rats. The outside columns represent the means ± SE. Numbers outside the columns represent the means ± SE. Number of animals = 10 in each group. The different letters denote the significance at ( $P < 0.05$ ), with  $T = 5.37$ ,  $P = 0.00004$

myoid cells, including spermatogonia and PS; Figure 1D shows normal seminiferous tubules with different spermatogenesis stages. At the base of the germinal epithelium are myoid cells, spermatogonia, PS, secondary spermatocytes, spermatids, spermatozoa, and sperms fill all the lumen space. Also, Leydig cells were evident.

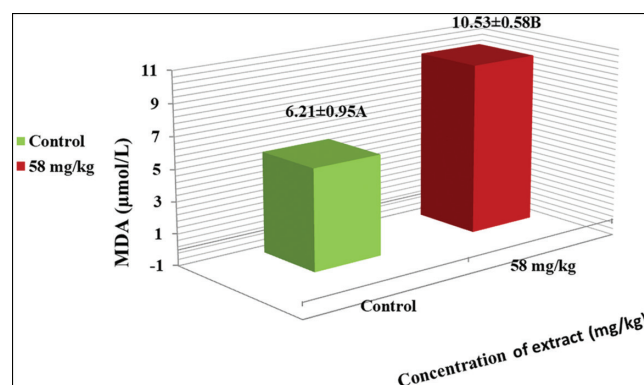
The dose-response curve statistical analysis yielded a MED<sub>50</sub> of 58 mg/kg body weight for the spinach extract. Figures 2–4 show that the results showed an increase in antioxidant enzymes (SOD, GPX, and CAT) with a significance level of ( $P < 0.05$ ). It was also noted that the level of MDA increased significantly ( $P < 0.05$ ) when compared to the control animals [Figure 5].

## DISCUSSION

The impact of several lifestyle factors, including age, weight, nutrient deficiencies,<sup>[21]</sup> smoking associated with diseases,<sup>[22,23]</sup> and exposure to environmental pollutants,<sup>[24]</sup> are impact on general health and adversely on reproductive performance. Various medicinal plants have been found to positively affect reproduction, like celery and garlic,<sup>[25]</sup> garden cress,<sup>[26]</sup> which have been shown to have reproductive importance for the male reproductive system. the most important benefit of herbs on male fertility is related to the plant's antioxidant



**Figure 4:** The effect of  $MED_{50}$  of the extract on CAT level of the male albino rats. The outside columns represent the means  $\pm$  SE. Numbers inside the columns represent the means  $\pm$  SE. Number of animals = 10 in each group. The different letters denote the significance ( $P < 0.05$ ) with  $T = 5.25$ ,  $P = 0.01317$



**Figure 5:** The effect of  $MED_{50}$  of the extract on MDA level of the male albino rats. The outside columns represent the means  $\pm$  SE. Numbers outside the columns represent the means  $\pm$  SE. Number of animals = 10 in each group. The different letters denote the significance ( $P < 0.05$ ) with  $T = 3.86$ ,  $P = 0.00115$

compound which enhances spermatogenesis.<sup>[27]</sup> Spinach, orange, and their derivatives have long been recognized as abundant stores of vitamins, minerals, and dietary fiber in addition to bioactive compounds like polyphenols, especially phenolic acids and flavonoids.<sup>[28]</sup> Additionally, they offer a strong antioxidant action.<sup>[29,30]</sup> Consumption of natural antioxidants and vitamins can protect sperm DNA from OS and improve male fertility.<sup>[31,32]</sup> Testicular tissues possess a high concentration of polyunsaturated fatty acids and a limited antioxidant defense system. Consequently, they are susceptible to oxidative damage caused by ROS. These ROS can oxidize proteins, lipids, and DNA, resulting in cellular harm. Recent findings suggest that peroxidative damage induced by ROS is a significant factor contributing to impaired sperm function, which can exhibit varying degrees of sensitivity to identical irradiation.<sup>[33]</sup> Our results in Figure 1 show standard and healthy testicular components, revealing different stages of sperm development within the tubules, and observing myoid cells, spermatogonia, PS, Sertoli cells, Leydig cells, and Reinke's crystals. While the results of Al-Tae<sup>[23]</sup> indicated that the seeds

of cross extract caused a low amount of sperm in the lumen, decreased height of the epithelial cells secreting seminal vesicles, and fewer stereocilia in the epididymis. According to research by Iqbal *et al.*,<sup>[34]</sup> reducing the negative effects of obesity-induced OS on testes and epididymis weight is possible through a diet supplement that includes spinach. The methanolic extract of spinach oleracea plant leaves contains the highest concentration of flavonoids and phenols and exhibits the highest antioxidant activities.

According to our result in [Figures 2–4], the  $MED_{50}$  of spinach extract caused an increase in the level of SOD, GPx, and CAT compared to the control group; this increase in antioxidant enzymes may be due to spinach being rich in phenolic compounds that act as antioxidants. SOD supplementation can potentially activate the body's internal antioxidant system to counteract an excessive amount of free radicals. As a result, it can be employed in various pathological conditions as well as the incorporation of naturally occurring antioxidants into widely used vegetable oils to improve their oxidative stability.<sup>[35]</sup> This confirms the results of earlier studies and adds to the importance of glutathione-rich foods as a healthful food choice.<sup>[36,37]</sup> Because spinach is an excellent provider of essential minerals such as iron, copper, phosphorous, zinc, CAT, an antioxidant enzyme exhibits the highest turnover rate among its counterparts. It is abundantly found in living tissues and plays a crucial role as a clinical enzyme in the decomposition of  $H_2O_2$  into water and molecular oxygen,<sup>[38]</sup> converting dangerous material into a safe state. Finally, malondialdehyde (MDA) serves as a dependable indicator for assessing the extent of damage inflicted upon a stressed plant. It is formed as a result of the peroxidation of polyunsaturated fatty acids within the cells. Consequently, MDA has gained significant popularity and widespread usage in the field. Selenium<sup>[39]</sup> may cause an increase in the concentration of MDA, as shown in the Figure 5, because spinach contains transitional elements, MDA level. This is not consistent with previous research, which shows an increase in the level of SOD and CAT and a decrease in MDA level.<sup>[40]</sup> Spinach is rich in transitional elements,<sup>[39]</sup> which in high concentrations encourage lipid peroxidation, subsequently increasing MDA levels. There are many degenerative diseases caused by OS, and the Fenton and Haber–Weiss reactions play a major role in this. All four macromolecules—DNA, proteins, lipids, and carbohydrates—are vulnerable to free radical damage when iron, copper, and aluminum are present, generating hydroxyl radicals through the Fenton reaction.<sup>[41,42]</sup>

## CONCLUSION

These observations suggest a possible positive impact of spinach extract on testicular tissue by increasing the concentrations of antioxidant enzymes in adult male rats. An increase in the concentration of MDA may be

due to the plant's transitional elements. Further studies are necessary for a more comprehensive understanding. Detailed investigations are required to identify the specific bioactive compounds in spinach responsible for these effects. Conducting long-term studies and assessing potential side effects or interactions with other medications or conditions are crucial steps for practical application in clinical settings.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

### REFERENCES

- Alahmar AT, Al Jothery AHT, Al-Daami QJ, Abbas A, Al-Hassnawi ATS. The effect of coenzyme Q10 on dexamethasone-induced oxidative stress in rats testes. *Med J Babylon* 2023;20:130-5.
- Hussein R, Abbas LH, Rayhaan S, Fadhil HA, AL-Mousawi ZR. The impact of adding melatonin and other antioxidants on post-thaw human sperm quality during cryopreservation. *Med J Babylon* 2023;20:18-23.
- Randhawa MA, Khan AA, Javed MS, Sajid MW. Green leafy vegetables: A health promoting source. *Nutrition, Diet, Lifestyle and Reproductive Health: Academic Press*; 2015. p. 205-20.
- Gil MI, Ferreres F, Tomás-Barberán FA. Effect of postharvest storage and processing on the antioxidant constituents (flavonoids and vitamin C) of fresh-cut spinach. *J Agric Food Chem* 1999;47:2213-7.
- Edenharder R, Keller G, Platt KL, Unger KK. Isolation and characterization of structurally novel antimutagenic flavonoids from spinach (*Spinacia oleracea*). *J Agric Food Chem* 2001;49:2767-73.
- Lomnitski L, Carbonatto M, Ben-Shaul V, Peano S, Conz A, Corradin L, *et al.* The prophylactic effects of natural water-soluble antioxidant from spinach and apocynin in a rabbit model of lipopolysaccharide-induced endotoxemia. *Toxicol Pathol* 2000;28:588-600.
- Roberts JL, Moreau R. Functional properties of spinach (*Spinacia oleracea* L.) phytochemicals and bioactives. *Food Funct* 2016;7:3337-53.
- Sáez G, Están-Capell N. Antioxidant enzymes. In: Schwab M, editor. *Encyclopedia of Cancer*. Berlin/Heidelberg, Germany: Springer; 2014.
- Guo Q, Li F, Duan Y, Wen C, Wang W, Zhang L, *et al.* Oxidative stress, nutritional antioxidants and beyond. *Sci China Life Sci* 2020;63:866-74.
- Holley AK, Bakthavatchalu V, Velez-Roman JM, Clair DKS. Manganese superoxide dismutase: Guardian of the powerhouse. *Int J Mol Sci* 2011;12:7114-62.
- Krishnamurthy P, Wadhvani A. Antioxidant enzymes and human health. *Antioxid Enzyme* 2012;1:3-18.
- Prior RL, Gu L, Wu X, Jacob RA, Sotoudeh G, Kader AA, *et al.* Plasma antioxidant capacity changes following a meal as a measure of the ability of a food to alter in vivo antioxidant status. *J Am Coll Nutr* 2007;26:170-81.
- Chen B, Tuuli MG, Longtine MS, Shin JS, Lawrence R, Inder T, *et al.* Pomegranate juice and punicalagin attenuate oxidative stress and apoptosis in human placenta and in human placental trophoblasts. *Am J Physiol Endocrinol Metab* 2012;302:E1142-52.
- Nantia E, Moundipa P, Monsees T, Carreau S. Medicinal plants as potential male anti-infertility agents: A review. *Andrologie* 2009;19:148-58.
- Susanti S, Dwiloka B, Bintoro VP, Hintono A, Nurwantoro N, Setiani BE. Antioxidant status, nutrition facts, and sensory of spinach extract fortified wet noodles. *Food Res* 2021;5:266-73.
- Eltoum I, Fredenburgh J, Myers RB, Grizzle WE. Introduction to the theory and practice of fixation of tissues. *J Histotechnol* 2001;24:173-90.
- Provo-Klimek JA, Troyer DL. A novel method for preparing histology slides to integrate the teaching of gross and microscopic anatomy. *J Vet Med Educ* 2002;29:137-41.
- Marklund S, Marklund G. Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* 1974;47:469-74.
- Ahmed AY, Aowda SA, Hadwan MH. A validated method to assess glutathione peroxidase enzyme activity. *Chem Pap* 2021;75:6625-37.
- Hadwan MH, Khabt H. Simple spectrophotometric method for analysis of serum catalase activity. *J Clin Diagn Res* 2018;12: 13-6.
- Al-Tae NSN. Physiological important of minerals in fertility of women: Comparison of pregnant women's minerals in urban and rural areas. *Med Legal Update* 2019;19:373-8.
- Al-Tae NSN. Physiological blood parameters of young university adults with blood glucose, blood pressure and smokers. *Indian J Public Health Res Dev* 2018;9:481-7.
- Al-Tae NSN. Physiological aspects of osmolality and cations of young university adults suffer from blood glucose, blood pressure and smokers. *Indian J Public Health Res Dev* 2019;10:980-110.
- AL-Mamoori NAH, Al-Tae NSN. Effects of cement dust on electrolytes and osmolality in serum and urine of kufa cement factory workers. *Indian J Forensic Med Toxicol* 2020;14:1605-9.
- Ghafil JS, Al-Tae NSN. Effect of garlic and celery extracts on lead toxicity in male mice. *Med Legal Update* 2020;20:614-8.
- Alalwany EAH, Altaee NSN, Al-Khamas AJH, Rashid KH. Histological changes in the testes, epididymis and seminal vesicles of adult male rabbits treated with garden cress (*Lepidium sativum* L.) seeds phenolic extract. *Biochem Cell Arch* 2021;21:2079-83.
- Taeponsorat L, Tangpraputgul P, Kitana N, Malaivijitnond S. Stimulating effects of quercetin on sperm quality and reproductive organs in adult male rats. *Asian J Androl* 2008;10:249-58.
- Al-Juhaimi FY. Citrus fruits by-products as sources of bioactive compounds with antioxidant potential. *Pak J Bot* 2014;46:1459-62.
- Derrien M, Badr A, Gosselin A, Desjardins Y, Angers P. Optimization of a green process for the extraction of lutein and chlorophyll from spinach by-products using response surface methodology (RSM). *LWT Food Sci Technol* 2017;79:170-7.
- Baenas N, Abellán Á, Rivera S, Moreno DA, García-Viguera C, Domínguez-Perles R. *Foods and Supplements*. Amsterdam, The Netherlands: Elsevier; 2018.
- Ozdemir N, Kantekin-Erdogan MN, Tat T, Tekin A. Effect of black cumin oil on the oxidative stability and sensory characteristics of mayonnaise. *J Food Sci Technol* 2018;55:1562-8.
- Mosher WD, Pratt WF. Fecundity and infertility in the United States: Incidence and trends. *Fertil Steril* 1991;56:192-3.
- Rooij DE, D G. Regulation of the proliferation of spermatogonial stem cells. *J Cell Sci* 1988;Supplement\_10:181-94.
- Iqbal S, Ali S, Siddiqui A. Antioxidant effects of spinach (*Spinacia Oleracea*) on testicular and epididymal weight of obese sprague dawley rats. *J Islam Int Med Coll* 2018;13:157-61.
- Rosa AC, Corsi D, Cavi N, Bruni N, Dosio F. Superoxide dismutase administration: A review of proposed human uses. *Molecules* 2021;26:1844.
- Tehseen M, Hina S, Nisa A, Zahra N. Study of antioxidant activity of spinach extract in stabilization of sunflower oil under accelerated storage conditions. *Pak J Agric Res* 2021;34:414-23.
- Nacca F, Cozzolino C, Carillo P, Woodrow P, Fuggi A, Ciarmiello LF. An HPLC-automated derivatization for glutathione and related thiols analysis in *Brassica rapa* L. *Agronomy* 2021;11:1157.
- Trivedi A, Singh N, Bhat SA, Gupta P, Kumar A. Redox biology of tuberculosis pathogenesis. *Adv Microb Physiol* 2012;60:263-324.
- Morales M, Munné-Bosch S. Malondialdehyde: Facts and artifacts. *Plant Physiol* 2019;180:1246-50.

40. Roughani A, Miri SM. Spinach: An important green leafy vegetable and medicinal herb. The 2nd International Conference on Medicinal Plants, Organic Farming, Natural and Pharmaceutical Ingredients. Mashhad, Iran; 2019. p. 1-6.
41. Rival T, Page RM, Chandraratna DS, Sendall TJ, Ryder E, Liu B, *et al.* Fenton chemistry and oxidative stress mediate the toxicity of the beta-amyloid peptide in a *Drosophila* model of Alzheimer's disease. *Eur J Neurosci* 2009;29:1335-47.
42. Everett J, Cespedes E, Shelford LR, Exley C, Collingwood JF, Dobson J, *et al.* Ferrous iron formation following the co-aggregation of ferric iron and the Alzheimer's disease peptide beta-amyloid(1-42). *J R Soc Interface* 2014;11:20140165.