

The Protective effect of water extract of fenugreek seeds, chicory and olive leaves on some biochemical parameters

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Abstract

Modern lifestyle causes a rise in consumption of fast foods which contain high calories. Fast foods contain high fats and carbohydrates with low protein; thus induce malnutrition status. This investigation aimed to study effectiveness of natural products against side effect of malnutrition. Water extracts of fenugreek seeds (raw, germinated and green leaves), chicory and olive leaves were chosen in the present study. Sprague-Dawley male rats (42 rats) were divided to 7 groups. Group 1 fed on basal diet as negative control. Groups 2 to 7 were fed on diet contained 20% vegetable oil and 10% protein as high fat diet (HFD) for 2 weeks before being treated by water extracts. Then, G2 fed on HFD during whole experimental period. Groups 3 to 7 were treated daily (2ml/rats/day) by prepared water extracts of fenugreek seeds (raw, germinated and green leaves), chicory and olive leaves for 8 weeks. At the end of experimental period, blood samples were collected. Rats were weighted, killed and organs removed, washed and weighted, too. Histopathological sections of the organs were examined. The results explained that, water extract of chicory had high levels of minerals and total flavonoids. Water extracts of fenugreek seeds improved serum glucose more than chicory and olive leaves. Water extract of olive leaves had the highest decrease of TG, LDL-C and as well as improvement of the liver functions. While, water extract of raw fenugreek seeds had the greatest improvement for TC, IA and urea. Concerning histopathological study, slides of the aorta and heart showed no histopathological changes for rats treated by prepared water extracts. Rats treated by water extracts of fenugreek seeds (raw, germinated and green leaves) showed a normal histopathological structure. Conclusion: water extracts of fenugreek seeds (raw, germinated and green leaves), chicory and olive leaves could improve serum lipid content and liver functions of malnourished rats.

Key words: fenugreek seeds - germinated fenugreek – green fenugreek leaves – chicory – olive leaves – blood lipid – liver and kidney functions – rats.

Introduction

People in developing countries depend on food rich in carbohydrates and fat with a little content of plant protein which can lead to malnutrition and obesity. The **world Health Organization** (WHO, 1998) who described obesity as an epidemic hazard worldwide, based on data analysis of body

mass index (BMI). Since that, obesity incidence increased at an alarming rate and is becoming major public health concern (**Pepkin, 2009**). Also, obesity facilitates the development of metabolic disorders such as diabetes, hypertension and cardiovascular disease (**Singlaet al., 2010**). **Prasad et al., (2016)** reported that vegetable oils increased the total cholesterol and decreased the antioxidant status. Also, all vegetable oils (coconut oils, olive oil, palm oil, sunflower oil and vanaspat oil) studied by the authors caused a decline of antioxidant status associated with observed increase in lipid peroxidation. The same authors found the coconut oil comparatively better. But, the presence of unsaturated fats (olive oil) in food meals had a good beneficial effect on blood lipid (**Al-Idrisset al., 2020**). Dietary fats intake and blood lipids contents are evidence for cardio vascular disease (CVD) as reported by (**Keys et al., 1950**).

Sánchez – Gutierrez et al., (2021) concluded that, olive leaves are considered as olive industry by-products. Whereas; it constitute a natural resource of valuable compounds. Olive leaves could be used in economy cycle, thus taking care of sustainability, environmental and socioeconomic issues in pursuit of the so-called bio-economy. The same authors reported that, olive leaves had a huge – value potential, mainly attributed to its content in phenolic compounds with demonstrated antioxidant and antimicrobial activity. Many studies reported that, an olive leaf has potentially positive effects on the parameters related with diabetes and cardiovascular diseases (**Nilüfer and Duygu, 2020**). Olive fruits and by products represent valuable sources for nutritional products with health benefits (**Rotondiet al., 2010**). In ancient Egypt, olive leaves were first used to treat several diseases such as fever, cough and cystitis (**Shain and Bilgin, 1998**).

Chicory (*cichoriunIntybus*) roots and leaves contain considerable amounts of phytochemicals and are good source of antioxidants. Leaves contain a higher amount of phytochemicals, antioxidants and antibacterial compounds than roots. **Kaur et al, (2016)** suggested that, chicory leaves would play an important role in antioxidant defense system against Endogenous free radicals and hence improve the human health. Chicory is a feasible source of biological elements (k, Fe, Ca), vitamins and bioactive compounds such as; inulin, sesquiterpene lactones, coumarin, cicharic acid, phenolic acids which exert potent pro- health effects on the human organism (**Janda et al, 2021**).

Fenugreek seeds (*TrigonellaFoenumgraecum*) are a good source of gum, fiber, alkaloid and saponin. Fenugreek could be used as food stabilizer, adhesive and emulsifying agent to change texture for some special purposes as results to high fiber content. Many studies suggest that fenugreek could be regarded, as antidiabetic, anticarcinogenic and remedy for hypocholestorlemia and hypoglycemia (**Khorshidianet al 2016**). According to (**Hefnawy and Ramadan 2011**) they mentioned, many food application of fenugreek such as emulsifier, prebiotic effect and thickening agents. Moreover, soaking and germination of fenugreek seeds caused a change in bioactive components and antioxidant activity. Whereas; soaked and germinated fenugreek seeds were increased about 13% and 55% more than raw fenugreek seed. Same authors concluded that germinated fenugreek was more health potential than raw fenugreek seeds (**Ojhaet al., 2018**).

This study aimed to investigate the protective effect of water extracts of chicory, olive leaves and fenugreek (raw seeds, germinated and green fenugreek) as available and cheap natural plants against induced obesity, blood lipids, kidney and liver functions.

Materials and methods

Materials:

Chicory and raw fenugreek seeds were purchased from local market in Giza, Egypt. Olive leaves was obtained from Horticulture Research Institute, Agricultural Research Centre, Giza, Egypt. Rats were purchased from the Laboratory Animal Department, Food Technology Research, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

Chemicals:

Folin-Ciocalteu phenol reagent (2N), Quercetin dihydrate (2-(3,4- dihydroxyphenyl)), and Gallic acid were purchased from Sigma–Aldrich (St. Louis, MO, USA). The kits were purchased from Gamma-Tread Company, Cairo, Egypt.

Methods:

Plant and Water Extract Preparation:

Ten grams of each plant was added to boiling tap water in a stainless-steel pot (10 %, W/V). Boiling continued for 10 minutes. Then, the pot was left to cool at room temperature. Then, it sieved through cotton cloths to superannuated solution according to (**Salem and Hassan, 2009**).

Antioxidants Contents:

Total phenols were determined by folin-ciocalteu's reagent as described by **Arnouset al., (2001)**. The total flavonoid content was determined by aluminum chloride method according to (**Chang et al., 2002**). Antioxidant activity was determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method according to **Brand-Williams et al., (1995)**. Azinobis-(3-ethylbenzothiazoline- 6- sulfonic acid (ABTS) assay was estimated as according to the method described by **Olszowy and Dawidowicz, (2018)**.

Determination of minerals:

For determination of (iron (Fe), zinc (Zn), calcium (Ca), potassium (K), sodium (Na) and chromium (Cr) in samples were digested by using microwave digestion system (Multiwave Go Plus) and determined by using microwave plasma Atomic Emission Spectroscopy (MP-AES)(model 4210, Agilent) as according to **A.O.A.C (2019)**.

Biological Study:

Animals

Forty two adult male Sprague-Dawley rats aged 4 weeks old (175.00±3 g) were obtained from the Laboratory Animal Department, Food Technology Research and housed in plastic cages and fed on basal diet according to (**Reeves et al., 1993**), and water was provided ad libitum for one week for adaptation period. The conditional animal room temperature was maintained at 21°C ± 2°C with timed lighting 12h and relative air humidity of 40% to 60%. Then, after the adaptation period rats were randomly divided into 7 groups. Group 1 (6 rats) continued on basal diet as negative control, groups 2 to 7 were fed on high fat diet (HFD) which is basal diet with modification to raise fat to 20% and decrease protein to 10% to induce malnutrition status as shown in (table 1).

Experimental Design:

Firstly, groups from G2 to G7 were fed on high fat diet (HFD) for 2 weeks. Then, the rats were treated by water extract of plants under study for 8 weeks (table 1). Blood samples were collected at the end of experimental period from eye plexuses into a dry clean centrifuge glass tube to coagulate and

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obtain serum. Blood samples were left cold for 15 minutes. Then, the tubes were centrifuged for 15 min at 3000 rpm. Finally, the collected supernatant serum was kept frozen at -20°C until analysis. Also, at end of the experimental period rats were weighed and anesthetized using ether and collection of tissue specimen were performed for further histological examination.

Table (1):
Experimental diets (g/100 g diet):

Ingredients	G1	G2	G3*	G4*	G5*	G6*	G7*
Corn starch	57.8	49.8	49.8	49.8	49.8	49.8	49.8
Protein	12	10	10	10	10	10	10
Sugar	10	10	10	10	10	10	10
Vegetables oil	10	20	20	20	20	20	20
Salt mixture	4	4	4	4	4	4	4
Vitamin mixture	1	1	1	1	1	1	1
Choline chloride	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Cellules	5	5	5	5	5	5	5
Water extract of raw fenugreek seeds	-	-	2ml	-	-	-	-
Water extract of germinated of fenugreek seeds	-	-	-	2ml	-	-	-
Water extract of green fenugreek leaves	-	-	-	-	2ml	-	-
Water extract of chicory	-	-	-	-	-	2ml	-
Water extract olive leaves	-	-	-	-	-	-	2ml

*2ml/rat/day.

Biochemical Analysis:

Serum glucose was determined according to **Barham and Trinder, (1972)**. Total cholesterol (TC) was determined according to **(Rifai, et al., 1999)**. Triglycerides (TG) were determined according to **Bucolo and David (1973)**. High-density lipoproteins (HDL) were analyzed according to **Assmann, (1979)**. Urea and BUN were determined according to **Tomas L. (1998a)**. Creatinine was determined according to **Tomas L. (1998b)**. uric acid was determined according to **Tietz (1990)**. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were estimated according to **Moss and Henderson (1999)**. Low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL) were stimulated according to **Lee and Nieman, (1996)**. Atherogenic index (AI) and coronary risk index (CRI) were calculated as according to **Adeneye, (2010)**.

Histopathological Examination:

Tissue specimens were collected from aorta, pulmonary blood vessels, heart, liver and kidney and preserved in 10% neutral buffered formalin, dehydrated in different grades of alcohol, cleared in xylene, embedded in paraffin, sectioned with microtome at 5µ thickness and finally stained with hematoxylin and eosin (H&E) and masson's trichrome (MTC) according to **Banchroft et. al., (1996)**.

On other hand tissue section of blood vessels were dewaxed in xylene, rehydrated and pretreated with 3 % hydrogen peroxide for blocking the activity of endogenous peroxidase. microwave assisted antigen retrieval was done for 20 minutes and sections were incubated overnight at 40 C with primary antibody for vascular endothelial growth factor (VEGF) (catalog MA1- 166629, Thermo

Scientific Co.,UK) was diluted with phosphate buffer saline (PBS)(1:50) then washed with PBS and incubated with biotinylated mouse secondary antibody (Cat No.32230,Thermo Scientific Co.,UK) and finally conjugated with streptavidin-peroxidase .sections were washed with PBS and incubated with diaminobenzidine (DAB) for 5 minutes and counterstained with Mayer`s hematoxylin .

Statistical Analysis:

Statistical analyses were carried out by SPSS19 program. Data were expressed as means. The Statistical analysis was performed using one-way analysis of variance followed by Duncan`s tests as outlined by *Snedecor and Cochran(1980)*.

Results

Some phytochemical and minerals in prepared water extracts:

Results in table (2) explained that, some phytochemical and minerals contents in prepared water extracts of fenugreek seeds (raw, germinated and green leaves), chicory and olive leaves. Water extract of chicory showed the greatest contents of total flavonoids and minerals. While, water extract of raw fenugreek seeds had a high level of total phenols and water extract of germinated fenugreek had a high Cr content.

Table (2):

Some phytochemical and minerals contents for water extracts of chicory, olive leaves and fenugreek (raw, germinated and green leaves).

Items	Water extracts of				
	Fenugreek seeds			Chicory	Olive leaves
	Raw	Germinated	Green leaves		
T. phenol (mg/100g)*	362.17 ^a	26.75 ^e	82.40 ^c	61.82 ^d	136.71 ^b
T. flavonoids (mg/100g)*	948.05 ^b	506.86 ^c	398.16 ^d	989.13 ^a	221.66 ^e
DPPH (%)	82.44 ^b	67.42 ^d	93.64 ^a	74.40 ^c	80.62 ^b
ABTS (%)	63.86 ^b	68.57 ^a	63.30 ^b	48.81 ^c	71.48 ^a
Some of minerals					
Cr (µg/100g)	25.50 ^b	75.30 ^a	49.10 ^{ab}	25.10 ^b	24.70 ^b
Fe (mg/100g)	6.16 ^b	4.84 ^c	2.58 ^d	17.05 ^a	2.58 ^d
Zn(mg/100g)	0.31 ^b	1.03 ^b	0.29 ^c	2.29 ^a	0.89 ^c
Ca(mg/100g)	42.99 ^e	51.23 ^d	96.25 ^c	188.54 ^a	139.23 ^b
Na(mg/100g)	25.80 ^d	17.20 ^e	88.40 ^b	429.86 ^a	56.80 ^c
K(mg/100g)	63.49 ^c	39.73 ^d	670.33 ^b	686.27 ^a	8.42 ^e

* Total phenols as Gallic acid and total flavonoids as Quercetin.

** Each value in a row followed by the same letter are not significantly different at (p ≤0.05).

On the other hands, the highest percentage of antioxidants activity found in water extract of green fenugreek leaves (93.64%) as determined by DPPH. While, it was found in water extract of olive leaves (71.48%) as determined by ABTS.

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The results in tables (3) showed the effect of fed HFD with treated by water extracts of chicory, olive leaves and fenugreek (raw, germinated and green leaves) on body weight gain (BWG). Generally, all rats fed on HFD with treated by water extracts showed decrease in BWG compared with the positive control. The rats fed on HFD and treated by water extract of olive leaves had the lowest increase of BWG (29.08%) and decreased about (37.78%) when compared to positive control. On the other hand, the rats fed on HFD with treated fenugreek resulted in decrease in BWG. While, water extract of raw fenugreek seed had a low BWG (34.9%) compared either germinated or green leaves of fenugreek (41.93 and 39.3%) respectively.

Effect of rats feeding on HFD treated by prepared water extracts on body weight and weight of organs:

The same results in table (3) showed that, water extracts of chicory and olive leaves caused low liver and kidney weight compared fenugreek groups. Also, rats treated by water extract of olive leaves had the lowest heart weight.

Table (3):

Effect of feeding rats on HFD treated by water extracts of chicory, olive leaves and fenugreek (raw, germinated and green leaves) on body weight and weight of organs.

Groups	Body weight changes				Weight of organs (gm)		
	Initial	Final	BWG (g)	BWG (%)	Liver	Kidney	Heart
Control (-)	175.00 ^a	242.00 ^{abc}	67	38.29	6.74 ^{ab}	1.06 ^d	0.78 ^b
Control (+)	174.60 ^a	256.66 ^a	82.06	47.00	7.06 ^a	1.18 ^{bc}	0.98 ^{ab}
Water extract of raw fenugreek seeds	175.20 ^a	236.33 ^{bc}	61.13	34.90	7.44 ^a	1.27 ^{ab}	0.96 ^{ab}
Water extract of germinated fenugreek seeds	175.20 ^a	248.66 ^{ab}	73.46	41.93	7.12 ^a	1.34 ^a	1.01 ^a
Water extract of green fenugreek leaves	175.40 ^a	244.33 ^{abc}	68.93	39.30	6.82 ^{ab}	1.28 ^{ab}	1.15 ^a
Water extract of chicory	175.60 ^a	226.66 ^c	51.06	29.08	5.87 ^c	1.07 ^d	1.05 ^a
Water extract of olive leaves	175.20 ^a	236.66 ^{bc}	61.46	35.08	6.35 ^{bc}	1.09 ^{cd}	0.80 ^b

* Each value in a column followed by the same letter are not significantly different at ($p \leq 0.05$)

Effect of feeding rats on HFD treated by prepared water extracts on serum glucose:

Fig (1) showed the effect of feeding rats on HFD and treated by water extracts of plants under study on serum glucose content. Generally, all water extracts under study caused to decrease in glucose level. Treating by fenugreek both raw and green leaves caused decrease in serum glucose more than chicory and olive leaves.

Effect of feeding rats on HFD treated by prepared water extracts on serum lipid profiles (table 4):

The results in table (4) showed that, the effect of feeding rats on HFD with treated by water extracts of chicory, olives leaves and fenugreek (raw, germinated and green leaves) on serum lipid profiles. Generally, all water extracts in this study improved serum lipid profiles. The rats fed on HFD treated by water extract of raw fenugreek seeds had the highest decrease for TC and LDL-C (39.26%

and 54.62%) compared to positive control. While, rats fed on HFD treated by water extract of olive leaves improved TG and VLDL – C (39. 56% and 41.10%, respectively) compared to control (+). Rats fed on HFD treated by water extract of green fenugreek leaves caused decrease of TG and VLDL- C more than both raw and germinated seeds. There are high significantly differences between groups for all lipid profiles.

Table (4):

Lipid profiles for rats fed on HFD and treated by water extracts of chicory, olive leaves and fenugreek (raw, germinated and green leaves).

Groups	TG (mg/dl)	TC (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	Risk factor	IA
Control (-)	104.00 ^a	105.33 ^b	30.66 ^a	53.73 ^{bc}	20.93 ^a	3.43 ^b	1.83 ^c
Control (+)	108.66 ^a	114.67 ^a	30.33 ^a	62.60 ^a	21.73 ^a	3.50 ^b	1.65 ^b
Water extract of raw fenugreek seeds	86.00 ^b	69.65 ^d	24.00 ^c	28.40 ^d	17.27 ^b	2.92 ^c	1.06 ^c
Water extract of germinated fenugreek seeds	104.33 ^a	106.00 ^b	29.00 ^{ab}	56.13 ^{ab}	20.87 ^a	3.66 ^{ab}	1.93 ^b
Water extract of fenugreek leaves	80.00 ^b	105.33 ^b	26.67 ^{bc}	62.67 ^a	16.00 ^b	3.96 ^a	2.78 ^a
Water extract of chicory	88.33 ^b	106.32 ^b	30.65 ^a	58.00 ^{ab}	17.66 ^b	3.47 ^b	2.02 ^b
Water extract of olive leaves	65.67 ^c	90.66 ^c	30.00 ^a	47.87 ^c	12.80 ^c	3.02 ^c	1.46 ^{bc}

* Each value in a column followed by the same letter are not significantly different at (p ≤0.05). **CRI; coronary risk index ***AI; athearogenic index

Effect of feeding rats on HFD and treated by prepared water extracts on liver and kidney functions(table 5):

Treating rats by water extract of raw fenugreek seed improved serum urea level . While, treating rats by water extract of chicory showed a decrease in serum urea content compared to rats in positive control.

Concerning of serum creatinine, the results in the table (5) showed that, rats fed on HFD with treated by water extract of green fenugreek leaves had the highest decrease of creatinine content compared to positive control (25.76%). There are high significant differences between groups for creatinine. Meanwhile, didn't show any significant differences between groups for uric acid.

Results in the same table (5) showed the effect of feeding on HFD and treated by water extracts of chicory olive leaves and fenugreek (raw, germinated and green leaves) on liver functions. Generally, the results showed water extracts of olive leaves improved liver functions, Thus, it decreased ALT and AST about (13.71% and 35.28%) compared to rats in positive control group.

Table (5):

Liver and kidney functions for rats fed on HFD with treated by water extracts of chicory, olive leaves and fenugreek (raw, germinated and green leaves).

Groups	Urea (mg/dl)	BUN* (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)	ALT (U/L)**	AST (U/L)**
Control (-)	28.67 ^c	13.39 ^c	0.70 ^{ab}	2.13 ^a	14.33 ^d	62.33 ^b
Control (+)	37.33 ^{ab}	17.43 ^{ab}	0.66 ^{ab}	2.13 ^a	19.33 ^{bc}	96.33 ^a
Water extract of raw fenugreek seeds	22.33 ^d	10.43 ^d	0.69 ^{ab}	2.17 ^a	18.00 ^{cd}	92.67 ^a
Water extract of germinated fenugreek seeds	32.66 ^{bc}	15.26 ^{bc}	0.64 ^b	2.17 ^a	22.67 ^b	95.00 ^a
Water extract of green fenugreek leaves	30.33 ^c	14.17 ^c	0.49 ^c	2.27 ^a	26.33 ^a	71.30 ^b
Water extract of chicory	29.33 ^c	13.70 ^c	0.74 ^a	2.17 ^a	17.33 ^{cd}	98.67 ^a
Water extract of olive leaves	39.00 ^a	18.21 ^a	0.69 ^{ab}	2.37 ^a	16.68 ^{cd}	62.34 ^b

* BUN; Urea nitrogen: To convert the result from urea to urea nitrogen multiply the result by 0.467

** ALT; Alanine aminotransferase, AST; Aspartate aminotransferase.

*** Each value in a column followed by the same letter is not significantly different at ($p \leq 0.05$).

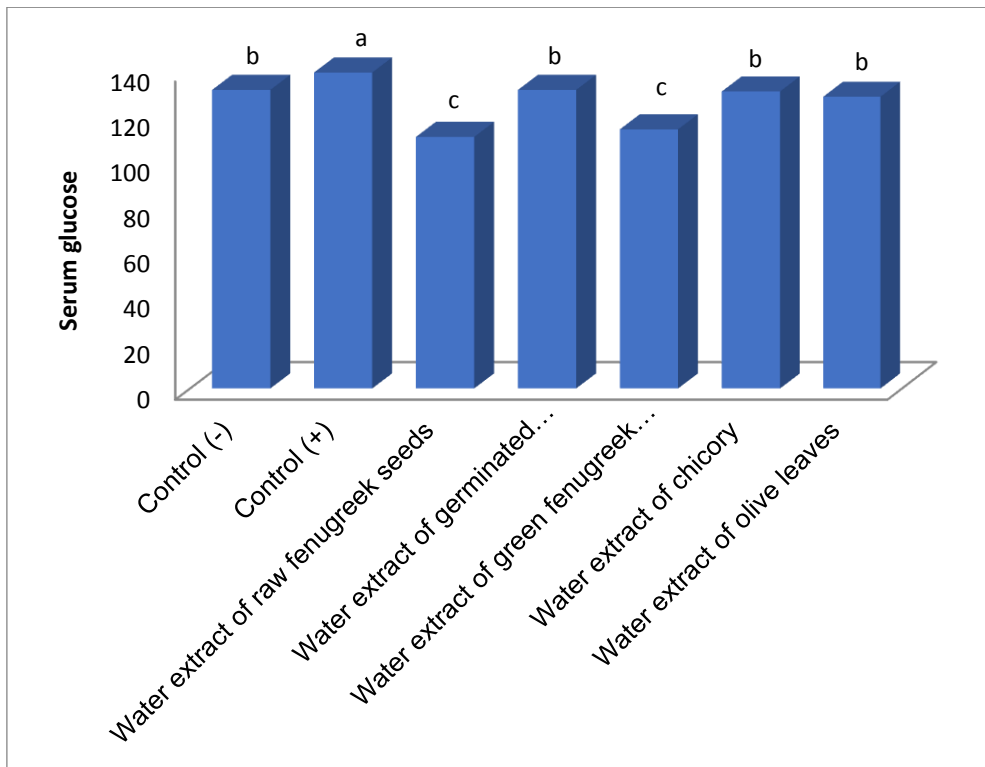


Fig (1).:

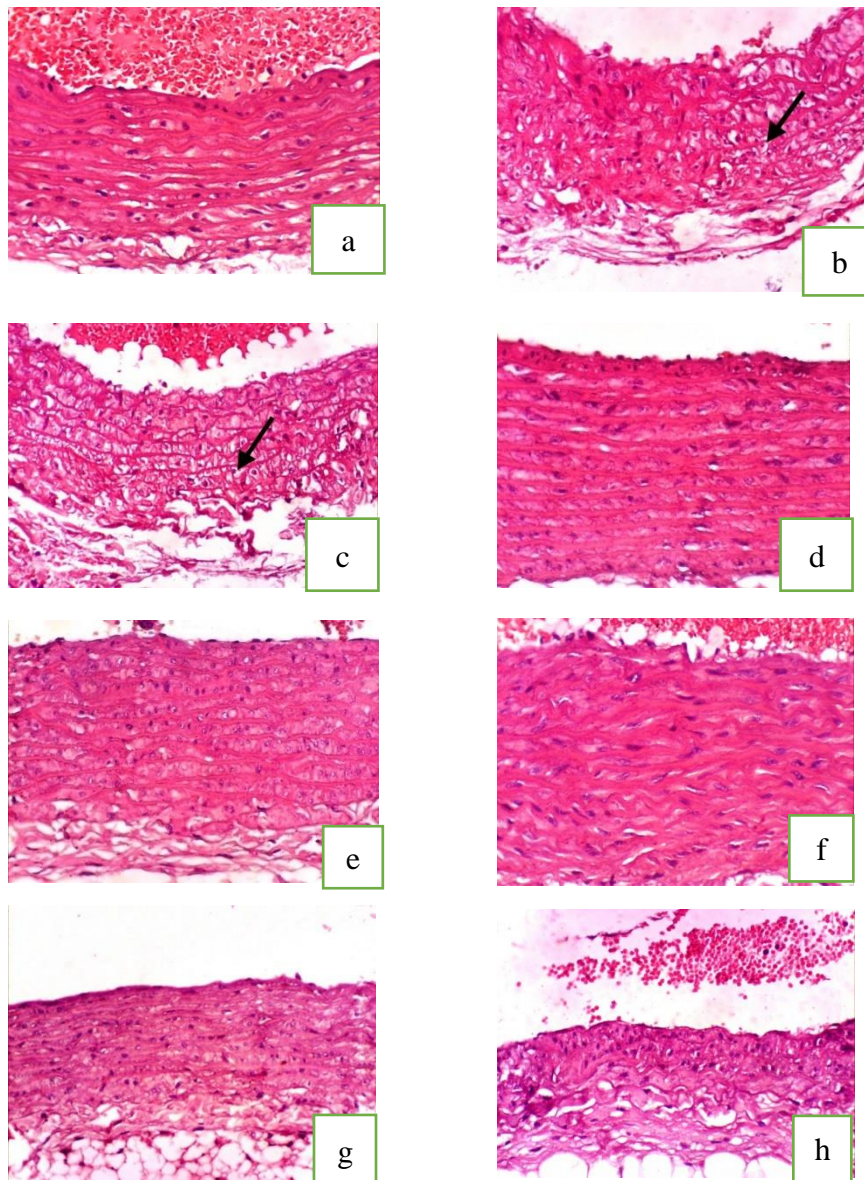


Fig (2):

Aorta of rats from groups under study: a) aorta of rats in negative control showing no histopathological changes (H&E); b & c) aorta of rats which fed on HFD as positive control showing vacuolation of the tunica media; d) aorta of rats which fed on HFD with 2ml/rat/day of water extract of raw fenugreek seeds showing no histopathological changes (H&E); e) aorta of rats which fed on HFD with 2ml/rat/day of water extract of germinated fenugreek seeds showing no histopathological changes (H&E); f) aorta of rats which fed on HFD with 2ml/rat/day of water extract of green leaves of fenugreek showing no histopathological changes (H&E); g) aorta of rats which fed on HFD with 2ml/rat/day of water extract of chicory showing no histopathological changes (H&E); and h) aorta of rats which fed on HFD with 2ml/rat/day of water extract of olive leaves showing no histopathological changes (H&E).

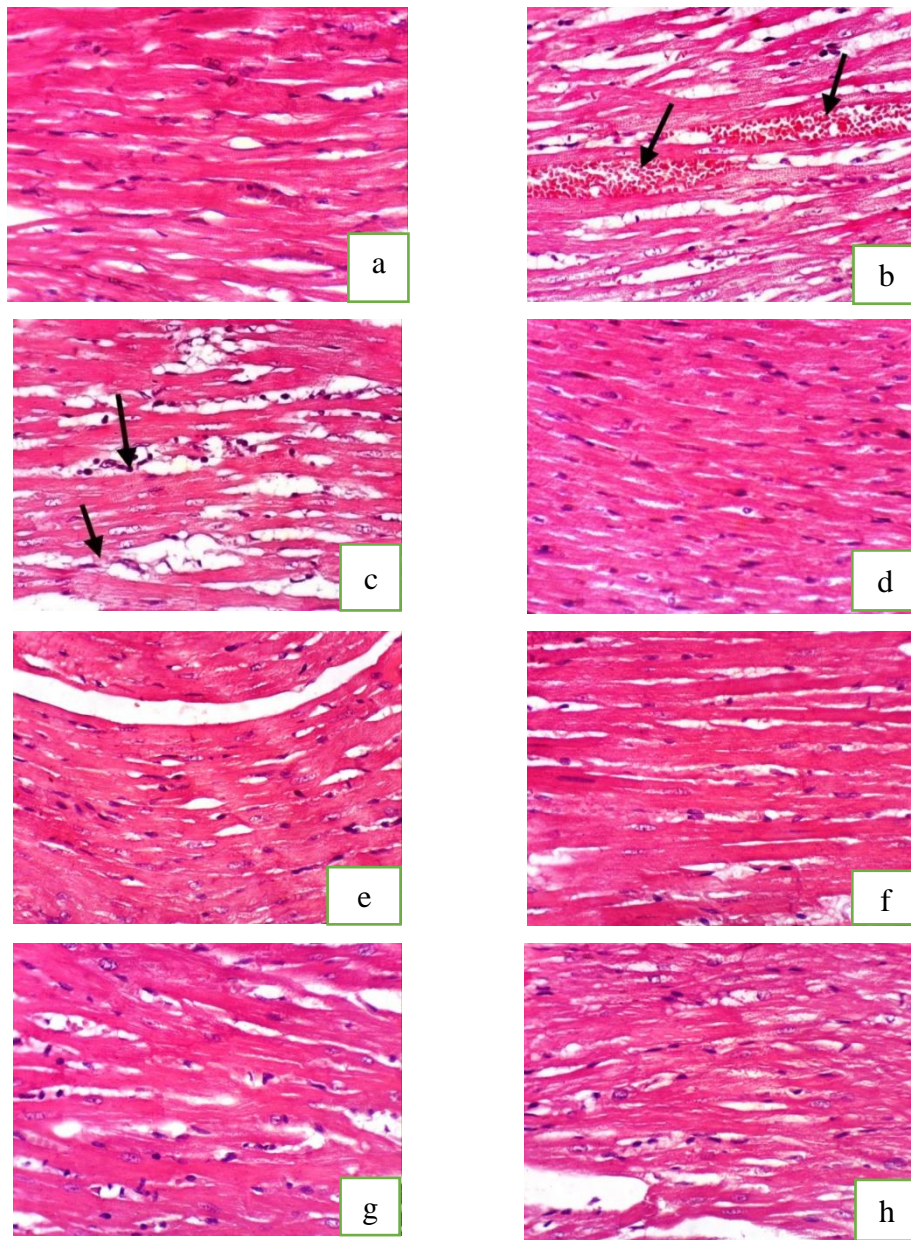


Fig (3):

Microscopical examination of heart for rats under study: a) heart of rats from negative control showing a normal histological structure (H&E); b & c) heart of rats fed on HFD as positive control showing congestion of myocardial blood vessels and showing intermuscular edema and inflammatory cell infiltration in between the cardiac myocytes (H&E); d) Heart of rat fed on HFD and 2ml/rat/day of water extract of raw fenugreek seeds showing no histopathological changes (H&E); e) Heart of rat fed on HFD and 2 ml/rat/day of germinated fenugreek seeds showing no histopathological changes (H&E); f) Heart of rat fed on HFD and 2m/rat/day of green fenugreek leaves showing no histopathological changes (H&E); g) Heart of rat fed on HFD and 2 ml/rat/day of water extract of chicory showing no histopathological changes (H&E) and h) Heart of rat fed on HFD and 2 ml/rat/day of water extract of olive leaves showing no histopathological changes (H&E);

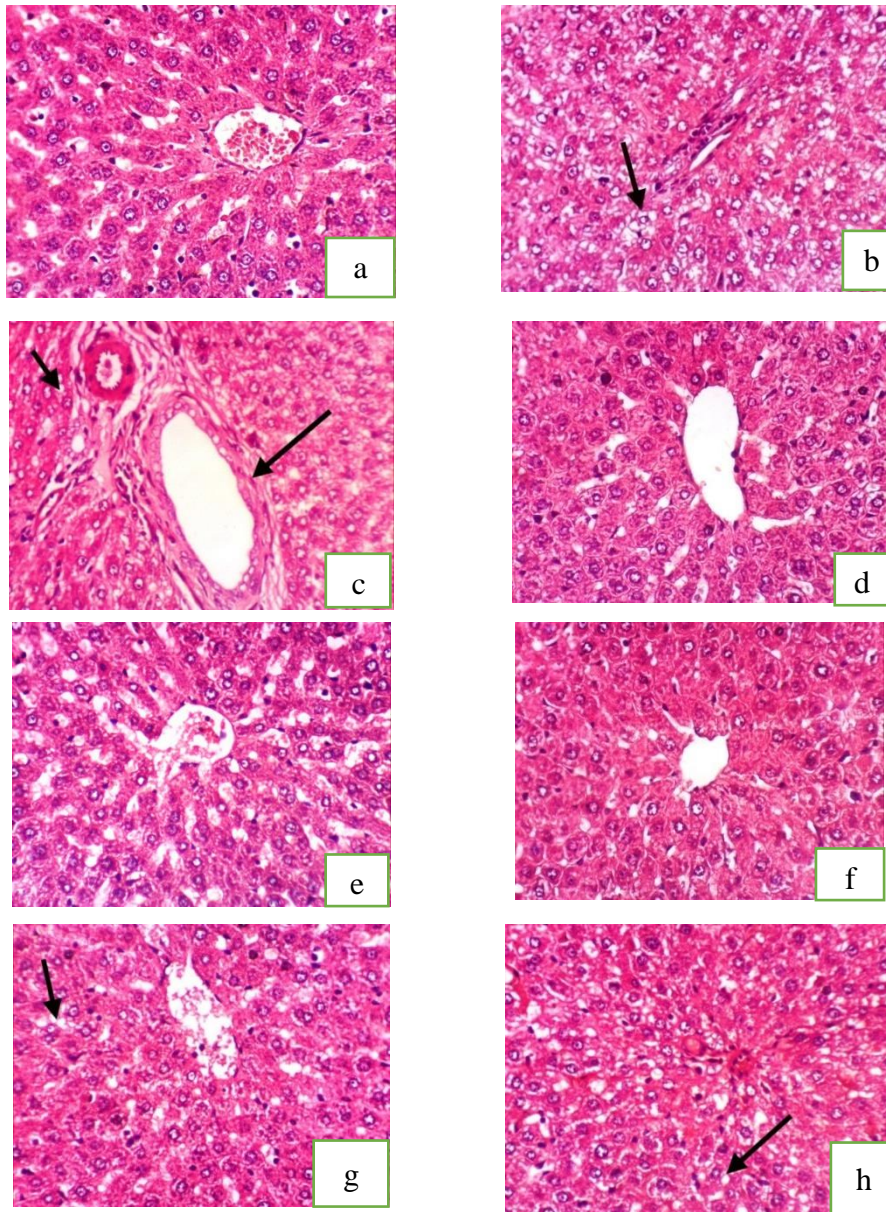


Fig (4).:

Microscopical examination of liver for rats under study a) Liver of rat from negative control showing the normal histological structure of hepatic lobule (H&E); b &c) Liver of rat fed on HFD as positive control showing cytoplasmic vacuolization of hepatocytes and cytoplasmic vacuolization of hepatocytes and fibroplasia in the portal triad around the bile duct (H&E); d) Liver of rat fed on HFD and 2ml/rat/day of water extract of raw fenugreek seeds showing no histopathological changes (H&E); e) Liver of rat fed on HFD and 2ml/rat/day of water extract of germinated fenugreek seeds showing no histopathological changes (H&E); f) Liver of rat fed on HFD and 2ml/rat/day of water extract of green fenugreek leaves showing no histopathological changes (H&E); g) Liver of rat fed on HFD and 2ml/rat/day of water extract of chicory showing slight cytoplasmic vacuolization of some hepatocytes (H&E) and h) Liver of

rat fed on HFD and 2ml/rat/day of water extract of olive leaves showing slight cytoplasmic vacuolization of some hepatocytes (H&E) .

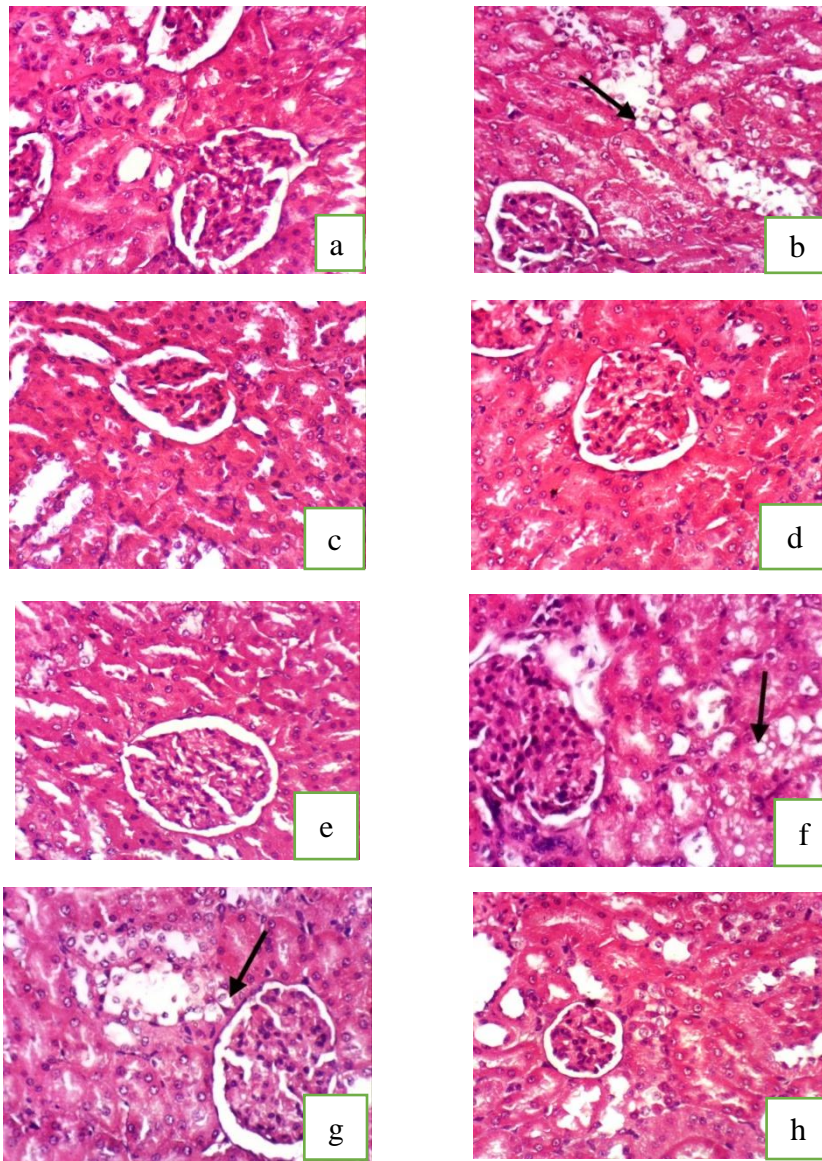


Fig (5):

Microscopical examination of kidney for rats under study a) Kidney of rat of negative control showing the normal histological structure of renal parenchyma (H&E); b) Kidney of rat fed HFD as positive control showing vacuolar degeneration of epithelial lining renal tubules (H&E); c) Kidney of rat fed on HFD and 2ml/rat/day of water extract of raw fenugreek seeds showing no histopathological alterations (H&E); d) Kidney of rat fed on HFD and 2ml/rat/day water extract of germinated fenugreek seeds showing no histopathological alterations (H&E); e) Kidney of rat fed on HFD and 2ml/rat/day water extract of green fenugreek leaves showing no histopathological alterations (H&E); f & g) Kidney of rat fed on HFD and 2ml/rat/day of water extract of chicory showing vacuolar degeneration of epithelial lining renal tubules (H&E) and h) Kidney of rat fed on HFD and 2ml/rat/day of water extract of olive leaves showing no histopathological alterations(H&E).

The slides of rats in negative control group revealed, normal histopathological structure of hepatic lobule from central vein and normal hepatocyte Fig. 4(a). Rats in the positive control group showed cytoplasmic vacuolization of hepatocytes and cytoplasmic vacuolization of hepatocytes and fibroplasia in the portal triad around the bile duct Fig. 4 (b and c). Rats fed on HFD and treated by water extracts of fenugreek seeds (raw, germinated and green leaves) showed no liver histopathological changes Fig.4 (d, e and f). While, rats fed on HFD and treated by water extracts of chicory and olive leaves showed slight cytoplasmic vacuolization of some hepatocytes Fig.4 (g and h).

Kidney findings are shown in Fig. (5). Negative control group had normal histopathological structure of renal parenchyma Fig. (5a). While, rats in the positive control which fed on HFD (20% vegetable oil) showed vacuolar degeneration of epithelial lining renal tubules (Fig. 5b). Rats in groups fed on HFD (20% vegetable oil) and treated by water extracts of fenugreek seeds (raw, germinated and green leaves) and olive leaves showed no histopathological changes in kidney tissues Fig. 5 (c, d, e and h). While, rats fed on HFD and treated by water extract of chicory showed vacuolar degeneration in epithelial lining of renal tubules Fig. 5 (f and g).

Discussion

The potential of natural products for treating obesity is still under study. The natural products may be a good alternative strategy for developing future effective, safe anti-obesity drugs (**Mayer et al, 2009**). **Hanet et al. (2005)** suggested that the natural products have phytochemical compounds in crude extracts or isolated pure natural compounds might cause loss of body weight and prevent effect of diet that induce obesity. Also, a wide variety of plant products such as saponins, polyphenols and flavonoids possess lipase inhibitory effects (**Shimoda et al., 2006**). Moreover, dietary phytochemical might be considered as anti-obesity agents to suppress the growth of adipose tissue and thereby reducing adipose tissue mass (**Mohamed et al., 2014**).

Results showed chicory was high in total flavonoids and minerals. This result is in line with **Kauret et al., (2016)** who recorded that, chicory has considerable amount of phytochemical and is good source of antioxidants. Also, **Janda et al., (2021)** reported that, chicory is a feasible source of biological elements (K, Fe and Ca). The present study explained that, total flavonoids were more than total phenols. This result may be due to heat treatment which increased the level of free flavonoids (**Stewart et al., 2000**).

The present study indicated that body weight decreased in groups fed on HFD treated by fenugreek (raw, germinated and green leaves). This result is in line with (**Wan-Li et al, 2007**) who found, a progressive reduction in body weight when treated the diabetic rats by water extract of fenugreek seed. The authors suggested that, fenugreek seeds may attenuate the toxicity of STZ and possibly cause a better utilization of nutrients in diet. According, **Mathernet et al., (2009)** reported that, galatomannan is one of the major soluble fibers of fenugreek seeds which decreases the bile salts uptake in the intestine thus reduces the digestion and absorption of starch in the body.

This study indicated that, water extract of chicory, olive leaves and fenugreek (raw, germinated and green leaves) caused a decrease in serum glucose. These results agreed with (**Wan-Li et al, 2007**) who studied effect of water extract of fenugreek on diabetic rats. Their results indicated that, diabetic rats treated with fenugreek decreased blood glucose. The reduction in blood glucose was

increased with the increase in fenugreek dosage. These results may be due to the soluble dietary fibers content in fenugreek which could inhibit absorption of glucose in the gastro intestinal tract (*Hannanaet al., 2003*). According, *Gorjipour et al., (2017)* who studied the effect of water extract of chicory on blood glucose in diabetic rats They found that blood glucose decreased compared untreated diabetic rats. *Bakhtuary (2011)* studied the effect of chicory on blood glucose for diabetic rats. His results indicated that chicory caused a decrease and had significant differences between rats.

On the other hand, serum glucose of rats treated by 2ml/ rat/ day of water extract of olive leaves were decreased compared to the positive control. This result is in the line with *Al –Azzawie and Alhamdani (2006)* who studied the effect of olive leaf and its extract on hyperglycemia in rabbits. they found that ethanol extract of olive leaf decreased blood glucose. Olive leaf extract may increase glucagon-like peptide – 1 secretion in vivo and in vitro environment and they recommended it use for nutrition treatment in type2 diabetes *Rafferty et al., (2011)*.

The present study showed decrease in lipid profile. These results are in line with (*Wan –Li et al., 2007*) who found that, rats treated by water extract of fenugreek caused a decrease in TC and TG and increase HDL- C. Reducing TC and TG by treating it with water extract of fenugreek may be due to decreasing the non – esterified fatty acids (NEFA), which could influence platelet aggregation and vascular changes by accelerating the rate of prostacyclin in plasma (*MacRuryet al., 1990*). The fiber contained in water extract of fenugreek might be due to inhibition of carbohydrate and fat absorption (*Eidiet al. 2006*). Also, *Sowmya and RajyalaRshmi (1999)* studied the effects of two doses of germinated fenugreek seeds powder for a month on lipid content of human, the authors reported that the germinated fenugreek powder caused a decrease in TC, TG, LDL-C and VLDL-C.

The study indicated that water extract of chicory caused a decrease in TC, TG, LDL- C and VLDL-C and increase in HDL-C levels. These results agreed with *Gorjipooret al.,(2017)* who found similar results for diabetic rats. Chicory contained inulin compound which is a soluble fiber. It has hypolipidemic effect through the growth of bifido bacteria and reducing fat absorption (*Kim and Shin, 1998*). Hypolipidemic effects of olive leaf extracts has been shown by *Lockyeret al., (2018)* who reported that, feeding rats on 50 and 100 mg/kg/ day of olive leaf extract may positively affect atherosclerosis by decreasing TC and LDL – C level.

Conclusion:

Water extracts of fenugreek seeds (raw, germinated and green leaves), chicory and olive leaves could improve serum lipid content and liver functions which resulted due to malnutrition status. Clinical studies on human might be conducted before generalizing the results.

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التأثير الواقي لمستخلصات بذور الحلبة و أوراق الشيكوريا و أوراق الزيتون على مستوى دهون الدم

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الملخص العربي

نظام الحياة الحديثة زاد من تناول الوجبات السريعة و التي تتميز بمحتواه من الكربوهيدرات و الدهون و ايضا انخفاضها من البروتين مسببة سوء التغذية و كذلك السمنة. لذا تهدف هذه الدراسة إلى تقليل الآثار السلبية الناتجة عنها. تم اختيار مستخلصات مائية المحضرة من بذور الحلبة سواء بذور خام او منبته و ايضا اوراق الحلبة الخضراء و اوراق الشيكوريا و أوراق الزيتون . استخدم 42 فأر ذكر لإجراء تجربة بيولوجية و تم تقسيمهم إلى 7 مجاميع الأولى مجموعة حاكمة و تم إعطائها الوجبة الحاكمة طول التجربة. أما المجاميع من 2 - 7 فتم تغذيتها على وجبة عالية الدهن (20%) و منخفضة البروتين (10%) لمدة اسبوعين ثم المعاملة بالمستخلصات لمدة 8 اسابيع . في نهاية مدة التجربة تم تحليل دهون الدم و وظائف الكبد و الكلى و كذلك تم عمل دراسة تشريحية للكبد و الكلى و القلب.

و أهم النتائج المتحصل عليها :

مستخلص الشيكوريا كان أغنى المستخلصات في الفلافونيدات الكلية و كذلك الأملاح المعدنية. مستخلصات بذور الحلبة الخام و أوراق الحلبة الخضراء كانوا أفضل في خفض سكر الدم من مستخلص الشيكوريا و اوراق الزيتون . بينما مستخلص ورق الزيتون حسن من مستوى الجليسيريدات الثلاثية و الليبوبروتين منخفض الكثافة و كذلك حسن وظائف الكبد . وجد ايضا أن مستخلص الحلبة الخام خفض الكوليسترول الكلى و معامل الخطورة الممرض و ايضا اليوريا. اثبتت الدراسة التشريحية عدم حدوث أى تغيرات فسيولوجية للقلب و الأورطى مع المعاملة بكل المستخلصات المدروسة. أيضا كانت الشرائح الكبد طبيعية دون أى تغير فى مجاميع المعطاه مستخلصات الحلبة (الخام و المنبته و ايضا اوراق الحلبة الخضراء).
لذا توصى الدراسة باستخدام هذه المستخلصات كمشروب للحد من تأثير سوء التغذية.