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# Protective Effect of Pan Bread Fortified with Ginger on Liver and Renal Function of Hepatotoxic and Nephrotoxic Albino Rats

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*In Loving Memory of Late Professor Doctor ""Mohamed Refaat Hussein Mahran""*

**Abstract**
Hepatotoxicity and nephrotoxicity are global health issues. Herbal therapy, such as ginger, still treats and prevents hepatotoxic and nephrotoxic functioning diseases. Five levels of pan bread supplemented with ginger (2, 4, 6, 10, and 12%) were sensory evaluated for the overall acceptability. The results showed that the overall acceptability of pan bread containing 2, 4, and 6% ginger was more acceptable than the levels of ginger 10 and 12%. Thirty-five male albino rats weighing (170 ± 10 gm) were used for biochemical study. The rats were distributed into five groups, each containing 7 rats. The first group (- ve) fed on wheat bread with a basal diet. The second group was fed on wheat pan bread with a basal diet until they received the dose of $CCL_4$ and kept as a control group (+ve). Group 3, 4, and 5: fed on pan wheat bread supplemented with (2%, 4%, and 6% ginger, respectively). After 4 weeks, the control group 2 (+ve) and groups (3, 4, and 5) were subcutaneously injected (twice a week) with a dose of 4ml $CCL_4$ in olive oil (50% v/v) /kg of body weight for another two weeks to induce chronic damage in the liver and kidney. The results of biochemical analysis of pan bread supplemented with ginger 6% treatment indicated an improvement of lipid profile, thyroid gland, antioxidant enzymes, kidney and liver functions in serum. Our biochemical studies corroborated the histology of liver and kidney tissues. The study suggests incorporating ginger into our daily diet as a functional food supplement for bread products.
*Keywords: Pan Bread, Ginger, Hepatotoxic, Nephrotoxic, Albino Rats*

## 1. Introduction

Numerous botanical species, such as "ginger, grapefruit, and chamomile", significantly promote human health and well-being. Zingiber officinale, commonly known as ginger, is commonly employed as a culinary additive in diverse global cuisines (Shahrajabian et al. 2019). Chemical analysis of ginger revealing the presence of more than 400 distinct chemicals. Ginger rhizomes mostly consist of carbohydrates (50–70%), lipids (3–8%), terpenes, and phenolic chemicals (Zhukovets & Özcan, 2020). Ginger has many terpene components such as "zingiberene, β-bisabolene, α-farnesene, β-sesquiphellandrene, and α-curcumene". Additionally, it contains phenolic compounds like gingerol, paradols, and shogaol. Gingerols (23–25%) and shogaol (18–25%) are more abundant than other compounds. In addition to these, the presence of amino acids, raw fiber, ash, protein, phytosterols, vitamins such as nicotinic acid and vitamin A, and minerals has been reported (Gonzalez-Gonzalez et al., 2023). According to Zhukovets and Özcan (2020), ginger is a rich source of antioxidants and contains numerous antioxidant components, making it a viable supplement with high antioxidant content. The chemical analysis of pan bread with ginger 6% showed the highest content of protein, fat, fiber, and ash compared with raw wheat and pan bread of wheat (Shawir et al., 2024). Attia et al. (2013) reported that ginger possesses potent antioxidant properties that can potentially mitigate or prevent the formation of free radicals and safeguard cells against lipid oxidation. Ginger has been found to have hepatoprotective and nephrotoxic properties and is commonly consumed by humans (Shakya 2016).

Triticum aestivum L., commonly known as common wheat, is a crucial constituent of the human diet. It manufactures food items such as bread, noodles, steamed bread, and cakes. The chemical analysis of raw wheat revealed that it has a protein content of 31.40%, a dietary fiber level of 18.50%, and a fat content of 7.0%. Additionally, it contains crucial amino acids, including isoleucine, leucine, and lysine (Akbari et al., 2022).

Bread is a popular cereal product and contains many nutrients, such as vitamins and minerals, especially phosphorus and copper (Ibrahim et al. 2015). The enrichment of wheat with ginger powder in making bread and cookies can potentially improve individuals' health and nutritional well-being, given

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the health advantages associated with these ingredients. Numerous investigations have documented the utilization of composite flour derived from wheat (Jukić et al. 2022).

Carbon tetrachloride is a chemical substance that can cause damage to the liver and kidneys in both humans and animals. The hepatotoxicity of carbon tetrachloride is attributed to its reduction by the cytochrome P450 enzyme system, as reported by Girish and Pradhan (Girish and Pradhan 2012).

The hepatotoxicity induced by the liver toxicant $CCl_4$ is primarily attributed to the lipid peroxidative degradation of biomembranes. The induction of chronic liver injury in rats can be observed upon the administration of carbon tetrachloride (Abdel Moneim 2016). The kidney, which is responsible for waste disposal, is a target for xenobiotic toxicants, including $CCl_4$, which can cause oxidative DNA damage and kidney cell death (Ma et al. 2014).

A healthy diet is beneficial in mitigating the adverse consequences of hepatotoxicity and nephrotoxicity, two significant global health issues. Using wheat and ginger powder as composite mixes in preparing pan bread may improve customers' nutritional and health status, given the health benefits of these ingredients. The study aimed to investigate the protective effect of pan bread fortified with ginger on the liver and renal function of hepatotoxic and nephrotoxic albino rats.

## 2. Materials

*2.1. Samples*

Ginger (Zingiber officinale). Wheat flour was extracted 72%, and salt and yeast were obtained from local markets in Alexandria, Egypt.

*2.2. Chemicals and Kits*

- All chemicals and reagents used were analytical grades obtained from EL- Goumhorya, Chemical Company, Egypt and Carbon tetrachloride ($CCL_4$) was purchased from Merck, Darmstadt.

- All kits for determining of the biochemical parameters were obtained from Sigma Chemical Company, Egypt.

*2.3. Animals*

A total of 35 male Albino rats of "the Sprague Dawley Strain" were acquired from the animal house of the Institute of Graduate Studies and Research, Alexandria University. The rats had a mean weight of 170 ±10 grams.

## 3. Methods

*3.1. Preparation of Ginger Powder*

The fresh ginger was rinsed with clean water to eliminate any impurities. Once the cleaning process was completed, the fresh ginger was sliced. The ginger slices were dehydrated in an oven at 40°C and milled using an electric grinder (Moulinex, France) to obtain a fine powder. The ginger powder was then sifted through a 0.2 mm sieve and stored in a polyethylene bag until it was ready to be used (Almasodi 2018).

*3.2. Preparation of pan bread supplemented with ginger*

The components of pan bread enhanced with ginger are presented in Table (1). Pan bread supplemented with ginger was prepared by mixing wheat flour, salt, yeast, and sugar. Ginger and warm water were added slowly, forming a dough that was incubated for 30 minutes before being cut into spherical pieces and rolled into a round shape. The dough was then fermented for another 20 minutes before being baked on medium heat in a Tefal frying pan until the loaves were fully cooked (Amjad et al., 2021).

*3.3. Sensory evaluation*

Sensory evaluation of pan bread and pan bread supplemented with different concentrations of ginger (2, 4, 6, 10, and 12%) were conducted in the Department of Home Economics, Faculty of Specific Education, Alexandria University. The samples were evaluated by using 40 panelists from the staff and students for color, taste, odor, texture, and overall acceptability, according to Almasodi (2018).*3.4. Biological study*

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*3.4.1. preparation of diets:*

The basal and experimental diets were prepared according to the method of Reeves et al. (1994) (Table 2).

Table (1): Ingredients used for preparation of pan bread supplemented with ginger

| Ingredients | Wheat Flour | Ginger | Sugar | Yeast | Salt | Water |
|---|---|---|---|---|---|---|
| Treatment | | | (g) | | | ml |
| Control Pan Bread (100%) wheat flour | 100 | - | 2 | 2.5 | 0.7 | 50 |
| Pan Bread (2% Ginger + 98% wheat flour) | 98 | 2 | 2 | 2.5 | 0.7 | 50 |
| Pan Bread (4% Ginger + 96% wheat flour) | 96 | 4 | 2 | 2.5 | 0.7 | 50 |
| Pan Bread (6% Ginger + 94% wheat flour) | 94 | 6 | 2 | 2.5 | 0.7 | 50 |
| Pan Bread (10% Ginger + 90% wheat flour) | 90 | 10 | 2 | 2.5 | 0.7 | 50 |

Table (2): The ingredients of the basal diet

| Ingredients | Amount (%) |
|---|---|
| Casein | 20 |
| Starch | 66.25 |
| Corn Oil | 4.0 |
| Choline Chloride | 0.25 |
| Vitamins Mix | 1.0 |
| Cellulose | 5.0 |
| Salt Mixture | 3.50 |
| Total | 100 |

*3.4.2. Experimental animal design:*

Thirty-five adult male albino rats weighing approximately 170 ±10 grams were housed in well-aerated cages under hygienic conditions and fed on a basal diet for two weeks for adaptation. After adaptation periods, rats were divided into five groups as follows:

**Group 1**: (n=7 rats) was fed wheat pan bread during the experimental period, and they were kept as a negative control group (-ve).

**Group 2:** (n=7 rats) was fed on wheat pan bread until it received the dose of CCL4 and kept as a positive control group (+ve)

**Group 3, 4 and 5:** fed on pan wheat bread supplemented with (2%, 4% and 6% of ginger powder, respectively).

After 4 weeks, the positive control group 2 (+ve) and groups (3,4 and 5) were subcutaneously injected (twice a week) with a dose of 4ml CCL4 in olive oil (50% v/v) /kg of body weight another two weeks to induce chronic damage in the liver and kidney. After that, blood samples were taken from rats to confirm the occurrence of hepatotoxicity and nephrotoxicity depending on the level of liver and kidney enzymes. After that, Rats of the control (+ve) were fed on wheat pan bread while groups (3, 4 and 5) were fed on pan bread supplemented with different concentrations of ginger for another 2 weeks.

*3.4.3. Blood sampling and organs*

After the 8-week experimental phase, rats were fasted overnight before being anesthetized by ether and dissected. Subsequently, blood samples were obtained and subjected to centrifugation at 3000 rpm to separate the serum, which was then utilized for biochemical analysis assessments. The measurement of total cholesterol followed the methodology described by Richmond (1973), triglyceride (T.G), (Fossati and Prencipe, 1982), "High-density lipoprotein" (HDL-c) (Lopes-Virella et al., 1977), "Low-density lipoprotein" (LDL-c) and "Very Low-density lipoprotein" VLDL-c were carried out according to Jaye et al. (2009). Serum thyroid stimulating hormone (TSH) (Hamouda et al., 2016). The liver enzymes, alkaline phosphates activity (ALP) (Lavie et al., 2018), "Aspartate transaminase activity" (AST) (Yagi et al., 1985), and Alanine transaminase (ALT) activity (Williamson, 1974). The antioxidant enzyme activity of Glutathione peroxidase (GPX) (Yokota et al., 1988), Catalase activity (CAT) (Hadwan and kadhum Ali, 2018), and superoxide dismutase activity (SOD) (Rigo et al., 1975). Urea (Wuepper et al., 2003), Creatinine (Shlipak et al., 2013). Histological data of liver and kidney tissues were examined under an Olympus light microscope, according to Gamble et al. (2008).

*3.7. Statistical analysis*

Data were analyzed using IBM SPSS software package version 23.0. Quantitative data was described using mean and standard deviation. The significance of the obtained results was judged at the 5% level. F-test (ANOVA) was used to compare between more than two groups, and the Post Hoc test (LSD) was used for pairwise comparison (Kirkpatrick 2015).

## 4. Results and Discussion

*4.1. Sensory evaluation*

The data in Table (3) and Fig. (1) showed the sensory evaluation of pan wheat pan bread and pan bread samples supplemented with different concentrations of ginger 2, 4, 6, 10 and 12%. The results showed that the mean values for color at a concentration of 6% gingerbread had the highest color acceptance score of 8.18, followed by bread supplemented with 4% gingerbread at 7.47, while the score was 6.24. Bread contains 12% ginger, which also had the lowest color values and acceptance. In the case of tasting, the mean values of acceptance showed that 6% of ginger pan bread had the highest value of taste acceptance, 8.47 compared with other treatments. The overall values of acceptance showed that 6% of ginger pan bread had the highest value of general acceptance, 8.24, followed by the treatment of pan bread with 4% ginger pan bread (7.47). In comparison, the treatment of pan bread with 10 and 12% added ginger had the lowest general acceptance values, 6.88 and 6.53. Thus, these concentrations were rejected, and 2, 4 and 6% concentrations of ginger (the highest acceptability values) were used for biochemical studies.

The present findings align with Almasodi (2018), which determined that substituting 3% gingerbread in wheat flour yields bread that is more palatable compared to bread made with an addition of 9% ginger. Furthermore, the findings align with the study conducted by Balestra et al. (2011), which proposed that incorporating ginger powder into the bread recipe would not enhance its acceptability. The sample containing the least amount of ginger powder exhibited the highest "overall acceptability" level.

*4.2. Lipid profile and thyroid-stimulating hormone*

"The data presented in Table (4) showed that the control (+ve) group significantly ($P<0.05$) increased the concentrations of all serum lipid profiles while HDL-c decreased. On the other hand, treatment of pan bread supplemented with ginger (2, 4, and 6%) decreased all serum lipid profiles while increasing HDL-c compared to the control (+ve) group". Moreover, increasing pan bread supplemented with ginger decreased the lipid profile or increased HDL-c. Moreover, the data indicated that the level of TSH in the data showed that the control (+ve) group had significantly ($P \leq 0.05$) higher 1.89 ng/ml compared to the control (-ve) 0.55 ng/ml and treatment groups of pan bread supplemented with (2, 4, 6%) of ginger. In contrast, the values of TSH were 1.42, 1.18, and 0.77 ng/ml for 2, 4, and 6% of ginger treatment groups, respectively. The present data showed a decrease in serum lipids, which appeared to be related to the effect of pan bread supplemented with different levels of ginger on the lipid profile of hepatotoxic and nephrotoxic rats. Ginger may reduce cholesterol absorption or lower blood cholesterol, LDL-c, and VLDL, thereby improving lipoprotein profiles. It also leads to a decrease in the percentage of the thyroid gland hormone, thus improving the gland's functioning well (Akinyemi et al. 2014).

The current results were matched with Hamouda et al. (2016), who reported that TC, "TG and LDL-C levels decreased in control compared with the CCl4 group". At the same time, HDL-C levels in the control were higher compared with the CCl4 group. Ginger enhances lipid metabolism by reducing the activity of key enzymes involved in hepatic fatty acid and triglyceride synthesis. This leads to improved total lipid and total cholesterol levels in the serum and liver (Lebda et al. 2012; Lai et al. 2016). The present findings align with those documented by Hamouda et al. (2016), in which TSH levels in the CCl4 group were higher at 0.19 µIU/ml compared with control and Ginger+CCl4 0.13 and 0.14 µIU/ml.

Table (3): Sensory evaluation of pan wheat bread and pan bread supplemented with different concentrations of ginger

| Variables<br>Treatment groups | Color | Taste | Odor | Textures | Acceptance |
|---|---|---|---|---|---|
| Pan wheat bread | 6.91$^{bc*}$ ±0.36 | 7.12$^{bcd}$ ±1.41 | 7.0$^{b}$ ±1.37 | 7.18$^{bc}$ ±0.88 | 7.24$^{bcd}$ ±1.39 |
| Pan Bread (Ginger 2% + wheat flour 98%) | 7.12$^{b}$ ±1.22 | 7.29$^{bc}$ ±0.59 | 7.29$^{bc}$ ±0.85 | 7.53$^{ab}$ ±0.87 | 7.35$^{bc}$ ±0.93 |
| Pan Bread (Ginger 4%+ wheat flour 96%) | 7.47$^{b}$ ±1.07 | 7.76$^{b}$ ±0.90 | 7.65$^{b}$ ±0.86 | 7.88$^{a}$ ±0.78 | 7.47$^{bc}$ ±1.23 |
| Pan Bread (Ginger 6%+ wheat flour 94%) | 8.18$^{a}$±0.53 | 8.47$^{a}$ ±0.51 | 8.35$^{a}$ ±0.61 | 8.24$^{a}$ ±0.75 | 8.24$^{a}$ ±0.56 |
| Pan Bread (Ginger 10% + wheat flour 90%) | 6.41$^{c}$ ±1.42 | 6.74$^{c}$ ±0.79 | 6.71$^{c}$ ±0.77 | 6.76$^{c}$ ±0.75 | 6.88$^{c}$ ±0.49 |
| Pan Bread (Ginger 12% + wheat flour 88%) | 6.24$^{c}$ ±0.97 | 6.47$^{cd}$ ±1.33 | 6.29$^{cd}$ ±1.31 | 6.35$^{cd}$ ±1.32 | 6.53$^{cd}$ ±1.33 |
| F | 8.659* | 9.228* | 8.947* | 9.986* | 5.154* |
| P | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* |
| LSD 5% | 0.680 | 0.670 | 0.682 | 0.623 | 0.716 |

*Data was expressed using Mean ± SD.

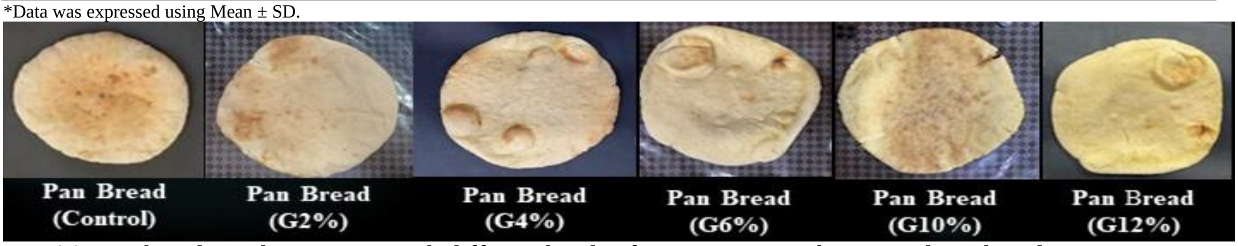


Fig. (1): Pan bread supplementation with different levels of ginger compared to control pan bread

Table (4): Effect of pan bread supplemented with different levels of ginger on the lipid profile and thyroid glands hormone of hepatotoxic and nephrotoxic rats

| Variables treatment groups | Cholesterol | TG | HDL | LDL | VLDL | TSH |
|---|---|---|---|---|---|---|
| | | | (mg/dl) | | | (ng/ml) |
| Control(-ve) | 87.36$^{d*}$ ±2.83 | 108.37$^{d}$ ±3.04 | 48.72$^{a}$ ±3.07 | 37.48$^{d}$ ±2.30 | 21.60$^{d}$ ±1.83 | 0.55$^{d}$ ±0.06 |
| **Control(+ve) | 226.27$^{a}$ ±2.76 | 257.07$^{a}$ ±3.10 | 20.67$^{d}$ ±2.52 | 153.96$^{a}$ ±2.96 | 51.40$^{a}$ ±1.91 | 1.89$^{a}$ ±0.19 |
| Rat fed on pan bread Pan Bread (2% ginger + 98% wheat flour) | 200.0$^{b}$ ±2.0 | 201.67$^{b}$ ±1.91 | 32.0$^{c}$ ±3.0 | 120.13$^{b}$ ±2.01 | 40.50$^{b}$ ±2.98 | 1.42$^{b}$ ±0.20 |
| Rat fed on Pan Bread (4% ginger + 96% wheat flour) | 145.08$^{c}$ ±3.13 | 165.26$^{c}$ ±2.52 | 37.73$^{b}$ ±3.39 | 74.77$^{c}$ ±2.98 | 33.0$^{c}$ ±3.0 | 1.18$^{bc}$ ±0.24 |
| Rat fed on Pan Pan Bread (6% ginger + 94% wheat flour) | 90.85$^{d}$ ±2.27 | 113.13$^{d}$ ±2.86 | 46.69$^{a}$ ±2.02 | 39.37$^{d}$ ±3.07 | 22.51$^{d}$ ±1.77 | 0.77$^{d}$ ±0.25 |
| F | 1710.353* | 1584.309* | 48.706* | 1074.367* | 84.545* | 21.020* |
| P | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* |
| LSD 5% | 4.785 | 4.951 | 5.167 | 4.912 | 4.308 | 0.364 |

*Data was expressed using Mean ± SD.
** Control+ $CCl_4$

*4.3. liver enzymes in serum*

The data presented in Table (5) showed the control (+ve) group had significantly (P≤0.05) higher AST 205.01 U/ml compared to the control (-ve) 136.33 U/ml and treatment groups of pan bread supplemented with (2, 4, 6%) of ginger. Also, AST decreased with the increase in ginger levels, whereas the values of AST were 172.51, 153.07 and 138.05 U/ml for 2, 4, and 6% of ginger treatment groups, respectively. The same trend was observed for ALT, whereas control (+ve) had a significantly higher 186.33 U/ml compared to the control (-ve) 69.07 U/ml. Also, the ALT decreased with the increase in ginger levels, whereas the values of ALT were 151.07, 129.33 and 73.17 U/ml for 2, 4, and 6% of ginger treatment groups, respectively. The same trend of the ALP enzyme was observed in which the control (+ve) group had significantly (P≤0.05) higher ALP (395.35 U/ml) compared to the control (-ve) 226.32 U/ml and treatment groups of pan bread supplemented with (2, 4, and 6%) of ginger. The levels of ALP decreased with the increase in ginger concentrations, whereas the activity of ALP was 281.33, 277.94 and 228.10 U/ml for 2, 4, and 6% of ginger treatment groups, respectively.

Assessing liver function can be done by measuring the activity of marker enzymes such as AST and ALT (Islam et al. 2011). The present findings demonstrate that the control+CCl4 group exhibited a notable increase in serum levels of ALP, AST, and ALT compared to the control group and the three ginger-treated groups. Conversely, the ginger-treated groups displayed a decrease in ALT, AST, and ALP levels, with the reduction being dependent on the quantity of ginger. The level of AST enzyme activity showed a substantial reduction (p<0.05) in the group of rats supplemented with 5% ginger compared to the control group treated with CCl4 (Kazeem et al. 2011). Ginger consumption preserves liver integrity and shields it from CCl4-induced damage (Bekkouch et al., 2022).

*4.4. Kidney Function*

The data presented in Table (5) showed the control (+ve) group had significantly (P≤0.05) higher levels of urea 40.43 mg/dL compared to the control (-ve) 21.63 mg/dL and treatment groups of pan bread supplemented with (2, 4, 6%) of ginger. Also, the level of urea decreases with the increase in ginger concentrations. At the same time, the level of urea was 31.33, 28.61 and 22.53 mg /dL for 2, 4, and 6% of ginger treatment groups, respectively. The same trend was observed for Creatinine, whereas control(+ve) had a significantly higher level of 1.55 mg/dL compared to the control (-ve) 0.54 mg/dL. The levels of Creatinine decreased with the increase in ginger concentrations, whereas the values of Creatinine were 0.85, 0.70 and 0.65 mg/dl for 2, 4, and 6% of ginger treatment groups, respectively.

"Serum creatinine and urea" are highly sensitive biochemical markers utilized to evaluate renal tissue damage due to their excretion through the kidneys. Consequently, in cases of cellular injury, there is a buildup of creatinine and urea in the bloodstream. The notable elevation in serum creatinine level indicates a decline in the renal tubules' capacity to eliminate waste products (Sindhu et al. 2015). The current results are consistent with Abd El-Ghany et al. (2012), who showed that the level of "Creatinine and urea" in the control positive rat group (CCl4) was increased (2.62 and 77.42 mg/dl) compared with ginger group 0.57and 39.4 mg/dl. Also, the treated groups of ginger + CCl4 showed a significant decrease in the serum levels of Creatinine and urea compared to the positive group (CCl4); this was similar to the results of Al-Yahya et al. (2013).

*4.5. Antioxidant enzymes in serum*

The data presented in Table (6) showed control (+ve) group had a significant (P≤0.05) decrease in SOD 15.37 U/ml compared to the control (-ve) 25.73 U/ml and treatment groups of pan bread supplemented with (2, 4, and 6%) of ginger. The level of SOD depended on ginger concentrations, whereas the values of SOD were 18.47, 19.57 and 24.00 U/ml for 2, 4, and 6% of ginger treatment groups, respectively. The same trend was observed for CAT, whereas control (+ve) had significantly decreased by 7.27 U/ml compared to the control (-ve) by 23.23 U/ml. The values of CAT were 11.90, 15.90 and 21.02 U/ml for 2, 4, and 6% of ginger treatment groups, respectively.

Table (5): Effect of pan bread supplemented with different levels of ginger on liver enzymes and kidney function in hepatotoxic and nephrotoxic rats

| Variables | AST | ALT | ALP | Urea | Creatinine |
|---|---|---|---|---|---|
| Treatment groups | (U/ml) | | | mg/dl | |
| Control(-ve) | 136.33$^{d*}$ ±3.13 | 69.07$^{d}$ ±2.0 | 226.32$^{d}$ ±3.30 | 21.63$^{d*}$ ±2.35 | 0.54$^{b}$ ±0.42 |
| **Control (+ve) | 205.01$^{a}$±2.45 | 186.33$^{a}$ ±2.14 | 395.35$^{a}$ ±3.36 | 40.43$^{a}$ ±1.73 | 1.55$^{a}$±0.52 |
| Pan Bread (2% Ginger + 98% wheat flour) | 172.51$^{b}$ ±2.06 | 151.07$^{b}$ ±1.96 | 281.33$^{b}$ ±2.56 | 31.33$^{b}$ ±2.52 | 0.85$^{b}$ ±0.06 |
| Pan Bread (4% Ginger + 96% wheat flour) | 153.07$^{c}$ ±1.98 | 129.33$^{c}$ ±2.75 | 277.94$^{b}$ ±2.45 | 28.61$^{bc}$ ±2.36 | 0.70$^{b}$ ±0.28 |
| Pan Bread (6% Ginger + 94% wheat flour) | 138.05$^{d}$ ±2.16 | 73.17$^{d}$±2.50 | 228.10$^{d}$ ±2.33 | 22.53$^{d}$ ±3.32 | 0.65$^{d}$ ±0.15 |
| F | 428.037* | 1462.133* | 1762.688* | 27.665* | 4.434* |
| P | <0.001* | <0.001* | <0.001* | <0.001* | 0.026* |
| LSD 5% | 4.355 | 4.168 | 5.157 | 4.56 | 0.60 |

*Data was expressed using Mean ± SD.
**Control+ $CCL_4$

Table (6): Effect of pan bread supplemented with different levels of ginger on the activities of antioxidant enzymes in the serum of hepatotoxic and nephrotoxic rats

| Variables | SOD | CAT | GPx |
|---|---|---|---|
| Treatment groups | U /mL | | mU/mL |
| Control(-ve) | 25.73 [a*] ±0.68 | 23.23 [a] ±0.29 | 24.28 [a] ±1.10 |
| **Control (+ve) | 15.37 [c] ±1.20 | 7.27 [e] ±1.47 | 12.30 [d] ±1.68 |
| Pan Bread (2% Ginger + 98% wheat flour) | 18.47 [b] ±1.59 | 11.90 [d] ±0.53 | 15.53 [c] ±0.40 |
| Pan Bread (4% Ginger + 96% wheat flour) | 19.57 [b] ±0.70 | 15.90 [c] ±1.40 | 19.27 [b] ±0.40 |
| Pan Bread (6% Ginger + 94% wheat flour) | 24.00 [a] ±0.79 | 21.02 [b] ±1.63 | 22.80 [a] ±0.87 |
| F | 47.714* | 89.427* | 72.485* |
| P | <0.001* | <0.001* | <0.001* |
| LSD 5% | 1.922 | 2.176 | 1.843 |

*Data was expressed using Mean ± SD.
**Control+ CCL4

Moreover, GPX enzyme activity was reduced in the control(+ve) group (12.30 mU/mL) compared to the control (-ve) 24.28 mU/mL. At the same time, the levels of GPX in ginger concentrations were increased depending on the concentration of ginger. In contrast, the values of GPX were 15.53, 19.27 and 22.80 mU/mL for 2, 4, and 6% of ginger treatment groups, respectively.

The current results revealed that pan bread supplemented with different levels of ginger elevates the activities of antioxidant enzymes in the serum of hepatotoxic and nephrotoxic rats. Consuming ginger may be effective in treating hepatotoxicity and nephrotoxicity. Ginger can potentially be a promising candidate for future utilization in nutritional applications (Ma et al. 2014). Fathi et al. (2021) reported that the control positive (CCl4) rat showed a significant decrease in SOD, GPX and CAT in the serum of rat groups compared with a normal control group and ginger groups. Moreover, Hussein et al. (2017) revealed a significant decrease in CAT and SOD activities of metalaxyl-intoxicated rats after four weeks compared to control.

Similarly, Mohammadi et al. (2020) suggested that ginger extract treatment leads to a significant increase in serum SOD and CAT activities compared to control positive. The high antioxidant activity of ginger was attributed to the high content of polyphenol compounds (6-gingerol and its derivatives) and diarylheptanoids, which have high antioxidant activity (Osae et al. 2019).

*4.8. Histology*

*4.8.1. Histology of liver tissue*

Photomicrograph of liver tissue from control (-ve) group showing the normal histological structure, regular characteristic hepatocytes with sinusoidal (S) voids arranged radially around the central vein (CV). The Liver cell shows normal basal nuclei and eosinophilic cytoplasm (→) Fig. (2 A). The liver tissue from a control (+ve) group showed blood-engorged pelvic veins, cellular infiltration, and Kupffer cells in sinus walls, causing liver lesions and fibrosis Fig. (2 B). However, the liver tissue from rats fed on pan bread supplemented with 6% ginger showed recovery of most liver tissue, normal liver structure (S), and the majority of hepatocyte nuclei have typical architecture (thin arrow) Fig. (2C).

The current results matched with (Mallikarjuna et al. 2008), the liver tissue of both the control and ginger groups exhibited hepatic plates arranged in a normal pattern, emanating from a central vein with a thin wall. These plates were divided by blood sinusoids bordered by endothelial cells. Also, the addition of ginger resulted in significant improvement of the liver's tissue structure and restoration of normal sinusoidal gaps. This could be attributed to the presence of antioxidant chemicals in ginger that regulate antioxidant enzymes in the context of Ni-induced liver damage.

Moreover, Badawi (2019) observed that ginger protected the architecture of hepatic tissue from damage by CCl4 that induced liver necrosis; this effect may be due to the presence of phenolic components such as gingerols and shogoals.

*4.10.2. Histology of kidney tissue*

Microscopic examination of the rat kidney cortex of a control (-ve) group showing normal glomerulus and glomerular mass, proximal (thick arrow) and distal (thin arrow) convoluted tubules. The Bowman's capsule comprises visceral epithelial cells surrounding the glomerulus and parietal epithelial cells enclosing the urinary space (Fig. 3A). The photomicrograph of rat kidney cortex tissue showed slight swelling, degenerated epithelial cells, and compression of glomeruli, leading to the widening of urinary spaces in the control (+ve) Fig. (3B). However, a photomicrograph image of the rat kidney cortex tissue from pan bread supplemented with 6% ginger showed normal glomerulus, mass, and space, with improved distal and proximal convoluted tubule structure Fig. (3C).

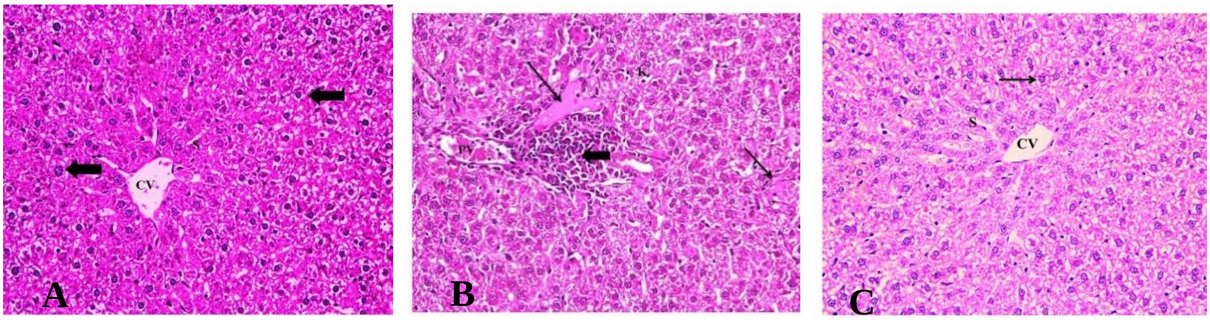

Fig. (2): Photomicrograph of the liver hepatotoxic in rats (H&E stain X 400). **A)** control (-ve) group, **B)** control (+ve), and **C)** pan bread supplemented with 6% ginger

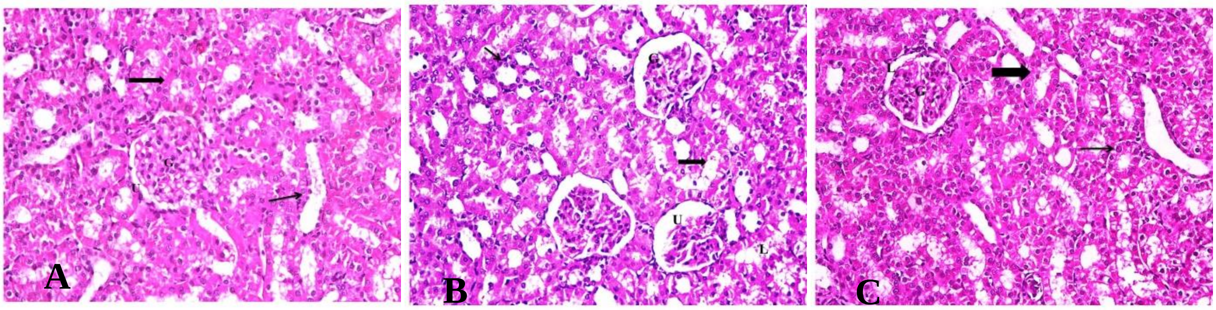

Fig. (3): Photomicrograph of the kidney nephrotoxic rats (H&E stain X 400). **A)** control (-ve) group, **B)** control (+ve) group, and **C)** pan bread supplemented with 6% ginger

Al-Qattan et al. (2008) stated ginger may delay renal failure by reducing structural nephropathies and inflammatory cell infiltration due to its antioxidant, anti-inflammatory, and free radical scavenging properties. The preventive effect of ginger is attributed to antioxidant, anti-inflammatory, and scavenging of free radicals (Yassin et al. 2021). Zingerone treatment confirmed the noticeable reduction of the histological character of renal injury at higher doses (Amin et al. 2021).

## 5. Conclusion

The sensory evaluation revealed that pan bread with 2, 4, and 6% ginger was more acceptable to committee members. Ginger supplementation reduces liver toxicity risk and improves lipid profile, thyroid hormones, antioxidant enzymes, kidney and liver, and renal functions. The liver and kidney histology were corroborated with the results of our biochemical studies. The obtained results recommended the usefulness of pan bread supplemented with 6% ginger in protection against hepatotoxicity and nephrotoxicity in rats.

## 6. Declarations

*Ethical Approval*

All study experiments were ethically approved by the Scientific Research Ethics Committee from the University of Alexandria, Animal Ethics Committee, Faculty of Medicine (Approval no. 04- SREC- 03-2022).

*Consent for publication: All authors agree to publication.*

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Availability of data and materials: The data supporting this study's findings are available from the corresponding author upon reasonable request.

Competing interests: The authors declare that they have no conflict of interest.

Authors' contributions

Conceptualization, S. M. S. S., and T. M. R. L.; methodology, A. A. M. E.; software S. M. S. S., and A. A. M. E.; validation, T. M. R. L.; formal analysis, S. M. S. S., and A. A. M. E.; investigation, A. A. M. E.; resources, A. A. M. E. and T. M. R. L.; data curation, A. A. M. E., writing—original draft preparation, S. M. S. S., A. A. M. E., and T. M. R. L.; writing—review and editing, T. M. R. L.; visualization, T. M. R. L.; supervision, S. M. S. S. and T. M. R. L.; project administration, T. M. R. L.; funding acquisition, A. A. M. E. All authors contributed to and approved the final version of the manuscript."

## 7. References

Abd El-Ghany MA, Ramadan AM, Ghozy SF (2012) Nutraceutical effects of curcuma, ginger, celery, yeast and honey on side effects of gentamicin induced nephrotoxicity in rats. World Appl Sci J 16:646–655

Abdel Moneim AE (2016) Prevention of carbon tetrachloride (CCl4)-induced toxicity in testes of rats treated with Physalis peruviana L. fruit. Toxicol Ind Health 32:1064–1073

Akbari B, Baghaei-Yazdi N, Bahmaie M, Mahdavi Abhari F (2022) The role of plant-derived natural antioxidants in reduction of oxidative stress. BioFactors 48:611–633

Akinyemi AJ, Ademiluyi AO, Oboh G (2014) Inhibition of angiotensin-1-converting enzyme activity by two varieties of ginger (Zingiber officinale) in rats fed a high cholesterol diet. J Med Food 17:317–323

Al-Qattan K, Thomson M, Ali M (2008) Garlic (Allium sativum) and ginger (Zingiber officinale) attenuate structural nephropathy progression in streptozotocin-induced diabetic rats. E Spen Eur E J Clin Nutr Metab 3:e62–e71

Al-Yahya M, Mothana R, Al-Said M, et al (2013) Attenuation of CCl 4-induced oxidative stress and hepatonephrotoxicity by Saudi Sidr honey in rats. Evidence-Based Complement Altern Med 2013:

Almasodi AGS (2018) Production and evaluation of some bakery products containing ginger powder. J Food Nutr Res 6:205–215

Amin I, Hussain I, Rehman MU, et al (2021) Zingerone prevents lead-induced toxicity in liver and kidney tissues by regulating the oxidative damage in Wistar rats. J Food Biochem 45:e13241

Amjad A, Sohaib M, Nawaz H, et al (2021) Assessment of rheological and quality characteristics of bread made by the addition of ginger powder in wheat flour. Food Sci Technol 42:e47820

Attia AM, Ibrahim FA, Nabil GM, Aziz SW (2013) Antioxidant effects of ginger (Zingiber officinale Roscoe) against lead acetate-induced hepatotoxicity in rats. Afr J Pharm Pharmacol 7:1213–1219

Badawi MS (2019) Histological study of the protective role of ginger on piroxicam-induced liver toxicity in mice. J Chinese Med Assoc 82:11–18

Balestra F, Cocci E, Pinnavaia G, Romani S (2011) Evaluation of antioxidant, rheological and sensorial properties of wheat flour dough and bread containing ginger powder. LWT-Food Sci Technol 44:700–705

Bekkouch O, Dalli M, Harnafi M, et al (2022) Ginger (Zingiber officinale Roscoe), lemon (Citrus limon L.) juices as preventive agents from chronic liver damage induced by CCl4: A Biochemical and Histological Study. Antioxidants 11:390

Fathi R, Akbari A, Nasiri K, Chardahcherik M (2021) Ginger (Zingiber officinale roscoe) extract could upregulate the renal expression of NRF2 and TNFα and prevents ethanol-induced toxicity in rat kidney. Avicenna J Phytomedicine 11:134

Fossati P, Prencipe L (1982) Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem 28:2077–2080

Gamble M, Banks I, Bancroft JD (2008) Managing the laboratory. Theory Pract Histol Tech 1:

Gonzalez-Gonzalez, M., Yerena-Prieto, B. J., Carrera, C., Vázquez-Espinosa, M., González-de-Peredo, A. V., García-Alvarado, M. Á., . . . Barbero, G. F. (2023). Optimization of an Ultrasound-Assisted Extraction Method for the Extraction of Gingerols and Shogaols from Ginger (Zingiber officinale). Agronomy, 13(7), 1787.

Girish C, Pradhan SC (2012) Hepatoprotective activities of picroliv, curcumin, and ellagic acid compared to silymarin on carbontetrachloride-induced liver toxicity in mice. J Pharmacol Pharmacother 3:149–155

Hadwan MH, kadhum Ali S (2018) New spectrophotometric assay for assessments of catalase activity in biological samples. Anal Biochem 542:29–33

Hamouda AF, Sameeh MY, Shrourou RM (2016) Effect of avocado (persea americana), cabbage (brassica oleracea) and ginger (zingiber officinale) on rat liver and thyroid injuries induced by CCl4 (carbon tetrachloride). J Pharm Pharmacol 4:108–118

Hussein SA, El Senosi YA, Mansour MK, Hassan MF (2017) Role of antioxidant and anti-inflammatory of Ginger (Zingiber officinal Roscoe) against metalaxyl induced oxidative stress in rats. Benha Vet Med J 33:504–516

Ibrahim UK, Salleh RM, Maqsood-ul-Haque SNS (2015) Bread towards functional food: an overview. Int J Food Eng 1:39–43

Islam K, Haque A, Karim R, et al (2011) Dose-response relationship between arsenic exposure and the serum enzymes for liver function tests in the individuals exposed to arsenic: a cross sectional study in Bangladesh. Environ Heal 10:1–11

Jaye M, Doan K-AT, Krawiec JA, et al (2009) Compositions and Methods for Effecting the Levels of High Density Lipoprotein (HDL) Cholesterol and Apolipoprotein AI, Very Low Density Lipoprotein (VLDL) Cholesterol and Low Density Lipoprotein (LDL) Cholesterol

Jukić M, Nakov G, Komlenić DK, et al (2022) Quality assessment of cookies made from composite flours containing malted barley flour and wheat flour. Plants 11:761

Kazeem MI, Bankole HA, Fatai AA (2011) Protective effect of ginger in normal and carbon-tetrachloride induced hepatotoxic rats. Ann Biol Res 2:1–8

Kirkpatrick LA (2015) A simple guide to IBM SPSS Statistics-Version 23.0. Cengage Learning

Lai Y-S, Lee W-C, Lin Y-E, et al (2016) Ginger essential oil ameliorates hepatic injury and lipid accumulation in high fat diet-induced nonalcoholic fatty liver disease. J Agric Food Chem 64:2062–2071

Lavie CJ, Laddu D, Arena R, et al (2018) Reprint of: healthy weight and obesity prevention: JACC health promotion series. J Am Coll Cardiol 72:3027–3052

Lebda MA, Taha NM, Korshom MA, et al (2012) Biochemical effect of ginger on some blood and liver parameters in male New Zealand rabbits. Online J Anim Feed Res 2:197–202

Lopes-Virella MFL, Stone PG, Colwell JA (1977) Serum high density lipoprotein in diabetic patients. Diabetologia 13:285–291

Ma J-Q, Ding J, Xiao Z-H, Liu C-M (2014) Ursolic acid ameliorates carbon tetrachloride-induced oxidative DNA damage and inflammation in mouse kidney by inhibiting the STAT3 and NF-κB activities. Int Immunopharmacol 21:389–395

Ma R-H, Ni Z-J, Zhu Y-Y, et al (2021) A recent update on the multifaceted health benefits associated with ginger and its bioactive components. Food Funct 12:519–542

Mallikarjuna K, Chetan PS, Reddy KS, Rajendra W (2008) Ethanol toxicity: Rehabilitation of hepatic antioxidant defense system with dietary ginger. Fitoterapia 79:174–178

Mohammadi G, Rashidian G, Hoseinifar SH, et al (2020) Ginger (Zingiber officinale) extract affects growth performance, body composition, haematology, serum and mucosal immune parameters in common carp (Cyprinus carpio). Fish Shellfish Immunol 99:267–273

Osae R, Zhou C, Xu B, et al (2019) Effects of ultrasound, osmotic dehydration, and osmosonication pretreatments on bioactive compounds, chemical characterization, enzyme inactivation, color, and antioxidant activity of dried ginger slices. J Food Biochem 43:e12832

Reeves PG, Rossow KL, Bobilya DJ (1994) Zinc-induced metallothionein and copper metabolism in intestinal mucosa, liver, and kidney of rats. Nutr Res 14:897–908

Richmond W (1973) Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clin Chem 19:1350–1356

Rigo A, Viglino P, Rotilio G (1975) Polarographic determination of superoxide dismutase. Anal Biochem 68:1–8

.

Samar M.S. Shawir , Aya A.M Eltayip., Tesby M.R Lotfy (2024). Characteristics of Pan Bread Fortified with Different Levels of Ginger; Technological Study. Journal of Specific Education Research, 2024(80).

Shahrajabian MH, Sun W, Cheng Q (2019) Clinical aspects and health benefits of ginger (Zingiber officinale) in both traditional Chinese medicine and modern industry. Acta Agric Scand Sect b—Soil Plant Sci 69:546–556

Shakya AK (2016) Medicinal plants: Future source of new drugs. Int J Herb Med 4:59–64

Shanmugam KR, Ramakrishna CH, Mallikarjuna K, Reddy KS (2010) Protective effect of ginger against alcohol-induced renal damage and antioxidant enzymes in male albino rats

Shlipak MG, Matsushita K, Ärnlöv J, et al (2013) Cystatin C versus creatinine in determining risk based on kidney function. N Engl J Med 369:932–943

Sindhu G, Nishanthi E, Sharmila R (2015) Nephroprotective effect of vanillic acid against cisplatin induced nephrotoxicity in wistar rats: a biochemical and molecular study. Environ Toxicol Pharmacol 39:392–404

Williamson DH (1974) L-Alanine determination with alanine dehydrogenase. In: Methods of enzymatic analysis. Elsevier, pp 1679–1685

Wuepper A, Tattersall J, Kraemer M, et al (2003) Determination of urea distribution volume for Kt/V assessed by conductivity monitoring. Kidney Int 64:2262–2271

Yagi T, Kagamiyama H, Nozaki M, Soda K (1985) [17] Glutamate-aspartate transaminase from microorganisms. In: Methods in Enzymology. Elsevier, pp 83–89

Yassin N, AbouZid SF, El-Kalaawy AM, et al (2021) Tackling of renal carcinogenesis in wistar rats by Silybum marianum total extract, silymarin, and silibinin via modulation of oxidative stress, apoptosis, Nrf2, PPARγ, NF-κB, and PI3K/Akt signaling pathways. Oxid Med Cell Longev 2021:

Yokota A, Shigeoka S, Onishi T, Kitaoka S (1988) Selenium as inducer of glutathione peroxidase in low-CO2-grown Chlamydomonas reinhardtii. Plant Physiol 86:649–651

Zhukovets T, Özcan MM (2020) A review: composition, use and bioactive properties of ginger (Zingiber officinale L.) rhizoms. J Agroaliment Process Technol 26:200–216