The Protective Effect of Chickpea and Lupine variety on rats with fatty liver

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ABSTRACT:

Effect of Chickpea *(Cicer arietinum L.)* and lupin *(Lupinus spp.)* on fatty liver, (56) rats were divided into two main groups. The first group (7 rats) fed on basal diet, as a control negative. The second group (49 rats) were randomly assigned to seven equal subgroups (1) fed on high fat diet (HFD) containing (carbohydrate 55.6% sucrose 30%, starch 21.6%, fructose 4%, Fat 27.5%, protein 8.2%, cellulose 4.2%, salt mixture 3.5% and vitamin mixture 1%) and used as a control positive group (+ve). (2) fed on (HFD) + 10% imported chickpea (3) fed on (HFD) + 10% balady chickpea. (4) fed on (HFD) + 10% sweet lupin (5) fed on (HFD) + 10% bitter lupin, (6) fed on (HFD) + 5% imported chickpea + 5% sweet lupin (7) fed on (HFD) + 5% balady chickpea + 5% bitter lupin. The results indicated that, treating rats with (HFD + 5% balady chickpea and 5% bitter lupin and the group fed on (HFD) + 10% bitter lupin led to significant decrease in body weight gain %, organs weight%, lipid profile including (cholesterol, triglycerides, LDL-c and VLDL-c), glucose, leptin and liver enzymes. while these treatments induced significant increase in feed intake, HDL-c and antioxidant enzymes. These results agreement with histopathological examination of liver from group (7) Showing no histopathological changes.

Key words: balady chickpea, imported chickpea, bitter lupin , sweet lupin, lipid profile, liver enzymes.

Introduction:

Chickpea (*Cicer arietinum* L.) is rich of high amount of polyunsaturated fatty acids (PUFA) (67%) **(Jukanti et al., 2012).** Specifically, it contains linoleic and oleic acids that are known with their anti-fatty liver effects by inhibition of fat deposition in hepatocytes **(Daley et al., 2010),** Additionally, *C. arietinum* is cholesterol free **(Jukanti et al., 2012)** and have several medicinal properties. In traditionally system of medicine, chickpea seeds were used as tonic, stimulant, aphrodisiac, anthelminthic, appetizer and for relieving burning sensation in stomach **(Zia et al., 2007).** In Chinese herbal medicine In Chinese herbal medicine, chickpea seeds were reported to have been employed for treating hypertension and diabetes mellitus for over the past 2500 years **(Li et al., 2008).**

Chickpeas have also been widely used to treat and prevent hypertension, hyperlipidemia, diabetes, itchy skin, flatulence, low libido, tumor formation and osteoporosis **(Liu et al.,1986).**

Lupins (*Lupinus spp.)* (sweet and bitter lupin) from the family of legume **(Yu et al., 2019**) and one of the Fabaceae family **(Citerne, Toby Pennington & Cronk, 2006)** and her native is Mediterranean area **(Kurzbaum, Safori, Monir, & Simsolo**, **2008).** Quinolizidine and piperidine alkaloids in lupin seeds have confer bitterness and toxicity to the alkaloid-rich lupin varieties (**Green, Welch, Panter & Lee, 2013)**. Many studies showed that lupin cause many useful health benefits, especially in the area of high blood sugar prevention **(Magni et al., 2004),** hypertension control, and cholesterol reduction **(Sirtori, Arnoldi &Johnson, 2004; Bettzieche et al., 2008)** and when consumption of lupin kernel fiber beneficially alter CVD risk factors cause of the high content of its water-soluble fiber and its high bile acid binding ability **(Belski, 2012)**. Because the lupin seeds contain antioxidants, **(Zielinska et al., 2008 and Martinez-Villalunga et al., 2009)** it effective to reduce cancer.

Fatty liver disease (FLD), is characterized by a progressive pattern of intrahepatic fat accumulation that can lead to hepatocellular lipotoxicity this disease affects about 1.80 billion people **(Younossi et al., 2016). (Alberti et al., 2005)** decided that hypertension, insulin resistance, obesity and dyslipidemia occur during fatty liver.

High-fat diets (HFD) increased body weight, body fat and liver fat levels which associated with insulin resistance **(Samuel et al., 2004).** On the other hand, **(Anstee and Goldin 2006**) reported that, feeding mouse models on HFD

does not produce liver fibrosis and only mild steatosis. While **(Kien, 2009 and Tanaka et al., 2008)** reported that, high intake of dietary fat which contain an increase the intake of saturated fatty acids associated with liver inflammation and fatty liver disease. Therefore, the present study aimed to evaluate the protective effect of diets containing chickpea and lupin on fatty liver disease in rats.

Material and Methods

Materials:

1- Casein, vitamins, minerals, cellulose and choline chloride obtained from El-Gomhorya Company, Cairo, Egypt.

2- balady, imported chickpeas *(Cicer arietinum L.*) And bitter, sweet lupin *(Lupinus spp)* obtained from in Agricultural research center, Giza, Egypt.

3- Starch obtained from local market, Cairo, Egypt.

4- Kits for biochemical analysis obtained from Alkan for pharmaceutical and chemical Dokki, Egypt.

Animals:

Male albino rat Sprague Dawley strain weighting 200 ± 10g purchased from Helwan farm of experimental animals, Ministry of Health and Population, Helwan, Cairo, Egypt.

Methods:

Chemical analysis:

Moisture, total protein, total lipid, carbohydrate and fiber were analyzed in chickpeas, lupins chemically according to **A.O.A.C,(2000).**

Preparation of dried sweet and bitter lupin:

Sweet and Bitter lupins was soaked in water for the later at a lupin. Water ratio of 1:10 (w/v). The soaking time were applied this variation of soaking time between bitter and sweet lupin is related to the level of bitter alkaloids. Thus, sweet lupin seeds, which contain a low level of alkaloids don t need a lot of time of soaking). After soaking the water was dried off and the seeds were dried at 50° C for 12 hr. in a hot air oven **Embaby, (2010)**

Preparation of dried chickpea.

The chickpea was coarsely ground to separate the outer crust by sieving through sieve of 200-300 pore size. The ingredients were mixed in a room with defused light and diet pellets were made and oven dried at a very low heat. These pellets were then stored in plastic containers with airtight fitted lids in a cool and dark place until further use **Ebrahim, (2018).**

Determination of total phenolic content:-

The total phenolic content (TPC) of chickpea and lipin was determined using the Folin-Ciocalteu's method in **Bhalodia et al., (2011).** The unknown sample concentration was determined in terms of mg GAE/g.

- Determination of DPPH activity:-

The antioxidant activity of extracts was calculated in terms of DPPH (2,2-diphenyl-1-picryl-hydrazin-hydrate) activity using the method of **Yamaguchi et al., (1998).**

Experimental Animals:

Fifty six male Albino rats of (Sprague – Dawley strain), weighting (195 ±10 g) each were used in this study. All rats were fed the control diet for 4 consecutive days for adaptation.

Stander diet: consists of casein (14%), corn oil (4%), choline chloride (0.25%), and vitamin mixture (1%), cellulose (5%) and the remainder is corn starch according to **Reeves et al, (1993),** salt mixture which used in these study according to **Hegsted et al., (1941)** and vitamin mixture prepared according to **A.O.A.C. (1975).**

After adaptation period, the rats divided into two main groups as follows: The first main group: (7 rats) fed on stander diet, as a control negative group. The second subgroup (49 rats) fed on high fat diet used to induce fatty liver disease according to **(Zarghani et al., 2016, Mohamed at al., 2020)**

Subgroup (1): fed on high fat diet (HFD) as a control positive group (+ve). Subgroup (2): fed on (HFD) + 10% imported chickpea. Subgroup (3) fed on (HFD) + 10% balady chickpea. Subgroup (4) fed on (HFD), + 10% sweet lupin. Subgroup (5) fed on (HFD) + 10% bitter lupin, Subgroup (6) fed on (HFD) + 5% imported chickpea + 5% sweet lupin Subgroup (7) fed on (HFD) + 5% balady chickpea + 5% bitter lupin. During the experimental period (8weeks), the diets consumed and body weights were recorded twice weekly, feed intake (FI), body weight gain (BWG %) and feed efficiency ratio (FER) were calculated according to **Chapman et al., (1959**). At the end of the experiment, the animals were fasted overnight, and then the rats were

anaesthetized and sacrificed. Liver, kidney and peritoneal fat pad were separated from each rat and weighted to calculate the liver, kidney and peritoneal fat pad / body weight % according to **Li et al., (2021).** Blood samples were collected. Blood samples were centrifuged and the serum was separated to estimate some biochemical parameters, i.e. cholesterol **Allain et al., (1974),** triglycerides **Foster and Dumns,(1973**), high density lipoprotein HDL-c **Lopes-Virella et al., (1977**), low density lipoprotein LDL-c and VLDL-c estimated according to **FriedWald et al., (1972**), serum leptin hormone according to **Guillaume and Bjorntorp (1996)**, glucose **Trinder, (1959**), Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) by **Reitman and Frankel, (1957),** alkaline phosphatase (ALP) by **Belfield and Goldberg (1971).** In the liver tissue homogenate, catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH) activities were measured according to the methods described by **(Aebi, 1984; Beauchamp and Fridovich 1971 and Ellman 1959**), respectively.

Histopathological study: Livers of the scarified rats removed, washed with normal saline and put in 10% formalin solution. The tissue specimens were cleared in xylene, embedded in paraffin, stained with Hematoxylen and Eosin (H and E) and then studied under an electronic microscope according to **Carleton (1979).**

Statistical analysis: Results are expressed as mean values with their standard deviation of the mean. Statistical differences between groups were evaluated using one-way ANOVA according to **Snedecor and Cochran (1986).**

Results and Discussion:

Chemical composition of chickpea and lupin.

Presented in Table (1). The data showed that proximate analysis of balady and imported chickpea. Balady chickpea had higher protein, fiber and fat content compared to imported. Imported chickpea on the other hand had higher ash content and moisture compared to balady chickpea. Both of balady and imported chickpea are rich on carbohydrate. This results are agree with several studied that reported chickpea is rich in carbohydrates; however, the chickpea variety can have up to 59% of carbohydrates **(Hamid and Kalsoom, 2017)** The protein ranges from 22-30% in both the varieties. This result is agree with **(Zia-Ul-Haq et al., 2007)** study whom reported that the level of protein can be vary depending on their subtype. Phenolic components were much higher in balady samples than imported. Isoflavones are diphenolic secondary metabolites that may lower the incidence of heart disease due to the inhibition of LDL-C oxidation, the inhibition of proliferation of aortic smooth muscle cells and the maintenance of the physical properties of arterial walls **(Panter et al. 2001).**

sweet and bitter lupine were chemically analyzed total protein recorded the largest percentage in sweet lupine (38.1%), While the lowest percentage in the bitter lupin (33.9%), While fat was found in a bitter lupine largest proportion, but lowest rate of fat present in sweet lupin. The highest ratio in fiber was bitter lupin was 45%, followed by sweet lupin 41%,phenolics analysis were high amount in bitter lupin than sweet lupin.

Table (1): Chemical composition of chickpea and lupin (g/100g Dry weight).

Samples / Nutrients		chickpea		lupin	
		balady	imported	bitter	sweet
Moisture%		$5.75^{c}\pm0.41$	$5.91^{c}\pm0.08$	$6.1^{c}\pm0.06$	$5.2^{c}\pm0.32$
Protein%		$27.31^{b}\pm0.44$	$23.41^{b}\pm0.43$	$33.9^{a}\pm0.31$	$38.1^{a}\pm0.32$
Fat%		$6.21^{c}\pm0.32$	$6.71^{c}\pm0.34$	$8.3^{c}\pm0.35$	$7.1^{c}\pm0.31$
Ash%		$2.54^{c}\pm0.51$	$2.81^{c}\pm0.45$	$3.8^{c}\pm0.43$	$3.1^{c}\pm0.34$
Fiber%		$11.10^{c}\pm0.23$	$7.38^{c}\pm0.22$	$10.1^{c}\pm0.24$	$9.6^{c}\pm0.25$
Carbohydrate%		$47.09^{a}\pm0.33$	$53.78^{a}\pm0.32$	$37.8^{a}\pm0.36$	$36.9^{a}\pm0.38$
phenolics analysis (mg/g)	total phenolics (mg GAE/g)	$52^{a}\pm0.04$	$33^{a}\pm0.06$	$55^{a}\pm0.08$	$34^{a}\pm0.07$
	Antioxidant activity (DPPH)	$22.1^{a}\pm0.16$	$14.1^{a}\pm0.18$	$20.8^{a}\pm0.17$	$15.7^{a}\pm0.13$

Each value is expressed as the mean± SD (n=3)

protective effect of balady, imported chickpea and bitter, sweet lupin on feed intake and body weight gain% of fatty liver disease in rats.

Presented in table (2). The mean value of feed intake (g/day/each rat) decreased in the control (+ve) (fatty liver disease FLD group) which fed on high fat diet HFD as compared to healthy rats fed on basal diet.

diets containing 10% balady chickpea, 10% sweet lupin and 5% sweet lupin +5% imported chickpea led to significant increase $P\leq0.05$ in the mean values of feed intake, as compared to the positive control groups. The highest increase in the mean value of feed intake recorded for diet with 5% balady chickpea and 5% bitter lupin, this treatment showed non-significant changes in feed intake, as compared to the negative control group.

Body weight gain % (BWG%) in (control +ve) increased significantly p≤ 0.05, as compared to healthy rats fed on basal diet. the mean value of BWG% decreased significantly p≤ 0.05, as compared to +ve. diets containing10% bitter lupin, 10% balady chickpea, 5% balady chickpea+5% bitter lupin and which provided the diet showed significant decrease in BWG%, as compared to the positive control group on the other hand, all treated groups showed significant decrease p≤0.05 in BWG%, except groups which treated with 10% imported chickpea and 10% sweet lupin which compared to the positive control group.

Table (2): protective effect of chickpea and lupin on feed intake and body weight gain% of fatty liver disease in rats.

Parameters / Groups		Feed intake (g/day/each rat)	BWG%	Feed efficiency ratio (FER)
Control (-ve)		18.900 ^{a}± 0.467	18.499 ^{f}± 0.405	2.38^{b}±0.641
Control (+ve)		15.988 ^{f}± 0.498	28.962 ^{a}± 0.838	4.25 ^{a}±0.731
HFD +	10% imported chickpea	17.198 $^{d\ e}$± 0.362	25.846 ^{b}± 0.793	3.37$^{ab\pm}$0.543
	10% balady chickpea	17.894 $^{b\ c}$± 0.513	21.975 ^{b} ± 0.862	2.85$^{b\pm}$0.671
	10% sweet lupin	17.786 $^{c\ d}$± 0.598	24.578 ^{c} ± 1.039	3.09^{ab}±0.561
	10% bitter lupin	17.903 $^{c\ d}$± 0.363	19.295 ^{de} ± 0.703	2.68^{b}±0.541
	5% imported chickpea and 5 % sweet lupin	17.574 $^{b\ c}$± 0.497	19.589 ^{d} ± 0.959	2.81^{b}±0.438
	5% balady chickpea and5% bitter lupin	18.505 $^{a\ b}$± 0.513	18.497 ^{e} ± 0.434	2.54^{b}±0.429

Least significant differences at P≤0.05.

Means with the same letter are insignificantly difference.

In this respect, some researchers reported that, an excess of body fat tissue may be related not only to energy intake and energy expenditure in humans **(Flatt,1995)** and **(Prentice, 1998)**, but also to the type of diet, especially high-fat diets (HFD), which may lead to various metabolic alterations such as hyperphagia in humans **(Westerterp et al., 2008)**, reduced lipolytic activity in fat tissue, reduction in leptin secretion and/or sensitivity, hypothalamic neuron apoptosis **(Moraes et al., 2009),** impairment of mitochondrial metabolism **(Pomplun et al., 2007),** insulin resistance, and obesity **(Wajchenberg, 2000)**. On the other side, ecological studies between populations tend to show a positive relationship between fat and obesity, especially if populations with low fat intakes **(Seidell, 1998).** In addition to, extremely low fat, high carbohydrate diets are also very effective for weight loss **(Ornish et al., 1990).**

Dietary fibers play an important role in body weight regulation, through both hunger suppression and diminished nutrient absorption **(Henness and Perry 2006)**. This result was within opinion whom reported that,chickpea high in fiber, low in energy density and glycemic load, and moderate in protein are thought to be particularly important for weight control **(Albete et al., 2010) (Luis et al., 2017).**

Gorecka et al. (2000) reported that, lupin seeds consumption did not significantly affect body weight and body mass index BMI ($P > 0.05$) The hull constitutes considerable part of the lupin seeds (20%) with a high content of dietary fibre and other valuable source of health promoting ingredients especially antioxidants.

protective effect of diets containing chickpea and lupin on some organs weight/body weight% and peritoneal fat pad/body weight % of fatty liver disease in rats presented in table (3).

The data presented in this table revealed that, the mean values of liver, kidney and peritoneal fat pad/body weight % increased significantly in the control (+ve) group as compared to the negative control group fed on basal diet. All treated groups showed significant decrease in the mean value of liver, kidney and peritoneal fat pad/body weight %, as compared to the control groups. The highest improvement in decreasing these organs and PFP recorded for the group treated with the combination of (balady chickpea and bitter lupin), followed by the group treated with (10% bitter lupin).

Table (3): protective effect of chickpea and lupin on organs and PFP on fatty liver disease in rats.

Parameters / Groups		Organs weight %		PFP%
		Liver	Kidney	
Control (-ve)		2.693 ^{e}± 0.058	0.479 ^{g}± 0.024	0.932 ^{g}± 0.089
Control(HFD) (+ve)		3.375 ^{a}± 0.058	0.798 ^{a}± 0.024	2.113 ^{a}± 0.056
HFD+	10% imported chickpea.	3.078 ^{c}±0.026	0.645 ^{c}± 0.015	1.714 ^{c}± 0.056
	10% balady chickpea.	3.012 $^{c\ d}$± 0.027	0.587 ^{d}± 0.023	1.516 $^{e\ f}$± 0.013
	10% sweet lupin.	3.071 ^{c}± 0.082	0.610 ^{d}± 0.030	1.612 $^{c\ d}$± 0.082
	10% bitter lupin.	2.976 ^{d}± 0.026	0.558 $^{e\ f}$± 0.032	1.493 $^{e\ f}$± 0.045
	5% imported chickpea and 5 % sweet lupin.	2.996 ^{d}± 0.026	0.568 $^{d\ e}$± 0.023	1.551 $^{d\ e}$± 0.031
	5% balady chickpea and5% bitter lupin.	2.940 ^{d}± 0.020	0.520 ^{f}± 0.023	1.424 ^{f}± 0.069

PFP: peritoneal fat pad Least significant differences at P≤0.05. Means with the same letter are insignificantly difference.at P≤0.05

Protective effect of diets containing chickpea, lupin on serum lipid profile on fatty liver disease in rats presented in tables (4a),(4b).

Total cholesterol, triglycerides, LDL-c and VLDL-c increased significantly p≤ 0.05, while HDL-c decreased in (control +ve), as compared to normal rats (control –ve group) fed on basal diet.

Diets with 10% (chickpea, lupin and their combination) caused significant decrease p≤0.05 in serum cholesterol, triglycerides, LDL-c and VLDL-c, while HDL-c recorded significant increase, as compared to the positive control groups.

Table (4a): protective effect of chickpea and lupin on serum lipid profile of fatty liver disease in rats.

Parameters / Groups		Ch.	Trig.
Control (-ve)		85.156 ^{g}± 3.231	52.502 ^{g}± 2.134
Control (+ve)		176.119 ^{a}± 4.286	99.360 ^{a}± 4.017
HFD+	10% imported chickpea.	141.743 ^{c}± 2.170	78.045 ^{c}± 1.986
	10% balady chickpea.	133.754 ^{d}± 2.216	73.713 ^{d}± 2.141
	10% sweet lupin.	134.431 ^{d}± 2.148	70.414 ^{e}± 1.515
	10% bitter lupin.	125.114 ^{e}± 1.467	68.645 ^{e}± 1.944
	5% imported chickpea and 5 % sweet lupin.	128.430 ^{e}± 2.525	64.517 ^{f}± 0.947
	5% balady chickpea and 5% bitter lupin.	116.413 ^{f}± 2.150	62.310 ^{f}± 2.593

Least significant differences at P≤0.05. Means with the same letter are insignificantly difference.

The results in these tables revealed that, lipid profile in the groups which treated with balady chickpea and bitter lupin recorded more improvement, than that of imported chickpea and sweet lupin groups. On the other hand, the best results in lipid profile recorded for group fed on containing 5% balady chickpea 5%+ biteer lupin, followed by the group treated 10% bitter lupin and the group which treated with 5% imported chickpea + 5% sweet lupin respectively.

(**Harisa and Alanazi, 2015)** studied that the beneficial roles of lupineus luteus and lifestyle changes in management of metabolic syndrome and found that administration of lupine with lifestyle changes is good intervention for prevention and treatment of metabolic syndrome. Also, it has been shown that dietary propionate reduce total plasma cholesterol**.**

(Koo, 1983) reported that saponins had been shown to prevent atherosclerosis in experimental animals. (**El Shewey, 2000)** indicated that rats receiving germinated lupine had significantly lowering levels of total cholesterol. It has been reported that lupin upregulates LDL receptors, and down regulates cholesterol biosynthesis genes (**Fontanari et al., 2012)**. The

same author added that lupine interferes with cholesterol enterohepatic circulation and decreases the accumulation of fat in the liver.

(Ebrahim, 2018) show that two types of chickpea had significantly (P<0.05) lower TG, cholesterol, LDL, VLDL and LDL levels. It may be due to both types of fiber had high amount of fiber. In general, increased consumption of soluble fiber from foods results in reduced serum total cholesterol and LDL-cholesterol (LDL-C) and has an inverse correlation with CHD mortality **(James et al., 2003)**.

Table (4b): protective effect of chickpea and lupin on serum lipid profile of fatty liver disease in rats.

Parameters / Groups		HDL-c	LDL-c	VLDL-c
Control (-ve)		53.467 ^{a}± 1.501	22.048^{h}± 1.997	11.310 ^{g}± 0.516
Control (+ve)		25.220 ^{g}± 2.647	130.02^{a}± 2.591	20.173 ^{a}± 0.411
HFD+	10% imported chickpea.	36.991 ^{e}± 2.293	89.081 ^{c}± 1.254	16.620 ^{c}± 0.299
	10% balady chickpea.	40.594 ^{d}± 1.676	78.743^{d}± 1.782	14.796 ^{d}± 0.988
	10% sweet lupin.	40.890 ^{cd}± 1.718	79.193^{d}± 1.659	13.838 ^{e}± 0.316
	10% bitter lupin.	43.497 ^{cd}± 2.184	67.192 ^{f}± 1.362	13.512 ^{e}± 0.312
	5% imported chickpea and 5 % sweet lupin.	44.492 ^{bc}± 1.865	70.219 ^{e}± 2.180	12.715 ^{f}± 0.197
	5% balady chickpea and 5% bitter lupin.	46.488 ^{b}± 2.216	56.653 ^{g}± 1.518	12.280 ^{f}± 0.518

Least significant differences at P≤0.05. Means with the same letter are insignificantly difference.

Protective effect of diets containing chickpea, lupin and on liver enzymes on fatty liver disease in rats presented in table (5).

Data in this Table showed that, feeding FLD rats on HFD increased the mean values of serum AST, ALT and ALP significantly, as compared to healthy rats fed on basal diet. Results in the present study indicated that, treating groups with HFD containing chickpea, lupin, combination of them improved the parameters of liver enzymes (AST, ALT and ALP), as compared to the group which treated with HFD only. The highest improvement in these

parameters recorded for the group which treated with the combination of 5% balady chickpea+5% bitter lupin , followed by the groups which treated with 10% bitter lupin

Consumption of high caloric diet led to lipid accumulation **(Asai and Miyazawa, 2001)**, increased the production of inflammatory cytokines, and the progression of liver disease **(Wei et al., 2007)**. On the other hand, **(Cao et al., 2010)** showed that HFD could induce the hyperlipidemia in rats, and hyperlipidemia could alter the related marker enzyme profiles in serum and liver tissue and progress to liver cirrhosis.

(Bouchoucha et al, 2016) this study showed a significant decrease in plasma AST and ALT activities after lupine supplementation. Likewise, **(harisa and Elanazi, 2015)** concluded to the beneficial effect of Lupinus luteus on plasma AST and ALT activities in individuals with metabolic syndrome. A decrease in the activity of these enzymes was also observed following Lupinus albus treatment in diabetic or hypercholesterolemic rats **(Neelakantan et al, 2014 ; Osman et al, 2011 ; El-Dakak et al, 2013) .**

Table (5): protective effect of chickpea and lupin on liver enzymes of fatty liver disease in rats.

Parameters / Groups		AST	ALT	ALP
		U/l		
Control (-ve)		72.102 ^{g}± 1.731	23.435 ^{g}± 1.264	96.168 ^{g}± 3.178
Control (+ve)		127.294 ^{a}± 4.284	79.894 ^{a}± 3.296	269.119 ^{a}± 6.267
HFD+	10% imported chickpea.	91.672 ^{c}± 2.215	59.980 ^{c}± 2.998	227.011 ^{c}± 1.511
	10% balady chickpea.	86.856 ^{d}± 2.135	55.979 ^{d}± 2.998	216.447 ^{d}± 3.118
	10% sweet lupin.	88.197 $^{c\,d}$± 2.171	54.313 ^{d}± 2.458	218.492 ^{d}± 2.512
	10% bitter lupin.	82.372 ^{e}± 2.511	49.017 ^{e}± 3.516	204.216 ^{e}± 2.957
	5% imported chickpea and 5 % sweet lupin.	83.442 ^{e}± 2.141	49.656 ^{e}± 2.538	206.323 ^{e}± 3.418
	5% balady chickpea and 5% bitter lupin.	78.124 ^{f}± 2.403	44.382 ^{f}± 3.395	196.391 ^{f}± 3.649

Least significant differences at P≤0.05. Means with the same letter are insignificantly difference.

protective effect of diets containing chickpea and lupin on serum leptin and glucose of fatty liver disease in rats presented in fig (1,2).

Leptin and serum glucose increased in group fed on HFD, as compared

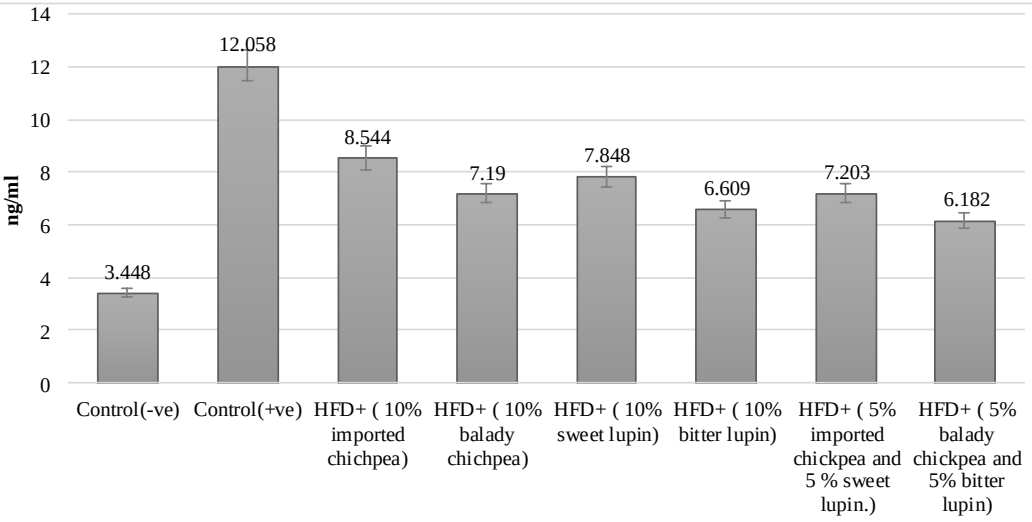


Fig (1) protective effect of chickpea and lupin on serum leptin on fatty liver disease in rats.

with healthy rats. diet with 10% chickpea or 10% lupin and their combination in the diet rats showed significant decrease $p \leq 0.05$ in leptin hormone and serum glucose, as compared to the positive control group, The best results in leptin hormone and serum glucose recorded for group which treated with the combination of balady chickpea and bitter lupin, followed by the group treated with bitter lupin, which provided the diet with 10%.

Our results are in agreement with **Fernandez-Riejos et al., (2010)** who reported that increased fat mass and larger adipocytes lead to elevated circulating leptin concentration. On the other hand, **Bluher and Mantozoros, (2004)** also reported that, human obesity is associated with increased level of leptin.

(Abdelrahman et al., 2017) reported that, diabetic rats fed on a diet supplemented with bitter lupin (5%, 10%) decreased the levels of blood glucose compared to the diabetic control. lupin contain high amount of monounsaturated fatty acid, the previous studies have suggested that a high monounsaturated fat diet improves glycemic control by exerting protective effect against β-cell death and augmenting insulin sensitivity.). Study suggests that both types of the chickpeas have a blood glucose lowering effect in the diabetic rats. It may be due to Chickpeas have an glycemic index of 10, which is significantly lower than other beans, including black beans, navy beans,

soybeans and lentils **(Hamid and Kalsoom, 2017)**.This result was within with **Tiwari et al. (2013)** study showed that chickpea is beneficial in diabetic rats.

Data in fig (3) showed protective effect of diets containing chickpea and lupin on the antioxidant enzymes on fatty liver disease in rats

The mean values of GSH, SOD and CAT of FLD group which was fed on HFD (control +ve) decreased significantly $p \leq 0.05$, as compared to the negative control group.

Diets containing chickpea, lupin, and combination of their led to significant increase in the antioxidant enzymes (GSH, SOD and CAT), as compared to the positive control groups (control -ve).

Bitter lupin showed more effect in increase the antioxidant enzymes, than that of imported chickpea, the best results in these enzymes recorded for the group treated

with combination of 5% balady chickpea and 5% bitter lupin together which provided the diet, followed by the groups fed on diet containing bitter lupin, which provided the diet with 10% and the combination of 5% imported chickpea and 5 % sweet lupin. respectively.

antioxidants are generally required for food, biological, and pharmaceutical systems. Plant constituents with antioxidant activity and free radical scavenging effects are regarded as the safe source for these antioxidants, as it was reported that synthetic antioxidants have shown adverse effects including mutagenic, carcinogenic, and toxic effects **(Kalın et al.,**

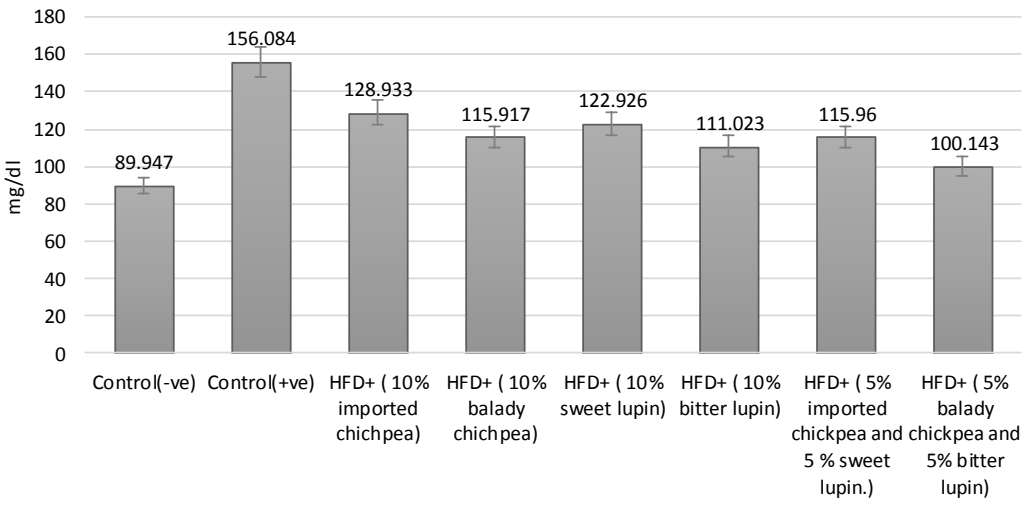


Fig (2) protective effect of chickpea and lupin on serum glucose on fatty liver disease in rats.

2015). Phenolic compounds, which can be obtained from Chickpea *(Cicer arietinum*), Isoflavones are diphenolic secondary metabolites that may lower the incidence of heart disease due to the inhibition of LDL-C oxidation, the inhibition of proliferation of aortic smooth muscle cells and the maintenance of the physical properties of arterial walls **(Panter et al. 2001).**

This study showed that in vitro antioxidant properties of row Chickpea and Cooked Chickpea. The cooking process significantly decreased ($p < 0.05$) the antioxidant activity of chickpea. Evaluated with ORAC, it decreased 48%, with the hydroxyl radical 32%, and with the superoxide radical 39%. Total phenolic compounds were reduced by 25%. This is due to their thermolability, water solubility, and hydrolyzability**(Wang et al .,2010).**

Reham ,et al.,20١٦ showed effect og chickpea in vivo antioxidant activity was determined via the assay of liver endogenous antioxidants viz. non enzymatic (GSH) and enzymatic (CAT and SOD), and MDA as a marker of lipid peroxidation. The administration of CCl4 significantly decreased the levels of endogenous antioxidants GSH by 63.6%, CAT by 65.5%, and SOD by 62.2% with the significant increase of hepatic level of MDA by 392.8% or four folds **(Breikaa et al., 2013; Akther et al., 2014)** The oral administration of chickpea 'Giza 1' extract (CA250 and CA500) significantly restored the levels of them nearly to values of the control group indicating in vivo antioxidant activity This complies with the data reported **by Sri Ramachandra et al. (2014)** on aerial parts of chickpea extract Consequently, the antioxidant and hepatoprotective activity could be associated with the phenolics present chickpea according to **Kinjo et al. (2006)**.

Osman, et al, 2011. Displayed The erythrocytes superoxide dismutase (SOD), plasma glutathione reductase (GR) and glutathione-Stransferase (GST) were significantly ($P<0.05$) inhibited (24.51%, 24.49% and 24.25%, respectively) in hypercholesterolemic rats (HC). A slight inhibition in the activities of erythrocytes glutathione peroxidase (GPx) and plasma catalase (CAT) was observed in the hypercholesterolemic group (HC) compared to the normal control group (NC). Hypercholesterolemiainduced diets supplemented with bitter lupin seeds improved the activities of these enzymes. However, no significant changes were observed for sweet lupin seed supplements. The effect of bitter lupin seeds in this context was even better since the levels of enzymes were brought almost too nearly that of the normal control (NC). Bitter lupin seed supplements stimulated SOD, GR and GST activities by 20.68%, 23.36% and 18.84%, respectively.

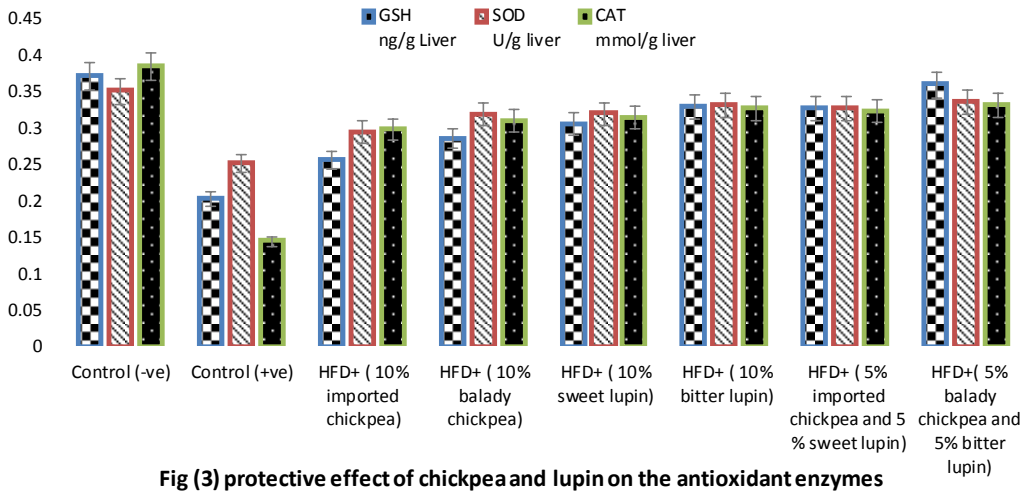


Fig (3) protective effect of chickpea and lupin on the antioxidant enzymes in liver tissue fatty liver disease rats.

Histopathological examination:

Liver of the negative control rats fed on basal diet revealed normal histological photo of hepatic lobule which consists of central vein surrounded by normal hepatocytes as shown in (photo. 1). Examination of liver of positive control showed of cytoplasmic vacuolization of hepatocytes as shown in photo (2), liver of rats from group (2:HFD+ 10% imported chickpea) Showing focal necrosis of hepatocytes as shown in photo (3), liver of rats from group (3:HFD+ 10% balady chickpea) Showing cytoplasmic vacuolization of hepatocytes as shown in photo (4), liver of rats from group (4: HFD+ 10% sweet lupin) showing kupffer cells activation and marked fibroplasia in the wall of bile duct as shown in photo (5),): liver of rats from group (5: HFD+ 10 %bitter lupin)Showing no histopathological changes as shown in photo(6), liver of rats from group (6: HFD+5%imported chickpea and 5% sweet lupin) showing cytoplasmic vacuolation of hepatocytes as shown in photo(7),): liver of rats from group (7 :HFD+ 5% balady chickpea and 5% bitter lupin) Showing no histopathological changes as shown in photo (8).These data agreement with (**El-tahan and Hanan, 2017).**

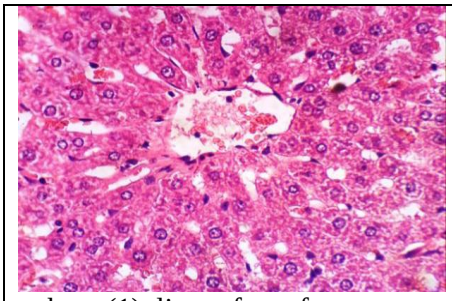


photo.(1): liver of rats from group ve- Showing the normal histological structure of hepatic lobule (H&E×400).

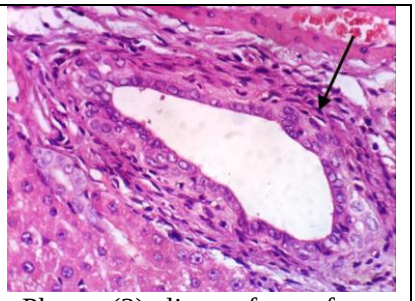


Photo. (2): liver of rats from group ve+ showing cytoplasmic vacuolization of hepatocytes (H&E×400).

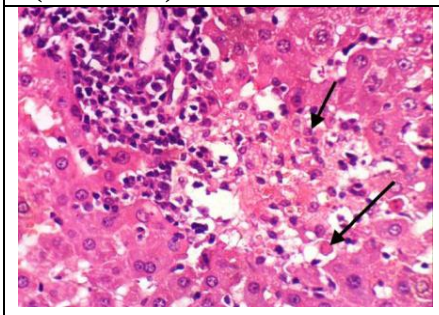


photo.(3): liver of rats from group 2 Showing focal necrosis of hepatocytes (H&E×400)

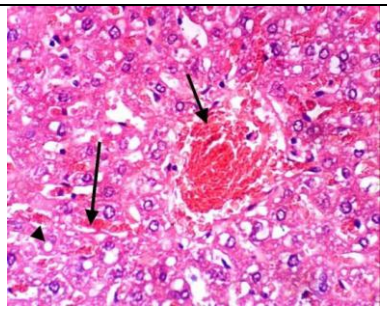


photo.(4): liver of rats from group 3 Showing cytoplasmic vacuolization of hepatocytes (H&E×400).

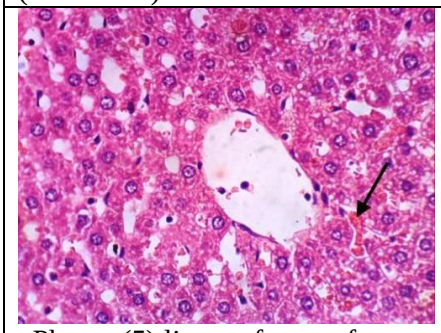


Photo (5):liver of rats from group 4showing kupffer cells activation and marked fibroplasia in the wall of bile duct (H&E×400).

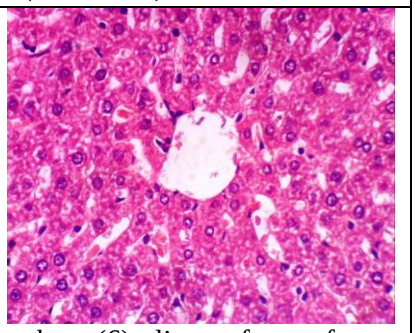
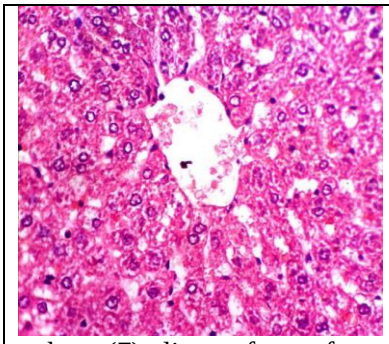
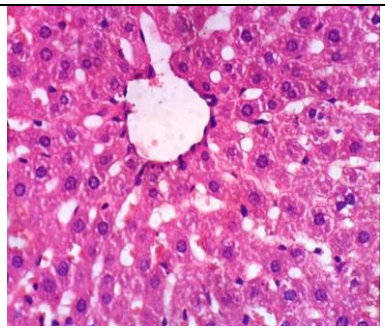


photo.(6): liver of rats from group 5
Showing no histopathological changes (H&E×400).

 photo.(7): liver of rats from group 6 showing cytoplasmic vacuolation of hepatocytes (H&E×400).	 photo(.8): liver of rats from group 7 Showing no histopathological changes(H&E×400).

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التأثير الوقائى للحمص والترمس بنوعيه على الفئران المصابة بالكبد الدهني

ايرينى ولسن نجيب

قسم الاقتصاد المنزلى – كلية التربية النوعية – جامعة عين شمس

المستخلص العربى

لمعرفة التأثير الوقائى للحمص والترمس علي مرض الكبد الدهني للفئران، تم استخدام عدد ٥٦ من ذكور ألالبينو البالغة تم تقسيم الفئران إلى مجموعتين رئيسيتين. المجموعة الأولى (7 فئران) تغذت على الغذائي الاساسي كمجموعة ضابطة سالبة. المجموعة الثانية (٤٩ فأرا) تم تقسيم الفئران بشكل عشوائي الى سبع مجموعات فرعية متساوية (١) تم تغذيتها على غذاء عالى الدهن (٥٥.٦% كربوهيدرات، ٢١.٦% نشا، ٣٠% سكروز، ٤% فركتوز، ٢٧.٥% دهون، ٨.٢% بروتين، ٤.٢% سيليلوز، ٣.٥١% املاح معدنية، ١% فيتامينات) كمجموعة ضابطة موجبة، (٢) تم تغذيتها علي غذاء عالى الدهن+ ١٠% حمص مستورد. (٣) تم تغذيتها علي غذاء عالى الدهن+ ١٠%حمص بلدى. (٤) تم تغذيتها علي غذاء عالى الدهن+ ١٠% ترمس حلو. (٥) تم تغذيتها علي غذاء عالى الدهن+ ١٠% ترمس مر (٦) تم تغذيتها علي غذاءعالى الدهن + (٥% حمص مستورد+ ٥% ترمس حلو) (٧) تم تغذيتها علي غذاء عالى الدهن،+(٥% حمص بلدى+ ٥% ترمس مر).اظهرت النتائج ان المجموعة الفرعية (٧) و (٥) أدت إلى حدوث تحسن في الوزن والنسبة المئوية لدهون الاحشاء الداخلية، اللبتين ، الجلوكوز ، صورة الدهن وأنزيمات الكبد ومضادات الاكسدة وتتفق هذه النتائج مع نتائج الفحص الهستوباثولوجى للكبد.

الكلمات المفتاحية: حمص بلدى،حمص مستورد،ترمس مر، ترمس حلو، صورة الدهون، انزيمات الكبد.