Protective Effects of Soaked, Boiled and Sprouted Red Kidney Beans against Oxidative Stress and Hepatotoxicity in Rats

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ABSTRACT

The liver is an organ that is exposed to tissue injury due to its contribution to several metabolic functions and removing toxins from it. Thus, it is constantly exposed to higher levels of endogenous and exogenous oxidants than other organs. Therefore, the present study aimed to examine the hepatoprotective influences of soaked, boiled, and sprouted red kidney beans. Thirty-five adult male albino rats, weighting 170 ± 10 g, were divided into 5 equal groups (n=7), the first group fed on a basal diet and served as the control (-ve) group. Another four groups were injected with CCl_4 to induce hepatotoxicity and oxidative stress and then classified into a positive control (+ve), soaked, boiled and sprouted red kidney bean groups, respectively. Chemical composition, mineral content and bioactive compounds of red kidney beans were measured. When the experiment ended, serum lipid analyses consisting of (TG, TC, LDL-c, VLDL-c, and HDL-c), liver enzyme (ALP, AST, and ALT), kidney function (creatinine, and urea), antioxidant enzymes (GPX, CAT and SOD) and thyroid stimulating hormone were also examined. Results indicate that sprouted red kidney beans have higher nutritional value and phenolic compounds than those soaked and boiled. So, germinated red kidney had the greatest impact on lipid profile, antioxidant enzymes, kidney, and liver functions. Additionally, the liver's histological analysis supported the biochemical measurements' findings. Thus, as potential protection against oxidative stress and liver damage, the study suggests including red kidney beans (boiled or sprouted) in our regular diet.

Key words: *Legumes, Liver disorder, Rats, Biochemical analysis, kidney function, liver function*.

التأثيرات الوقائية للفاصوليا الحمراء المنقوعة والمسلوقة والمستنبتة ضد الإجهاد التأكسدي والسمية الكبدية في الفئران

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الملخص العربى

الكبد عضو قابل لتلف الانسجة ويرجع ذلك لمشاركته في العديد من وظائف التمثيل الغذائي وإزالة السموم منها ولهذا السبب فهو يتعرض باستمرار لمستويات أعلى من المواد المؤكسدة الداخلية والخارجية مقارنة بالأعضاء الأخرى. ولذلك، تهدف الدراسة الحالية إلى دراسة التأثيرات الوقائية فى الكبد للفاصوليا الحمراء المنقوعة والمسلوقة والمنبتة. تم تقسيم خمسة وثلاثين من ذكور الجرذان البيضاء البالغة، بوزن ١٧٠ ± ١٠ جرام، إلى ٥ مجموعات متساوية (العدد = ٧)، المجموعة الأولى تغذت على نظام غذائي أساسي وكانت بمثابة (المجموعة الضابطة السالبة -ve). تم حقن الأربع مجموعات الاخرى بمادة رابع كلوريد الكربون للحث على التسمم الكبدي والإجهاد التأكسدي ثم تم تقسيمهم إلى المجموعة الضابطة الموجبة (+ve)، الفاصوليا الحمراء المنقوعة والمسلوقة والمنبتة، على التوالي. تم قياس التركيب الكيميائي والمحتوى المعدني والمركبات النشطة بيولوجيا للفاصوليا الحمراء. وعند انتهاء التجربة، تم تحليل الدهون في الدم (TG، TC، LDL-c، VLDL-c، وHDL-c)، إنزيمات الكبد (ALP، AST، ALT)، وظائف الكلى (كرياتينين، ويوريا)، . كما تم فحص الإنزيمات المضادة للأكسدة (GPX، CAT، وSOD) وهرمون الغدة الدرقية. تشير النتائج إلى أن الفاصوليا الحمراء المنبتة لها قيمة غذائية ومركبات فينولية أعلى من المنقوعة والمسلوقة. لذلك، كان للفاصوليا الحمراء النابتة التأثير الأكبر على مستوى الدهون، والإنزيمات المضادة للأكسدة، ووظائف الكلى والكبد. بالإضافة إلى ذلك، دعم التحليل النسيجي للكبد نتائج القياسات البيوكيميائية. وبالتالي، كحماية محتملة ضد الإجهاد التأكسدي وتلف الكبد، تقترح الدراسة إدراج الفاصوليا الحمراء (المسلوقة أو المنبتة) في نظامنا الغذائي المعتاد.

الكلمات الافتتاحية : *البقوليات، اضطرابات الكبد، الفئران، التحليل الكيميائي الحيوي، وظائف الكلى، وظائف الكبد.*

1. Introduction

The liver is a sensitive organ that processes and metabolizes various chemical agents and drugs, which can lead to acute liver injury (ALI). Hepatic damage shows an essential and potentially lethal risk **(Hirao et al., 2020).** The etiology of ALI incorporates an intricate combination of inflammatory disorders, oxidative stress, and necrosis **(Lyu et al., 2020).** It generally occurs due to pharmaceuticals, illnesses, and hepatic ischemia-reperfusion harm, amid various other factors (**Zhao et al., 2021**).

Carbon tetrachloride (CCl_4) is common for producing hepatic injury in rats' acute liver injury models, which reflect hepatic damage from chemicals in humans **(Lee et al., 2020).** CCl_4 is converted into the extremely active trichloromethyl radical (•CCl3) and trichloromethyl peroxy radical (•OOCCl3) in liver tissue by cytochrome P450, which results in cellular damage and lipid peroxidation (**Unsal et al., 2021**). Oxidative radicals stimulate macrophages to produce tumor necrosis factor α (TNF-α) and supplementary pro-inflammatory cytokines (**Li et al., 2021b**). Consequently, enhancing the antioxidant system could potentially act as an essential technique for safeguarding the liver during acute oxidative stress **(Li et al., 2021a).**

Oxidative stress is characterized by an elevated processing of reactive oxygen species (ROS) that surpasses the capacity of antioxidant systems to defend against it (**Noh and Ha, 2011**). As well, the term ""oxidative stress" can be described as "an imbalance of oxidants and antioxidants preferring the oxidants, resulting in breakdown of the redox signaling and control, as well as structural damage" **(Sies et al., 2017).**

Legumes are notably rich in protein and dietary fiber. Protein-derived bioactive peptides from legumes play a significant role as health-promoting chemicals, particularly through their interaction with amino acids of disease-related enzymes. The presence of bioactive peptides in legumes can enhance food value **(Ortiz-Martinez et al., 2014).** Legumes are regarded as economically sustainable and more nutritious alternatives to animal-based products. Furthermore, people are progressively consuming plant-based diets for improved health **(Sharma and Sahni, 2021).** Legumes is typically ingested following several processing methods, which enhance the antinutritional components. Traditional processing procedures like sprouting, hydration, thermal processing (heating and autoclaving) and fermentation have been demonstrated to be successful in the elimination of the antinutritional elements **(Khattab *et al.* 2009).**

Red kidney beans (*Phaseolus vulgaris,* L), also known as navy, pintored kidney, or French beans, serve as an important source of nutrition for people worldwide. They are acknowledged as a vital element of balanced diets because their substantial nutritional content (high in protein and low in fat) and their functional attributes. Carbohydrates, phenolics, lectins, phytates, vitamins and soluble fiber are the primary functional components of commonly consumed beans. Phenolics, encompassing phenolic acids, proanthocyanidins and flavonoids are particularly significant due to their robust antioxidant effects **(Curran, 2012).** Red kidney beans are recognized for their high protein content (29.1%), fiber (4%), vitamin E and unsaturated fatty acids **(Imran *et al*., 2014).** It exhibits superior antioxidant activity relative to other bean varieties **(Luo *et al.* 2016)**. Furthermore, they were documented to have chemopreventive, antioxidant, antibacterial properties, anti-inflammatory , and antimutagenic **(Gan et al., 2016)**. Consequently, ordinary beans offer significant health advantages as a dietary element.

Legumes are generally consumed after various processes like soaking, boiling and sprouting and with processing, the antinutritional factors are improved. The sprouting process is currently a bioprocessing process technology extensively utilized by researchers globally to create nutritional legume-based products. According to **Avilés-Gaxiola et al. (2018),** it boosts protein digestibility while reducing the effects of antinutritional elements. The sprouting process usually commences with the whole seed, and studies indicate that removing the hull may substantially enhance sprouting percentages (**Pedrini et al., 2019).** The sprouting includes the breakdown of distinct components during the enzyme activation process, resulting in elevated nutritious quality of legumes. This process is necessary for the nutrition of humans (**Chethan Kumar *et al*.,2022**).

This study was carried out to investigate the Protective effect of processed (soaked, boiled and sprouted) red kidney beans against oxidative stress and hepatotoxicity in Rats. This will provide useful information for liver patients and others alike about the benefits of different preparation processes for legumes, whether nutritional or therapeutic

2. MATERIALS

Red kidney bean (*Phaseolus vulgaris*, L) samples were obtained via a local market in Alexandria, Egypt. All chemicals and reagents used were analytical grades obtained from EL- Gomhouria, Chemical Company, Egypt and Carbon tetrachloride (CCL_4) was purchased from Sigma Aldrich Chemical Company. All kits were obtained via Sigma Chemical Company, Egypt. A total of 35 male Albino rats of "the Sprague-Dawley Strain" were acquired via the house animal of the Institute of Graduate Studies and Research, Alex. Uni. The rats had a mean weight of 170 ±10 grams.

3. Methods

3.1. Processing of red kidney beans:

3.1.1. Soaking process

The process delineated by **Onwuka (2005)** was utilized. Initially, the red kidney bean was cleaned; grit and broken beans were removed. Then the red kidney beans were placed in a plastic bowl and washed two times. After that, samples were divided into two parts as follows: the first dried without any processes to prepare powder and the second part was used for soaking. The soaking process was continued for 24 h in tap water (1:5 w/v) at a temperature (about 20-30°C). The soaked samples were subsequently cleaned in the running water followed by splitting into three equal portions as follows: the first part was used as soaked, while the second part was used for the boiling process and the third part was used for the sprouting process.

3.1.2. Boiling process

The boiling process was performed in a beaker of water (1:3 w/v) beans: water. Samples were cooked until they became soft for about 25 minutes according to the method outlined by **Audu and Aremu (2011)**.

3.1.3. Sprouting process

The soaked seeds have sprouted in a plastic container via a moistened flannel fabric below the basket, followed by covering via a dark fabric and the sprouting temperature is usually maintained at (20-30˚C). The sprouting process was carried out for 0, 10, 20, and 30 hours at room temperature, producing sprouts that ranged from 0.5 to 1.5 cm, with no branched roots present. Every eight hours,10 mL of water was sprinkled evenly on 50 g. of red kidney beans to need a moderate humidity to facilitate sprouting, and the water should be changed often, typically twice daily, to eliminate the microbial growth. The sprouting process was conducted as outlined in the approach described by **Tania et al. (2022)**.

All samples were dried at 35°C for 16 h in an oven to reach 8% moisture content, and then ground to a fine consistency by using a grinder (MX-151SG1, Panasonic Co., Ltd., China) and sieved through a 300-µm mesh to separate the hulls from the powder .All samples were preserved at 4°C., for subsequent analysis and biological study.

3.2. Chemical composition

The chemical composition includes moisture, crude protein, ash, crude fats and crude fibers were analyzed in samples of red beans according to the approach utilized by **AOAC (2010).** The nitrogen-free extract (NFE) was determined on a moisture-free basis by difference, calculated as equation NFE%=100-(crude protein%+crude fat%+crude fiber%+ash%). Total dietary fiber content in the samples of red kidney beans was outlined according to the method described by **Asp et al. (1983).** Minerals were analyzed in samples using an atomic absorption spectrophotometer according to **(AOAC, 2010).**

3.3. Measurement of bioactive elements and antioxidant activity

3.3.1. Total phenols

The total amount of phenolic compounds (TPC) for each sample was assessed utilizing the method of **Mena et al. (2011)**. The results presented as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

3.3.2. Total Flavonoids

The total amount of flavonoid compounds (TFC) for each sample was assessed utilizing the method of **Zhishen et al.(1999)**. The findings were measured as milligrams of catechin equivalents per gram of dry weight (mg CE/g DW).

3.3.3. Antioxidant activity

The free radical neutralization assay for DPPH and ferric reducing antioxidant power (FRAP) evaluation were conducted in accordance with the methodologies outlined by **Espín et al. (2000)**. Values for each method were reported as micromoles of trolox, which is equivalents per gram of dry mass (µM TE/g DM).

3.4. Biological study

3.4.1 The design of experimental animals:

35 male albino rats ranging from 170 to 200 grams were acquired from the Institute of Graduate Studies and Research's house of animals at Alex. Uni. The standard diet was prepared according to (**Reeves et al., 1993).**

The rats were divided into two main groups after one week of adaptation. The first group(n=7) was fed on the standard diet and was maintained as the negative control group(-ve). The second group(n=28rats) was intraperitoneally (IP) injected with a dose of 0.5mg CCl_4/Kg., body weight twice weekly. 100 ml of CCL_4 was dissolved in 100 mL of olive oil according to **Iredale et al. (1998)**. Subsequently, blood samples were collected from rats to verify the presence of hepatotoxicity and oxidative stress based on the measurement of liver and kidney enzymes. The hepatotoxicity group was split into four subgroups (n=7 rats each): group 2 was used as a positive control and fed on the standard diet (+ve), whilst groups 3, 4, and 5 were fed on the standard diet enriched with red kidney beans (soaked, boiled, and sprouted, respectively) for four weeks. The groups (3, 4, 5) fed on soaked, boiled and sprouted red kidney beans by 15% by oral gavage. The ratio of red kidney bean given to the rats were determined according to a pretest to reach the best proportion that led to an improvement in liver function. The red kidney beans samples were meticulously mixed and diluted with a little amount of distilled water to ease oral ingestion.

3.4.2. Blood sampling

Following the 4-week experimental phase, the rats underwent an overnight fasting period prior to being anaesthetized with ether and subsequently dissected. Following this, blood samples were collected and centrifuged at 3000 rpm to isolate the serum. The serum was then utilized for biochemical analysis measurements.

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).** According to **Friedewald et al. (1972)**, the calculation for low-density lipoprotein (LDL.) was measured by the equation (cholesterol-(TG/5+HDL). By the method of **Crook (2006)** very low-density lipoprotein (VLDL.) was calculated by dividing the TG levels by a factor of 5.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 (CAT) **(Hadwan and Ali, 2018),** and superoxide dismutase (SOD) **(Rigo et al., 1975).** Urea **(Wuepper et al., 2003)** and Creatinine **(Shlipak et al., 2013).**

3.4.3. Statistical analysis

Version 23.0 of the IBM SPSS program was used to analysis the data. Quantitative data was described using means and standard deviations. The 5% level was used to assess the results' significance. For comparisons between more than two groups, the F-test (ANOVA) was employed, and for pairwise comparisons, the Post Hoc test (LSD) was used **(Kirkpatrick, 2015).**

4. Results and Discussion

4.1. Chemical composition

The gross chemical composition of raw, soaked, boiled and sprouted red kidney beans is presented in Table 1. The results present that the percentages of moisture contents of raw, soaked, boiled and sprouted red kidney beans were 5.02, 6.05, 7.98, and 6.95 g/100g, respectively. It can be noted that soaking and boiling in water slightly increase the water content. This is due to the absorption of water by the cells. Also, during germination, whole legumes absorb a large amount of water, which affects the structure of the legumes and increases the metabolic process **(Azeez et al., 2022).**

The data displayed that sprouted red beans had a significantly ($P \leq 0.05$) higher protein content than boiled, soaked and raw beans, reaching (35.81, 30.78, 23.35, 20.75 g/100 g, respectively). The process of boiling and sprouting increases the activity of protease enzymes and the formation of free amino acids that break down bound proteins into their constituent amino acids and peptides, leading to an increase in the protein level. Thus, sprouting enhances the nutritional availability of amino acids and proteins, this corresponds with findings from prior studies **(Sofi et al., 2020)**. The elevated protein values identified in this study suggest that kidney beans are a commendable protein source, comparable to those of other legumes such as cowpeas and white kidney beans **(Olanipekun et al., 2015)**.

Concerning fat content, the percentage of fat was found to be significantly low (*P*≤0.05) to be fairly low in boiled and sprouted red kidney beans (1.10 and 1.48 g/100g) compared to soaked and raw (1.72 and 1.69 g/100g.The activity of lipolytic enzymes increases as they break down fats and use them as an energy source during germination, which leads to a decrease in fat content (**Sofi et al.2020**). Thus, germination can lead to a substantial decrease in fat content in legume flours. The fat content for all samples is inferior to that reported for red kidney beans **(Audu and Aremu, 2011)**. These findings are matching with **Olanipekun et al.(2015)** who found a reduction in fat content by boiling or sprouting.

The results shown in Table (1) presented that the ash had significant (*P*≤0.05) differences between raw and sprouted red kidney beans which the ash content was found to be 3.85 and 4.95 g/100g, respectively. In contrast, the ash had non-significant (*P*≤0.05) differences between raw, soaked, and boiled red kidney beans followed as 3.85, 4.02 and 4.14 g/100g respectively. This is in accordance with **Olanipekun et al.(2015)** who found that raw seeds contain 3.29% ash, while boiled seeds contain 4.29%. This percentage also converges with **Audu and Aremu (2011)** who found that red beans contain 4.4% ash. Antinutrients may affect the mineral composition of the raw sample, leading to a decrease in the ash value.

Concerning crude fiber, the results demonstrated that sprouted red kidney beans had a significantly (*P*≤0.05) higher amounts of crude fiber (15.45 g/100g) compared with soaking and boiling as follows (12.79 and, 8.45g/100g, respectively). During sprouting, cellular components and new cells are formed (like cellulose, hemicellulose, and lignin), which leads to an increase in the crude fiber content **(Nkhata et al., 2018)**.The previous values are in agreement with **Srenuja et al. (2023)** who found a high fiber content in whole kidney beans after germination at 24 hrs, 48 hrs and 72 hrs (8.73,9.38 and 11.54 g/100g, respectively). In contrast, boiling samples had lower crude fiber content, likely due to the boiling process, which removes fiber. Fiber plays a very important role in human nutrition, as it prevents colon cancer and helps maintain the health of the digestive system **(Audu and Aremu, 2011)**.

The N-free extract content is listed in **Table 1**. showed that sprouted red kidney beans had significantly ($P \leq 0.05$) lower amounts (42.31 g/100g) compared with boiling, soaking and raw as follows (55.53, 58.12 and 59.64 g/100g, respectively). Meanwhile, the research demonstrated that are maximum N-free extract was detected in raw kidney beans. N-free extract value identified in this investigation aligns with the value of 60.65 g /100g that reported by **Sasanam et al. (2011)** in red kidney bean and values of 58.64 g /100g for dark red kidney bean**.**

Means for the total dietary fiber in different samples are presented in **Table 1**, also indicated that the total dietary fiber of sprouted red kidney beans (28.56g /100g) was significantly different ($P \leq 0.05$) when compared with boiled, soaked and raw (12.17, 20.17 and 23.54 g/100g, respectively). Furthermore, the study revealed that minimum total dietary fiber was detected in boiling process. According to**Margareta and Nyman (2003)**, during thermal processing total dietary fiber was modified, some glycosidic connections may be disrupted and the alimentary fiber polysaccharides of the cellular wall architecture may be depolymerized by establishing protein-fiber complexes, **(Bressani, 1993)** or influencing the solubility. Moreover, through germination, the increase in total dietary fiber content (SDF and IDF) may be due to the formation of new cells and cellular components during germination **(Nkhata et al., 2018).**

Notable, the rising was observed in boiled and sprouted samples may be as a result of the reduction in anti-nutrition factors such as saponin, tannin and phytic acid. This may result from the stimulation of the anti-nutritional enzyme mechanism, which transforms phytate phosphorus into inositol monophosphate, as well as the liberation of phytic acid during the pre-germination soaking and germination procedure **(Sofi et al. 2020).** Additionally, as thermal processes i.e boiling and roasting of legumes break down the anti-nutrition enzyme system.

Table (1): Gross Chemical composition of raw, soaked, boiled and sprouted red kidney beans

	Parameters						
	Moisture	Crude protein	Crude fat (g/100g)	Ash	Crude fiber	* N-free extract	Total dietary fiber
Raw	5.02 [d] ±0.04	20.75 [c]±1.89	1.69 [a] ±0.09	3.85 [b] ±0.16	14.07[ab]±0.82	59.64[a]±2.96	23.54 [b]±1.68
Soaked	6.05[c] ±0.46	23.35 [c]±1.97	1.72 [a] ±0.26	4.02 [ab] ±0.95	12.79 [b]±1.61	58.12[a] ±4.79	20.17 [c]±1.88
Boiled	7.98 [a] ±0.39	30.78 [b]±1.68	1.10 [b] ±0.05	4.14 [ab] ±0.15	8.45 [c] ±0.82	55.53[a] ±2.70	12.17 [d]±1.16
Sprouted	6.95[b]±0.62	35.81[a]±1.28	1.48[a] ±0.06	4.95 [a] ±0.17	15.45 [a]±1.06	42.31[b] ±2.57	28.56 [a]±1.98
F	**25.702***	**47.737***	**12.319***	**2.907**	**21.700***	**16.477***	**48.865***
P	**<0.001***	**<0.001***	**0.002***	**0.101**	**<0.001***	**<0.001***	**<0.001***
LSD 5%	**0.813**	**3.253**	**0.265**	**0.933**	**2.120**	**6.355**	**3.212**

Data were presented using Mean ±SD. of three replicate

*: Statistically significant at $P \leq 0.05$

4.2. Mineral content

The results regarding the mineral composition of raw, soaked, boiled and sprouted red kidney beans are depicted in Table (2). These results showed that different processes (soaking, boiling, and sprouting) increased the mineral content. Interestingly, the sprouting process increased the content of Na, Mg, K, Ca, Mn, Fe and Zn (63.21, 723.5, 36.73, 74.31, 11.32, 27.56 and 8.68 mg, respectively) compared with raw (53.29, 643.68, 25.09, 58.60, 4.99, 18.62 and 4.51 mg, respectively). Also, the soaking and boiling processes increased the content of Na, Mg, K, Ca, Mn, Fe and Zn compared with raw red kidney beans.

The current results are matched with **Agamy et al. (2023)** who found that minerals increased during soaking and boiling. The minerals don't destroy by exposure to heat and this may be due to the decrease in the anti-nutritional factors with increasing soaking time. Furthermore, the ensue of **Rizvi et al. (2024)** pointed out that the concentration of anti-nutritional factors decreases in bean seeds as a result of their leakage into the soaking water, so the minerals are released via their organically attached complexes owing to their diminished concentration, as these anti-nutritional elements adhere to the minerals.

In contrast, some studies reported a reduction in mineral concentration due to leaching of minerals into the boiling water, and removal or destroying the seed coat of kidney beans. Removal of seed coat might be concerned with mineral reduction in grains as reported by **Damodaran and Parkin (2008).** Otherwise, these results were close to **Srinoja et al. (2023)** who observed that reducing anti-nutritional compounds during germination increases mineral content.

These results showed that soaked, boiled and sprouted red beans contain relatively higher amounts of magnesium, sodium, potassium, calcium and iron, while zinc and manganese contain lower amounts. magnesium was identified as the most prevalent mineral in the samples, which contrasts with the findings of **Margier et al. (2018)** who found that potassium is the element with the greatest content in beans. The mineral analysis results are consistent with those of **Audu and Aremu (2011),** Nevertheless, minor variations may arise from differing cultivars and geographical conditions.

Table (2): Mean mineral content of raw, soaked, boiled and sprouted red kidney beans

Parameters	**Na**	**Mg**	**K**	**Ca**	**Mn**	**Fe**	**Zn**
				(mg/100g)			
Raw	53.29 [c] ±1.38	643.68 [d] ±6.18	25.09 [d] ±0.64	58.60 [c] ±1.50	4.99 [d] ±0.65	18.62 [d] ±0.79	4.51 [d] ±0.33
Soaked	56.63 [b] ±1.71	672.75 [c] ±4.30	29.31 [c] ±0.96	66.06 [b] ±1.98	6.70 [c] ±0.58	22.09 [c] ±0.64	5.61 [c] ±0.22
Boiled	59.50 [b] ±1.09	692.3 [b] ±6.15	31.95 [b] ±1.65	68.98 [b] ±1.95	8.72 [b] ±0.73	24.45 [b] ±0.89	7.40 [b] ±0.27
Sprouted	63.21[a]±2.26	723.5 [a] ±8.19	36.73 [a] ±1.71	74.31 [a] ±1.62	11.32 [a] ±1.06	27.56 [a] ±1.12	8.68 [a] ±0.18
F	19.134*	83.651*	40.737*	40.809*	36.846*	55.812*	156.660*
P	0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD 5%	3.144	11.965	2.492	3.344	1.464	1.649	0.483

Data were presented using Mean ±SD. of three replicate

*: Statistically significant at $P \leq 0.05$

4.3. Bioactive compounds and antioxidant activity

Data of bioactive compounds and antioxidant activity of raw, soaked, boiled and sprouted red kidney beans are tabulated in **Table (3)**. The results obtained showed that the process of soaking, boiling and sprouting led to an increase in bioactive compounds and antioxidant activity compared to raw beans. The data displayed that sprouted red beans had a significantly ($P \leq 0.05$) higher TPC, TFC, DPPH and FRAP content than boiled, soaked and raw beans, reaching (7.32, 7.72, 20.23, 20.02, respectively). On the other hand, the raw have the lowest content of TPC, TFC, DPPH, and

FRAP (4.35, 4.64, 14.12 and 14.62, respectively). The results in accordance with **Boateng et al. (2008)** who indicated that soaking method affected the flavonoid, total phenolic and antioxidant contents (DPPH., FRAP.) in kidney beans. On the contrary, **Pathak and Kulshrestha (2017)** observed a decrease in the concentration of total phenolics by 9.31% during soaking of small red beans while the total phenolic content in the local variety decreased by 2.9% after soaking.

This difference may be due to the difference in soaking time and type of water used. According to a study **(Casas-Forero et al., 2020)** which showed that a higher concentration of phenols, such as gallic or syringic acids, which have a stronger ability to scavenge free radicals like the DPPH, was found in pea extracts. On the other hand, germination enhances antioxidant activity, as phenolic compounds and dietary fibers are associated, which leads to improving the health and nutritional properties of legumes **(Duenas et al., 2016**).

Besides, these results were consistent with **(Faid, 2019)** who found that germinated red bean contain the highest amount of polyphenols, as the phenolic compounds in the seeds increased during germination. On the other hand ,these results are in contrast to previous studies conducted by (**Abioye et al., 2018**) who found that Leaching of water-soluble proanthocyanidins from the test and pericarp when preparing finger millet, as well as the formation of complex bonds with proteins during germination leads to a decrease in TPC.

Table (3): Bioactive compounds and antioxidant activity of raw, soaked, boiled and sprouted red kidney beans

Parameters	TPC (mg GAE/g DW)	TFC (mg CE/g DW)	DPPH (µMTE/g DW)	FRAP (µMTE/g DW)
Raw	4.35 [d] ±0.24	4.64 [d] ±0.20	14.12 [d] ±0.44	14.62 [c] ±0.06
Soaked	5.35 [c] ±0.15	5.67 [c] ±0.25	15.81 [c] ±0.64	16.65 [b] ±0.68
Boiled	6.24 [b] ±0.43	6.85 [b] ±0.38	17.43 [b] ±0.59	17.89 [b] ±0.72
Sprouted	7.32[a]±0.48	7.72[a]±0.13	20.23 [a] ±0.75	20.02 [a] ±0.96
F	38.556*	81.773*	53.631*	32.072*
P	<0.001*	<0.001*	<0.001*	<0.001*
LSD 5%	0.665	0.485	1.156	1.302

Data were presented using Mean ±SD. of three replicate *: Statistically significant at $P \leq 0.05$

TPC : Total phenolic compounds
TFC: Total flavonoids compounds
FRAP: ferric reducing antioxidant power
DPPH: 2,2-diphenyl-1-picrylhydrazyl

4.4. The result of biological study

4.4.1. Lipid profile and thyroid-stimulating hormone

The data presented in Table (4) showed that treatment with CCl_4 alone caused a significant increase ($P \leq 0.05$) in serum cholesterol, TG, LDL-C and VLDL-C, while HDL-C was decreased ($P \leq 0.05$) compared to the control group (-ve). On the other hand, treatment with soaked, boiled and sprouted red kidney beans caused a significant decrease ($P \leq 0.05$) in the concentration of all serum lipid profile, except HDL-C which showed a significant increase ($P \leq 0.05$) in male rats compared to the control group (+ve). On the other hand, using sprouted red beans with CCl_4 returned the previous parameter values to the control group (-ve) better than soaked and boiled beans. Moreover, the data indicated that the level of TSH in the control (+ve) group was significantly ($P \leq 0.05$) higher 1.95 ng/ml compared to the control (-ve) 0.61 ng/ml and treatment groups with soaked, boiled and sprouted red kidney bean improved as follow (1.52, 1.28 , 0.81 ng/ml, respectively).

CCl_4 causes liver injuries and lipid peroxidation that in turn affect thyroid function. These results are consistent with previous findings that found CCl_4 treatment significantly ($P \leq 0.05$) increases total lipid with previous findings that found CCl_4 treatment significantly ($P \leq 0.05$) increases total lipid, liver tissue weight, TC and TG where CCl_4 is induced fatty liver where lipogenesis is produced (**Abdel-Hamid, 2006**).

The impairment in liver function resulted in a dysfunction of thyroid function, as the liver is essential for the metabolism of thyroid hormones. **Khan (2012)** found that treatment with CCl_4 significantly raised TSH levels and generated oxidative stress in rat thyroid tissue. Furthermore, increased TSH after CCl_4 administration has been corroborated by prior research indicating various abnormalities in thyroid function tests among individuals with chronic liver disorders (**Krishna et al, 2024**).

The result illustrates the protective effect of soaked, boiled and sprouted levels of red kidney beans on lipid profile and thyroid which may be related to their chemical composition and processing improved the hypocholesterolemic ability of red kidney beans. The hypocholesterolemic impact of soluble fibers has been documented in both experimental animal models and people (**Pande et al., 2012**). Legumes are often abundant in polyphenols, which have been demonstrated to safeguard LDL from oxidation and diminish LDL absorption (**Rudrapal et al., 2022**). Soluble fibers physically bind to bile acids and entrap cholesterol resulting in lowered cholesterol absorption, increased bile acid synthesis, decreased hepatic cholesterol, increased LDL clearance, and upregulated LDL receptors (**Bakr and Farag, 2023**). Also, **Velissaridou et al. (2024)** explained that consumption of legumes and nuts resulted in an increase in HDL-C levels. In particular, consumption of legumes has been associated with lower LDL-C levels.

Many researches have proven that germination improves the nutritional value of legumes due to the availability of vitamins, minerals, reducing anti-nutrients, increasing digestibility, and improving the nutritional quality of proteins by decomposing them into essential amino acids and absorbable polypeptides (**Satya et al., 2013**). Also, (**Yao et al., 2014**) found that dietary proteins with appropriate amino acid composition and sequence reduced the serum cholesterol level in rats. Sulfur-containing amino acids have also been shown to have a decreasing effect on LDL-C levels and increasing effect on HDL-C in serum.

The same results were found by **Olanipekun et al.(2016)** who found that boiled and sprouted kidney been diets improved HDL-C concentrations. Germination also leads to the activation of hydrolases (carbohydrase, lipase, and protease). Protease rapidly transforms protein molecules into amino acids, while carbohydrase degrades starch deposits into maltose and subsequently glucose, and lipase disassembles fat content into fatty acids (**Ali and Elozeiri, 2017**). Therefore, the amino acids, glucose and fatty acids contents of red kidney bean sprouts are higher than those of unsprouted red kidney beans.

Table (4): Effect of soaked, boiled and sprouted red kidney beans on the lipid profile and thyroid glands hormone of hepatotoxic rats

groups	Cholesterol	TG	HDL (mg/dl)	LDL	VLDL	TSH (ng/ml)
Control(-ve)	90.38[e]±1.69	112.57[e] ±2.97	50.27[a] ±1.97	17.60[e] ±0.87	22.51[e] ±0.59	0.61[d] ±0.09
**Control(+ve)	231.57[a] ±3.49	263.17[a] ±2.69	25.67[d] ±1.88	153.27[a] ±1.07	52.63[a] ±0.54	1.95[a] ±0.16
Rat fed on soaked	207.01[b] ±2.97	211.67[b] ±2.97	37.12[c] ±1.58	127.56[b] ±0.80	42.33[b] ±0.59	1.52[b] ±0.12
Rat fed on boiled	150.08[c] ±2.94	172.26[c] ±2.47	42.73[b] ±1.83	72.90[c] ±0.62	34.45[c] ±0.49	1.28[c] ±0.15
Rat fed on spourted	98.95[d] ±1.93	120.13[d] ±3.59	48.69[a] ±1.29	26.23[d] ±0.08	24.03[d] ±0.72	0.72[d] ±0.07
F	**1647.052***	**1371.057***	**100.068***	**18565.942***	**1370.318***	**62.216***
P	**<0.001***	**<0.001***	**<0.001***	**<0.001***	**<0.001***	**<0.001***
LSD 5%	**5.068**	**5.575**	**3.252**	**1.439**	**1.115**	**0.231**

Data were presented using Mean ±SD. *: Statistically significant at P ≤ 0.05

TG : triglyceride **HDL**: high density lipoprotein **LDL**: low density lipoprotein

VLDL: very low density lipoprotein **TSH**: thyroid stimulating hormone

4.4.2. Liver and Kidney Function

The data presented in Table 5. showed significant (P≤0.05) increases AST, ALT and ALP. in control (+ve) (215.33, 196.33 and 401.35 U/ml, respectively) compared with the control (-ve) (141.55, 75.07 and 232.32 U/ml, respectively). The values of AST were decreased after using soaked, boiled and sprouted red kidney beans, whereas the values were 182.52, 164.08 and 148.05 U/ml, respectively. The same trend was observed for ALT and ALP, whereas the values were (161.17, 139.33 and 83.17 U/ml) (292.08, 280.85 and 240.18 U/ml), respectively.

Once the plasma membrane of a liver cell is damaged, a variety of enzymes normally found in the cytosol are released into the bloodstream. Membrane damage or necrosis releases the enzymes ALT, AST and ALP into the circulation; therefore, these can be measured in the serum. Increased AST levels signify an imbalance in liver functionality, as seen in viral hepatitis, as well as myocardia infraction and muscular damage. ALT catalyzes the conversion of alanine to glutamate and pyruvate and is released in a similar manner (**Contreras-Zentella and Hernández-Muñoz, 2015**). Consequently, ALT exhibits greater specificity for the liver, making it a superior indicator for identifying liver damage.

An elevated concentration of plasma liver biomarker enzymes (AST, ALT, and ALP) in rats administered CCL_4 signifies hepatocellular impairment and the extent of necrotic liver injury. The results of the current study were in consistent with the study of **Almohmadi et al. (2024)** who stated that treatment of rats with CCl_4 caused liver damage and significantly raised AST, ALT and ALP levels.

The treatment with soaked, boiled and sprouted red kidney beans significantly altered the severity of CCl_4-induced liver damage. The restoration of enzyme levels to near-normal in treated rats indicates that soaked, boiled, and sprouted red kidney beans can maintain liver cell membranes and inhibit enzyme leakage. The prevention of free radical formation, their neutralization, and the protective potential of soaked, boiled, and sprouted red kidney beans against hepatotoxins may contribute to their therapeutic effects. Sprouts also are beneficial for the liver because they contain antioxidants and phytochemicals that prevent hepatic steatosis and apoptosis.

The data presented in Table 5. showed that control (+ve) had significantly($P \leq 0.05$) higher levels of urea and creatinine (50.43 and 1.65 mg/dL) compared to the control (-ve) (31.63 and 0.64 mg/dL). At the same time, the levels of urea and creatinine were improved after using soaked, boiled and sprouted red kidney beans as follows (42.82, 38.61 and 33.53 mg/dl) (0.95, 0.80 and 0.73 mg/dl), respectively. The treated groups with CCl_4 showed a significant rise in the serum levels of urea and creatinine this was similar to the results of **Zahira et al. (2023).** Sprouted legumes are classified as alkaline, which helps reduce diet acidity and relieve renal stress. Moreover, red kidney beans which are rich in potassium decrease calcium loss and prevent the formation of kidney stones (**Díaz et al., 2019).**

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. This finding was consistent with the views of **Şenlik and Alkan (2023)** who reported that germination enhance the quality of legumes. Germination not only reduced anti-nutrients but also increased the contents of dietary fiber, carbohydrates, amino acids, and bioactive compounds such as phenolic compounds.

Table (5): Effect of soaked, boiled and sprouted red kidney beans on liver and kidney function of hepatotoxic rats

groups	AST	ALT	ALP	Urea	Creatinine
		(U/ml)		mg/dl	
Control(-ve)	141.55 [e] ±3.65	75.07 [d] ±2.69	232.32 [e] ±4.30	31.63 [d] ±1.94	0.64 [d] ±0.05
**Control(+ve)	215.33 [a] ±3.90	196.33 [a] ±3.0	401.35 [a] ±3.68	50.43 [a] ±1.59	1.65 [a] ±0.10
Rat fed on soaked	182.52 [b] ±2.65	161.17 [b] ±2.98	292.08 [b] ±1.15	42.82 [b] ±0.48	0.95 [b] ±0.09
Rat fed on boiled	164.08 [c] ±2.16	139.33 [c] ±2.70	280.85 [c] ±0.58	38.61 [c] ±1.29	0.80 [c] ±0.05
Rat fed on spourted	148.05 [d] ±2.99	83.17 [d] ±1.29	240.18 [d] ±0.10	33.53 [d] ±1.98	0.73 [cd] ±0.04
F	**270.171***	**1173.498***	**2034.245***	**70.905***	**104.390***
P	**<0.001***	**<0.001***	**<0.001***	**<0.001***	**<0.001***
LSD 5%	**5.900**	**4.915**	**4.890**	**2.929**	**0.129**

Data were presented using Mean ±SD. *: Statistically significant at $P \leq 0.05$

AST: Aspartate transaminase **ALT**: Alanine transaminase **ALP**: alkaline phosphates

4.4.3. Antioxidant enzymes in serum

A data of antioxidant enzymes is pointed out in Table (6). Values show that the control (+ve) group had a significant (*P*≤0.05) decrease in SOD 11.04 U/ml compared to the control (-ve) 30.40 U/ml. The level of SOD improved after using soaked, boiled and sprouted red kidney bean, whereas the values were 18.87, 25.00 and 27.44 U /mL**,** respectively compared to Control(+ve). The same trend was observed for CAT, whereas control (+ve) had significantly (*P*≤0.05) decreased by 9.27 U/ml compared to the control (-ve) by 25.35 U/ml. The values of CAT were 13.90, 17.96 and 22.02 U /ml for soaked, boiled and sprouted red kidney bean, respectively. Farther more, the result of GPX was in the same line for all groups. The group of sprouted red kidney beans gave the best result.

This study demonstrated that the treatment of CCl_4 on normal rats resulted in hepatic and renal toxicity, as CCl_4 facilitated lipid peroxidation, increased reactive oxygen species (ROS), and led to a decrease of tissue protein content due to subcellular damage. This result is consistent with **El-Boshy et al.(2017)** who reported that a positive control rat (CCl_4) pointed out a significant decrease in SOD, GPX and CAT in the serum of rat groups compared with a normal control group.

The scientific explanation for this decrease may be due to the hepatotoxic effect of CCl_4, which is directly cleaved by cytochrome P450 in hepatocytes, producing trichloromethyl radicals that induce lipid peroxidation and ultimately result in membrane damage. Activated Kupffer cells produce toxic metabolites (reactive oxygen intermediates and inflammatory cytokines) that lead to hepatic parenchymal cells injury according to **El-Boshy et al.(2017)**. It clear that antioxidant markers are altered in rat livers following CCl_4 intoxication.

The current results revealed that the groups fed on soaked, boiled and sprouted red kidney beans elevated the activities of antioxidant enzymes in the serum of hepatotoxic rats especially the group that consumed sprouted red kidney beans. 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**). When reacted with oxide substances, these compounds can be used as hydrogen or electron donors. Moreover, saponins in legumes also have antioxidant activities due to their ability to scavenge free radicals (**Timilsena et al. 2023**). Nutrition containing polyphenolic compounds has been associated with reducing various diseases and oxidative stress and improving human health. Red kidney beans can potentially be a promising candidate for future utilization in nutritional applications (**Gadallah et al., 2023).**

Table (6): Effect of soaked, boiled and sprouted red kidney beans on the activities of antioxidant enzymes of hepatotoxic rats

groups	SOD U /mL	CAT	GPx mU/mL
Control(-ve)	30.40 [a] ±0.60	25.35 [a] ±1.08	28.28 [a] ±1.04
**Control(+ve)	11.04 [e] ±0.58	9.27 [e] ±0.85	14.30 [d] ±1.03
Rat fed on soaked	18.87 [d] ±0.59	13.90 [d] ±1.05	17.53 [c] ±1.59
Rat fed on boiled	25.00 [c] ±2.10	17.96 [c] ±1.09	21.27 [b] ±2.49
Rat fed on spourted	27.44 [b] ±0.55	22.02 [b] ±1.10	22.80 [b] ±1.03
F	**155.304***	**113.388***	**35.577***
P	**<0.001***	**<0.001***	**<0.001***
LSD 5%	**2.017**	**1.954**	**2.907**

Data were presented using Mean ±SD. *: Statistically significant at $P \le 0.05$

SOD: superoxide dismutase **CAT**: Catalase **GPX**: Glutathione peroxidase

Histopathological Study

Photos (A, B, C, D, E and F) showed the effect of control(-ve), control(+ve), soaked, boiled and sprouted red kidney beans on the changes of histopathological in the liver tissue of hepatotoxic rats.

The control group (-ve) exhibited normal cellular architecture characterized by discrete liver cells, sinusoidal spaces, and a central vein as shown in photo (A). Photos (B and C) for control group (+ve) showed that carbon tetrachloride -treatment induced severe histopathological changes in liver; The majority of the intrahepatic blood vessels, particularly the central veins, exhibited dilation and congestion. In certain regions, necrosis invaded by mononuclear cells was also observed. Furthermore, the hepatocytes exhibited a loss of their typical architecture and fatty vacuolization with pyknotic nuclei appeared in the cytoplasm. These findings align with those of **EL Sayed et al. (2019).** The liver of animals fed with soaked, boiled and sprouted red kidney beans (phto. D, E, F) displayed an improvement but few central veins were still congested and few lymphocytes were present.

The results of the histopathological study support the results of the biochemical parameters. This result is consistent with **Yeap et al . (2015)** who reported that the presence of bioactive compounds in legumes such as phenolic compounds and other compounds with antioxidant activity, lowers oxidative stress, free radical production and reduces liver damage.

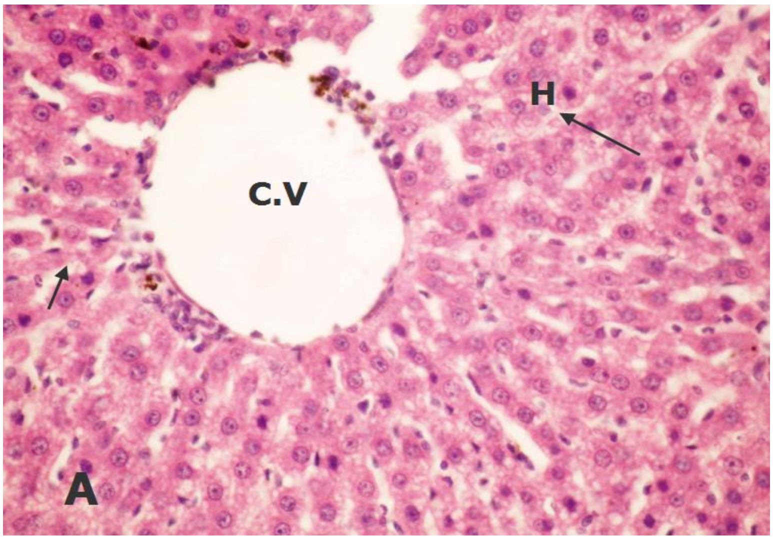

Photo (A) . Liver section of rats of control group (-), exhibiting radically distributed hepatic cords surrounding the central vein (cv) and normal hepatocytes (H) with centrally positioned nuclei and normal hepatic sinusoids (→).

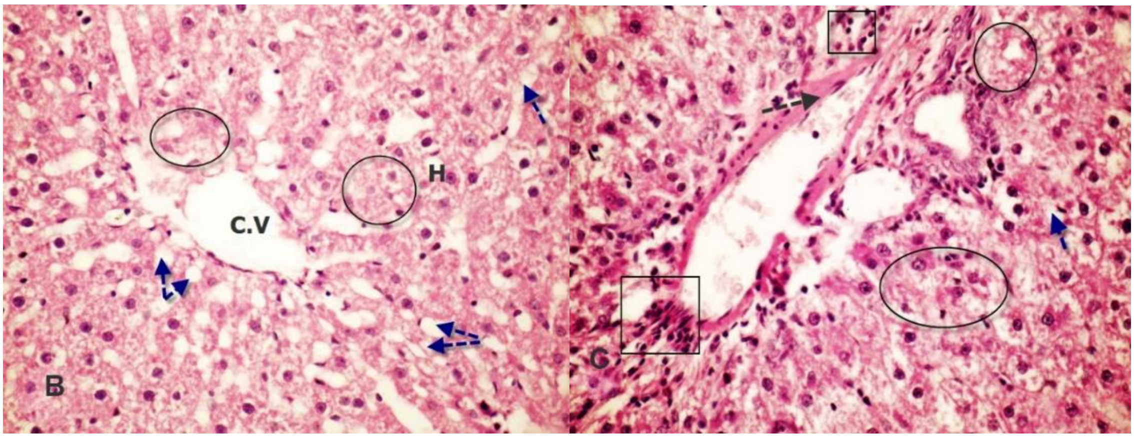

Photo (B, C). Liver section of rats treated with carbon tetrachloride. Fatty degeneration ( ), degenerated hepatocytes with pyknotic nuclei ( ), dilatation and congestion in central veins ( ) and the presence of lymphocytes within the hepatic tissue ( ) were observed in the carbon tetrachloride- treated rats, in contrast, the central vein displaying normal hepatocyte architecture in the control group.

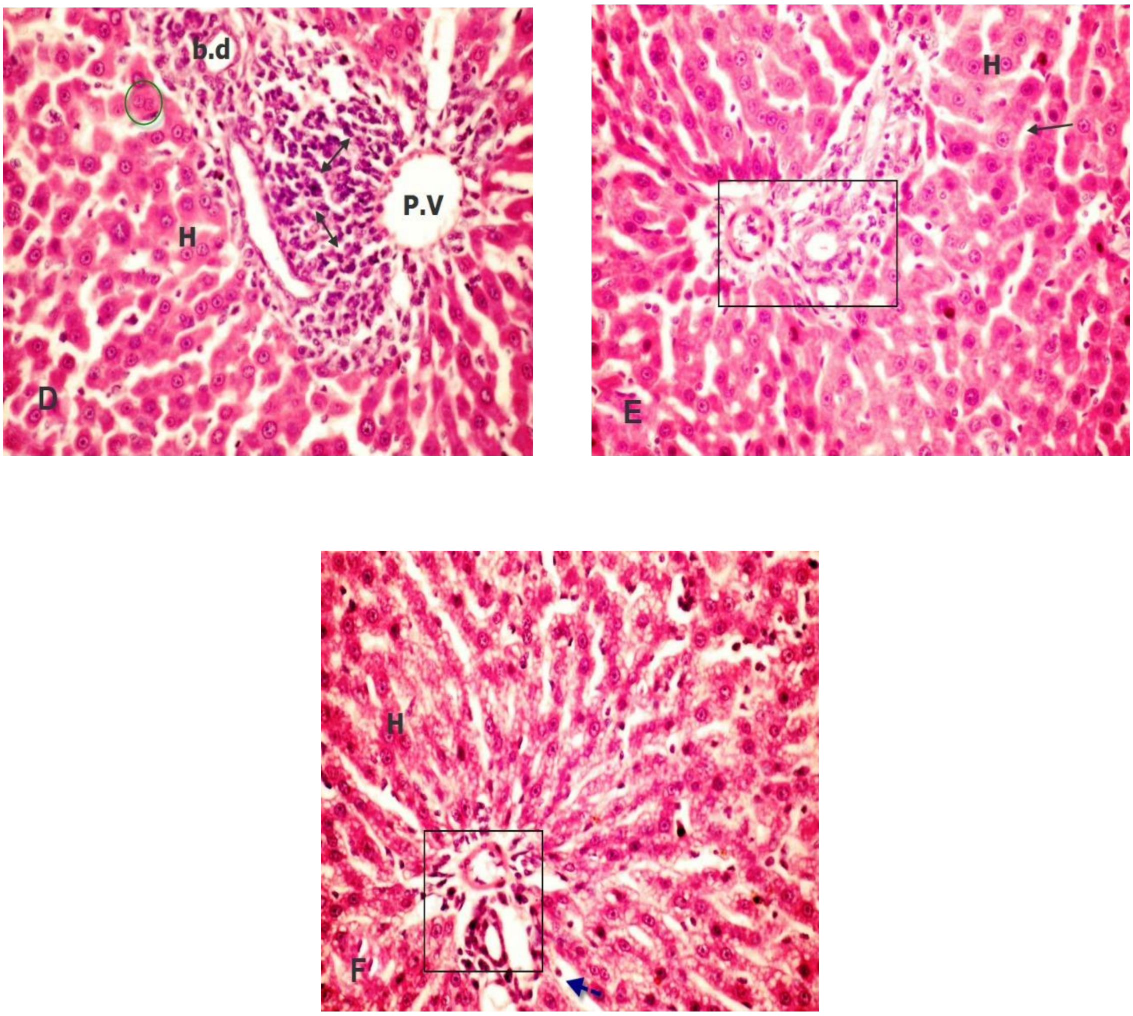

Photo (D, E, F). Liver section of rats treated with soaked beans (D), boiled beans (E) and sprouted red kidney beans (F). Histological alterations generated by carbon tetrachloride treatments were significantly decreased in the groups D, E and F, which exhibited moderate enhancement in hepatic cells. (H&E, ×400)

4. Conclusions

These findings underline the potential of sprouting to enhance the nutritional composition of red kidney beans and its implications for the development of more nutritious food products. Consequently, sprouted red kidney beans enrich protein and dietary fibers in various foods, expanding the basket of plant-based foods. As well, the kidney reduces liver toxicity risk and improves lipid profile, thyroid hormones, antioxidant enzymes, kidney and liver, and renal functions. The liver histology was corroborated with the results of our biochemical studies. The results recommended Sprouted's usefulness in protecting against hepatotoxicity and oxidative stress in rats.

5. Ethical Approval

All study experiments were ethically approved by the Scientific Research Ethics Committee from the University of Alex., Animal Ethics Committee, Faculty of Medicine (Approval no. 021- SREC- 09- 2024).

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