

The Protective Effect of Seeds and Sprouts of Fenugreek and Alfalfa on Rats Exposed to Lead Poisoning

***Rasha M. Bahnasy, Eman El-Sayed Abd-Elhady
and Mai Hussein Abd-Elfatah***

¹Nutrition and Food Science Dept. Faculty of Home Economics
Al- Azhar University

Abstract

This study was conducted on rats to investigate the effectiveness of Fenugreek seeds, Fenugreek sprouts, Alfalfa seeds and Alfalfa sprouts as nutraceuticals for hepatointoxication induced by lead acetate. Thirty six healthy adult male albino rats "*Sprague Dawley*" strain weighing (200± 10 gm.), were used and divided into 6 equal groups, one was kept as a (-ve) control group, while the other groups received daily 0.4% lead acetate in distilled water to induce hepatotoxicity. Four groups were given the experimental seeds and their sprouts in the form of powder mixed with the basal diet (Fenugreek seeds 5%, Fenugreek sprouts 5%, Alfalfa seeds 2% and Alfalfa sprouts 2%) respectively for eight weeks. The sixth group of rats were kept as (+ve) control group received basal diet only. At the end of the experiment, feed intake (FI), body weight gain (BWG), feed efficiency ratio (FER) and relative weights of liver, kidneys, heart, spleen were calculated. Also, Liver enzymes (AST, ALT & ALP), serum total protein, albumin & globulin and MAO were also determined in serum, lead was determined in whole blood, antioxidant enzymes (SOD & α-GST) were determined in the liver tissue. The results indicated that rats that received lead acetate and fed on basal diet only recorded significant changes for all above biological and biochemical parameters. While the treatment with seeds and their sprouts revealed significant improvement in all liver function biochemical parameters and antioxidant enzymes. The study concluded that; seeds of Fenugreek and Alfalfa and their sprouts were effective in improving liver dysfunction and other side effects. This may be attributed to the high content of phenolic compounds in these seeds.

Key word: Hepatotoxicity – liver enzyme – lead acetate – Fenugreek seeds - Alfalfa seeds - Antioxidant enzymes

Introduction

The environmental contamination by heavy metals has increased drastically along with the rapid development of modern industry. Among these metals is lead, of which its levels have increased substantially during the last few years (*Bilandžić et al., 2009*). Lead (Pb) a soft, grey-blue heavy metal is a common cause of poisoning of domestic animals throughout the world (*Khan et al., 2008*). Workers were exposed to lead in several ways daily. e.g. those work in gasoline additives, food can soldering, lead based paints, ceramic glazes, drinking water systems and folk remedies (*Markowitz 2000*). Health hazards from increased lead exposure was found to produce wide range of biochemical and physiological dysfunctions (*Courtois et al., 2003*).

Rasha M. Bahnasy, et al

Liver is the largest gland inside the body and some other animals; it lies below the diaphragm in the abdominal-pelvic region of the abdomen, where it performs a wide range of metabolic activities necessary for homeostasis, nutrition and immune defense (**Ezejindu et al., 2013**). Also, it has many functions including making bile, changing food into energy, cleaning poisons from the blood, etc. (**Mahan and Escott-Stump, 2008**). Most of the toxic compounds in the body are metabolized in liver. The entry of these toxicants into the body is principally via the gastrointestinal tract, and after absorption, they are transported through the hepatic vein to the liver (**Doherty, 2000**). Liver plays a major role in lead metabolism, and it is in special risk due to the oxidative action of this xenobiotic as there was unquestionable evidence that lead induced lipid peroxidation of hepatic cellular membranes (**Sivaprasad et al., 2004**).

Herbs have been used in all parts of the world not only as food but also as potent drugs for thousands of years. They do not work like chemical drugs and they are not substitute of them (**Vuorela et al., 2004; Horne, 2013**). Medicinal plants are used by 80 % of the world population especially in developing countries to cure and improve the general health, principally due to the common belief that plant-derived drugs are without any side effects along with being economical and locally accessible (**Gupta and Raina, 1998**).

Fenugreek (*Trigonella foenum-graecum* L.) is a member of the leguminous family (**Board, 2010**) which is an annual plant found primarily in Mediterranean countries, the Middle East, and India. Fenugreek is an important spice and its dried seeds have wide application in food and beverages. Fenugreek has been reported to be an important medicinal plant with a large number of medicinal properties such as restorative and nutritive properties (appetite stimulant) with hypocholesterolemic, antidiabetic, antileukemic and antimicrobial effects (**Acharya et al., 2008; Murlidhar and Goswami, 2012**). The chemical composition of fenugreek seed (FS) has been thoroughly studied and its medicinal properties are associated with its phytochemicals such as galactomannans, phenolic compounds, alkaloids, proteins, vitamins (A precursors B1, C and nicotinic acid) and volatile oils (**Acharya et al., 2008**). Germinated fenugreek seeds rich in bioactive antioxidant substances are also used extensively as an important ingredient in daily food preparations and herbal formulations (**Khole et al., 2014**).

Alfalfa and called *Medicago sativa* L. (the genus *Medicago*), belonging to the family of legume (Fabaceae), known as "the king of forage", is cultivated as a perennial forage crop in temperate zones worldwide (**Ma et al., 2016**). *M. sativa* is consumed as a vegetable in the form of salads, tortillas, croquettes, puddings, soups and casseroles all over the world (**Martínez et al., 2016**). As a functional food, it is mentioned in many ancient medical books in China, such as the Compendium of Materia Medica, and was regarded as the alfac-facah (the father of all food) by the ancients (**Ehsanpour and Razavizadeh, 2005**). In the form of crops and vegetables, *M. sativa* contains abundant nutrients with potential health-promoting effects, including antioxidant, anti-inflammatory, anticancer, antibacterial, anxiolytic, immunomodulatory and memory-improving activities, etc. (**Bora and Sharma, 2011; Chen et al., 2015; Rafińska et al., 2017**). Extensive phytochemical studies of *M. sativa* have led to the isolation of a number of flavonoids, saponins, alkaloids, coumarins, fatty acids and essential oils, with the flavonoids being the chemical constituents (**Abbruscato et al., 2014; Rafińska et al., 2017**). Because of the excellent quality of the nutritional components and various biological activities in *M. sativa*, it has been approved by the European Food Safety Authority as a kind of dietary supplement (**Gatouillat et al., 2014**).

Materials and Methods

Materials :

Alfalfa seeds and Fenugreek seeds were obtained from The Agriculture Seeds, Herbs and Medicinal Plants Company, Cairo, Egypt. Lead acetate, casein (85%), vitamins mixture and salt mixture were obtained from El- Gomhoryia Company for Chemical Industries, Cairo, Egypt. Barn, corn starch and corn oil purchase from local market. Thirty-six adult male albino rats of "Sprague Dawley" Strain was obtained from the laboratory animal colony. Ministry of Health and Population, Helwan, Cairo, Egypt.

Methods :

Alfalfa seeds and Fenugreek seeds were cleaned manually to remove broken seeds, dust and other extraneous materials and then drained and washed with Purified water. All seeds were dried under shade at room temperature then grinded using a blender into fine powder and were kept in dark glass containers in a refrigerator till use. To prepare the sprouts seeds were washed with purified water and then soaked in purified water for 12 h at ambient temperature. A seed to water ratio of 1:10 (W/V) was used. The unimbibed water was discarded. The soaked seeds were rinsed twice in water. The soaked seeds were allowed to germinate under a wet muslin cloth for 2 : 4 day with or without light at 37°C and 99% relative humidity , with frequent watering as described by (**Obizoba and Atii, 1994**). The sprouts were rinsed in purified water and dried by dry air at room temperature then grinded using a blender into fine powder and were kept in dark glass containers in a refrigerator till use.

Biological experiment

Male albino rats (n = 36) of Sprague Dawley strain weighing (200± 10 g.) were kept in single well aerated wire cages with wire bottoms under hygienic conditions and fed on basal diet for one week for adaptation. The diet was introduced to the rats in special food containers to avoid scattering of food. In addition, water was provided to the rats by glass tube projection through the wire cages. Food and water were provided *ad-libitum* and checked daily.

After adaptation for one week, the rats were divided into six groups (6 ratseach) as follow : group (1) were fed on basal diet (as a negative control group). From group two to group six, rats received 0.4% lead acetate in distilled water (**Sivaprasad et al., 2004**). group (2) were fed on basal diet (as a positive control group). Group (3) were fed on basal diet supplemented with 5% Fenugreek seeds. Group (4) were fed on basal diet supplemented with 5 % Fenugreek sprouts. Group (5) were fed basal diet supplemented with 2% Alfalfa seeds. Group (6) were fed basal diet supplemented with 2% Alfalfa sprouts, added on the expense of corn starch.

The experimental period lasted for 8 weeks. At the end of the experimental period, rats were fasted overnight before sacrificed and blood samples were collected from each rat and centrifuged to obtain the serum. Serum was carefully separated and transferred into dry clean Eppendorf tubes and kept frozen at -20°C for analysis as described by (**Schermer, 1967**). Liver, kidneys, spleen and heart were removed from each rat by careful dissection, cleaned from the adhesive matter by a saline solution (0.9%), dried by filter paper and weighed. At the end of the experiment, feed intake, body weight gain, organs weight as a percent of total body weight and Feed Efficiency Ratio were determined. According to (**Chapman et al., 1959**).

AST and ALT were determined in the serum according to the method described by **(Reitman and Frankel, 1957)**. Serum alkaline phosphatase determination was performed according to the colorimetric method of **(Roy, 1970)**. Protein was estimated by Biuret reaction in which peptide bonds of protein react with alkaline copper solution to give a violet coloration as described by **(Sonnenwirth and Jarett, 1980)**. Albumin was determined according to the method described by **(Drupt, 1974)** and modified by **(Spencer and Price, 1977)**. Serum globulin was calculated according to **(Chary and Sharma, 2004)**. Lead is determined according to the method described by **(Villeda-Hernandez et al., 2001)**. Monoamine oxidase (MAO) was determined according to **(Holt et al., 1997)**.

After rats being sacrificed, the liver was excised immediately for biochemical investigation. Each sample was washed in saline solution, dried by filter paper and weighed then, was placed in a dry clean aluminum foil and kept frozen till estimated antioxidant enzymes levels. Superoxide dismutase (SOD) activity was determined in liver tissue according to **(Misra and Fridovich, 1972)**. α -glutathion S-transferases was determined in liver tissue according to **(Habig et al., 1974)**. Statistical analysis was carried out using the programme of Statistical Package for the Social Sciences (SPSS), PC statistical software (Version 20; Untitled-SPSS Data Editor). The results were expressed as mean \pm standard deviation (mean \pm SD). Data were analyzed using one-way classification, analysis of variance (ANOVA). **(Snedecor and Cochran, 1979)**.

Results and discussion

Effect of Fenugreek and Alfalfa seeds on body weight gain, feed intake and feed efficiency ratio.

As shown in table (1) feed intake (FI) recorded significant decrease of (+ve) control group (15.20 ± 1.09) when compared to (-ve) control group (18.2 ± 1.3). Rats received diet supplemented with fenugreek seeds (5%), fenugreek sprouts (5%), alfalfa seeds (2%) and alfalfa sprouts (2%) showed significant increases ($P < 0.05$) in FI when compared with (+ve) control group. All of them recorded no significant difference with the (-ve) control group. The best results were recorded by alfalfa seeds (2%) group which was the nearest value to (-ve) control group (17.80 ± 0.44) & (18.2 ± 1.3) respectively.

Results for body weight gain (BWG) recorded significant decrease of (+ve) control group (27.54 ± 3.85) when compared to (-ve) control group (37.7 ± 6.84). Rats that received diet supplemented with fenugreek seeds (5%) and fenugreek sprouts (5%) showed no significant difference ($P < 0.05$) in BWG when compared with the (+ve) control group but the other which received diet supplemented with alfalfa seeds (2%) and alfalfa sprouts (2%) showed significant increases ($P < 0.05$) in BWG compared to (+ve) control group. The best results were recorded by alfalfa sprouts (2%) group which was the nearest value to (-ve) control group (35.42 ± 4.41) & (37.72 ± 6.84) respectively.

Feed efficiency ratio (FER) recorded non-significant difference of the (+ve) control group when compared with the (-ve) control group. Rats that received diet supplemented with fenugreek sprouts (5%) and alfalfa sprouts (2%) showed significant decreases ($P < 0.05$) in FER when compared with the (-ve) control group, the best result was recorded by the group that fed on alfalfa (2%) which was the nearest value to the (-ve) control group.

These findings are in agreement with **(Ibrahim et al., 2011)** who found that lead caused decreases in rats' growth rate when fed on it. Also, agreed with **(Seddik et al., 2010)** who reported that chronic Pb exposure for 90 days was associated with a decrease in body weight gains when compared

to control rats. (*El-Nekeety et al., 2009*) indicated that animals treated with lead acetate (LA) showed a significant decrease in feed intake and consequently the body weight gain. The loss in body weight may be caused by the toxic ions associated with other factors, causing imbalance in metabolism produced by impairing zinc-dependent enzymes which are necessary for many metabolic processes. (*Hwang and Wang, 2001*).

Also, it is known that Pb induced growth depression due to reduced food consumption through the contact of lead with appetite-depressant receptors in the gastrointestinal tract (*Sharifi et al., 2002*). Some studies indicated that fenugreek seed extract supplementation may reduce the body and adipose tissue weight (*Chevassus et al., 2010; Geetha et al., 2011*). The probable mechanism may be that fenugreek flushes out the carbohydrates from the body before they enter the blood stream resulting in weight loss. Also, fenugreek seeds contain a high proportion (40 %) of soluble fiber. This fiber forms a gelatinous structure (similar to guar gum) which may have effects on slowing the digestion and absorption of food from the intestine and creates a sense of fullness in the abdomen, thus suppresses appetite and promotes weight loss (*Geetha et al., 2011*). (*Raju et al., 2001*) showed that Trigonella seed powder treatment caused a significant and consistent weight gain over the period of administration.

Table (1):
Effect of Fenugreek and Alfalfa seeds on body weight gain, feed intake and feed efficiency ratio in hepatic intoxicated rats.

Parameters	FI (g/day)	BWG*	FER
(-ve) Control	18.2 ^a ± 1.3	37.72 ^a ± 6.84	0.103 ^a ± 0.0024
(+ve) Control	15.2 ^b ± 1.09	27.54 ^d ± 3.85	0.073 ^{abc} ± 0.0033
Fenugreek seeds 5%	17.40 ^a ± 0.89	29.09 ^{bcd} ± 4.63	0.078 ^{abc} ± 0.013
Fenugreek sprouts 5%	17.20 ^a ± 0.38	28.05 ^{cd} ± 0.79	0.052 ^c ± 0.028
Alfalfa seeds 2%	17.80 ^a ± 0.44	34.64 ^{abc} ± 6.37	0.094 ^{ab} ± 0.019
Alfalfa sprouts 2%	17.60 ^a ± 0.89	35.42 ^{ab} ± 4.41	0.062 ^{bc} ± 0.029

Different superscript letters in the column denote high significant differences ($P < 0.05$). gm during the whole period.

Effect of Fenugreek and Alfalfa seeds on relative organs weight.

As shown in table (2) relative organs weight values showed significant increase in (+ve) control group as compared with (-ve) control group. Rats that received diets supplemented with fenugreek seeds (5%), fenugreek sprouts (5%), alfalfa seeds (2%) and alfalfa sprouts (2%) showed significant decrease ($P < 0.05$) in relative liver weight when compared with (+ve) control group. The best results recorded by alfalfa sprouts 2% group which was similar to (-ve) control group (2.70 ± 0.291) and (2.69 ± 0.311) respectively.

Rats that received diets supplemented with fenugreek seeds (5%), fenugreek sprouts (5%) and alfalfa sprouts (2%) showed significant decrease ($P < 0.05$) in relative heart weight when compared with (+ve) control group. but there was no-significant decrease ($P < 0.05$) in Alfalfa seeds (2%) when compared with (+ve) control group. The best result was recorded by Alfalfa sprouts (2%) group which was the nearest value to (-ve) control group (0.332 ± 0.07) & (0.29 ± 0.02) respectively.

Rats that received diets supplemented with fenugreek seeds (5%), fenugreek sprouts (5%), alfalfa seeds (2%) and alfalfa sprouts (2%) showed significant decrease ($P < 0.05$) in relative

Rasha M. Bahnasy, et al

spleen weight when compared with (+ve) control group. The best result was recorded by Fenugreek sprouts (5%) group and was the nearest value to (-ve) control group (0.35 ± 0.11) & (0.26 ± 0.03) respectively. Rats which received diets supplemented with Fenugreek seeds (5%), Fenugreek sprouts (5%), Alfalfa seeds (2%) and Alfalfa sprouts (2%) showed significant decrease ($P < 0.05$) in relative kidney weight when compared with (+ve) control group. The best result was recorded by Alfalfa sprouts (2%) group and was the nearest value to (-ve) control group (0.74 ± 0.08) & (0.64 ± 0.05) respectively.

These results are in agreement with (*Ibrahim et al., 2012*) who reported that the relative weights of the four examined organs (liver, kidneys, heart, and spleen) were affected by lead acetate ingestion. The detected elevation in the organs' weight or ratio is thought to be due to the necrosis and apoptosis and could be attributed to the accumulation of the lipids in the four organs. (*Martínez et al., 2016*) showed that, Alfalfa intake managed to reduce both liver weight and total fat content, possibly due to its phenolic compounds that may have inhibited the early stages of hepatic steatosis, increasing hepatic β -oxidation and decreasing adipogenesis or lipogenesis.

Table (2):

Effect of Fenugreek and Alfalfa seeds on relative organs weight in hepatic intoxicated rats.

Parameters Groups	Liver	Heart	Spleen	Kidneys
(-ve) Control	$2.69^c \pm 0.311$	$0.29^b \pm 0.02$	$0.26^c \pm 0.03$	$0.64^c \pm 0.05$
(+ve) Control	$4.66^a \pm 0.15$	$1.06^a \pm 0.45$	$1.19^a \pm 0.28$	$1.54^a \pm 0.37$
Fenugreek seeds 5%	$2.96^{bc} \pm 0.12$	$0.33^b \pm 0.01$	$0.68^b \pm 0.03$	$0.77^c \pm 0.13$
Fenugreek sprouts 5%	$2.69^c \pm 0.20$	$0.44^b \pm 0.19$	$0.35^c \pm 0.11$	$0.89^{bc} \pm 0.23$
Alfalfa seeds 2%	$3.06^b \pm 0.085$	$0.94^a \pm 0.37$	$0.65^b \pm 0.22$	$1.17^b \pm 0.34$
Alfalfa sprouts 2%	$2.70^c \pm 0.291$	$0.332^b \pm 0.07$	$0.40^c \pm 0.18$	$0.74^c \pm 0.08$

Different superscript letters in the column denote high significant differences ($P < 0.05$).

Effect of Fenugreek and Alfalfa seeds on serum AST, ALT and ALP for hepatic intoxicated rats.

As shown in table (3) results for serum AST, ALT and ALP recorded significant increase for the (+ve) control group when compared to (-ve) control group. All treated groups showed significant decrease ($P < 0.05$) when compared with (+ve) control group. Better results were found in alfalfa sprouts (2%) group, fenugreek seeds (5%) group and fenugreek sprouts (5%) group that recorded no significant difference compared to (-ve) control group.

Sivaprasad et al., (2003) demonstrated similar results, that lead changes the liver functions because of inducing fatty liver hence an increase of AST, ALT and ALP levels. On the other hand the findings of this work are in agreement with (*Kaviarasan and Anuradha, 2007*) who concluded the hepatoprotective effect of fenugreek seed polyphenolic extract (FPEt) against ethanol-induced hepatic injury and apoptosis in rats. Treatment with FPEt restored the levels of markers of liver injury and mitigated the alterations in alcohol metabolizing and detoxification enzymes. Also, the results are in agreement with (*Anwar et al., 2011*) who concluded that treatment with an alcoholic extract of fenugreek seeds significantly decreased serum AST, and ALT levels in diabetic liver. (*Ahmad et al., 2013*) observed that treatment of rats with low, as well as high, dose of ethanolic extract of *M. sativa* had no adverse effects on the kidney or the liver functions. According to (*Annie et al., 2006*) phytoestrogens can be used in the treatment of various liver disorders. Also the results agreed with (*Martínez et al., 2016*) who found that impaired hepatic function involves an increase in the plasma AST, ALT, γ -GT and ALP activities, which was observed in the spontaneously hypertensive rats (SHR)

model. The intake of alfalfa in these animals returned γ -GT and ALP activities to values close to those found in Wistar–Kyoto rats (WKY) animals. Similar findings were described by (Al-Dosari, 2012), who found that alfalfa prevented the increase of ALP activity in serum of CCl₄-treated rats. Moreover, the results are in agreement with (Servatyari et al., 2017) who showed that use of the *Medicago sativa* extract decreased significantly the serum levels of ALP and ALT and in the experimental groups, compared to the control group.

Table(3):
Effect of Fenugreek and Alfalfa seeds on serum AST, ALT and ALP in hepatic intoxicated rats

Parameters	AST (U/L)	ALT (U/L)	ALP (U/L)
(-ve) Control	72.20 ^c ± 3.49	83.8 ^c ± 3.96	185.6 ^d ± 4.03
(+ve) Control	149.60 ^a ± 7.70	171.40 ^a ± 7.40	241.2 ^a ± 2.58
Fenugreek seeds 5%	81.40 ^b ± 6.02	84.20 ^c ± 3.70	205.6 ^c ± 6.96
Fenugreek sprouts 5%	80.60 ^b ± 7.12	95.40 ^b ± 2.70	189.20 ^d ± 4.76
Alfalfa seeds 2%.	81.80 ^b ± 1.30	91.00 ^b ± 2.5	218.0 ^b ± 5.70
Alfalfa sprouts 2%	77.40 ^{bc} ± 3.78	90.6 ^b ± 1.67	209 ^c ± 3.93

Different superscript letters in the column denote high significant differences (P<0.05).

Effect of Fenugreek and Alfalfa seeds on total protein, albumin and globulin of hepatic intoxicated rats.

Data presented in table (4) showed total protein (g/dl) albumin and globulin recorded significant decrease for (+ve) control group when compared with the (-ve) control group. Rats received diets supplemented with Fenugreek seeds (5%), fenugreek sprouts (5%), alfalfa seeds (2%) and alfalfa sprouts showed significant increase (P<0.05) in total protein, albumin and globulin when compared with (+ve) control group, with no significant difference among all treated groups. Better results were obtained on feeding rats fenugreek seeds (5%) and alfalfa seeds (2%). (Sivaprasad et al., 2003) have demonstrated similar results, that lead caused fatty liver and changes the liver functions, that results in decreased albumin/globulin ratio. Serum total proteins were higher in rats treated with high dose of ethanolic extract of *M. sativa*, while no such increase was seen with low dose when compared with the control group (Ahmad et al., 2013). Also the results agreed with (Servatyari et al., 2017) who showed that use of alfalfa increased total protein and fibrinogen in a dose dependent manner in the experimental groups (p <0.01, p <0.001).

Table (4):
Effect of Fenugreek and Alfalfa seeds on serum proteins in hepatic intoxicated rats.

Parameters	T. Pro. (g/dl)	Alb. (g/dl)	Glob. (g/dl)
(-ve) Control	5.32 ^a ± 0.23	3.68 ^a ± 0.23	1.64 ^a ± 0.11
(+ve) Control	3.6 ^c ± 0.35	2.76 ^c ± 0.32	0.84 ^d ± 0.15
Fenugreek seeds 5%	4.48 ^b ± 0.22	3.16 ^b ± 0.20	1.32 ^b ± 0.13
Fenugreek sprouts 5%.	4.28 ^b ± 0.10	3.00 ^{bc} ± 0.12	1.28 ^b ± 0.13
Alfalfa seeds 2%.	4.28 ^b ± 0.20	3.20 ^b ± 0.22	1.08 ^c ± 0.08
Alfalfa sprouts 2%	4.34 ^b ± 0.11	3.12 ^b ± 0.14	1.22 ^{bc} ± 0.08

Different superscript letters in the column denote high significant differences (P<0.05).

Effect of Fenugreek and Alfalfa seeds on SOD and α-GST in hepatic intoxicated rats.

Data presented in table (5) showed that results for SOD (U/L) and α-GST (ng/ml) recorded significant decrease for (+ve) control group when compared to (-ve) control group. Rats received diets supplemented with fenugreek seeds(5%), fenugreek sprouts (5%), alfalfa seeds(2%) and alfalfa sprouts(2%) showed significant increase (P<0.05) when compared with (+ve) control group. The best result was recorded by Alfalfa sprouts (2%)group which was the nearest value to (-ve) control group. The results agreed with **(Sivaprasad et al., 2003)** who reported that rats treated with Lead had lowered activities for catalase, superoxide dismutase, glutathione peroxidase and reduced glutathione. Moreover **(Tripathi and Chandra, 2009)** reported that Fenugreek extract caused significant reduction in LPO and increase in SOD and GST when used in combination with *Momordicacharantia* (MC) in alloxan-induced diabetic rats. In addition, **(Sushma and Devasena, 2010)** studied the role of fenugreek in ameliorating the hepato- and nephro-toxicity induced by cypermethrin in rats. They reported that fenugreek reduce the levels of the antioxidant enzymes, such as SOD, and α-GST, which were found to be increased in the liver and kidneys. Authors postulate mechanism of action might be due to the flavonoids present in fenugreek and their ability to scavenge the free radicals induced by cypermethrin metabolism. Also, **(Sharma et al., 2011)** reported that Lead exposure induced a significant decrease (P<0.001) SOD and α - GST. **(Baquer et al., 2011)** mentioned that the fenugreek administration protected the liver and kidney of diabetic rats from alteration of levels of antioxidant enzymes (SOD, GPx, CAT) and attributed by the fact that fenugreek husk is a valuable and effective source of natural antioxidants and natural ingredients.

Findings are in agreement with **(El Kader et al., 2012)** who found that the treatment with LA significantly decreased the activities of antioxidant enzymes for SOD content as compared to the control animals (P<0.05). The result of the present study found that sprouting for fenugreek or alfalfa were more effective than seeds. This result agreed with **(Moradi kor et al., 2013)** who noted that germination or sprouting of fenugreek seeds increased their antioxidant profile.

The findings in this study are in agreement with **(Shi et al., 2014)** who concluded that the SOD activity in serum, liver, spleen, and muscle increased linearly. Liver SOD activity were affected by treatment of alfalfa saponin extract. Also, the results agreed with **(Martínez et al., 2016)** who found a decreased activity of both SOD and GPx in the liver of SHR, whereas the dietary administration of alfalfa was an efficient strategy to return GPx activity back to values even higher than those of normal animals. This potential has been attributed to different polyphenols and polysaccharides found in alfalfa **(Al-Dosari, 2012; Wang et al., 2014)** as well as to uronic acid which is capable of inhibiting the formation of hydroxyl and superoxide radicals **(Chen et al., 2004)**.

**Table (5):
Effect of Fenugreek and Alfalfa seeds on SOD and α-GST in hepatic intoxicated rats.**

Groups	Parameters	SOD (U/L)	α-GST(ng/ml)
(-ve) Control		0.305 ^a ± 0.0005	0.457 ^a ± 0.003
(+ve) Control		0.087 ^f ± 0.003	0.108 ^f ± 0.001
Fenugreek seeds 5%		0.113 ^e ± 0.0025	0.128 ^g ± 0.001
Fenugreek sprouts5%.		0.176 ^d ± 0.003	0.195 ^d ± 0.002
Alfalfa seeds 2%.		0.213 ^c ± 0.001	0.262 ^c ± 0.002
Alfalfa sprouts 2%		0.229 ^b ± 0.0005	0.305 ^b ± 0.001

Different superscript letters in the column denote high significant differences (P<0.05).

Effect of Fenugreek and Alfalfa seeds on MAO and LEAD in hepatic intoxicated rats

Data presented in table (6) showed that serum MAO (mu/ml) recorded significant decrease for (+ve) control group when compared with the (-ve) control group. Rats that received diets supplemented with Fenugreek seeds (5%), Fenugreek sprouts(5%), Alfalfa seeds (2%) and Alfalfa sprouts(2%) showed significant increase (P<0.05) when compared with the (+ve) control group. The best result was recorded by Alfalfa sprouts (2%) group which was the nearest value to (-ve) control group.

Results for lead (mcq/l) recorded significant increase for (+ve) control group when compared to the (-ve) control group. Rats that received diets supplemented with Fenugreek seeds(5%), Fenugreek sprouts (5%), Alfalfa seeds(2%) and Alfalfa sprouts (2%)showed significant decrease (P<0.05) when compared with (+ve) control group. The best result was found in Alfalfa sprouts (2%)group which was the nearest value to (-ve) control group. (*Haleagrahara et al., 2011*) showed a significant increase in blood and bone marrow lead levels following the consumption of lead acetate in drinking water for 21 days. Lead acetate absorbed from the gastrointestinal tract is carried via blood, mainly in the erythrocytes, to the bone where it accumulates.Also,the results agreed with(*El Kader et al., 2012*) where Lead concentration was significantly (P<0.01) increased in rats treated with LA alone for 7 days comparing with the control rats. (*Devi et al., 2005*) found that on giving young and adult rats Pb in water, they reposted the activity of the enzyme MAO and other catecholamine in the brain, they concluded Pb- exposure perturbs the aminergic system in the cerebral cortex, cerebellum and hippocampus and may contribute to the cognitive and behavioral impairments observed in Pb-exposed rats.

Table(6):

Effect of Fenugreek and Alfalfa seeds on MAO and Lead in hepatic intoxicated rats

Groups	Parameters	MAO (mu/ml)	Lead(mcq/l)
(-ve) Control		0.29 ^a ± 0.001	70.97 ^f ± 0.44
(+ve) Control		0.118 ^f ± 0.001	211.37 ^a ± 3.2
Fenugreek seeds 5%		0.12 ^b ± 0.001	171.17 ^b ± 4.1
Fenugreek sprouts5%.		0.185 ^d ± 0.001	122.74 ^d ± 0.48
Alfalfa seeds 2%.		0.237 ^c ± 0.001	133.78 ^c ± 0.28
Alfalfa sprouts 2%		0.261 ^b ± 0.001	111.54 ^e ± 1.1

Different superscript letters in the column denote high significant differences (P<0.05).

Conclusion

According to these findings It could be concluded that Fenugreek seeds, Fenugreek sprouts, Alfalfa seeds and Alfalfa sprouts are considered as a potential source of natural antioxidant with hepatoprotective activity. The hepatoprotective effect of seeds and their sprouts of the above mentioned may be attributed to phenolic compounds, which is known for their antioxidant activity. Further detailed investigations on these seeds are needed in order to identify and isolate the hepatoprotective components in these seeds and to justify their use in the treatment of liver disorders. The authors recommend the public education to increase awareness of the potential toxic effects of lead pollution .

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التأثير الوقائي لبذور وبراعم الحلبة والبرسيم الحجازي على الجرذان المعرضة للتسمم بالرصاص

رشا محمد بهنسي ، ايمان السيد عبد الهادي ، مي حسين عبد الفتاح

قسم التغذية وعلوم الأطعمة- كلية الاقتصاد المنزلي-جامعة الأزهر

المستخلص العربي

أجريت هذه الدراسة لبحث تأثير المعالجة ببذور الحلبة، بذور الحلبة المنبته، بذور البرسيم الحجازي وبذور البرسيم الحجازي المنبت على الجرذان المصابة بالتسمم الكبدى المحدث بأسيتات الرصاص. وقد تم استخدام 36 جرذ ذكر بالغ ألبينو من سلالة (*Sprague Dawley*) تبلغ أوزانهم (10 ± 200 جرام) حيث تم تقسيمهم عشوائى الي 6 مجموعات متساوية تركت احداها كمجموعة ضابطة سالبة في حين تم إعطاء المجموعات الأخرى يوميا طوال فترة التجربة (ثمانى أسابيع) أسيتات الرصاص بتركيز 0.4% في مياه الشرب المقطرة لإحداث الإصابة بالتسمم الكبدى وتم الاحتفاظ بمجموعة ضابطة موجبة تتناول الغذاء الأساسى فقط في نفس الوقت أعطيت باقى المجموعات بذور الحلبة 5% وبذور الحلبة المنبته 5% وبذور البرسيم الحجازى 2% وبذور البرسيم الحجازى المنبت 2% كل على حدة في صورة مسحوق يدعم به الغذاء لمدة 8 أسابيع مع الاحتفاظ بالمجموعة السادسة كمجموعة ضابطة موجبة تتناول الغذاء الأساسى فقط وفي نهاية التجربة تم حساب المآخوذ الغذائى (FI) ومعدل الزيادة المكتسبة في وزن الجسم (BWG) ومعدل كفاءة الغذاء (FER) والوزن النسبى لبعض الأعضاء (الكبد والكلى والطحال والقلب) كما تم تقدير البروتين الكلى والألبومين والجلوبيولين وبعض انزيمات الكبد (AST,ALT,ALP) ومستوى انزيم MAO في مصل الدم ومستوى الرصاص في الدم والانزيمات المضادة للأكسدة (SOD,α-GST) في انسجة الكبد. وقد أشارت النتائج بأن الجرذان المعاملة بأسيتات الرصاص سجلت تغيرات كبيرة في مستويات المؤشرات البيولوجية والبيوكيميائية المذكورة أعلاه. ومن ناحية أخرى أدت المعالجة بالبذور والبذور المنبته الي تحسن كبير في نشاط انزيمات الكبد والانزيمات المضادة للأكسدة ومستوى انزيم MAO ومستوى الرصاص. ويمكن أن نستنتج أن البذور المختبرة كان لها تأثير فعال في معالجة الخلل الوظيفى للكبد الناجم عن اسيتات الرصاص وتأثيراته الأخرى وربما يرجع ذلك لمحتواها المرتفع من بعض المركبات الفينولية.

الكلمات المفتاحية سمية الكبد –انزيمات الكبد – أسيتات الرصاص – بذور الحلبة – بذور الحلبة المنبته – بذور البرسيم الحجازى – بذور البرسيم الحجازى المنبته –الانزيمات المضادة للأكسدة.