The Protective Effects of Faba Bean Technological Treatments on Liver Toxicity

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Abstract: The aim of this investigation is to evaluate antioxidative and hepatoprotective effects of germinated faba bean on CcL_4 induced liver injury in male *albino* rats. Physio-chemical properties and antioxidant activity of germinated and ungerminated faba bean were investigated. Raw and germinated faba bean seeds for 24, 48 and 72 hrs incorporated diets were investigated against CcL_4 induced liver damage. Germination for 48 hrs increased protein digestibility to 92.6%, total flavonoids 13.6 (mg Quercetin/g DW), total phenolic compounds 48.4 (mg GAE/g DW) and antioxidant activity 84.5% compared to dry seeds. Administration of Ccl_4 at a dose of 1 mg/kg body weight significantly increased the activities of alanine amino transferase, aspartate amino transferase, total cholesterol and triglycerides. Body weight and weight gain were recorded. Serum liver enzymes, albumin and globulins were analyzed. Hematological parameters and lipid profile were improved by increasing in germination time. Rats fed on germinated faba bean diets decreased AST 68.51 U/L and ALT 39.55 U/L. On the other hand, total cholesterol, triglycerides and LDL-C were decreased while HDL-C was increased. Albumin and globulins were increased, also kidney functions were improved. ***In conclusion***, it was suggested that faba bean seeds could protect the liver cells from CcL_4 induced liver damage, may be due to their antioxidative effect against toxic metabolites of CcL_4 and increase of protein digestibility as compared to dry seeds.
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1. Introduction

The liver is the key organ involved in numerous metabolic functions and detoxification of hazardous substances, so it's a frequent target of number of toxicants **(Meyer and Kulkarni, 2001).** Number of environmental pollutants can cause cellular damage through metabolic activation of the compounds to highly free radical products which induce lipid peroxidation and cell membrane damage. Nutrition plays an important role in patients suffering from liver disease. Natural food contains bioactive compounds that lead to regeneration of liver cells and improve liver functions. Some beans properties as antimutagenic effects of their natural phenolic and flavonoids compounds, e.g. black bean (*phaseolus vulgaris* L.) is a protective agent against DNA damage **(Azevedo *et al*., 2003).**

Legume proteins are also a good source for what do called bioactive proteins which benefit liver cells regeneration. Legume protein quality is attributable to the high level of albumin plus globulin fractions which are rich in the essential amino acid lysine. Faba bean (*Vicia faba*) is the most important legume crop in North Africa. Several national dishes are prepared from the mature dry seeds by cooking the whole seeds (medammis), frying dough (bean berger or flafel), cooking crushed and decoated seeds to a paste (bisara). The 2010 world production of faba bean was 4.3 M T from 2.55 Mha**.** Egyptian production of faba bean in 2012 was 185000 tones according to **(FAOSTAT, 2010) and (FAOSTAT, 2012).**

Several investigators recorded the molecular mass of subunits of antioxidant proteins isolated from different plants such as 18 KDa, 43 KDa and 9KDa **(Balamurugan and Menon, 2009).** In human, proteins serve many functions including structural, kinetic, catalytic and singling roles. Also, proteins maintain fluid balance, acid - base balance and form antibodies to protect the body against diseases **(Whiteny and Rolfes, 2005).** Therefore, to achieve optimal body functions the consumption of the diet must be that contains an adequate amount of protein from different sources such as plant and animal proteins.

Germination became common in Western Europe as the sprouts meet the requirements of the modern nutrition. During germination the nutritive quality of legume foods were improved, high molecular weight protein fractions broke down and low molecular weight and small peptides were detected**,** also the antinutritional factors were decreased and protein digestibility was improved **(Juo and Stotzky, 1970)**. The aim of the present

study is to evaluate germination of faba bean seeds during different times as a functional food and investigate their antioxidantive and hepatoprotective effects on CcL_4 induced liver injury in rats.

2. Material and Methods

Material.

Faba bean seeds, starch and oil were purchased from local market at Giza. Casein, minerals, vitamins and cellulose were purchased from Technogein company.CcL_4 (98.8% purity) was purchased from El-Nasr Pharmaceutical Chemical Company (Egypt). Adult male albino rats were obtained from animal house, Food Technology Research Institute, Agriculture Research Center, Giza, Egypt. All kits were purchased from Bio diagnostic Company, Dokki, Giza, Egypt.

Methods

Germination.

Faba bean germination was carried out according to the method of **(Ghavidel and Prakash, 2007)** with some modification, seeds were cleaned, washed and disinfected with 0.07% sodium hypochloride for 15 min at room temperature and washed thoroughly in running tap water. Then the seeds were soaked in 4-5 volumes of water at room temperature for 12 hrs. At the end of this period, the water was drained and the bean samples were allowed to germinate under a wet cheese cloth for 24, 48 and 72hrs. Soaked and germinated seeds were dried in an hot air oven at 40°C until constant moisture then ground into powder using a grinder. Ungerminated seeds served as control. Samples were stored at 40°C until analysis.

Physical properties.

Physical properties were measured according to the methods of **(AOAC, 2000)**.

Chemical analysis.

Moisture, protein, crude lipids, ash and crude fibers were measured according to the methods of **(AOAC, 2000)**. Total carbohydrates were calculated by difference. Total phenols were estimated by the Folin-Ciocalteu method reported in **(Elfalleh *et al.*, 2009).** The amount of total flavonoids was measured spectrophotometrically by the method of **(Nasri *et al.*, 2011)**. The DPPH (2,2 diphenyl-1- picric hydrazyl) radical scavenging activity of methanolic extracts was determined following the method reported by **(Okonogi *et al.*, 2007).** *In-vitro* protein digestibility of faba bean treatments was determined according to **(Saunders *et al.*,1973).** Firmness by penetration of ungerminated and germinated faba bean seeds is measured with (quality technology LTD Test) by (Cometech, B type, Taiwan) texture analyzer according to the method described by **(Bourne, 2003)**.

Protein extraction of faba bean.

Extraction of faba bean protein fractions was performed according to the method of **(Landry and Moureaux, 1970).** Two grams of faba bean were sequentially extracted with 20 ml demonized water for 20 min at 25°C then centrifuged at 18.900 g for 10 min at 4°C (albumin fraction) and the pellet sequentially extracted with 20 ml of 0.5 M NaCl for 60 min at 25°C and centrifuged at 18.900 g for 10 min at 4°C (globulin fraction).

Electrophoresis.

Capillary electrophoresis was performed with (Capillary's 2 Flex Piercing Analyzer Sebia) multi parameter instrument for serum protein and protein extract of faba bean analysis on parallel capillaries according to **(Oda *et al.*, 1997).** The capillaries protein (E) 6 kits (migration buffer, wash solution, dilution segments and reference no.2003) control and standard were used. A sample dilution with buffer is prepared and injected by aspiration at the anodic end of the capillary. A high voltage protein separation is performed and direct detection of the proteins is made at 200 nm at the catholic end of the capillary, globulin and albumin were detected.

Experimental.

Forty two male albino adult rats were obtained from Animal House, Food Technology Research Institute, Agriculture Research Center, Giza, Egypt. The rats were kept under normal laboratory conditions (temperature remain 25± 2^0C) for one week before the beginning of experiment. During this period, the rats were allowed free access of water and basal diet. Body weight was recorded for each rat. The basal diet prepared according to **(A.O.A.C., 2000)**; corn starch 70 %, casein 10%, corn seed oil 10%, salts mixture 4%, vitamins mixture 1% and cellulose 5%.

Experimental design.

Animals were divided into seven groups (6 rats) in each. Group (1) fed on basal diet and served as Negative control (NC). The rest groups were given carbon tetrachloride (CcL_4) by injection to produce acute liver damage. CcL_4 was diluted in (1:1 v/v) paraffin oil and subcutaneously injected in the first and the second day experiment at a dose of 1 mg/kg body weight **(Manoj and Aqueed, 2003)**. These groups fed on diets according to the results of the faba bean composition analysis and formulated so that each diet provided approximately the same amount of carbohydrates, lipids, vitamins, minerals and fibers. Group (1) Negative control (NC): fed on basal diet, Group (2) Positive control (PC): fed on basal diet, Group (3): fed on basal diet containing faba bean raw, Group (4): fed on basal diet containing faba bean soaked, Group (5): fed on basal diet containing faba bean germinated for 24 hrs,

Group (6): fed on basal diet containing faba bean germinated for 84 hrs, Group (7): fed on basal diet containing faba bean germinated for 72 hrs. Germinated and ungerminated faba bean seeds were formulated diets as half percent of casein.

Biochemical analysis:

Blood hemoglobin (Hb), Hematocrite (Ht) and platelets were determined using a whole blood sample and the method described by **(Dacie and Lewis, 1984)**. Red blood cells (RBCs) and White blood cells (WBCs) were measured as recommended by **(Riley, 1960)**. Serum triglycerides, total cholesterol, HDL and LDL were determined according to the methods of **(Fossati and Principe, 1982)**, **(Allain *et al.*, 1974)**, **(Lopes-Virella *et al.*, 1977)** and **(Steinberg, 1981)** respectively. Alanine-amino transferase (ALT) and aspartate-amino transferase (AST) were determined according to the method described by **(Reitman and Frankel, 1957)**. Uric acid, creatinine and urea were estimated according to the methods described by (**Barham and Trider, 1972)**, **(Bartles *et al.*, 1972), (Fawcett and Soctt, 1960)** respectively.

Histopathological examination of the liver:

The post-mortem examination was done as soon as possible and the liver was collected. Fixation was done in 10% natural formalin then dehydrated cleared, and ended paraffin then sectioned at 7µm and stained with harries hematoxylin and eosin for histopathological examination **(Carleon, 1967)**.

Statistical analysis

The obtained data were exposed to analysis of variance. Duncan's multiple range tests at ($P \leq 0.05$) level was used to compare between means. The analysis was carried out using the PRO ANOVA procedure of Statistical Analysis System **(SAS Program, 1996).**

3. Results

Physical properties of dry, soaked and germinated faba bean seeds are shown in Table (1). Results revealed that 100-seeds weight was 97.12±1.0 for dry bean and significantly increased to 161.7±0.8 after 12 hrs soaking in water by increment 66.5%. Germination of faba bean for 24, 48 and 72 hrs increased 100-seeds weight by 78.34%, 90.51% and 104.28%, respectively. On the other hand, true density and bulk density values were of significant increase after 12 hrs soaking and different germination times compared to dry beans. True density values were 1.11±0.03 and 6.03 for dry and soaked beans, respectively. True density reached to the maximum values after 48 and 72 hrs germination (15.3±0.8 and 15.5±0.9 respectively). No significant differences between bulk density values during 12 hrs soaking and germination times.

From the results in Table (1), it could be noticed that absorption rate increased after soaking and reached to the maximum after 48 hrs germination time (215.30±1.16%). Weights of hull and splits were gradually significantly increased after soaking and during germination times to reach to the maximum weights (32.3±0.9 and 166.4±1.22, respectively), after 72 hrs germination compared to dry seeds. The same table showed that sprout length was affected by increasing germination time. The sprout lengths (cm) of bean were 0.3±0.11, 0.7±0.1 and 1.2±0.13 after 24, 48 and 72 hrs germination, respectively.

Table (1): Physical properties of dry, soaked and germinated seeds.

Properties	Dry bean	Soaking 12hrs	Germination time (hrs)			*LSD*
			24	48	72	
100-seed weight	97.12 ±1.0^{e}	161.7 ±0.84^{d}	173.2 ±1.12^{c}	185.4 ±1.23^{b}	198.4 ±0.91^{a}	1.8709
True density (g/cm^3)	1.11 ±0.03^{d}	6.03 ±0.15^{c}	11.2 ±0.9^{b}	15.3 ±0.8^{a}	15.5 ±0.97^{a}	1.1987
Bulk density (g/cm^3)	0.40 ±0.05^{b}	0.64 ±0.04^{a}	0.59 ±0.02^{a}	0.59 ±0.2^{a}	0.6 ±0.01^{a}	0.5656
Absorption rate (%)	0.0 ±0.0^{e}	195.2 ±0.53^{d}	201.3 ±1.01^{c}	215.3 ±1.16^{a}	205.5 ±1.22^{b}	1.6534
Hull (g)	13.5 ±1.1^{c}	28.7 ±0.92^{b}	31.5 ±0.87^{a}	30.7 ±1.1^{a}	32.3 ±0.9^{a}	1.7757
Splits (g)	83.52 ±0.88^{e}	133.3 ±0.86^{d}	140.7 ±1.2^{c}	155.5 ±1.23^{b}	166.4 ±1.22^{a}	1.9926
Sprout length (cm)	0.0 ±0.0^{d}	0.0 ±0.0^{d}	0.3 ±0.11^{c}	0.7 ±0.1^{b}	1.2 ±0.13^{a}	0.1409

Values are means ± SD. Different letters are significant ($p < 0.05$).

Chemical composition of germinated and ungerminated faba bean seeds is presented in table (2). Results showed that significant increases were found in protein from 32.52±1.01 to 35.28±0.97 and crude fiber from 6.23±0.08 to 8.55±0.15 for faba bean germinated for 72 hrs compared with dry bean.

On the other hand, significant decreases were occurred in lipid, ash and total carbohydrate (1.73±0.08 to 1.15±0.05, 3.67±0.12 to 3.34±0.09 and 54.6±1.1 to 51.6±0.43, respectively) after 72 hrs germination compared with dry bean. Germination of faba bean increased protein digestibility where as the highest value was (92.61%) after 72 hrs followed by 48 hrs germination time (87.21%) compared to dry bean (63.34%) and soaked bean (64.19%).

Firmness by penetration of germinated bean seeds decreased form 26.41±0.84 for dry seeds to 7.75±1.44 and 9.20±2.25 after 72 hrs and 48 hrs germination times, respectively.

Table (2): Chemical composition (g/100g) (On dry basis)**, digestibility (%) and penetration** (Max. force) **of dry, soaked and germinated seeds.**

Properties	Dry beans	Soaking 12 (hrs)	Germination time (hrs)			*LSD*
			24	48	72	
Protein	32.52 ±1.01^{c}	33.37 ±0.9^{bc}	34.39 ±1.11^{ab}	35.29 ±0.81^{a}	35.28 ±0.97^{a}	1.7551
Lipid	1.73 ±0.08^{a}	1.62 ±0.09^{a}	1.44 ±0.06^{b}	1.32 ±0.08^{b}	1.15 ±0.05^{c}	0.1533
Fiber	6.23 ±0.08^{d}	6.36 ±0.22^{d}	7.34 ±0.1^{c}	7.86 ±0.07^{b}	8.55 ±0.15^{a}	0.2528
Ash	3.67 ±0.12^{a}	3.48 ±0.12^{b}	3.32 ±0.08^{bc}	3.24 ±0.07^{c}	3.34 ±0.09^{c}	0.1828
Total carbohydrates	54.6 ±1.1^{a}	54.4 ±1.0^{ab}	53.8 ±0.96^{ab}	52.6 ±1.05^{bc}	51.6 ±0.43^{c}	1.6992
Digestibility (%)	63.34 ±0.89^{d}	64.19 ±1.1^{d}	75.17 ±0.64^{c}	87.21 ±1.16^{b}	92.61 ±0.79^{a}	1.7035
Penetration Max. force (N)	26.41 ±0.84^{a}	18.16± 1.03^{b}	11.67 ±1.43^{c}	9.20 ±2.25^{cd}	7.75 ±1.44^{d}	2.6997

Values are means ± SD. Different letters are significant ($p < 0.05$). N: Newton

Total phenolic compounds, total flavonoids and DPPH radical scavenging activity for dry, soaked and germinated bean are shown in Table (3). Total phenolic content of methanolic extract of dry bean was found to be 30.4±0.2 mg gallic acid equivalent/g extract DM. This value decreased after 12 hrs soaking to reach 25.5±0.3 and increased again after 24 hrs germination (33.7±0.3 mg GAE/g DW). The highest value of phenolics was 48.4±0.26 mg GAE/g DW) after 72 hrs germination. However, germination significantly ($p<0.05$) increased total flavonoids content to reach the highest value after 48 hrs germination (13.6±0.4 mg quercetin/g DW) compared to dry bean (9.5±0.2 mg quercetin/g DW). On germination DPPH radical scavenging activity increased significantly ($p<0.05$). No significant differences between DPPH (%) of dry and soaked seeds (63.2±1.9% and 62.5±1.7%, respectively). The highest DPPH was recorded after 48 hrs (84.5±2.0%) followed by 72 hrs germination (77.3±2.3%).

Table (3): Total phenolic, total flavonoids and antioxidant activity of dry, soaked and germinated seeds.

Properties	Dry beans	Soaking 12 hrs	germination time (hrs)			*LSD*
			24	48	72	
Total phenolic (mg GAE/g DW)	30.4 ±0.2^{d}	25.5 ±0.3^{e}	33.7 ±0.41^{c}	42.6 ±0.25^{b}	48.4 ±0.26^{a}	0.4121
Total flavonoids (mg quercetin/g DW)	9.5 ±0.2^{d}	8.4 ±0.31^{e}	11.5 ±0.28^{c}	13.6 ±0.40^{a}	12.6 ±0.26^{b}	0.4505
DPPH (%)	63.2 ±1.9^{d}	62.5 ±1.7^{d}	72.4 ±1.9^{c}	84.5 ±2.0^{a}	77.3 ±2.3^{b}	3.6449

Values are means ± SD. Different letters are significant ($p < 0.05$).

Body weight changes, gain and daily gain of rats fed diets containing dry, soaked and germinated faba bean are shown in Table (4). The results indicated that positive control group had a lower weight gain as compared to the negative control group. The body weight gain was observed in 48, 72 and 24 hrs germinated bean diets fed groups compared to positive control group.

Hemoglobin, hematocrite, RBCs, WBCs and platelets of rats fed germinated and ungerminated diets are shown in table (5). Results showed that positive control group had lower values of hematological parameters than negative control group. Rats fed on diets containing 48, 72 and 24 hrs germinated seeds respectively had better values of the previous parameters than positive group. The diets containing faba bean germinated for 48 hrs fed group

was the best one for hemoglobin (15.6±0.3 gm/dL), RBCs (6.8±0.1 M/mm), WBCs (19.7±1.05 cmm) and platelets (729.7±10.01 cmm) compared to the other groups.

Table (4): Effect of different diets containing dry, soaked and germinated seeds on body weight in rats (g).

Groups	Body weight			
	Initial	Finial	Gain	Daily gain
Negative control	152.4±0.86^{a}	191.8±1.4^{a}	39.3±2.3^{a}	0.65±0.03^{a}
Positive control	153.5±0.65^{ab}	160.4±0.76^{f}	6.8±0.75^{f}	0.11±0.01^{f}
Dry beans	153.6±1.11^{ab}	161±1.61^{f}	7.4±0.5^{f}	0.12±0.04^{f}
Soaking (12 hrs)	154.5±0.81^{ab}	164.6±0.86^{e}	10.1±0.26^{e}	0.17±0.04^{e}
Germinated (24 hrs)	152.4±1.2^{ab}	169.3±0.9^{d}	16.7±2.1^{d}	0.28±0.03^{d}
Germinated (48 hrs)	152.4±1.1^{ab}	175.5±0.79^{b}	22.9±1.34^{b}	0.38±0.02^{b}
Germinated (72 hrs)	153.5±0.78^{ab}	173.5±1.1^{c}	19.9±1.8^{c}	0.33±0.03^{c}
LSD	1.6476	1.9448	2.6159	0.0436

Values are means ± SD. Different letters are significant ($p < 0.05$).

Table (5): Effect of different diets containing dry, soaked and germinated seeds on blood picture in rats.

Groups	HB (gm/dL)	HT %	RBCs (M/mm)	WBCs (cmm)	Platelets (cmm)
Negative control	16.3±0.55^{a}	47.5±1.32^{a}	7.3±0.2^{a}	20.4±0.2^{a}	772.0±10.6^{a}
Positive control	11.6±0.45^{e}	35.7±2.38^{c}	5.5±0.1^{e}	11.03±0.75^{e}	480.6±6.02^{g}
Dry beans	13.5±0.25^{d}	39.9±0.15^{b}	5.8±0.1^{d}	13.86±0.92^{d}	508.3±1.52^{f}
Soaking (12 hrs)	13.5±0.35^{d}	39.4±1.11^{b}	6.4±0.1^{c}	15.63±1.35^{c}	584.3±3.1^{e}
Germinated (24 hrs)	14.4±0.26^{c}	41.2±2.35^{b}	6.5±0.15^{c}	17.53±1.22^{b}	629.7±5.03^{d}
Germinated (48 hrs)	15.6±0.3^{b}	46.5±1.09^{a}	6.8±0.1^{b}	19.7±1.05^{a}	729.7±10.01^{b}
Germinated (72 hrs)	14.6±0.32^{c}	44.9±0.65^{a}	6.6±0.1b^{c}	17.1±0.75^{bc}	685.6±5.13^{c}
LSD	0.6473	2.6362	0.2057	1.6853	11.6914

Values are means ± SD. Different letters are significant ($p < 0.05$).

Serum alanine and aspartate amino transferases concentrations of rats fed germinated and ungerminated diets are shown in Table (6). Significant ($p \leq 0.05$) increase of serum ALT and AST concentrations was found in Ccl4-intoxicated rats group compared to negative control group. Positive control group recorded the highest levels of ALT (83.48±2.18 U/L) and AST (120.6±5.15 U/L). Feeding on germinated and ungerminated faba bean containing diets caused significant inhibition of ALT and AST concentration compared to positive group. Germination of faba bean for 48 and 72 hrs had the better values of ALT and AST (39.55±1.85U/L and 68.51±1.82 U/L) and (46.58±1.99 U/L and 70.33±1.91U/L), respectively than dry bean (74.31±1.9 U/L) and (101.2±1.22 U/L), respectively.

Table (6): Effect of different diets containing dry, soaked and germinated seeds on liver functions in rats (U/L).

Groups	AST(U/L)	ALT(U/L)
Negative control	39.25±2.05^{e}	27.43±0.88^{g}
Positive control	120.6±5.15^{a}	83.48±2.19^{a}
Dry beans	101.2±1.22^{b}	74.31±1.90^{b}
Soaking (12 hrs)	98.48±2.24^{b}	62.42±2.11^{c}
Germinated (24 hrs)	81.49±2.18^{c}	57.52±2.16^{d}
Germinated (48 hrs)	68.51±1.82^{d}	39.55±1.85^{f}
Germinated (72 hrs)	70.33±1.91^{d}	46.58±1.99^{e}
LSD	4.6314	3.3578

Values are means ± SD. Different letters are significant ($p < 0.05$).

Serum lipid profile resulted in CcL_4-induced liver toxicity and feeding rats on germinated and ungerminated containing diets are listed in Table (7). Serum triglycerides and total cholesterol were significantly ($p \leq 0.05$) higher in positive control group (125.8±1.77 mg/dL and 90.63±2.44 mg/dL) than negative control group (114.5±2.05 mg/dL and 70.27±2.19 mg/dL). Diets contained germinated and ungerminated faba bean improved triglycerides and total cholesterol levels compared to positive control group. No significant differences between dry and soaked faba bean diets for triglycerides and cholesterol levels. Feeding on diet containing faba bean germinated for 48 hrs and 72 hrs normalized triglycerides levels (115.4±1.05 mg/dL and 116.5±1.25 mg/dL) compared to negative control. Rats fed on diets containing germinated seeds (24, 48 and 72 hrs) showed significant inhibition of total cholesterol levels (78.13±1.53, 74.69±0.89 and 76.59±1.25 mg/dL, respectively compared to positive control rats.

Positive control group exhibited a significant reduction (42.95%) of HDL-C level compared to untreated rats (negative group). Rats fed on diets containing germinated faba bean exhibited increased HDL-C levels, particularly which fed on 48 hrs germinated faba bean (44.6±1.49 mg/dL) compared to positive group (30.42±0.81 mg/dL). LDL-C levels were significantly lower ($p \leq 0.05$) in CcL_4 groups that fed on different diets containing germinated faba bean than positive control (60.53±0.81 mg/dL). The lowest significant value of LDL-C (28.68±0.96 mg/dl) occurred in rats fed on 48 hrs germinated faba bean diet followed by rats fed on 72 hrs germinated faba bean diet (33.64±0.87 mg/dL).

Table (7): Effect of different diets containing dry, soaked and germinated seeds on lipid profile in rats (mg/dL).

Groups	Triglycerides	Total Cholesterol	HDL-C	LDL-C
Negative control	114.5±2.05^{e}	70.27±2.19^{d}	53.32±1.91^{a}	16.48±1.9^{g}
Positive control	125.8±1.77^{a}	90.63±2.44^{a}	30.42±0.81^{e}	60.53±0.81^{a}
Dry beans	121.4±1.05^{b}	82.25±4.05^{b}	35.58±1.71^{d}	54.03±1.19^{b}
Soaking (12 hrs)	119.5±0.95^{bc}	85.5±1.01^{b}	37.36±1.39^{cd}	49.69±0.66^{c}
Germinated (24 hrs)	118.3±0.95^{cd}	78.13±1.53^{c}	41.0±4.22^{cd}	39.41±0.76^{d}
Germinated (48 hrs)	115.4±1.05^{e}	74.69±0.89^{c}	44.6±1.95^{b}	28.68±0.96^{f}
Germinated (72 hrs)	116.5±1.25^{de}	76.59±1.25^{c}	43.6±1.49^{b}	33.64±0.87^{e}
LSD	2.3861	3.8032	3.9132	1.9216

Values are means ± SD. Different letters are significant ($p < 0.05$).

Data presented in Table (8) showed serum levels of urea, creatinine and uric acid of rats treated with CcL_4 and fed on diets containing dry, soaked and germinated faba bean compared to positive and negative controls. Significant increases were observed in urea and creatinine levels of positive control rats (41.5±1.92 and 1.14±o.11 mg/dL) compared to negative control rats (33.5±1.80 and 0.67±0.02 mg/dL), respectively. On the contrary, feeding on diets containing germinated faba bean normalized values of urea, creatinine and uric acid compared to positive control group.

Table (8): Effect of different diets containing dry, soaked and germinated seeds on kidney functions in rats (mg/dL).

Groups	Urea	Creatinine	Uric acid
Negative control	33.5±1.80^{d}	0.67±0.02^{d}	3.54±0.22^{a}
Positive control	41.5±1.92^{a}	1.14±0.11^{a}	3.01±0.45^{cd}
Dry beans	39.5±2.35^{ab}	1.04±0.17^{ab}	2.74±0.2^{d}
Soaking (12 hrs)	37.6±1.95^{bc}	0.94±0.19^{abc}	2.93±0.2^{cd}
Germinated (24 hrs)	36.4±0.76^{bcd}	0.92±0.12^{abc}	3.23±0.08^{abc}
Germinated (48 hrs)	34.7±1.21^{cd}	0.74±0.11^{cd}	3.44±0.16^{ab}
Germinated (72 hrs)	36.3±1.13^{bcd}	0.84±0.59^{bcd}	3.33±0.22^{abc}
LSD	2.9348	0.2191	0.4273

Values are means ± SD. Different letters are significant ($p < 0.05$).

Data presented in Table (9) showed the changes of albumin, globulins and A/G ratio of faba bean during germination time and serum blood of rats which fed on diets containing these seeds. From this table it could be observed that albumin fraction in dry faba bean was o.6 g/100g (1.85%), this value

increased after 48 hrs of germination to 0.85 g/100g (2.41%). On the other hand germination for 48 and 72 hrs increased globulin fraction from 24.2 g/100g (74.4%) for dry bean to 26.85 g/100g (76.08%) and 27.54 g/100g (78.06%), respectively. Concurrently, A/G ratio was increased to 0.031 by increasing time of germination to 48 hrs compared to dry bean (0.025).

At the same line, serum albumin and globulin fractions of rats fed on diets containing dry, soaked and germinated seeds increased. Germination for 48 hrs recorded the highest value of albumin and globulin (3.45 and 3.8 g/dL) respectively, compared to dry bean (2.5 and 3.5 g/dL) and positive control (2.3 and 3.18 g/dL), respectively. A/G ratio of serum was o.91 at 48 hrs higher than dry bean o.71.

Table (9): Albumin, Globulins and A/G Ratio of faba bean treatments (g/100g) and serum of blood (g/dL).

Properties	dry bean	soaking (12 hrs)	Germination time (hrs)			NC	PC
			24	48	72		
Faba bean (g/100g)							
Albumin	0.6	0.66	0.71	0.85	0.81	-	-
Globulins	24.2	24.0	25.05	26.85	27.54	-	-
A/G Ratio	0.025	0.027	0.028	0.031	0.029	-	-
Serum of blood (g/dL)							
Albumin	2.5	2.7	2.8	3.45	3.15	3.5	2.3
Globulins	3.5	3.2	3.6	3.8	3.55	3.6	3.18
A/G Ratio	0.71	0.84	0.78	0.91	0.89	0.97	0.69

NC: Negative control PC: Positive control

Data in Table (10) for histopathological examination of livers is actually quite similar to that of the former table, assuming a sort of relation between the liver histopathology and its function as the result of feeding on germinated faba bean. Microscopically, livers of rats from negative control showed no histopathological changes. Livers of rats from positive control revealed severe necrosis, apoptosis, infiltration of inflammatory and fatty changes. Rats fed on germinated faba bean (24, 48 and72 hrs) containing diets showed no histopatholoical changes of the livers compared to dry and soaked beans diets.

Table (10): Histopathological changes in liver of rats fed on different diets containing dry, soaked and germinated seeds.

Groups	Necrosis of hepatocytes	apoptosis of hepatocytes	Infiltration of inflammatory	Fatty changes of hepatocytes
Negative control	-	-	-	-
Positive control	+++	+++	+++	+++
Dry beans	-	+	+	+
Soaking (12 hrs)	-	+	-	+
Germinated (24 hrs)	-	-	-	-
Germinated (48 hrs)	-	-	-	-
Germinated (72 hrs)	-	-	-	-

(-) No changes; (+) very mild; (++) mild; (++++) severe

4. Discussion

Faba bean (*Vicia faba L.*) contributes to human nutrition as a result of its high protein content and other essential nutrients. Many of reports have finished their data on the changes in the protein content of germinated legume seeds and reported that germination improves the nutritive quality of the legume foods. Our results showed that protein content increased as the germination time increased. This could be as a result of biochemical changes by sprouting leading to an increase in free amino acids **(Obatolu *et al.*, 2001)** or at least in part of the loss of non protein dry matter **(Rumiyati *et al.*, 2012)**.

In the present study crude fiber content was found to increase at the later stage of germination, the seed coats may contribute to their fiber content. These results agree with **(Trugo *et al.*, 2000)** who reported that a high concentration 36% of fiber content of lupine was after 2 days of germination. These changes may be due to changes in polysaccharides found in the cell wall such as cellulose, glucose and mannose, and an increase in

the cellular structure of the seeds during germination **(Martin-Cabrejas *et al*., 2003)**.

Lipid value of faba bean seeds in the present study was observed to decrease as the germination time increased, this may be due to increased lipolytic enzymes and using the fatty acids as energy source during germination. Lipid content has also been reported to decrease during germination **(Mostafa and Rahma, 1987)**.

Ash content significantly decreased at the later stage of faba bean germination (72hrs) in parallel to observations of **(Wang *et al.,* 1997)** and **(Tatsadjieu *et al.*, 2004)** who reported that the differences of ash content after soaking was due to loss in minerals due to rootlet and washing of faba bean seeds in water reduce the sour smell during the period of germination.

It was observed that dry faba bean seeds had significantly more carbohydrates than germinated seeds. These results agree with **(Inyang and Zakari, 2008)**. Carbohydrates was used as a source of energy for embryonic growth which could explained the changes of carbohydrates after germination and this may be due to β-amylase activity that hydrolyzes the starch into simple carbohydrates **(Nonogaki *et al*., 2010)**.

The proteolytic resistance of dry seeds has been attributed to the presence of antinutritional compounds which affect protein digestibility **(Periage *et al*., 1996)**. In the present study, germination of faba bean seeds increased protein digestibility. This may be due to loss of antinutritional factor such as phytates and trypsin inhibitors during soaking and germination of the seeds **(Negi *et al.,* 2001).** These results are similar to **(Correia *et al*., 2010)** who found that germination causes activation of intrinsic analyses, proteases, phytases which led to an increase in *in vitro* protein digestibility.

Firmness by penetration of germinated seeds defined as the maximum force required to shear the germinated seeds. In our study, firmness of germinated seeds decreased by increasing of germination time, these effects may be due to physiochemical and texture changes as a result of biosynthesis and degradation of faba bean seeds biochemical compounds during germination process.

Qualitative morphological properties of faba bean seeds are resulted with their physical properties such as weight of hundred seeds, true and bulk densities, absorption rate and sprout length during germination process. The gain in weight was observed in table (1), an increase in weight of 100-seeds observed were mainly due to the water that has shrunk. These results agree with **(Jim *et al.,* 2012)** who reported that the total increase in weight was three times plus from the initial weight of the seeds. Absorption rate of faba beans also increased by increasing in germination time, this may be due to the more porosity of the hull and splits increase.

Phenolic compounds not only effectively prevent the oxidation in foods, they also act as protective factors against oxidative damage in the human body **(Lopez-Amoros *et al*., 2006).** Faba bean raw seeds contain low and high macular weight phenolic compounds such as flavonoids and proanthocyandins **(Borowska *et al*., 2003) and (Siah *et al*., 2012)**. Our results indicated that total phenolic and total flavonoids increased by increasing in germination time, this may be due to appearance of new phenolic compounds as a result of break down and synthesis during germination. These results agree with **(Lopez-Amoros *et al*., 2006)**, who reported that germination modifies the quantities and qualitative phenolic compounds of legumes and the changes depend on the type of legume and germination conditions.

Reserves within the storage tissues of the seeds are mobilized during germination to support seedling growth. From this moment the seed breaks dormancy, protective response emerge through the synthesis of phenolics and the other compounds. The current upsurge of interest about the efficiency and function of natural antioxidation food and biological systems, the testing of antioxidant activity has much attention **(Gharachorloo *et al*., 2012)**. Germination led to increase in antioxidant activity compared to dry faba bean seeds this may be faba bean germination seeds contained higher amounts of total phenolic and flavonoids compounds and other bioactive compounds such as vitamins, low molecular weight proteins and small peptides than dry seeds which make them act as reducing agents, hydrogen donors, and metallic chelating potential **(Yahia *et al*., 2013)**. The potent antioxidant capacity of faba bean seeds largely contributed by the proanthcyanidins or highly polymerized tannins **(Siah *et al*., 2012)**.

Body weight decreased as the results of CcL_4 injection and considered to be the result of direct toxicity of CcL_4 or indirect to the liver disorders. Body weight changes a result of CcL_4 injection have been used as indicator of CcL_4–related organ damage **(Pradeep *et al.,* 2005)**. In the present experiment, the body weight increased in the rats fed on faba bean germinated seeds containing diets compared to ungerminated seed diets. The increase of body weight may be due to the germination of faba bean seeds improved palatability of these seeds and the nutritive utilization of protein and carbohydrates **(Urbano *et al*., 2005)**.

Haematological parameters; haemoglobin, haematocrit, RBCs, WBCs and platelets are relevant

and vital indices to toxicity assessment. The results of our study indicated that significant (P<0.05) decrease in levels of previous parameters in toxin control group compared to the negative one which could be due to excessive destruction of erythrocytes, disturbed haematopoiesis and reduction in the rate of their formation **(Mada *et al*., 2014)**. On the other hand feeding on faba bean germinated seeds containing diets stimulated haematopoiesis and restored Hb and platelets towards normal values.

These effects may be due to phytic acid, tannin contents and α-galactosides which reduced by germination of faba bean seeds and improved the bioavlability of minerals (**Vidal-Valverde *et al*., 1998)**, especially iron ion **(McDonald *et al.,* 1996)**.

Liver is a pivotal inflammatory organ that involved in metabolism, storage and excretion of metabolites. Carbon-tetrachloride (CcL_4) is a model for studying free radicals that can induce liver injury. That principle effect of CcL_4 is hepatic damage induction by increasing lipid peroxidation, decreasing activities of antioxidant enzymes, generation of free radicals and elevation of hepatic enzymes such as ALT and AST **(Fahmy and Soliman, 2007)**. Germination has been well associated with elevated amount of antioxidants content **(Fernandez-Orozco *et al*., 2008)**.

Hepatoprotective and antioxidantive effects of faba bean by germination for 48, 72, 24 hrs, respectively, may be due to the presence of flavonoids and phenolic bioactive compounds, which increased during germination **(Sikirc *et al*., 1993)**. In the present study, feeding rats on germinated faba bean diets resulted in significant decrease in ALT and AST activity in serum as compared to dry faba bean diet and positive group. These decreases were considered as indicators of the improvement in the functional status of liver cells that may be due to increase of protein digestibility, degradation of high molecular weight proteins to small molecular weight, simple peptides and amino acids which act as power antioxidants against free radicals scavenging activities.

In our experiment, carbon-tetrachloride (CcL_4) induction caused significant higher levels of total cholesterol LDL-cholesterol and triglycerides in rats, these agree with **(Gopal and Senaottuvelu, 2008)** who indicated that this effect may be due to the presence of damage in the liver. Feeding rats on dry faba bean based diet decreased total cholesterol, triglycerides and LDL-cholesterol compared to casein diet. On the other hand, HDL-cholesterol were increased, these effects may be due to the reduction in cholesterol absorption induced by fiber contained in whole faba bean seeds. Feeding the faba bean protein diet resulted in an increase in cholesterol excretion in faces as compared with hypercholesterolemic diet may be due to an enhanced biliary cholesterol excretion **(Macarulla *et al.,* 2001)**.

On the other hand, rats fed on germinated faba bean seeds diets normalized triglycerides levels and caused reduction of total cholesterol compared to positive control group, may be due to the presence of flavonoids and phenolic bioactive compounds which highly detected in germinated products with hepatoprotective and antioxidantive effects **(Fernandez-Orozco *et al.,* 2008)**. Also, these effects may be due to the hydrolyzed high molecular weight protein of faba bean to low molecular weight protein, increase in peptides and amino acid which have antioxidant activity and regulate cholesterol synthesis by the liver **(Je *et al*., 2007)**.

Positive control rats had higher value of uric acid than negative group, this may be due to increase in oxidative stress and increase purine synthesis **(Sato *et al.,* 1995)**. Rats fed on germinated faba bean diets normalized uric acid value compared to positive group, this effect may be due to the increase of antioxidant activity (DPPH) of germinated than ungerminated seeds.

On the other hand, significant decrease of urea and creatinine levels were observed in rats fed on germinated faba bean diets compared to ungerminated diets. These effects may be due to increase in glomerular filtration rate or increase of protein digestibility of faba bean seeds which improved the digestion of the rats and regulation of protein synthesis and break down in over all muscles **(Kevin *et al*., 2007).**

In the present study, the results revealed increase in albumin and globulin fractions of faba bean seeds after 48 and 72 hrs germinations. This may be due to the high proteolytic activity of stored protein during germination. Our results agree with **(Abu Baker *et al*., 2010)**, who reported that, albumin and globulin were increased after germination.

On the other hand, feeding rats on germinated faba bean diets increased serum albumin and globulins, may be due to improvement of protein digestibility of faba bean which reflected on albumin biosynthesis by the liver compared to ungerminated seeds.

In our study, the pathological changes observed in CcL_4 liver injury were studied. CcL_4 injection caused elevation of liver function biomarkers and severe necrosis, apoptosis, infiltration of inflammatory and fatty changes in the liver.

However, possible hepatoprotective effect of germinated faba bean was observed when attenuated liver was treated with germinated faba bean seeds diets. This effect may be due to increase in

antioxidant amino acids, phenolic compounds and other bioactive compounds **(Norikura *et al*., 2007).**

Conclusion

Our results suggested that faba bean could protect the liver cells from CcL_4 induced damage and recommended that germination of faba bean seeds is better for decreasing the cooking time of this legume and utilization for patient suffering from liver diseases due to their antioxidantive, hepatoprotective and hypocholestrolemic effects.

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