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BIOLOGICAL EVALUATION OF STABILIZED WHEAT GERM IN HYPERCHOLESTEROLEMIA RATS

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Abstract:
There is a global rise in the prevalence of diseases such as atherosclerosis and cardiovascular diseases. Therefore, this study was performed to investigate the effect of feeding on stabilized wheat germ (SWG) at levels 25, 50,75 and 100% for casein in hypercholesterolemic (hyper-c) rats. Results showed that, replacing a (hyper-c) diet with (SWG) significantly reduced serum total cholesterol (T.C), total triglycerides (T.G), low density lipoproteins (LDL), very low density lipoproteins (v.LDL), while high density lipoproteins (HDL) increased .Furthermore, (hyper-c) diet with (SWG at levels 75 and 100% for casein) replacement also recorded the best and nearest of HDL-C to the (control–ve). Feeding on (hyper-c) diets substituted with SWG (G6 and G7) led to reduce the levels of serum liver function enzymes and increase the activity levels of antioxidant enzymes comparing with (hyper-c) control (G2) (control +ve). Histopathological examination showed that feeding diets supplemented with SWG to the hyper-c rats lowered the degree of liver lesions. So, it can be suggested that, SWG has a clear effect in lowering serum cholesterol levels and may be beneficial for patients with liver and cholesterol diseases.

Keywords: *Rats, lipid profile, stabilized wheat germ, Hypercholesterolemic*

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INTRODUCTION:

Consumption of large amounts of simple sugar and saturated fat (i.e. Western diet) is a risk to develop obesity, insulin resistance (IR), and coronary heart disease (C.V.D.)[1]. Increased consumption of high energy diets contributes to accumulation of lipids in tissues other than adiposis such as liver, heart and musculoskeletal system, leading to metabolic disorders[2]. Hypercholesterolemic rats have shown a significant increase in serum total lipids, TC, LDL-C, albumin, uric acid, glucose, and decrease in the antioxidant defense system. It also showed a reduction in the activities of catalase and superoxide dismutase and increases the content of lipid peroxide[3]. Wheat is the world's most widely grown cereal. It is the main ingredient in many diets and contains a large proportion of the daily intake of energy[4]. Wheat germ (WG) is a by-product of the flour milling industry and it is one of the most potential and excellent sources of relatively low-cost nutrients, minerals, fiber and proteins[5]. It is often used in wheat germ oil (W.G.O.) processing or in the introduction of bulk into animal feed. It also includes many bioactive compounds, each with antioxidant properties, including large amounts of tocopheroles, carotenoids, flavonoids and phytosterols[6,7]. WG is widely recognized as a nutritious raw material to be added in or as a product in nutrition formulations. It is typically used as bread enriched with germs, cookies, sweets, and supplements to breakfast cereals, and for production of wheat-germ oil. Wheat germ is used as a source of oil, mainly in food, medical and cosmetic industries with about 8 % -14% oil (average 10 %)[8,9], high lipase and lipoxygenase activities are also present in wheat germ. These activities favor sensitivity to oxidation, and the consequent destruction of essential fatty acids and vitamins[10]. Besides, lipase and lipoxygenase activities determine the poor and unstable sensory properties of baked goods made of wheat flour containing the germ, especially during storage[11]. Oxidation may be prevented by inactivating enzymes under heat, microwave and extrusion cooking treatments; removing the oil fraction from wheat germ and using antioxidants[11].

WGO is a great source of polyinsaturated fatty acids and vitamin E. It is one of the best natural sources of α-tocopherol, the most vitamin E active form of tocopherol. In contrast, WGO was related to lower plasma and hepatic cholesterol, increased physical resistance and delayed aging of animals. In addition, blood sugar reduction and cholesterol reduction in diabetic patients[12,13]. WGO lowers the total lipids, serum cholesterol and LDL-C significantly indicating its pronounced hypolipidemic and antiatherosclerosis effects in cholesterol fed rabbits[14]. High amount from wheat germ in sausage formula have shown a reduction in hypercholesterolemia in rats compared to groups which were fed differently [15].

The fact that the rats received high fat-cholesterol diet supplemented with wheat germ resulted in lower prevalence of liver steatosis which might be secondary to different mechanisms such as lower intestinal absorption of dietary lipids, impaired hepatic absorption of intestinal lipoproteins, or reduced biosynthesis. Recent studies have shown that apos E receptors, responsible for hepatic absorption of chylomicron, is unregulated unlike the apos B, LDL-specific receptors[16].

Therefore, this study was designed to investigate the effect of SWG on hypercholesterolemic rats.

MATERIALS AND METHODS:

Materials:

Fresh wheat germ was obtained from Middle and West Delta Milling Company at Banha, Egypt during the season of 2019.The animals used in this study were male albino rats weighting between (156g-160g) which were obtained from experimental animal house of Food Technology Research Institute, Agric., Res., Center, Giza, Egypt. In addition, same chemical substances were used.

Methods:

Preparation of wheat germ flours (WGF):

Wheat germ contains several enzymes, such as dipeptidase, proteinase, lipase, lipoxidase and phytase. In order to stabilize them, wheat germs were treated by autoclave stabilized wheat germ (SWG), wheat germs were steamed by autoclave under atmospheric pressure for 20 mins[17]. Finally, the stabilized wheat germs were milled by a laboratory mill. There sulting flour was sieved through a 60-mesh screen and stored in polyethylene bags and saved at -20°C until further analysis had been achieved.

Gross chemical composition of stabilized wheat germ flours:

Moisture, ash, crude protein, ether extract and crude fiber contents determined while total carbohydrates content differences were measured[18].

Biological Evaluation:

Animal feeding experiment of Hypercholesterolemia:

Forty-two animals of adult male albino rats (156.69-158.69g) were taken from Food Technol. Res. Instit. Agric. Res. Giza, Egypt. Animals were kept in screen-bottomed cages and fed under laboratory conditions for seven days as a basal diet. The experimental period of 12 weeks after adaptation period had free access to food and water for rats. Six groups of rats were divided into ten with a similar body weight. Rats were feed according to the following scheme:

Group 1 (G1): Fed on the basal diet (negative control).
Group 2 (G2): Fed on Hypercholesterolemic (hyper-c) diet (Positive control).
Group 3 (G3): Fed on (hyper-c) diet replacement 25% stabilized wheat germ (S-WGF) for casein.
Group 4 (G4): Fed on (hyper-c) diet replacement 50% (S-WGF) for casein.
Group 5 (G5): Fed on (hyper-c) diet replacement 75% (S-WGF) for casein.
Group 6 (G6): Fed on (hyper-c) diet replacement 100% (S-WGF) for casein.
The composition of different experimental diets shown in **Table (A).** Rats fasted over the night before sacrificing at the end of the experimental period (12 weeks). Centrifuged and collected blood. Serum was separated for analysis[19]**.**

Table (A): Composition of different experimental Hypercholesterolemic diets (g/kg):

Groups Ingredients	G1	G2	G3	G4	G5	G6
S-WGF	0	0	154.32	308.64	462.96	617.28
Corn starch	660.5	548.0	452.21	356.42	260.64	164.85
Casein	140	140	105.00	70.00	35.00	0
Corn oil	100	100	82.41	64.82	47.22	29.63
Cellulose	50	50	44.06	38.12	32.18	26.24
Mineral mixture	35	35	35	35	35	35
Vitamin mixture	10	10	10	10	10	10
Cholesterol	0	10	10	10	10	10
Beef tallow	0	100	100	100	100	100
Bile salt	0	2.5	2.5	2.5	2.5	2.5
L-cystine	2	2	2	2	2	2
Choline chloride	2.5	2.5	2.5	2.5	2.5	2.5

G1: Feeding on the basal diet (negative control).
G2: Feeding on (hyper-c) diet (Positive control).
G3: Feeding on (hyper-c) diet replaced 25% (S- WGF) for casein.
G4: Feeding on (hyper-c) diet replaced 50% (S- WGF) for casein.
G5: Feeding on (hyper-c) diet replaced 75% (S- WGF) for casein.
G6: Feeding on (hyper-c) diet replaced 100% (S- WGF) for casein.

S-WGF: autoclave stabilized wheat germ flours.
Every week all rats have been weighed so as food intake during the study. Body weight (B.W.G.) and Food Efficiency Ratio (F.E.R.) were calculated as follows [20]:

B.W.G. % = [(Final Weight) - (Initial Weight)] / [(Initial Weight)] x 100.
F.E.R. = [Gain in body (g/day)/ Feed intake (g/day)] x 100.

Blood sampling:
At the end of the experiment, blood samples were taken from all the experimental rats. After 12 hours of fasting, blood samples were obtained from the eyes venous plexuses and then centrifuged at 3000 r.p.m. For 15 minutes to extract the aspirated serum into dry plastic pipes and kept frozen by-180C for biochemical analysis[21].

Collection of organs:
Each animal's liver, kidney and heart were removed and instantly weighed. By using this formula, a relative organ weight (R.O.W.) was determined: **(R.O.W.) = (Organ weight / Animal weight) x 100.**
Biochemical Analysis and Enzymes Assays: -
Triglycerides were calculated by the method of **Fossati and Prancipe [22].** In accordance with the **Richmond [23]** method total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) was studied Mathematical calculation and conducted using the method for low lipoprotein cholesterol concentration (LDL-C) and very low (VLDL-C) cholesterol density[24].
LDL-Cholesterol =Total cholesterol - (HDL-Cholesterol -Triglycerides / 5). VLDL - C = Triglycerides/ 5.

In addition to that, the activity of serum glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) were measured[25].

Determination of Glucose:
The Blood Glucose was measured using the **Triender[26]** method.

Liver function tests:
The activity of Glutamic pyruvic transaminase (GPT) or alanine amino transferase (ALT) and glutamic-oxaloacetic transaminase (GOT) or aspartate amino transferase (AST) activities were calculated by using commercial Pasteur Lab kits, Paris, France and alkaline phosphatase enzymes (ALP)[27].

Determination of kidneys functions:
Uric acid method has been determined using a commercial kit (Biomed Company, Germany) [26].
Creatinine concentrations were calculated using a colorimetric enzyme kit (Biolabo, Maizy, France)[29].

Histopathological examination:
Tissue samples of the heart, liver and kidneys of different groups were collected after the postmortem examinations. Two days after, samples are exposed to 10% neutral formalin, then washed by running water overnight. The dehydration of the washed samples was achieved by using an increasing concentration of ethyl alcohol from 70% to absolute alcohol. The samples were cleared by immersing in xylol for 2 hr. The specimens were collected in a closed down bottle of 50 % xylol paraffin at 37 ° C for 3 hours. Samples have subsequently been put in paraffeine melting and kept 2 hours at 48 ° C and tissue sections have been then finally blocked in hard paraffin and microtomed into a 5-micron thickness section. Section were stained by Haematoxylin and Eosin (H & E). stained sections were mounted with Canada balsam and covered with cover slip to be ready for histopathological examinations **[30].**

Statistical analysis:
The data were analyzed statistically by analyzed using SPSS (Windows, Version 22,2013), using a one-way variance analysis (ANOVA) of 5 % ($p<0.05$) probability [30].

RESULTS AND DISCUSSION:

Proximate chemical composition of stabilized wheat germ:
Gross chemical composition of stabilized wheat germ (SWG) presented in Table (1). It is clear that, SWG contains 22.68 % protein, 11.40% ether extract and 3.50 % ash and Crude Fiber 3.85%. These results were approximately in agreement with those of **Mahmoud *et al.*, (2015)[7] and de Vasconcelos et *al.*, (2013)[32] .**

Table (1): Gross chemical composition of stabilized wheat germ (g/100 g on dry weight basis).

Components%	Moisture	Crude protein	Ether extract	Ash content	Crude fiber	Total carbo-hydrates
Stabilized wheat germ	12.90	22.68	11.40	3.50	3.85	62.42

Each value was an average of three determinations.

Effect of feeding at different levels of SWG for casein in hypercholesterolemic rats:

(A) - feeding and growth parameters.

The effect of SWG on feed intake (F.I.), body weight gain % (B.W.G.) and feed efficiency ratio (F.E.R.) of hyper-c rats for 12 weeks is shown in Table (2). Initial weights of the rat groups were almost the same with no significant difference with range of 156.81 - 158.69 g. Moreover; at the end of the experiment (after 12 weeks), the final weights of hyper-c rat G2 (control +ve) were higher than the negative groups G1 (control -ve). Also, found that (B.W.G.) of all groups showed a significant decrease compared to hyper-c G2 (control+ve) may be caused the wheat germ flour had contained rich amounts in dietary fiber. The study results are similar to previous similar experiments[33;34]. The mechanisms of dietary fiber that contribute to weight loss: delayed gastric emptying, decrease glucose diffusion and prohibition fat absorption were shown in previous experiments. It was also found that a significant decrease of (F.I.) and (F.E.R.) in comparison with those of hypercholesterolemia rats in group2 (control+ve).

Table (2): Effect of feeding at different levels of stabilized wheat germ on feeding and growth parameters of hypercholesterolemic rats.

Groups	Initial weight(g)	Final weight (g)	Body weight gain (B.W.G.)		Feed Intake	F.E.R.
			G	%		
G1	157.90 ^{a} ±1.00	189.45^{e} ±1.41	31.55^{d} ±0.84	19.98	1145.1^{b} ±1.01	2.75^{d} ±0.07
G2	156.81 ^{a} ±0.78	260.51 ^{a} ±1.47	103.70^{a} ±0.56	66.13	1253 ^{a} ±1.02	8.27 ^{a} ±0.04
G3	158.49 ^{a} ±0.73	202.23^{b} ±2.14	43.74 ^{b} ±3.71	27.59	1132 ^{c} ±2.83	3.86 ^{b} ±0.32
G4	158.21 ^{a} ±1.08	199.23^{c} ±2.14	41.02^{bc} ±3.38	25.92	1120 ^{d} ±2.84	3.66 ^{bc} ±0.30
G5	157.7 ^{a} ±0.97	195.12^{d} ±2.15	37.42 ^{c} ±3.32	23.73	1109e ±2.83	3.37c ±0.30
G6	158.69 ^{a} ±0.89	191.13^{e} ±2.15	32.44 ^{d} ±3.88	20.44	1120 ^{d} ±2.80	2.89 ^{d} ±0.34

Each value is an average of seven determinations ± standard deviation.
Values followed by the same seven letters in columns are not significantly different at $p \leq 0.05$.
G1, G2 … etc. were as given in Table (A).

B - Relative organs weight:

The liver, kidneys and heart of rats fed on basal diet and other treatments were weighted at the end of experimental period (12 weeks) and the ratio of each organ to final body weight was calculated. The results in Table (3) reveals that, the weight of liver G 2 (control +ve) had the highest liver weight with average of (6.61g) in comparsion to other groups which had an average liver weight (2.53). this difference is likely secondary to accumulation of fat in the liver[35].

G1 (control -ve) had the lowest value in liver weight and relatively liver weight. In addition, the liver weight of rats fed with replacement with SWG for casein in the diet after hypercholesterolemia were lower than those of G2(control +ve). On the other hand, there was no significant difference in kidneys and heart weights, of neither (control – ve) nor (control + ve)in all tested groups. These results are similar to previous studies.

Table (3): Effect of feeding at different levels of stabilized wheat germ on the relative organs weight in hypercholesterolemia rats.

Groups	Relative weight (gm %) of						Final body weight (g)
	Liver		Heart		Kidney		
	Weight (g)	R.O. W	Weight (g)	R.O. W	Weight (g)	R.O. W	
G1	5.02^{b} ±0.11	2.65	0.75^{a} ±0.10	0.39	1.47^{a} ±0.10	0.77	189.45^{e} ±1.41
G2	6.61^{a} ±010	2.53	0.82^{a} ±0.10	0.31	1.51^{a} ±0.10	0.58	260.51^{a} ±1.47
G3	5.10^{b} ±0.12	2.52	0.73^{a} ±0.12	0.36	1.46^{a} ±0.12	0.72	202.23^{b} ±2.14
G4	5.13^{b} ±0.13	2.57	0.72^{a} ±0.12	0.36	1.44^{a} ±0.12	0.72	199.23^{c} ±2.14
G5	5.08^{b} ±0.12	2.60	0.74^{a} ±0.13	0.38	1.44^{a} ±0.12	0.74	195.12^{d} ±2.15
G6	5.15^{b} ±0.16	2.69	0.72^{a} ±0. 12	0.38	1.44^{a} ±0.12	0.75	191.13^{e} ±2.15

Each value is an average of seven determinations ± standard deviation.

Values followed by the same letter in columns are not significantly different at $p \leq 0.05$.
G1, G2 … etc. have been as given in Table (A). R.O.W.: relative weight.

C- serum lipids parameter.

Results in Table (4) indicate that, the total cholesterol content at the end of experimental period for the G1 (control -ve) was 122.34 mg/dl, while total cholesterol contents of G 2 (control +ve) was 254.67 mg/dl.

On the other hand, G_3, G_4, G_5 and G_6 which feed on hyper-c diet replaced with SWG (25, 50,75 and 100%) of diets showed values of 159.10, 152. 46, 149.71 and 146.61 mg/dl respectively. It could be noted that, hyper-c rats fed on SWG had a significant lower serum cholesterol compared with positive control G2. Previous studies concluded that the phytosterol in wheat germ might have been the beneficial component in lowering cholesterol absorption and potentially improve cholesterol concentrations, that some effects on cholesterol metabolism might be mediated by dietary fiber. Furthermore, **Leghari *et al.* (2018)[40]** observed the reduction in total cholesterol of rats fed with raw wheat germ in their diets and attributed the reduction in total cholesterol to tocopherols, octacosanol and polyunsaturated fatty acids of wheat germ. Results also illustrated that, total triglycerides (TG) for the G1 (control -ve) was 117.78 mg /dl after 12weeks. Observed increased to 219.13 mg/dl in hyper-c rats which fed on hyper-c diet (G_2) while, the total triglycerides contents for G_3, G_4, G5 and G_6 fed on hyper-c diet replacement with SWG (25,50,75 and 100%) showed values of 137.93 , 133.97,131.93 and 128.58 mg/dl respectively. Results showed that, supplementation of feeding hyper-c diet with SWG led to improvement in the HDL-C. Furthermore, hyper-c diet with SWG replacement at 75% and 100% also recorded the best and nearest of HDLC to the negative control. These values were significantly different comparing with that recorded in positive control. It was observed also that, LDL-C value of G1 (control -ve) was 34.08 mg/dl, but the value of the hypercholesterolemic G2 (control +ve) was 165.66 mg/dl. On the other hand, the LDL-C of rats fed on hyper-c diet substitution with SWG at the ratio of 25,50.75 and 100% (G3, G4, G5 and G6) being 70.30, 62.46,58.45 and 54.76 mg/dl, respectively. The wheat germ oil contains the highest content of octacosanol which effects on hypercholesterolemia have been related to its LDL cholesterol decreasing and HDL cholesterol increasing trends[42]. In addition to fiber and antioxidants from wheat germ prevented LDL particles from becoming oxidized and reduction of cholesterol absorption[42,43]. Data of **v.LDL** cholesterol for fed rats on basal, hyper-c diets summarized in Table (4) was observed that, vLDL cholesterol value of G1(control -ve) was 23.55 mg/dl, while the value of G2 (control +ve) was 43.82 mg/dl. The vLDL cholesterol rats fed on hyper-c diet substitution with SWG 25,50,75 and 100% (G4,G5,G6andG7) produced 27.58, 26.79, 26.38 and 25.71mg/dl , respectively. It was observed that, hyper-c rats fed on hyper-c diet substitution with SWG 25, 50 75 and 100% relative to basal diets had a significant lower serum total cholesterol, total triglycerides, LDL cholesterol and VLDL cholesterol compared with hyper-c G2 (control +ve). In contrary, these groups had significantly high level HDL-C at ($P \leq 0.05$). The mechanism of cholesterol lowering by wheat germ include (1) the inhibition of pancreatic lipase activity by soluble proteins present on wheat germ[44] (2) the reduction in triglyceride lipolysis and (3) reduction in cholesterol absorption by the endogenous wheat germ phytosterols. Table (4) Effect of feeding on stabilized wheat germ on serum lipids parameter and Blood sugar level in rats after 12 weeks.

Groups	Total Cholesterol mg/dl	Triglyceride mg/dl	HDL-C mg/dl	LDL- C mg/dl	vLDL - C mg/dl	Blood sugar (mg/dl)
G1	122.34^{f} ±0.30	117.78^{f} ±1.11	64.74^{b} ±0.50	34.03^{f} ±0.58	23.55^{f} ±0.22	104.95^{e} ±1.12
G2	254.67^{a} ±2.02	219.13^{a} ±0.99	45.17^{e} ±0.65	165.66^{a} ±1.46	43.82^{a} ±0.19	161.91^{a} ±1.16
G3	159.10^{b} ±0.77	137.93^{b} ±0.95	61.19^{d} ±0.73	70.3^{b} ±0.86	27.58^{b} ±0.19	116.97^{b} ±0.94
G4	152.46^{c} ±1.29	133.97^{c} ±0.60	63.20^{c} ±0.73	62.46^{c} ±1.24	26.79^{c} ±0.12	111.99^{c} ±0.97
G5	149.71^{d} ±0.90	131.93^{d} ±0.95	64.86^{b} ±0.41	58.45^{d} ±1.01	26.38^{d} ±0.19	109.0^{d} ±0.95
G6	146.61^{e} ±0.87	128.58^{e} ±1.05	66.13^{a} ±0.70	54.76^{e} ±1.62	25.71^{e} ±0.21	103.0^{f} ±0.98

Each value is an average of seven determinations ± standard deviation.
Values followed by the same letter in columns are not significantly different at $p \leq 0.05$.
G1, G2 … etc. were as given in Table (A).

The same table illustrated that blood sugar levels of hyper-c groups were markedly higher than the G1 (control -ve), also different levels of SWG for casein led to a significant decreased in blood glucose level of the hyper-c rat groups (G_3, G_4, G_5 and G_6), comparing with the G2 (control +ve), this may be due to that wheat germ is a good source of pyhtosterol can reduce oxidative stress, improve lipid metabolism, decrease the high blood sugar and cholesterol levels with.

D- Liver function activities.

The effect of feeding on SWG for casein at the level of, alanine amino transferase (ALT), aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) enzymes in serum of hyper-c rats for 12 weeks recorded in Table (**5**). At the final of experiment (12 weeks), the concentration value of AL.T. was significantly increased for the hyper-c control G2 (control +ve).The concentration value of (ALT) enzyme in hyper-c rats (G2) was 52.76 U/L, while G 1 (control -ve) was 30.38 U/L. Data in the same table showed that, the feeding on hyper-c diets substitution with SWG (G_3, G_4,G_5 and G_6) led to a more reduction at level 25,50,75 and 100% were 37.36, 35.19, 34.15 and 32.31 U/L, respectively comparing with hyper-c control (G2). AST activity was significantly increased for hyper-c control G_2. The liver AST activity of hyper-c rats was 73.29 U/L relative to G1 (control -ve) was 52.32 U/L. Data in the same table showed that, the rats fed on substitution of SWG for casein at 25, 50, 75 and100% were 58.04, 56.90, 53.87 and 52.25 U/L, respectively for (G3, G4, G5 and G6). The (ALP) activity of the negative control (G1) was 95.75 U/L while (ALP) activity of hyper-c diets positive control (G2) was 134.42 U/L. hyper-c rats fed on SWG which substitutes 25, 50, 75 and 100% for casein showed significant decreases comparing with hyper-c control (G2).

Table (5): Effect of feeding on stabilized wheat germ on Liver function activities (AL.T.), (AS.T.) and AL.P.(U/L) in hypercholesterolemic rats.

Groups	**ALT**	**AST**	**ALP**
G1	30.38 ^{e} ±1.09	52.32 ^{d} ±1.17	95.75 ^{e} ±1.12
G2	52.76 ^{a} ±1.11	73.29 ^{a} ±1.12	134.42 ^{a} ±1.72
G3	37.36 ^{b} ±0.48	58.04 ^{b} ±0.49	111.18^{b} ±1.60
G4	35.19 ^{c} ±0.48	56.90 ^{b} ±0.58	104.60 ^{c} ±1.58
G5	34.15 ^{c} ±0.48	53.87 ^{c} ±0.45	98.80 ^{d} ±1.60
G6	32.31 ^{d} ±0.49	52.25 ^{d} ±0.48	95.99 ^{e} ±1.58

Each value is an average of seven determinations ± standard deviation.
Values followed by the same letter in columns are not significantly different at $p \leq 0.05$.
G1, G2 … etc. were as given in Table (A).

E- Antioxidant enzymes.

Results given in Table (6) showed that, hyper-c rats had significantly lower levels of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) antioxidant enzymes activity in compared with (G_1). The data in the following table clearly show that substitution of SWG at level 25,50,75 and 100% in hyper-c diet improved the activity levels of (GPx), (SOD) and (CAT) antioxidant enzymes in compared with positive group (G_2).

Table (6): Effect of feeding on stabilized wheat germ on glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) enzymes in serum.

Groups	GPx(nmole)	CAT (nmole)	SOD(U/ml)
G1	18.97[a] ±0.55	55.93a ±0.75	81.60a ±0.26
G2	8.08[f] ±0.17	35.21e ±0.90	54.20e ±0.24
G3	11.80[e] ±0.17	46.83d ±0.42	71.70d ±0.16
G4	12.91[d] ±0.14	51.10c ±0.64	75.40c ±0.14
G5	14.48[c] ±0.16	53.42b ±0.64	78.90b ±0.15
G6	16.97[b] ±0.15	55.07a ±0.65	81.20a ±0.14

Each value is an average of seven determinations ± standard deviation.
Values followed by the same letter in columns are not significantly different at p ≤ 0.05.
G1, G2 … etc. were as given in Table (A).

F- kidney functions.

The results of urea, uric acid and creatinine in serum of negative control (G1) and hyper-c positive control (G2), at the end of experimental period after feeding for 12 weeks are shown in Table (7).

The obtained results illustrated that, urea at the end of experimental period for (G1) was 29.30 mg/dl. The same table presented that urea content of hyper-c positive control (G2) showed that value of 52.51 mg/dl in serum, while the hyper-c rats fed on (SWG) at 25, 50, 75 and 100 % (G3, G4, G5 and G6) gave 38.73, 36.46, 34.66 and 32.81mg/dl respectively. The results showed that, the urea contents were decreased in rats fed on hyper-c diets substitution with (SWG) 25,50,75and100 % compared to hyper-c control (G2). The obtained results (Table 7) illustrated that uric acid contents at the end of experimental period fornegative control (G1) it was1.73 mg/dl. The same Table showed that, uric acid contents of hyper-c positive control (G2) showed a value 4.66 mg/dl. while the hyper-c diets of G3, G4, G5 andG6 substitution with (SWG) 25, 50, 75 and 100 % were 3.05, 2.46, 1.91 and 1.67 mg/dl, respectively. The obtained results illustrated that creatinine contents at the end of experimental period for negative control was 0.52 mg/dl, the same Table presented that creatinine contents of hyper-c positive control showed that a value of 1.15 mg/dl, while hyper-c diets G3, G4, G5 and G6 fed on (SWG) 25, 50, 75 and 100 % were 0.78, 0.70, 0.67 and 0.63mg/dl, respectively. It could be seen from the data present in Table (7) illustrated that hyper-c rats fed (SWG) 25,50,75 and 100% had significantly lower serum urea, uric acid and creatinine compared with hyper-c group G2 (P≤0.05). Meanwhile, negative group G1 fed on basal diet had a significantly lower mean value for urea, uric acid and creatinine.

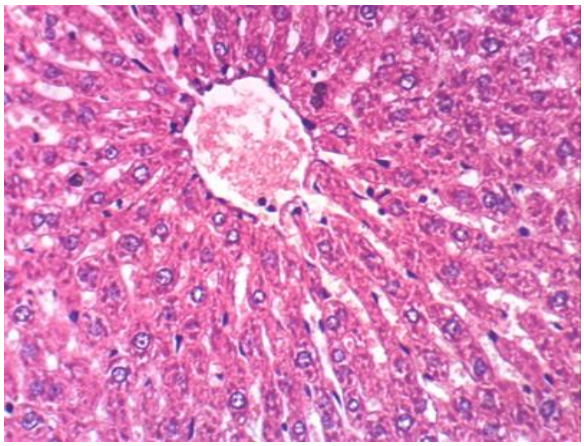
Table (7): Effect of feeding on stabilized wheat germ on kidney function activities (Urea, Uric acid and Creatinine) in hyper-C rats.

Groups	Urea mg/dl	Uric acid mg/dl	Creatinine mg/dl
G1	29.30 [e]±1.17	1.73 [d]±0.33	0.52 [c]±0.021
G2	52.51 [a]±1.07	4.66 [a]±0.32	1.15 [a]±0.010
G3	38.73 [b]±1.11	3.05 [b]±0.41	0.78 [b]±0.085
G4	36.46[c]±1.10	2.46 [bc]±0.39	0.70 [bc]±0.097
G5	34.66 [cd]±1.12	1.91 [cd]±0.39	0.67 [bc]±0.105
G6	32.81 [d]±1.10	1.67 [d]±0.39	0.63 [bc]±0.133

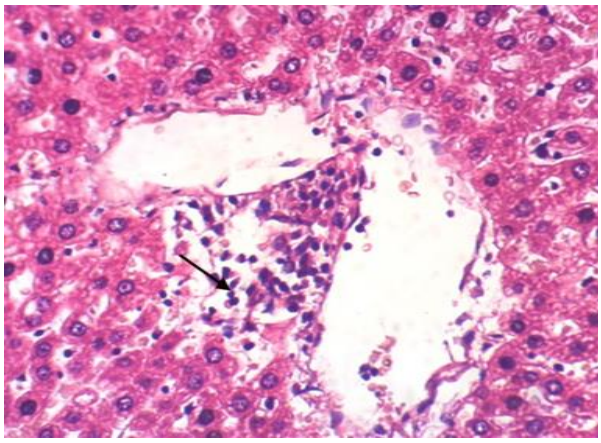
Each value is an average of seven determinations ± standard deviation.

Values followed by the same letter in columns are not significantly different at p ≤ 0.05.
G1, G2 … etc. were as given in Table (A).
***Histological* examination:**

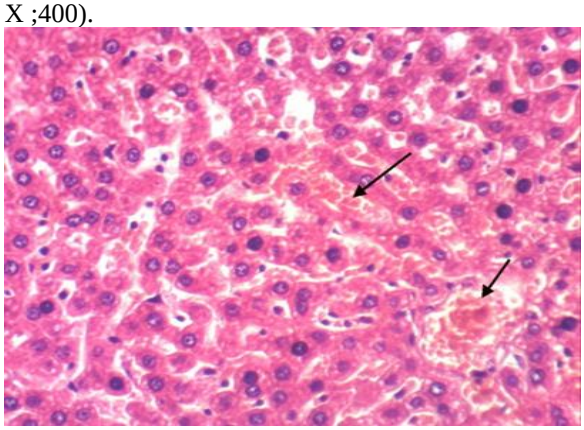
The influence of stabilized wheat germ on the liver, heart and kidney tissues of male albino rats were studied and the detected histopathological alterations were showed in slides, from No. 1 to 24 and Table (8) of the examined organs in various treatments.



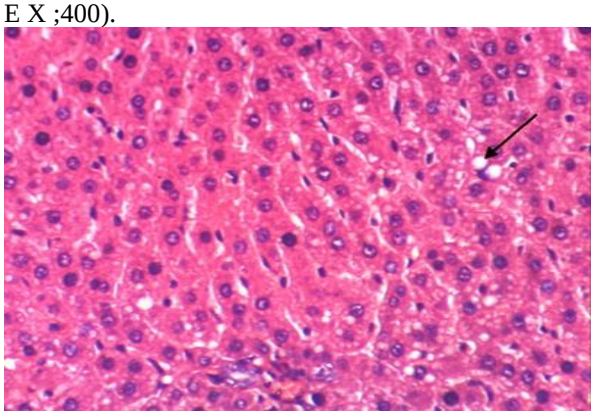
Slide (1): Liver of rat from group 1 showing the normal histological structure of hepatic lobule (H & E X ;400).



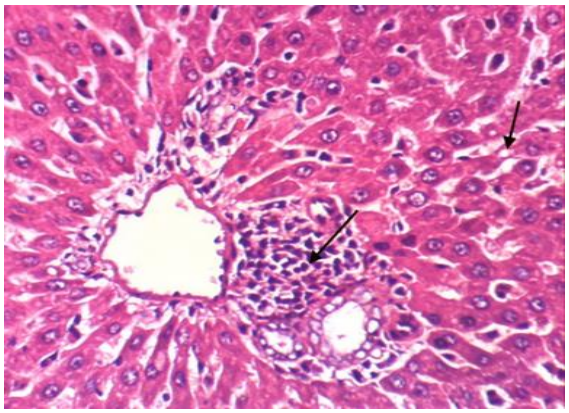
Slide (2): Liver of rat from group 2 showing congestion of central vein and hepatic sinusoids (H & E X ;400).



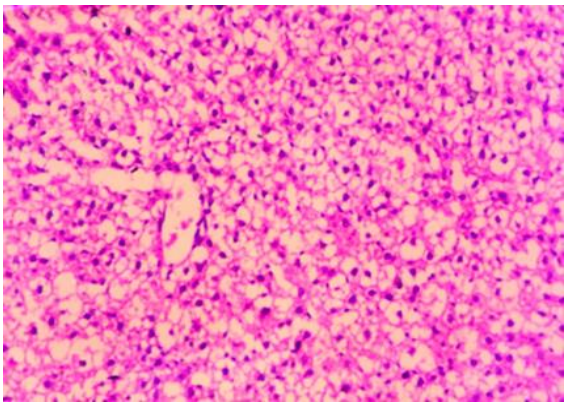
Slide (3): Liver of rat from group 2 showing cytoplasmic vacuolization of hepatocytes (H & E X ;40



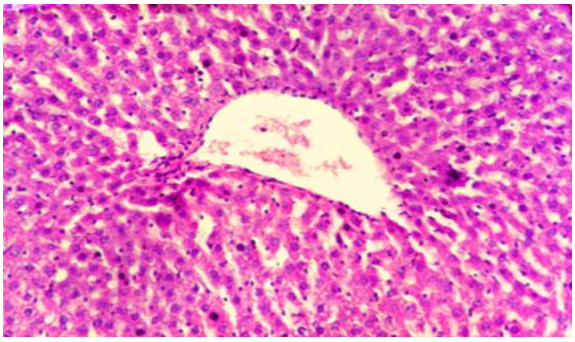
Slide (4): Liver of rat from group 2 showing focal hepatocellular necrosis associated with mononuclear inflammatory cells infiltration (H & E X ;400).



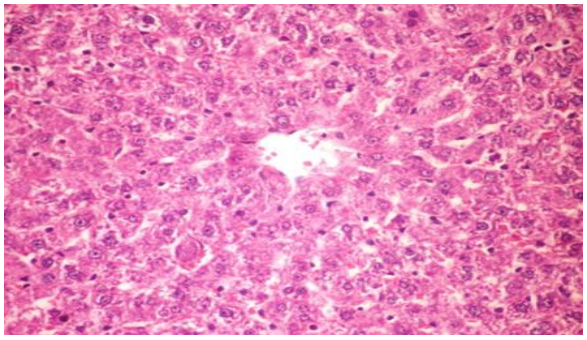
Slide (5): liver of rat from group 3 showing vacuolization of hepatocytes and portal infiltration with few inflammatory cells (H and E X 400).



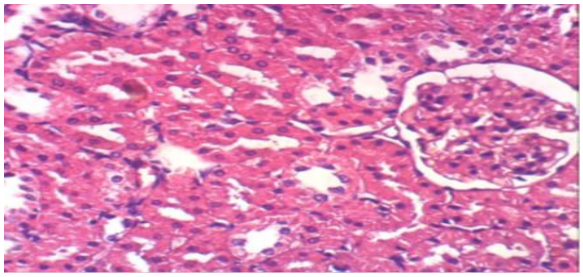
Slide 6): liver of rat from group 4 showing vacuolardegeneration of hepatocytes (H and E X 400).



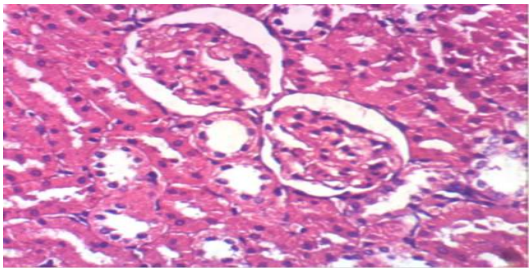
Slide (7): liver of rat from group 5 showing no histopathological change (H and E X 400).



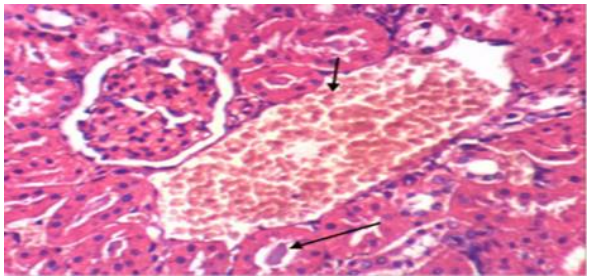
Slide (8): liver of rat from group 6 showing no histopathological change (H and E X 400).



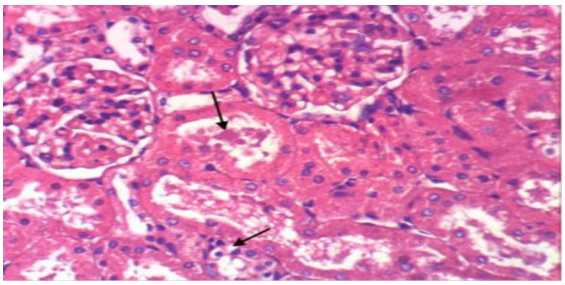
Slide (9): Kidney of rat from group 1 showing the normal histological structure of renal parenchyma (H & E X 400).



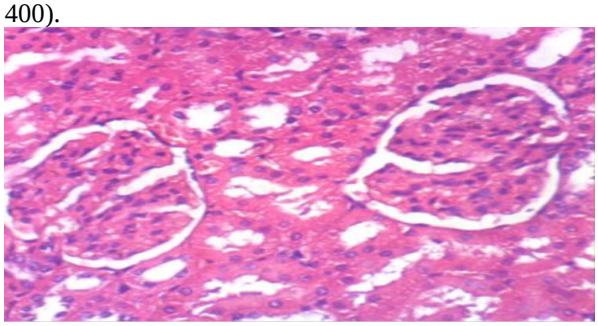
Slide (10): Kidney of rat from group 1 showing the normal histological structure of renal parenchyma (H & E X 400).



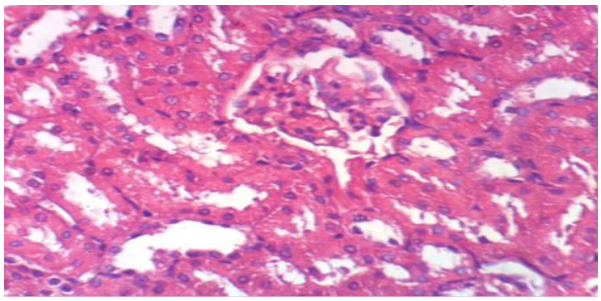
Slide (11): Kidney of rat from group 2 showing congestion of renal blood vessel and proteinaceous material in the lumen of some renal tubules (H & E X 400).



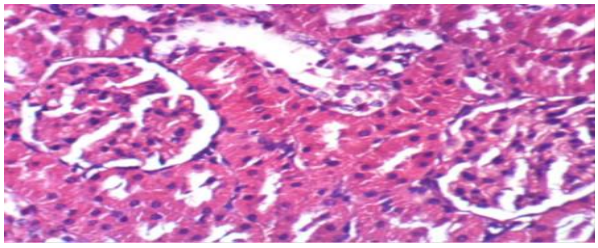
Slide (12): Kidney of rat from group 2 showing proteinaceous material in the lumen of some renal tubules (H & E X X 400).



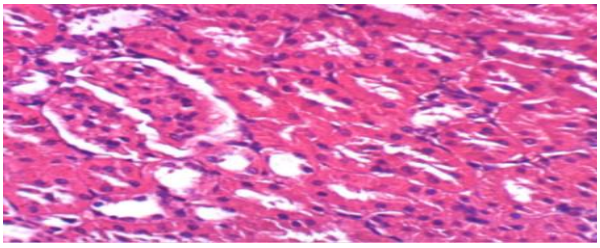
Slide (13): Kidney of rat from group 3 showing no histopathological alterations (H & E X 400).



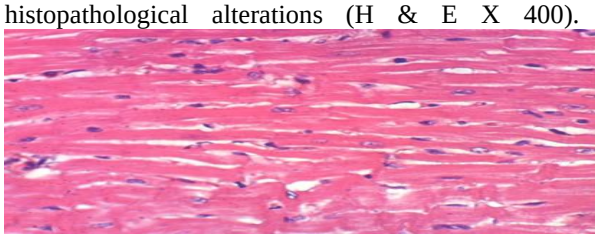
Slide (14): Kidney of rat from group 4 showing no histopathological alterations (H & E X 400).



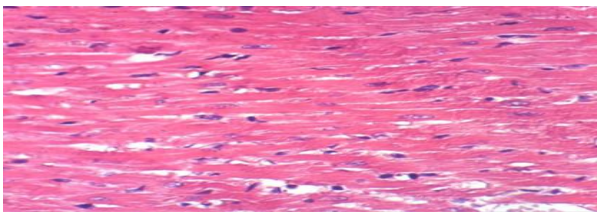
Slide (15): Kidney of rat from group 5 showing no histopathological alterations (H & E X 400).



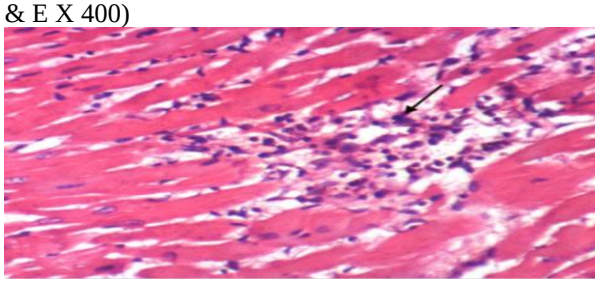
Slide (16): Kidney of rat from group 6 showing no histopathological alterations (H & E X 400)



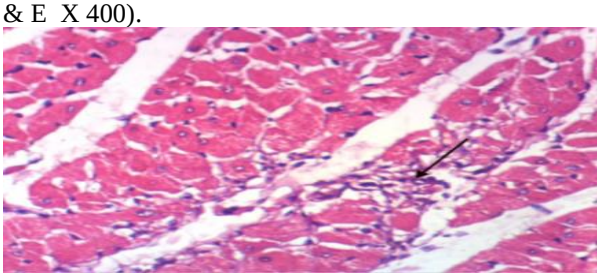
Slide (17): Heart of rat from group 1 showing the normal histological structure of cardiac myocytes (H & E X 400)



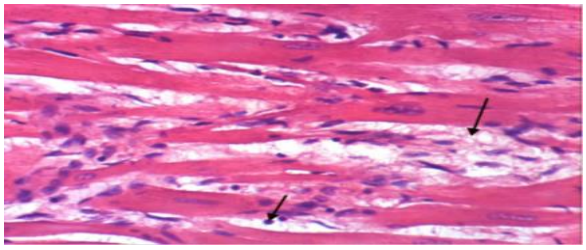
Slide (18): Heart of rat from group 1 showing the normal histological structure of cardiac myocytes (H & E X 400).



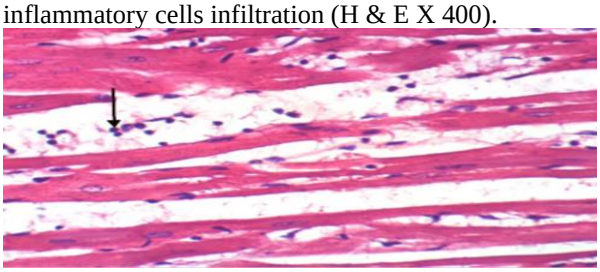
Slide (19): Heart of rat from group 2 showing focal necrosis of cardiac myocytes associated with inflammatory cells infiltration (H & E X 400).



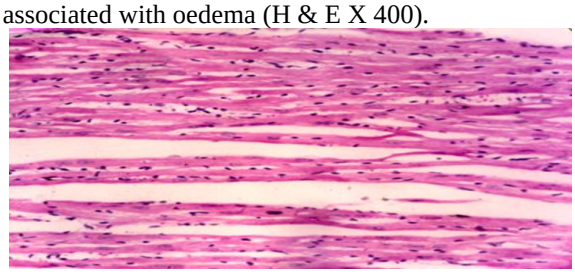
Slide (20): Heart of rat from group 2 showing focal necrosis of cardiac myocytes associated with inflammatory cells infiltration (H & E X 400).



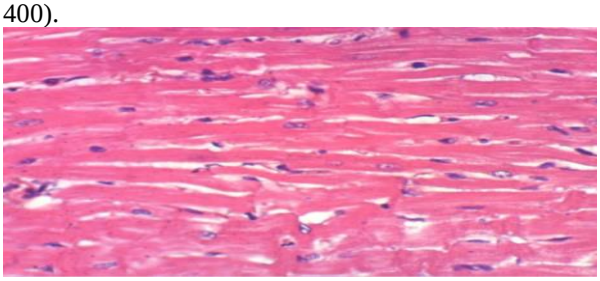
Slide (21): Heart of rat from group 3 showing intermuscular inflammatory cells infiltration associated with oedema (H & E X 400).



Slide (22): Heart of rat from group 4 showing intermuscular inflammatory cells infiltration (H & E X 400).



Slide (23): Heart of rat from group 5 showing showing no histopathological changes (H & E X 400).



Slide (24): Heart of rat from group 6 showing showing no histopathological changes (H & E X 400).

Table (8): Histopathlogical changes in liver, kidney and heart of rats fed on different levels of stabilized wheat germ for 12 weeks.

Groups	Liver	Kideny	Heart
G1	Normal histological structure of hepatic lobule.	Normal histological structure of renal parenchyma.	Normal histological structure of cardiac myocytes.
G2	showing congestion of central vein and hepatic sinusoids, cytoplasmic vacuolization of hepatocytes and focal hepatocellular necrosis associated with mononuclear inflammatory cells infiltration.	showing congestion of renal blood vessel and proteinaceous material in the lumen of some renal tubules and proteinaceous material in the lumen of some renal tubules.	focal necrosis of cardiac myocytes associated with inflammatory cells infiltration.
G3	showing vacuolization of hepatocytes and portal infiltration with few inflammatory cells.	No histopathological alterations.	intermuscular inflammatory cells infiltration associated with oedema.
G4	showing vacuolar degeneration of hepatocytes.	No histopathological alterations.	intermuscular inflammatory cells infiltration.
G5	No histopathological change.	No histopathological alterations.	No histopathological change.
G6	No histopathological change.	No histopathological alterations.	No histopathological change.

CONCLUSION:

In this study, feeding rats which had hypercholesteremia with meals containing SWG of 75-100% was resulted in decrease liver enzymes and antioxidant. The histology result showed that SWG was linked to a reduction in severity of fatty liver. Furthermore, SWG had a clear effect on decreasing hypercholesteremia. For which, further studies are recommended to evaluate the effect of SWG in humans with fatty liver and hypercholesteremia.

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