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Effect of melatonin from some sprouted legumes on oxidative stress in rats

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ABSTRACT

Melatonin (MT), a neuropeptide hormone secreted by the pineal gland, is a potent free radical scavenger and small molecule drug discovered at multiple sites in the body. MT, a natural antioxidant, has been shown to inhibit oxidative stress and stabilize endothelial function, providing cardiovascular protection. It was found that legume seeds were a good dietary source of melatonin, serotonin, and free tryptophan. This study aimed to evaluate the effect of melatonin on oxidative stress in rats. Animals were weighed and randomly assigned into two main groups. Group 1 (6 rats) fed a basal diet as a negative control, while Group 2 (24 rats) received oral caffeine (100 g/kg body weight/day for two weeks) to determine oxidative stress. Mice of group (2) were divided into four subgroups, each with 6 mice. Subgroup (1) fed a basal diet as a positive control. Subgroup (2) received a basal diet supplemented with 20% (w/w) dry sprouted lupin powder. Subgroup (3) received a basal diet supplemented with 20% (w/w) dry sprouted chickpea powder. Subgroup (4) received a basal diet supplemented with melatonin at doses of 10 mg/kg body weight. The results showed that caffeine significantly increased the risk of oxidative stress ($P \leq 0.05$). However, the results of treated subgroups (2, 3 & 4) indicated that there was improved weight gain, decreased liver enzymes, decreased MDA, and increased GSH and CAT levels, which are associated with oxidative stress, compared to the oxidative stress subgroup (1). On the other hand, histological examination of the positive control subgroup (1) after two months of treatment revealed improved heart and liver tissues in the treated rats, especially in the subgroup (that consumed sprouted chickpeas. More specifically, the melatonin-enriched diet reduced the harmful effects of oxidative stress.

Keywords: Melatonin, Lupine, antioxidant, free radicals, Oxidative stress, Legumes, chickpeas, Albino rats, Liver Enzymes; Histopathology.

INTRODUCTION

Free radicals are chemical entities containing at least one unpaired electron in the outer shell which usually gives them high reactivity. The most frequently occurring free radicals and reactive molecules in biological systems are derived from oxygen (Reactive Oxygen Species, ROS) and nitrogen (Reactive Nitrogen Species, RNS). ROS or RNS are formed during electron transfer reactions, by losing

or accepting electron(s) (Jomova *et al.,* 2023).

Oxidative Stress (ROS) is important players in cellular proliferation, differentiation, migration, apoptosis, and necrosis (Shoaib *et al.,* 2021). Low to intermediate levels of ROS and RNS are necessary for the maintenance of many important physiological functions, redox homeostasis, and the regulation of key transcription factors. In contrast, excessive formation of ROS is responsible for disrupted redox homeostasis which in turn leads to Oxidative stress and ROS-mediated damage to all important biomolecules including DNA, proteins, and membranes (Liguori *et al.,* 2018, Dengxiong *et al.,* 2024).

ROS is a biochemical process that disrupts the redox balance due to an excess of oxidized substances within the cell. Oxidative stress is closely associated with a multitude of diseases and health issues, including cancer, diabetes, cardiovascular diseases, neurodegenerative disorders, inflammatory conditions, and aging. Therefore, the developing of antioxidant treatment strategies has emerged as a pivotal area of medical research (Dama *et al.,* 2024).

Melatonin (MT), a pineal gland-secreted neurohormone peptide, is a powerful free radical scavenger and small-molecule drug detected at multiple sites in the body. It regulates mesenchymal stem cells (MSCs) proliferation, apoptosis and stemness (Gu *et al.,* 2024). Also, it plays a role in cardiovascular protection (Zhang *et al.,* 2023). In addition, the intake of melatonin containing foods could significantly increase the melatonin concentration in human serum, indicating melatonin could provide beneficial effects on health through foods (Meng *et al.,* 2017).

MT has been identified and qualified in many foods. Its content is higher in eggs and fish than that in meat in animal foods, while in plant foods, the highest contents of melatonin was found in nuts, legumes and some cereals and germinated legumes. Mushrooms are also good dietary sources of melatonin.

Various studies indicated that legume seeds were a good dietary source of melatonin, serotonin and free tryptophan. However, under saline condition, the levels of melatonin, serotonin, Total Phenolic Compounds (TPC) and antioxidant activity were enhanced in soybeans, black beans and lentils, while chickpeas and red kidney beans exhibited higher levels of melatonin, TPC and antioxidant activity (Nontasan *et al.,* 2021).

Lupine seeds are good sources of protein which can regulate intestinal absorption of glucose and attenuate risk of Oxidative stress (free radicals/oxidants) generation, Antioxidants depletion and T2DM diseases (Metwally *et al.,* 2023).

Legumes, one of the most important sources of food in the world, play an important role in human nutrition in many countries. Some biotechnological processes and methods such as germination are considered both simple and economical to improve the nutritive value of legumes by causing desirable changes in the nutrient availability, texture and organoleptic characteristics. Extensive breakdown of seed-storage compounds and synthesis of structural proteins and other cell components take place during the germination. Vitamins and secondary compounds, many of which are considered beneficial as antioxidants, often change dramatically during the germination (Tarzia *et al.,* 2012).

The current study aims to assess the impact of melatonin from some sprouted legumes on oxidative stress in rats.

MATERIALS AND METHODS

Materials and animals:

Lupine and chickpea seeds were obtained from local market. Caffeine and melatonin were obtained as food grads from El-Gomhouria Chemical Company, Cairo, Egypt. The chemicals purchased from El-Nasr Pharmaceutical Chemicals, Egypt. Thirty-two male Albino rats (Sprague - Dawley Strain) weighing about 170±5g was obtained from Agricultural Research Center, Giza, Egypt.

Experimental procedures:

1. Germination of lupine and chickpea seeds

Sorted sound seeds (50 g) were cleaned and soaked in distilled water for eight hours in a dark room at 25°C with and without 67 mM sodium chloride (NaCl) for 72 h. The soaking water was then decanted, and the seeds were germinated on dishes lined with a layer of sterile sheet cloth in a dark seed germinator at 28°C and 80% relative humidity. Three replicates were performed for each treatment. The seeds were sprayed with 100 ml of sterile distilled water or NaCl solution daily. The NaCl solution was freshly prepared before use. Germinated legumes were collected, immediately freeze dried, and ground into fine powder. The powder was passed through a 50 µ mesh sieve and kept in a tightly closed and dark container at -20 °C until analysis (Khalil & Mansour, 1995).

2. Analytical methods:

- **Reduced glutathione (GSH):** was determined by Ellman, (1959) using spectrophotometer (model DU 4700) adjusted at wavelength 412 nm.
- **Catalase Activity:** was determined by Aebi, (1984) using spectrophotometer (model DU 4700) adjusted at wavelength 240 nm.
- **Determination of malondialdehyde (MDA)** (µMol/g.Tissue): Hepatic malondialdehyde (MDA) content was estimated according to the method of Albro *et al*., (1986).
- **Determination of (ALT and AST)**: were determined calorimetrically using spectrophotometer (model DU 4700) at 505 nm according to the method of Reitman and Frankel, (1957).
- Chemical constituent of dry sprouted lupine and chickpea and un sprouted were measured according to the method of (AOAC,1975).
- Amino acids of the investigated samples were carried out as described by the method of the Association of Official Analytical Chemists (AOAC, 2012) using Amino acid analyzer biochrom 30 U.K.

3. Determination of melatonin by HPLC-FD

HPLC method with fluorescence detection (FLD) was validated to analyse the melatonin content in lupine and chickpeas. The system consisted of a Hewlett Packard 1100 HPLC system equipped with a quaternary pump (HP 1311A module), an online degasser (HP G1322A) and a fluorescence detector (HP G131A module) set at 285/345 excitation/emission wavelengths. Separation was performed on a Kinetex C-18 column (2.6 µm particles,150 mm × 4.6 mm, Phenomenex, USA) kept at 25 ◦C (Jasco column oven) with a flow rate of 1.0 mL/min. A mobile phase binary gradient was performed with two solutions: (A) 60 % acetonitrile containing 0.1 % (v:v) formic acid and (B) 0.1 % formic acid. The elution profile was15%–60% A from 0 to 15 min, 60%–90% A from 15 to 20 min and 90 % to 15 % A from 20 to 25 min to recover the initial conditions. HP 1100 Chemical Station software was used for acquisition and integration of the chromatograms. Quantification was performed by comparing peak areas with those of melatonin standards

dissolved in 5% acetonitrile containing 0.1 % formic acid (Verde *et al.,* 2022).

4. Animal, housing and diets

The animal groups were kept in an atmosphere of filtered, pathogen-free air, water, and a temperature of 20-25ºC for 8 weeks, with a 12-hour light/dark cycle and a light cycle (8-20 h) and a relative humidity of 50%. For one week, all rats were fed a basal diet. The basal diet was designed to contain 14% casein, 10% sucrose, 4% corn oil, 5% 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 acclimatizing for seven days prior to the study. Rats were weighed, and randomly allocated into two main groups. Group (1) (6 rats) fed on basal diet, as a control negative group, while the second main group: (24 rats) received orally caffeine (100 g/kg body weight/day for two weeks) to establish oxidative stress models according to Emmanuel *et al.* (2017) then, blood samples were taken randomly to analyze liver enzymes to confirm oxidative stress. Then rats were divided into four subgroups; subgroup (1) fed on basal diet, as a positive control, subgroup (2) fed on basal diet + 20% (w/w in diet) dry sprouted lupine powder, subgroup (3) fed on basal diet + 20% (w/w diet) dry sprouted chickpea powder, subgroup (4) fed on basal diet + melatonin at doses of 10 mg/kg body weight.

Statistical Analysis:

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

RESULTS AND DISCUSSION

I. Physicochemical properties of sprouted and un sprouted lupine and chickpea:

1. Chemical analyses

Germination, among other technological processes, has been widely used for its ability to decrease levels of antinutritional factors present in legume seeds, at the same time improving the concentration and bioavailability of their nutrients (Urbano *et al.,* 2005).

Table (1) showed that the highest protein, fat, and ash contents (41.3, 12.33, 3.79, respectively) was recorded in the sprouted lupine sample, followed by sprouted chickpeas (23.3, 7.71, 2.84, respectively), compared to un sprouted samples of lupine (25.2, 9.3, 3.6, respectively) and un sprouted chickpeas (19.5, 6.7, and 2.7, respectively). The increase in protein may be due to loss of dry weight as some carbohydrates and fats are utilized during respiration but also some amino acids are synthesized during germination (Jan *et al.,* 2017).

Sprouting, however, resulted in a slight decrease in moisture, fiber, and carbohydrate contents in the lupine samples (4.7, 4.40, 33.48, respectively), as well as in the chickpea samples (6.3, 3.2, 56.65, respectively), compared to the un sprouted group of lupine (9.7, 18.3, 33.9, respectively) and un sprouted chickpeas (8.7, 16, and 46.4, respectively). These results were consistent with the findings of Ertaş (2015) who showed that the ash

content of lupine samples decreased with germination, while increasing with sprouting. The same upward trend was observed in protein content. Germination resulted in a slight, but significant ($P<0.05$) increase in protein content. Gulewicz *et al.* (2008) identified upward trends in ash and protein. The highest protein content was found with a 6-day germination period. The current study provides evidence that sprouted chickpeas can be a useful ingredient in the production of fortified foods. Industrial-scale sprouted chickpea flour demonstrated increased bioavailability of relevant micronutrients and a significant reduction in antinutritional factors (Marengo *et al.,* 2016).

Nkhata *et al.* (2018) concluded that PAL activity increases during germination. It is an enzyme that catalyzes pathways responsible for the biosynthesis of various phytochemicals, leading to increased antioxidant activity.

Table (1): Chemical constituent of dry sprouted lupine and chickpea and un sprouted (%).

Constituents	Samples (g)			
	Lupine		Chickpea	
	Un sprouted %	Sprouted %	Un sprouted %	Sprouted%
Proteins	25.2	41.3	19.5	23.3
Fat	9.3	12.33	6.7	7.71
Ash	3.6	3.79	2.7	2.84
Moisture	9.7	4.7	8.7	6.3
Fiber	18.3	4.40	16	3.2
Carbohydrates	33.9	33.48	46.4	56.65

These numbers were the meaning of triplicate assessing

2. Amino acids

Data in Table (2) showed 18 amino acids in sprouted and un sprouted lupine and chickpea, 10 were classified as essential amino acids, and 8 as non-essential amino acids. Concerning lupine, the essential amino acids Tyrosine was the highest value, while Methionine was the least value. Leucine was the highest value followed by Lysine in sprouted lupine, however Methionine was the least value. White lupine seeds have a minimum content of protein with anti-nutritive properties and a higher content of arginine, lysine, leucine, and phenylalanine, which makes white lupine seeds more valuable than other species regarding nutrition standards. White lupine contains more amino acids (AA), including essential amino acids (EAA), and is also characterised by a higher index of essential amino acids (EAAI) and protein efficiency ratio (PER) (Prusinski, 2017).

In chickpea, Leucine was the highest value followed by Lysine, while Cystine was the least valued. Leucine was the highest value followed by Lysine In sprouted chickpea, however Cystine was the least value.

Regarding the ratio of total essential amino acid (TEAA) in different treatments, sprouted lupine was the best sample because it contains the highest ratio of essential amino acids (15.36 g/100g dry matter). Meanwhile sprouted lupine recorded the highest percentage of total non-essential amino acids (TNEAA) (24.76 g/100g dry matter). Chickpea protein was rich in essential amino acids such as isoleucine, lysine, total aromatic amino acids and tryptophan. Therefore, chickpea protein

could complement well those protein sources that are low in lysine and tryptophan. However, leucine, total sulfur amino acids, threonine and valine were slightly deficient in chickpea protein (El-Adawy, 2002). In the current study the eight recorded amino acids (Cys, Ala, Ile, Leu, Trp, Val, Lys, and His) suppressed oxidative stress induced inflammatory response and therefore can act as cellular antioxidant defense systems, including nonenzymatic antioxidants (Katayama and Mine, 2007).

Table (2): Comparison between essential and non-essential amino acids in dry sprouted lupine and chickpea and un sprouted.

Amino Acids * (A.A)	Samples (g/100mg)				FAO*
	Lupine		Chickpea		
	Un sprouted	Sprouted	Un sprouted	sprouted	
Essential amino acid					
- Leucin	0.49	2.86	1.45	1.63	6.6
- Isoleucine	0.47	1.73	0.75	0.97	2.8
- Lysine	0.40	1.92	1.44	1.51	5.8
- Methionine	0.11	0.40	0.14	0.44	-
- Cystine	0.21	0.83	0.19	0.51	-
- Phenylalanine	0.34	1.70	1.51	1.33	-
-Tyrosine	0.26	1.88	0.40	0.74	-
-Therionine	0.31	1.48	1.05	0.83	3.4
- Valine	0.54	1.70	0.97	1.03	3.5
- Tryptophan	0.14	0.86	1.1	1.7	1
Total E.A. A	3.63	15.36	8.64	10.69	-
Non-essential amino acid					
- Alanine	0.85	1.49	0.79	1.00	-
- Aspartic	3.16	5.71	0.38	2.65	-
- Glutamic	5.03	7.75	1.47	3.91	1.9
- Glycine	1.49	1.54	1.26	0.88	-
- Histadine	0.19	0.99	0.29	0.59	-
- Proline	5.91	1.51	0.87	0.89	-
- Serine	0.90	1.93	0.82	1.03	-
- Arginine	0.39	3.84	1.26	2.04	-
Total non E.A. A	13.6	24.76	7.14	12.99	-

* g amino acid per 100 g protein.
** FAO/WHO, (1985**)** amino acid reference pattern of protein for children (2–5 years old) diet.

3. Melatonin

Data in Table (3) indicated that there was a significant difference in melatonin levels in un sprouted legumes compared to sprouted legumes, and its percentage was higher in sprouted legumes than in un sprouted ones. Its percentage in un sprouted lupine was (15.42%), while it was (155.50%) in sprouted lupine. However, it was in un sprouted chickpeas (26.46%), while in sprouted chickpeas (361.49%). This study indicates that legumes exhibited significant differences in melatonin concentrations, total phenolics, total flavonoids, and antioxidant activity. Melatonin, total

phenolics, total flavonoids, and antioxidant activity levels in legume cotyledons, radicles, and seed coats increased with the progression of germination (Saleh *et al.,* 2019).

Results indicated that legume seeds were a good dietary source of melatonin, serotonin and free tryptophan, as well as TPC. Germination of legume seeds, regardless of NaCl treatment, would stimulate melatonin, serotonin and tryptophan content, and exhibited stronger antioxidant activity than un germinated seeds (Nontasan *et al.,* 2021).

Table (3): Comparison between % of melatonin in dry sprouted and un sprouted lupine and chickpea.

legumes	Melatonin %	
	un sprouted	Sprouted
lupine	15.42	155.50
chickpea	26.46	361.49

II. Biological evaluation of experimental rats induced with oxidative stress and treated with melatonin, sprout chickpea or lupine

1. Body weight gain:

At the beginning of the investigation, all rats had approximately the same weight (170.60 ± 6 g). While final body weight of all rats' groups was not significantly different except inducted group (+ve) (Caffeine) was decreased in significant (P≤0.05) 203.20 ± 12.25 (Table 4). On the other hand, (sprouted lupine), (Melatonin) and (sprouted chickpea) respectively occurred higher increased in body weight gain (BWG) (243.20±5.98, 245.20± 25.73, 253.80± 23.38). It was noticed that the positive control group recorded the lowest weekly body weight gain. Hanafi, (2025) recorded the highest weekly body weight gain in the soybean seed sprouts group, followed by the un sprouted soybean seed group.

Table (4): Mean organ weight/body weight (%) of experimental rats which treated with melatonin, sprouted lupin or chickpeas.

Body weight (g)	Control (-ve)	Control (+ve)	Melatonin	Sprouted lupine	sprouted chickpea
IBW	171.00 a ± 9.31	175.40 a ± 8.03	176.60 a ± 6.99	170.60 a ± 3.63	175.20 a ± 13.09
BW at Wk2	182.00 a ± 9.73	176.80 ab ± 8.43	172.00 abc ± 6.56	174.4 abc ± 5.31	168.40 bc ± 9.60
BW at Wk3	205.60 a ± 18.43	177.00 c ± 10.12	195.40 ab ± 12.68	189.00 bc ± 3.96	196.40ab ± 14.75
BW at Wk4	225.00 a ± 12.29	179.80 c ± 11.20	209.80 ab ± 17.91	209.40 ab ± 7.99	213.20 ^{ab} ± 23.18
BW at Wk5	236.80 a ± 12.23	185.20 c ± 12.37	218.60 ab ± 21.84	214.80 b ± 11.67	231.60 ^{ab} ± 26.17
BW at Wk6	249.80 a ± 10.56	191.60 c ± 12.39	230.00 b ± 22.10	228.20 b ± 7.03	243.80 ^{ab} ± 27.04
BW at Wk7	263.80 a ± 15.16	197.00 c ± 12.87	238.20 b ± 25.69	236.00 b ± 6.00	247.40 ^{ab}± 24.72
FBW	274.00 a ± 14.80	203.20 c ± 12.25	245.20 b ± 25.73	243.20 b ± 5.98	253.80 b ± 23.38
BWG/wk %	60.36 ^{a} ± 5.80	15.84 ^{d} ± 4.35	38.56 ^{c} ± 10.11	42.56 ^{bc} ± 2.05	44.81 ^{bc} ± 6.49

Data are presented as means ± SDM (*n=8*).

a, b, c and d: Means with different letter among treatments in the same column are significantly different ($P \leq 0.05$)

IBW= Initial body weight; FBW= Final body weight; BWG= Body Weight gain; Wk: Week

2. Organs weight / body weight % of experimental rats treated with melatonin, sprouted lupin or chickpeas

It could be noticed from Table (5) that, liver weight/body weight % of the (+ve) control subgroup showed a significant increase (P<0.05) than the (-ve) control group (5.43 ± 0.32 and 3.33 ± 0.20, respectively). Significant difference (P>0.05) was recorded in liver weight/body weight % among the (+ve) control subgroup and the subgroups treated with (sprouted chickpea), (Melatonin), and (sprouted lupine) (4.15 + 0.35, 4.08 ± 0.36, 3.4 ± 0.16, respectively).

The observation that measuring the liver-to-body weight ratio is a more accurate method to determine changes in liver size compared to measuring liver weight alone because the liver weight's dependence on the size of the rat (Turtle *et al.,* 2013).

Regarding the mean value of heart weight/body weight %, it could be noticed that the (+ve) control subgroup showed a significant increase (P<0.05) than the (-ve) control group (0.46 ± 0.05 and 0.35 ± 0.03, respectively). All treated subgroups showed significant decreased P<0.05 in heart weight/body weight % as compared to the (+ve) control group. The best result was recorded for rats fed on diet supplemented with sprouted lupine (0.36 ± 0.03).

Table (5): Mean organ weight/body weight (%) of experimental rats which treated with melatonin, sprouted lupin, and sprouted chickpeas

Organs weight (%)	Control (-ve)	Control (+ve)	Melatonin	sprouted lupine	sprouted chickpea
Liver	3.33 e ± 0.20	5.43 a ± 0.32	4.08 bc ± 0.36	3.47 de ± 0.16	4.15 b ± 0.35
Heart	0.35cd ± 0.03	0.46 a ± 0.05	0.37 bc ± 0.04	0.36 bcd ± 0.03	0.40 b ± 0.03

Data are presented as means ± SDM (*n=8*).

A, B, C and D: Means with different letter among treatments in the same row are significantly different ($P \leq 0.05$).

3. Liver enzymes of experimental rats which treated with melatonin, sprouted lupine, and sprouted chickpeas

From Table (6) rats induced by Caffeine control (ve +) showed significant increase (P≤0.05) in the activities of ALT and AST (85.66±1.72, 158.38±2.16, respectively) compared with the control (ve) group (32.32±1.73, 67.5±1.91, respectively). After treatment, there was a significant decrease (P ≤ 0.05) in ALT and AST activities, with the best result in favor of the sprouted chickpea subgroup (25.36±1.3, 68.33±3.5, respectively).

The enzymes Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Gamma glutamate transferase (GGT) are important enzymes that are often employed in assessing liver injury (Obi *et al.,* 1998). Because the liver is furnished with machineries for synthesizing serum proteins excluding γ-globulins, thus, liver damage is characterized by hypoproteinemia and decreased albumin which can affect the whole physiological status of animals (Kanchana and Mohammed, 2011). Attention has been developed on the protective biochemical function of the natural antioxidants contained in dietary plants that are candidates for prevention or protection of oxidative damage caused by free radicals' species (Vincent *et al.,* 2004). Farther more, Ihemeje *et al.* (2018) found that boiling, soaking and sprouting had improved the soluble fiber content in red kidney beans. In addition, it was shown that

sprouted red beans resulted in a significant ($P \leq 0.05$) decrease in the level of liver enzymes and kidney function compared to the positive control sample, soaked and boiled red kidney beans.

Table (6): Liver enzymes (U/l) of experimental rats which treated with melatonin, sprouted lupin, and sprouted chickpeas.

Parameters	Control (Ve-)	Control (Ve+)	Melatonin	sprouted lupine	sprouted chickpea
AST	$67.5^{d}\pm1.91$	$158.38^{a}\pm2.16$	$110.17^{b}\pm2.01$	$68.18^{d}\pm3.4$	$68.33^{c}\pm3.5$
ALT	$32.32^{d}\pm1.73$	$85.66^{a}\pm1.72$	$55.36^{ab}\pm1.21$	$30.66^{d}\pm2.25$	$25.36^{c}\pm1.3$

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

4. Lipid peroxidation and antioxidants status (MDA, GSH and CAT) levels in liver and heart tissues

As shown in Table (7) the control (ve+) subgroup induced with oxidative stress by caffeine showed highly significant ($P \leq 0.05$) elevation of MDA (142.24±9.11) and significant ($p \leq 0.05$) decrease in GSH (170.44±3.26) and CAT (49.27±0.34) levels in liver compared with ve- control group. Similar results were recorded for GSH (100.17±3.67) and CAT (1.30±0.29) levels in heart tissues compared with ve- control group. After treatment, there was a significant decrease ($P \leq 0.05$) in MDA level for the sprouted chickpea subgroup (85.61±7.14) in liver and (70.11±4.22) in heart tissues. On the other hand, the levels of GSH and CAT in liver and heart tissues were significantly increased compare to the ve+ subgroup (Table 7). Given the beneficial effects of preventing these diseases resulting from Regarding cellular oxidation processes and reactive oxygen species, antioxidants, which are essential for preventing the formation and inhibiting the activity of nitrogen and oxygen radicals, have become essential compounds with health benefits that should be included in the diet (Dastmalchi *et al.,* 2020).

The activities of SOD., CAT., and Gpx of the serum of rats were used as indicators of oxidative stress. A daily consumption of red kidney beans could enhance the liver antioxidant status as shown by the increased levels of SOD, CAT and Gpx. This improvement may be due to the content of red kidney beans which considered a rich source of phenolic compounds. Many studies have shown that phenolic compounds have strong antioxidant capacity (Ouamnina, 2024).

Table (7): Lipid peroxidation and antioxidants status in liver, heart and testis tissues in experimental rats.

Parameters	Control (ve-)	Control (ve+)	Melatonin	sprouted lupine	sprouted chickpea
_Liver					
MDA (μ mol/g. tissue)	98.22^{d}±4	142.24^{d}±9.11	121.13±0.5	89.27a±4.12	85.61^{ab}±7.14
GSH (μ mol/g. tissue)	376.03^{a}±2.40	170.44^{d}±3.26	312.32^{c}±4.50	354.19^{a}±3.52	371.73^{ab}±5.12
CAT ($s^{-1}g^{-1}$)	183.99^{a}±3.69	49.27^{d}±0.34	123.99^{c}±4.30	175.17^{a}±3.6	186.08^{ab}±2.19
Heart					
MDA (μ mol/g. tissue)	74.18^{d}±2.2	189.13^{a}±2.90	70.99^{b}±4.21	71.22^{d}±2.9	70.11^{d}±4.22
GSH (μ mol/g. tissue)	279.12^{a}±1.9	100.17^{d}±3.67	214.23^{c}±5.3	250.18^{a}±4.3	269.44^{a}±3.29
CAT ($s^{-1}g^{-1}$)	6.01^{a}±0.3	1.30^{d}±0.29	4.12^{c}±0.23	5.00^{a}±0.29	5.95^{b}±0.17

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

REFERENCES

Aebi, H. (1984). Catalase in vitro. In Methods in enzymology (Vol. 105, pp. 121-126). Academic press.

Albro, P.W.; Corbett, J.T. and Schroeder, J.L. (1986). Application of the thiobarbiturate assay to the measurement of lipid peroxidation products in microsomes. J. Biochemical and Biophysical Methods, 13(3):185-194.

AOAC (2011). Official methods of analysis of AOAC International. 18th edition, revision 4, in: Horwitz W and Jr. Latimer GW (eds.) AOAC International, Gaithersburg, Maryland, US

AOAC. Association of Official Analytical Chemists (1975). Official methods of analysis (11th ed., pp. 927–928). Washington, DC: AOAC

Aydın, S.; Kaya, T.; Erman, O. and Yılmaz, Ö. (2021). The protective effects of lupine (*Lupinus albus*) fruit extract against to destructive effects of hyperglycemia in type i diabetic rats. Bentham Science Publishers, 21(8):1466-1475.

Banchroft, J.D.; Sevens, A. and Turner, D.R. (1996). Theory and practice of histological techniques, Fourth ed. Churchill Livingstone, New York, London, San Francisco, Tokyo.

Bruno, J.; Feldman, C.; Konas, D.; Kerrihard, L. and Matthews, L. (2019). Incorporating sprouted chickpea flour in pasta increases brachial artery flow-mediated dilation, 106(3):130

Dama, A.; Shpati, K.; Daliu, P.; Dumur, S.; Gorica, E. and Santini, A. (2024). Targeting metabolic diseases: the role of nutraceuticals in modulating oxidative stress and inflammation. Nutrients, 16(4):507.

Dastmalchia, N.; Baradaranb, B.; Latifi-Navidc, B.; Safaralizadeha, R.; Khojasteha, S.; Aminib. M.; Roshanid, E. and Lotfinejadb, B. (2020). Antioxidants with two faces toward cancer, N. Dastmalchi, et al. Life Sciences 258 (2020) 118186.

Dengxiong, L.; Qingxin, Y.; Ruicheng, W.; Zhouting, T.; Wang, J.; Luxia, Y.; Shao, F.; Chaipanichkul, P.; Yoo, K.; Wei, W.; Okoli, U.; Deng, S.; Mang, K.; William, C.; Heavey, S. and Feng, D. (2024). Interactions between oxidative stress and senescence in cancer: Mechanisms, therapeutic implications, and future perspectives. Redox Biology, 73:103208.

El-Adawy, T. (2002). Nutritional composition and antinutritional factors of chickpeas (*Cicer arietinum* L.) undergoing different cooking methods and germination. Plant Foods for Human Nutrition, 57: 83–97.

Ellman, G.L. (1959). Determination of Sulfhdryl group. Arch. Biochem. Biophys, 82: 70-74.

Emmanuel, A.; Majesty, D.; Benjamin, A.; Peter, A. and Ugwokaegbe, P. (2017). Effect of caffeine on some selected biochemical parameters using rat model. Hindawi, Advances in Biology, Volume 2017, Article ID 9303276, 8 pages

Ertaş, N. (2015). Technological and chemical characteristics of breads made with lupin sprouts. Quality Assurance and Safety of Crops & Foods, 7 (3):313-319.

FAO (1985). Energy and protein requirements. Report of a joint FAO/WHO/UNU Expert Consultation. Geneva: WHO Technical Report Series no. 724. Geneva: WHO.

Farag, A.; Aboul Naser, A.; Zayed, A. and Sharaf El-Dine, M. (2022). Comparative insights into four major legume sprouts efficacies for diabetes management and its complications: untargeted versus targeted NMR Biochemometrics approach. Metabolites, 13(1):63.

Fedor, S.; Tomas, B.; Ludovit, P. and Russel, J. (2016). Elevated heart rate and nondipping heart rate as potential targets for melatonin: a review. Journal of Pineal Research, 61(2): 127-137.

Gu, K.; Xiao-Mei, F.; Sun, S.; Xing-Yao, H. and Yong, W. (2024). Yes-associated protein-mediated melatonin regulates the function of periodontal ligament stem cells under oxidative stress conditions. World J Stem Cells, 16(11):926–943.

Gulewicz, P.; Martinez-Villaluenga, C.; Frias, J.; Ciesiolka, D.; Gulewicz, K. and Vidal-Valverde, C. (2008). Effect of germination on the protein fraction composition of different lupin seeds. Food Chemistry, 107: 830-844.

Hanafi, M. (2025). Effect of seeds and sprouts of red radish and soybeans on oxidative stress caused by paracetamol on rats. J. Specialized Education Studies and Res., 29:2974-4423.

Ihemeje, A.; Amadi, J. and Afam-Anene, O. (2018). Nutrient and Phytochemical Composition of Jackfruit (Artocarpus heterophyllus) Pulp, Seeds and Leaves, Int. J. Innovative Food, Nutrition & Sustainable Agriculture 6(3):27-32.

Jan, R.; Saxena, D. and Singh, S. (2017). Physicochemical, textural, sensory and antioxidant characteristics of gluten e free cookies made from raw and germinated Chenopodium (*Chenopodium album*) flour. LWT - Food Science and Technology, 71:281–287.

Jomova, K.; Raptova, R.; Suliman, Y.; Saleh, A.; Alwasel, H.; Nepovimova, E.; Kamil, K. and Valko, M. (2023).

Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. Archives of Toxicology, (2023) 97:2499–2574.

Kanchana, N.A. and Mohammed, S. (2011). Hepatoprotective effect of Plumbago zylanica on paracetamol induced liver toxicity in rats. Int. J. Pharmacy and Pharm Sci., 3(1): 151-154.

Katayama, S. and Mine, Y. (2007). Antioxidative activity of amino acids on tissue oxidative stress in human intestinal epithelial cell model. J. Agric. Food Chem., 55: 8458–8464.

Khalil, A. and Mansour, E. (1995). The effects of cooking autoclaving and germination on the nutrition quality of Fabe beans. Food chem., 54:177-182.

Krzysztof, C.; Witold, B. and Ewa, G. (2014). Acute caffeine poisoning resulting in atrial fibrillation after guarana extract overdose. Toxicology and Environmental Diseases, 31:531

Liguori, I.; Russo, G.; Curcio, F.; Bulli, G.; Aran, L. and Della-Morte, D. (2018). Oxidative stress, aging, and diseases. Clinical Interventions in Aging 2018:13 757–772.

Marengo, M.; Carpen, A.; Bonomi, F.; Casiraghi, M.; Meroni, E.; Quaglia, L.; Iametti, S.; Pagani, M. and Marti, A. (2016). Macromolecular and micronutrient profiles of sprouted chickpeas to be used for integrating cereal-based food. Cereal Chem., 94(1):82–88.

Meng, X.; Li, Y.; Li, S.; Zhou, Y.; Gan, R.; Xu, D. and Hua-Bin, L. (2017). Dietary sources and bioactivities of melatonin. Nutrients, 9:367.

Metwally, M.; El-Bawab, E.; Abdel, M. and Abdallah, A. (2023). Conglutin gamma, a lupin seed protein reduces oxidative stress in diabetics. J. Global Scientific Res., 8(2):2866-2883.

Nabeel, M.; Aly, K, Sanjota, N.; Shagufta, A.; Abdul, S.; Hadl, R. and Moin, H. (2024). Impact of caffeine on the histology of liver and blood microflora of albino rats. E-Publication: Online Open Access, Vol: 67 Issue 03.

Nkhata, S.; Ayua, E.; Kamau, E. and Shingiro, J. (2018). Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. Food Sci. Nutr., 6:2446–2458.

Nontasan, S.; Chottanom, P. and Moongngarm, A. (2021). Melatonin, its precursors, total phenolic content and antioxidant activity in legumes germinated under normal and saline conditions. J. Sustainability Science and Management, 16(2):53-66.

Obi, F.O.; Usenu, I.A. and Osayande, J.O. (1998). Prevention of carbon tetrachloride-induced hepatotoxicity in the rat by *H. rosasinensis* anthocyanin extract administered in ethanol. Toxicology, 131(2-3):93-98.

Ouamnina, A.; Alahyane, A.; Elateri, I.; Boutasknit, A. and Abderrazik, M. (2024). Relationship between phenolic compounds and antioxidant activity of some moroccan date palm fruit varieties (*Phoenix dactylifera* L.): A Two-Year Study. Plants (Basel), 13(8):1119.

Prusinski J. (2017). White lupin (*Lupinus albus* L.) – nutritional and health values in human nutrition – a review. Czech J. Food Sci., 35: 95–105.

Reeves, P.G.; Nielsen, F.H. and Fahey, G.C. (1993). AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the

reformulation of the AIN76A rodent diet. J. Nutr., 123(11):1939-51.

Reitman, S., and Frankel, S. (1957): A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28(1):56-63.

Saleh, H.; Hassan, A.; Mansour, A.; Fahmy, A. and El-Bedawey, A. (2019). Melatonin, phenolics content and antioxidant activity of germinated selected legumes and their fractions. J. Saudi Society of Agricultural Sciences, 18(3):294-301.

Shoaib, M.; Kim, N.; Choudhary, R.; Yin, T.; Shinozaki, T.; Becker, L. and Kim, J. (2021). Increased plasma disequilibrium between pro- and anti-oxidants during the early phase resuscitation after cardiac arrest is associated with increased levels of oxidative stress end-products. Mol. Med., 27:135.

Sirtori, R.; Lovati, M.; Manzoni, C.; Castiglioni, S.; Duranti, M.; Magni, C.; Morandi, S.; D'Agostina, A. and Arnoldi, A. (2004). Proteins of white lupin seed, a naturally isoflavone-poor legume, reduce cholesterolemia in rats and increase ldl receptor activity in HepG2 Cells. J. Nutr., 134(1):18-23.

Snedecor, G. and Cochran, W. (1980). Statistical methods Iowa State University press, Ames. Statistical methods, 7th ed.

Tarzia, B.; Gharachorlooa, M.; Bahariniab, M. and Mortazavic, S. (2012). The effect of germination on phenolic content and antioxidant activity of chickpea. Iranian J. Pharmaceutical Res.11(4):1137-1143.

Turtle, E. J.; Dear, J. W. and Webb, D.J. (2013). A systematic review of the effect of paracetamol on blood pressure in hypertensive and non-hypertensive subjects. British J. Clin.Ppharmacol., 75(6):1396-1405.

Urbano, G.; Aranda, P.; Vilchez, A.; Aranda, C.; Cabrera, L. and Porres, J. *et al.,* (2005): Effects of germination on the composition and nutritive value of proteins in *Pisum sativum*, L. Food Chem., 93:671–679.

Verde, A.; Míguez, J.; Leao-Martins, J.; Gago-Martínez, A. and Gallardo, M. (2022). Melatonin content in walnuts and other commercial nuts. Influence of cultivar, ripening and processing (roasting). J. Food Composition and Analysis 105:104180.

Vincent, A.M.; Russell, J.W.; Low, P. and Feldman, E.L. (2004). Oxidative stress in the pathogenesis of diabetic neuropathy. Endocrine reviews, 25(4):612-628.

Zhang, Z.; Zhang, X.; Chen, Y.; Jiang, W.; Zhang, J.; Wang, J.; Wu, Y.; Wang, S.; Yang, X.; Liu, M. and Zhang, Y. (2023). Understanding the mechanism of red light induced melatonin biosynthesis facilitates the engineering of melatonin-enriched tomatoes. Nature Communications, 14:5525.

تأثير الميلاتونين لبعض البقوليات المنبتة على الإجهاد التأكسدي لدى الفئران

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المستخلص

الميلاتونين وهو هرمون نيوروببتيدي تفرزه الغدة الصنوبرية، هو كاسح قوي للجذور الحرة ودواء جزيئي صغير تم اكتشافه في مواقع متعددة في الجسم. وقد ثبت أن الميلاتونين، وهو مضاد للأكسدة الطبيعية، يثبط الإجهاد التأكسدي ويثبت وظيفة بطانة الأوعية الدموية، مما يوفر حماية للقلب والأوعية الدموية. وقد وُجد أن بذور البقوليات المنبت مصدر غذائي جيد للميلاتونين والسيروتونين والتريبتوفان الحر. تهدف هذه الدراسة إلى تقييم تأثير الميلاتونين على الإجهاد التأكسدي في الفئران. تم وزن الحيوانات وتوزيعها عشوائيًا في مجموعتين رئيسيتين. المجموعة 1 (6 فئران) تغذت على نظام غذائي أساسي كعنصر تحكم سلبي، بينما تلقت المجموعة 2 (24 فأرًا) الكافيين عن طريق الفم (100 جم / كجم من وزن الجسم / يوم لمدة أسبوعين) لاصابة بالإجهاد التأكسدي. تم تقسيم فئران المجموعة (2) إلى أربع مجموعات فرعية، تحتوي كل منها على 6 فئران. المجموعة الفرعية (1) تغذت على نظام غذائي أساسي كعنصر تحكم إيجابي. تلقت المجموعة الفرعية (2) نظامًا غذائيًا أساسيًا مضافًا إليه 20% (من وزن الوجبة) من مسحوق الترمس المنبت الجاف. تلقت المجموعة الفرعية (3) نظامًا غذائيًا أساسيًا مضافًا إليه 20% (من وزن الوجبة) من مسحوق الحمص المنبت الجاف. تلقت المجموعة الفرعية (4) نظامًا غذائيًا أساسيًا مضافًا إليه الميلاتونين بجرعات 10 ملغم/كغم من وزن الجسم. أظهرت النتائج أن الكافيين زاد بشكل ملحوظ من خطر الإجهاد التأكسدي ومع ذلك، أشارت نتائج المجموعات الفرعية المعالجة (2 و3 و4) إلى تحسن في زيادة الوزن، وانخفاض في إنزيمات الكبد، وانخفاض في مادة المالونديالدهيد، وزيادة في مستويات الجلوتاثيون و الكاتالاز، المرتبطة بالإجهاد التأكسدي، مقارنةً بالمجموعة الفرعية للإجهاد التأكسدي (1). من ناحية أخرى، أظهر الفحص النسيجي للمجموعة الفرعية الضابطة الإيجابية (1) بعد شهرين من العلاج تحسنًا في أنسجة القلب والكبد لدى الفئران المعالجة، وخاصة في المجموعة الفرعية (التي تناولت الحمص المنبت). وبشكل أكثر تحديدًا، قلل النظام الغذائي الغني بالميلاتونين من التأثيرات الضارة للإجهاد التأكسدي.

الكلمات المفتاحية: الميلاتونين، الترمس، مضادات الأكسدة، الجذور الحرة، الإجهاد التأكسدي، البقوليات، الحمص، الجرذان البيضاء، إنزيمات الكبد؛ علم الأمراض النسيجي.