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Green Walnut Husk Ameliorating the Adverse Effects Induced by High Fat Diet in Rats

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A B S T R A C T

This study was designed to investigate the ameliorating effect of methanolic extract of green walnut husk (GWH) in hypercholesterolemic rats. A total of thirty male Albino Wistar rats (*Rattus norvegicus domestica*) were divided randomly into six equal groups. Group 1, negative control, fed on a standard rat diet whereas groups 2–6, hypercholesterolemic rats, fed a high-fat diet (1% cholesterol in a standard diet). Group 2, positive control, was left untreated, whereas the groups 3–5 treated orally with methanolic extract of GWH at 200, 400, and 800 mg/kg/day BW, respectively. Group 6, treatment control, received atorvastatin intraperitoneally at a dosage rate of 0.8 mg/kg/day. The treatment lasted for 84 days. Lipid profiles, biomarkers for liver and kidney functions, some hematological parameters, and liver histopathological assessment were performed. No significant variation was observed on lipid profile values after 42 days of GWH intake; while after 84 days, there was significant reduction (P<0.05) in cholesterol, LDL, and triglycerides and significant increase (P<0.05) in HDL. On day 42, the GWH intake revealed no ameliorating effect on ALT, AST, ALP, serum creatinine, and blood urea nitrogen (BUN); while on day 84, the GWH at 400 and 800 mg/kg BW reduced liver injury enzymes and serum creatinine levels but not the BUN. The GWH showed no significant effect on RBC, HGH, HCV, MCH, and MCHC counts; however, the WBCs count of all experimental groups showed significant (P<0.05) increase when compared to negative control. In comparison with other experimental groups, the 800 mg/kg GWH group and the treatment control group exhibited significant decrease (P<0.05) in HCT. The histopathological findings of the liver showed that the 800 mg/kg BW dosage rate of GWH was efficient in ameliorating the adverse tissue changes noticed in the positive control and other experimental groups. It can be inferred that GWH at dosage rate 200, 400, and 800 mg/kg BW have a potential antidyslipidemic effect in dose and period dependent manner. Further investigation to identify the safety of GWH for long standing using against hyperlipidemic patients is required.

Keywords: green walnut husk, hyperlipidemia, cholesterol, albino Wister rats

INTRODUCTION

Cholesterol is a waxy material found in the body and cells, synthesized mainly by the liver, which travels in the body or blood as bundled packets which are called lipoproteins. It is the most abundant steroid in animals and is mainly a cell membrane and lipoproteins' constituent (1). Cholesterol is a lipid compound consisting of four hydrocarbon rings (designated as A to D) attached to an

eight-carbon, branched hydrocarbon chain at carbon 17 (2). Ring A has a hydroxyl group at carbon 3, and ring B has a double bond between carbons 5 and 6 (3). Cholesterol is a structural constituent of animal cell membranes, regulating their fluidity, and is a building block of vitamin D, bile acids, and steroid hormones (4). Therefore, it is essential for and should be supplied to the body (5). The total cholesterol (TC) is divided into chylomicron, high-density lipoprotein (HDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), and triglycerides (1).

Hypercholesterolemia (high cholesterol) has a role in the development of atherosclerosis, which is one of the leading causes of death globally (6). Hypercholesterolemia occurs in the body when the level of cholesterol exceeds the plasma cholesterol levels. The risk of cardiovascular diseases (CVD) increases when too much cholesterol circulates in the blood. CVDs include heart disease, heart attack, stroke, atherosclerosis, peripheral vascular disease (PVD), and coronary artery diseases. Cholesterol can create sticky deposit material that is called plaque through the artery walls. Plaque causes stenosis in blood vessels, which reduces or prevents blood flow to the brain, heart, and other vital organs (7). Hypercholesterolemia is related to endothelial cell dysfunction because cholesterol alters vascular structure and function as it builds within the vascular wall's lining and can interlope with endothelial function. These effects cause lesions, plaques, occlusion, emboli, and delayed healing and recovery due to endothelial cell dysfunction (8). Antioxidants are one of the most efficient approaches to prevent lipid oxidation in hypercholesterolemia patients. Lately, some studies have been established, reporting the adverse effects of synthetic antioxidants such as carcinogenesis (9). Therefore, they turn toward the use of natural antioxidants from plants as a replacement for synthetic antioxidants.

Plant-based materials, particularly nuts, have gotten a lot of attention and interest in recent years. Different plants have many beneficial medical compounds and can be used as antioxidant, antibacterial, antiviral, and antiradical agents (10). The green husk of *Juglans mandshurica* Maxim. is also beneficial in medicine due to its antioxidant, antitumor, and antibacterial characteristics (11). Green walnut husk (GWH) contains diarylheptanoids, flavonoids, hydrolyzable tannins, hydroxybenzoic acids, hydroxycinnamic acids, naphthalene, naphthoquinone glycosides, naphthoquinones, α-tetralone dimers, α-tetralones glycosides, α-tetralones, ceramides, alkanes, steroids, triterpenoids, sesquiterpenes, and neolignans (12). The medicinal benefits of the GWH as an antihyperlipidemic natural compound have not been investigated. Accordingly, this study was designed to elucidate the effects of oral GWH extract on rats fed on a high-fat diet. The extract's effects on hematological profiles, serum lipid profiles, and enzymatic activity in high-cholesterol diet-induced hyperlipidemic rats were also investigated. The study also assessed the pathological changes in the liver after administering different GWH extract dosage rate.

MATERIALS AND METHODS

Experimental Animals

The experiment was conducted on 30 healthy male Albino Wistar rats (*Rattus norvegicus domestica*), weighing 150-180 g and aged 10–12 weeks. All animals were housed in polypropylene cages in a temperature-controlled room (22–24°C) on a light/dark cycle of 12 h. All rats had free access to food and water. The feeding pots and water bottles were washed and refilled daily, and the rats were kept for one week before the experiments to acclimatize. The animals were provided by the College of Veterinary Medicine, University of Sulaimani, Kurdistan, Iraq. The Ethics Committee at the College of Veterinary Medicine, University of Sulaimani, reviewed and approved the experimental protocol (approval number 2020/18).

Preparation of GWH Methanolic Extract

The ground husk was soaked in 80% methanol (Fisher Chemical™) at a rate of 1:12 (w/v). The mixture was put in an automatic shaker incubator (Lab Tech, Model: LCB-901V, Korea) at 25°C and 200 rpm for 48–72 h, after which it was filtered using filter paper (Whatman No. 4). The filtered yellow solution was put in the rotary evaporator (Heidolph, Germany) at 35°C and 5 rpm to remove the methanol. The aqueous residue was put into a dark container at -20°C. After that, the aqueous residue was lyophilized for 48 h in a freeze dryer (Christ LCG, Germany) to obtain the powder (13).

Preparation of 1% High-Cholesterol Diet

Cholesterol (Sigma-Aldrich, USA) was added to rat pellets at a rate of 1% to make hypercholesterolemic diets (HCDs). A freshly prepared cholesterol-rich diet was served daily, rat chow diet water and a 1% cholesterol-rich diet/head/day (14).

The Dosage Rate of Atorvastatin

The dosage rate for humans is one tablet (10 mg/70 kg) every day. As a result, the dosing rate is around 0.14 mg/kg. Through multiplication by 6.2, this dose is converted to an animal equivalent dose (AED). Thus, the dosing rate for a rat was around 0.8 mg/kg**.**

Preparation of Atorvastatin Solution for Injection

Atorvastatin (Zocor®; Merck and Co., Inc, Whitehouse Station, NJ, USA) solution was prepared fresh daily by dissolving a 10 mg tablet in 12.5 mL of sterilized distilled

water, making a final concentration of 0.8 mg/mL, and the dosing volume is 0.1 mL/100 g BW rat. The tablet was dissolved by shaking for a few min.

Experimental Protocols

Before starting the experiment, thirty rats were divided into six groups, each containing five rats. The first group (negative control) was given normal saline alone. Groups 2–6 fed a high-fat diet (HFD) to produce hyperlipidemia. Group 2 (positive control) was left without treatment, while groups 3–5 (treatment groups) received orally the methanolic extract of GWH at dosage rates of 200, 400, and 800 mg/kg/day BW, respectively. Group 6 (treatment control) was treated with 0.8 mg/kg atorvastatin. The total duration of the study was 84 days.

Blood Samples

On day 42, blood samples (2 mL) were collected from the periorbital sac, while on day 84, the blood was collected from the caudal vena cava after anesthetizing the rats by intraperitoneal injection of a mixture of ketamine (Ketalar, 75 mg/kg), and xylazine (AnaSed, 2 mg/kg). The blood was collected into two types of tubes: plain tubes for the biochemical analyses of serum, and ethylenediaminetetraacetic acid (EDTA) tubes for hematological analyses. The internal organs were inspected for gross pathological changes. The liver was washed with normal saline, weighed, and processed for histopathological examination (15).

Hematology

Blood samples collected from the periorbital sac and caudal vena cava in EDTA tube were used for hematological studies. The analyzed parameters were erythrocytes count, hematocrit, hemoglobin concentration, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), platelets and total leukocytes count using an automated hematology analyzer (Abbott CELL-DYN® 3700, USA).

Serum Biochemical Tests

The degree of hypercholesterolemia in all rats was measured after 42- and 84-days intervals of the experiment. Serum was collected from blood samples in plain tubes by centrifugation of the blood at 3000 rpm. The serum samples were analyzed for lipid profiles (total cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), biomarkers for liver (alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and kidney function (creatinine, blood urea nitrogen (BUN)) using a Hitachi 902 automatic chemistry analyzer (Hitachi Ltd, Tokyo, Japan) and standard reagents from Roche.

Histopathology

Histopathological examination of liver was performed on day 84. Liver samples were excised and sliced into small sections with a thickness of about 0.5–2.0 cm and fixed for 48 h in 10% formalin. Following that, the samples were appropriately washed with tap water, dehydrated using a series of increasing alcohol concentrations, and cleared by xylene solution, followed by paraffin liquid infiltration, and embedding in molten paraffin liquid. Sections of 4 µm were obtained using a semi-automated microtome (Leica Jung, 2045 Multicut, Germany). The liver sections were deparaffinized by immersing twice in xylene (five min per round) and rehydrated by three ethanol grades (100%, 90%, and 70%) for five min each. The sections were stained with Harris's hematoxylin and eosin (Sigma-Aldrich) method. Finally, the slides were ready for microscopic examination and evaluation with different light microscopy (Olympus, Tokyo, Japan) magnifying powers (16).

Statistical Analysis

Data were shown as mean ± SEM (standard error mean), and statistical analysis was done using IBM SPSS software for Windows version 24 (IBM Corp., Armonk, NY, USA). A one-way ANOVA followed by Duncan's post-hoc multiple comparisons test was used, and differences were regarded as significant at $P \leq 0.05$.

RESULTS

Lipid Profile on Day 42

There were significant increases ($P<0.05$) in cholesterol, triglyceride, LDL, and VLDL observed in the positive control rats compared to the negative control group (Table 1). In contrast, a significant decrease in HDL was observed in the positive control. Moreover, all other groups revealed notable increases in cholesterol, triglyceride, LDL, and VLDL, while the HDL levels decreased significantly after 42 days of feeding on a high-cholesterol diet.

Lipid Profiles on Day 84

The positive hyperlipidemic control group revealed a significant increase ($P<0.05$) in cholesterol, triglyceride, LDL, and VLDL compared to the negative control group. The positive hyperlipidemic control group showed a significant decrease in the HDL level compared to the negative control. Moreover, the groups treated with different doses of GWH extract and atorvastatin revealed a prominent decrease in cholesterol, triglyceride, LDL, and VLDL levels. In contrast, the HDL level increased significantly in the treated hyperlipidemic groups (Table 2).

Table 1. Effect of methanolic extract green walnut husk (GWH) on lipid profiles after 42 days of the experiment[1]

Groups	Parameters				
	Cholesterol(mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Negative Control	75.0±4.15 [b]	51.8±2.03 [b]	18.2±1.52 [ab]	16.0±1.04 [ab]	10.4±0.40 [c]
Positive Control	361±13.4 [a]	290±15.6 [a]	10.8±1.28 [b]	24.4±2.20 [a]	57.9±3.12 [b]
200 mg/kg GWH	366±8.71 [a]	333±14.2 [a]	12.0±1.30 [b]	22.6±1.69 [ab]	66.5±2.84 [a]
400 mg/kg GWH	359±20.1 [a]	355±10.6 [a]	11.0±1.40 [b]	22.0±0.89 [a]	71.0±2.11 [a]
800 mg/kg GWH	341±20.9 [a]	332±16.3 [a]	12.6±1.60 [b]	23.6±1.72 [a]	66.4±3.26 [a]
0.8 mg/kg atorvastatin	361±13.7 [a]	329±12.2 [a]	13.0±1.87 [b]	25.6±2.18 [a]	65.8±2.44 [a]

[1]Mean±SEM, n=5. [a-e]Means within a column lacking a common superscript differ significantly ($P \leq 0.05$)

Table 2. Effect of methanolic extract green walnut husk (GWH) on lipid profiles after 84 days of the experiment[1]

Groups	Parameters				
	Cholesterol(mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Negative Control	74.8±6.14 [d]	81.0±4.14 [d]	19.4±0.50 [b]	12.6±1.20 [d]	16.2±0.82 [ad]
Positive Control	495±22.5 [a]	388±3.20 [a]	10.2±1.15 [c]	30.6±0.92 [a]	77.6±0.64 [a]
200 mg/kg GWH	446±22.0 [b]	333±13.6 [b]	11.4±0.92 [c]	26.2±1.39 [b]	66.6±2.71 [b]
400 mg/kg GWH	280±17.4 [c]	279±24.2 [b]	12.4±0.67 [c]	20.4±1.02 [c]	55.8±4.84 [c]
800 mg/kg GWH	244±17.3 [c]	285±26.0 [c]	16.2±1.65 [ab]	18.4±1.91 [c]	60.7±4.56 [bc]
0.8 mg/kg atorvastatin	104±6.30 [d]	96.2±4.46 [d]	29.0±4.32 [a]	12.4±1.69 [d]	19.2±0.89 [d]

[1]Mean±SEM, n=5. [a-e]Means within a column lacking a common superscript differ significantly ($P \leq 0.05$)

Complete Blood Count on Day 84

Results of complete blood counts are presented in Table 3. There were no significant ($P>0.05$) differences in RBC, HGH, HCV, MCH, and MCHC of all experimental groups. WBCs of positive control, groups treated with 200, 400, 800 mg/kg BW GWH, and atorvastatin treated group showed significant ($P<0.05$) increase when compared to negative control (Table 3). Additionally, only animal group administered 800 mg/kg BW GWH methanolic extract, and atorvastatin treated group at dose 0.8 mg/kg exhibited significant decrease ($P<0.05$) in HCT in comparison with other experimental groups.

Liver and Kidney Functions on Day 42

The positive hyperlipidemic control rats exhibited significant ($P<0.05$) increases in the serum activity of ALT, AST, ALP, serum creatinine, and BUN compared to negative control rats (Table 4). The high-fat caused significant increases in ALT, AST, and ALP in rat groups treated with 200, 400, and 800 mg/kg/day BW. However, serum creatinineand BUN did not increase significantly ($P<0.05$).

Liver and Kidney Functions on Day 84

The untreated hyperlipidemic rats (group 2) exhibited significant ($P<0.05$) increases in the serum activity of ALT, AST, ALP, serum creatinine, and BUN when compared to negative control rats (Table 5). The hyperlipidemic treated groups with GWH methanolic extract at an 800 mg/kg BW dose showed significant ($P<0.05$) reductions in ALT, AST, ALP, and serum creatinine compared to those treated with 200 and 400 mg/kg BW GWH methanolic extract. However, no significant differences were observed between the groups treated with 200 and 400 mg/kg BW GWH methanolic extract. The hyperlipidemic treated group with atorvastatin showed significant decreases in ALT, AST, ALP, and serum creatinine compared to rat groups treated by the GWH methanolic extract.

Table 3. Effect of methanolic extract of green walnut husk (GWH) on hematological parameters of all groups after 84 days of the experiment[1]

Groups	Parameters						
	WBC (10^9/L)	RBC (10^{12}/L)	HGH(g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
Negative Control	7.49±0.16 [b]	7.01±0.35 [a]	14.0±0.32 [a]	41±0.27 [bc]	55.3±1.57 [a]	19.7±0.61 [a]	34.3±0.43 [a]
Positive Control	10.6±0.87 [a]	7.51±0.33 [a]	13.9±0.64 [a]	43±1.16 [ab]	56.9±1.54 [a]	20.8±0.34 [a]	33.6±0.22 [a]
200 mg/kg GWH	10.8±0.41 [a]	7.16±0.33 [a]	14.0±0.23 [a]	42±0.47 [abc]	56.0±0.97 [a]	19.9±0.55 [a]	34.1±0.56 [a]
400 mg/kg GWH	10.3±0.52 [a]	6.99±0.37 [a]	13.5±0.27 [a]	41±1.92 [c]	55.3±0.65 [a]	19.3±0.65 [a]	34.8±0.98 [a]
800 mg/kg GWH	9.76±0.53 [a]	7.10±0.18 [a]	13.4±0.89 [a]	37±0.95 [d]	55.1±1.44 [a]	19.9±0.77 [a]	33.2±0.49 [a]
0.8 mg/kg atorvastatin	10.2±0.45 [a]	7.41±0.17 [a]	12.8±1.09 [a]	36±3.24 [d]	59.2±2.42 [a]	20.6±0.71 [a]	33.4±1.25 [a]

[1]Mean±standard error mean, n=5. [a-e]Means within a column lacking a common superscript differ significantly ($P \leq 0.05$)

Table 4. Effect of methanolic extract of green walnut husk (GWH) on liver and kidney functions of all groups after 42 days of the experiment[1]

Groups	Parameters				
	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Creatinine(mg/L)	BUN (mg/L)
Negative Control	71.2±4.24 ^{e}	161±12.2 ^{e}	213±26.7 ^{e}	0.40±0.03 ^{a}	52.8±2.08 ^{a}
Positive Control	290±38.0 ^{bc}	286±8.32 ^{c}	354±14.6 ^{b}	0.46±0.02 ^{a}	55.4±5.04 ^{a}
200 mg/kg GWH	273±19.6 ^{c}	288±25.5 ^{c}	335±29.5 ^{cb}	0.44±0.02 ^{a}	60.6±2.67 ^{a}
400 mg/kg GWH	296±26.1 ^{bc}	342±8.59 ^{bd}	355±6.84 ^{b}	0.46±0.03 ^{a}	56.8±1.85 ^{a}
800 mg/kg GWH	319±16.3 ^{a}	367±25.2 ^{a}	374±21.8 ^{a}	0.46±0.02 ^{a}	57.8±2.20 ^{a}
0.8 mg/kg atorvastatin	214±44.1 ^{d}	255±36.9 ^{d}	307±30.2 ^{d}	0.46±0.04 ^{a}	58.2±1.93 ^{a}

[1]Mean±standard error mean, n=5. $^{a-e}$Means within a column lacking a common superscript differ significantly (P≤0.05)

Table 5. Effect of green methanolic extract of walnut husk (GWH) on liver and kidney functions of all groups after 84 days of the experiment[1]

Groups	Parameters				
	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Creatinine(mg/L)	BUN (mg/L)
Negative Control	122±21.2 ^{e}	187±21.9 ^{e}	273±22.8 ^{d}	0.44±0.02 ^{c}	59.2±1.77 ^{b}
Positive Control	382±48.7 ^{a}	439±42.6 ^{a}	508±55.1 ^{a}	0.56±0.02 ^{a}	69.2±2.78 ^{a}
200 mg/kg GWH	369±17.2 ^{a}	400±28.1 ^{b}	489±25.6 ^{a}	0.56±0.02 ^{a}	69.0±1.76 ^{a}
400 mg/kg GWH	292±24.7 ^{b}	315±19.5 ^{c}	360±10.2 ^{c}	0.50±0.03 ^{a}	60.8±2.63 ^{b}
800 mg/kg GWH	245±31.0 ^{c}	309±22.2 ^{c}	452±29.3 ^{b}	0.48±0.02 ^{b}	62.4±5.76 ^{b}
0.8 mg/kg atorvastatin	215±37.4 ^{d}	254±40.4 ^{d}	328±45.5 ^{c}	0.50±0.04 ^{b}	57.6±2.97 ^{c}

[1]Mean±standard error mean, n=5. $^{a-e}$Means within a column lacking a common superscript differ significantly (*P*≤0.05)

Histopathological Findings

The microscopic examination of liver sections of the negative control rat exhibited normal hepatolobular structure represented by normal hepatocytes, Kupffer cells, central vein, sinusoids, and portal tracts containing branches of the portal vein, bile duct and hepatic artery (Figure 1). Liver sections of the positive control group showed congestion of the central and portal veins associated with focal infiltration of mononuclear inflammatory cells and marked narrowing of the sinusoids due to severe swelling of the hepatocytes which showed marked cytoplasmic vacuolation (Figure 2). The hyperlipidemic rats treated with 200 mg/kg BW GWH methanolic extract showed hepatocellular swelling associated with cytoplasmic vacuolation, sinusoidal narrowing and focal infiltration of mononuclear inflammatory cells (Figure3). The hyperlipidemic rats treated with 400 mg/kg BW GWH methanolic extract showed slight hepatocellular swelling, bile duct hyperplasia and focal infiltration by mononuclear inflammatory cells (Figure 4). The liver section of hyperlipidemic rats treated with 800 mg/kg BW GWH methanolic extract of showed mild congestion of the central vein, sinusoids, hepatic artery and portal vein and focal infiltration of the periportal area by mononuclear inflammatory cells (Figure 5). Hyperlipidemic rats treated with atorvastatin medication (treatment control) showed slight congestion of the portal veins, lack of vacuolar degeneration of parenchymal cells, and no inflammatory cells were present in the peri-portal area (Figure 6).

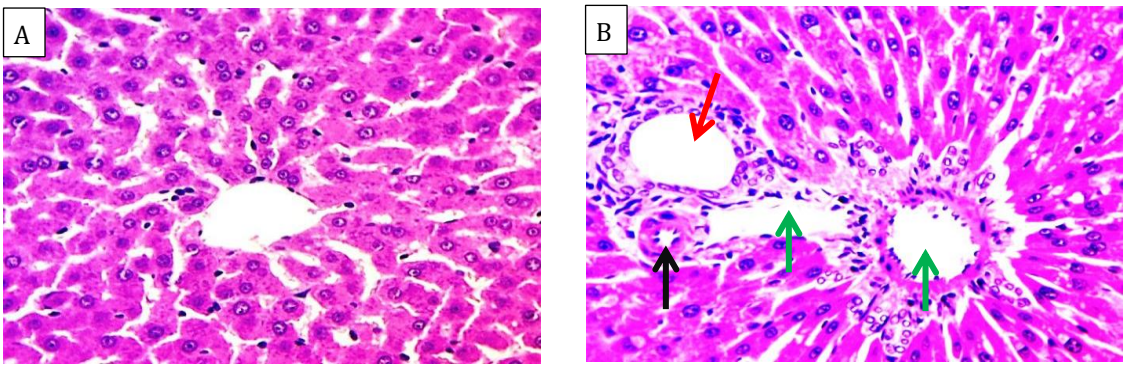

Figure 1. Liver sections of control negative rats (Group 1, fed on a standard rat diet) showing normal hepatolobular structures. A: Normal hepatocytes and Kupffer cells surrounding the central vein in the centrilobular area. B: Normal hepatocytes surrounding branches of the hepatic artery (black arrow), portal vein (green arrow) and bile duct (red arrow) in the portal area. Moderate infiltration of inflammatory cells around blood vessels at the portal area is observed. H&E, 40

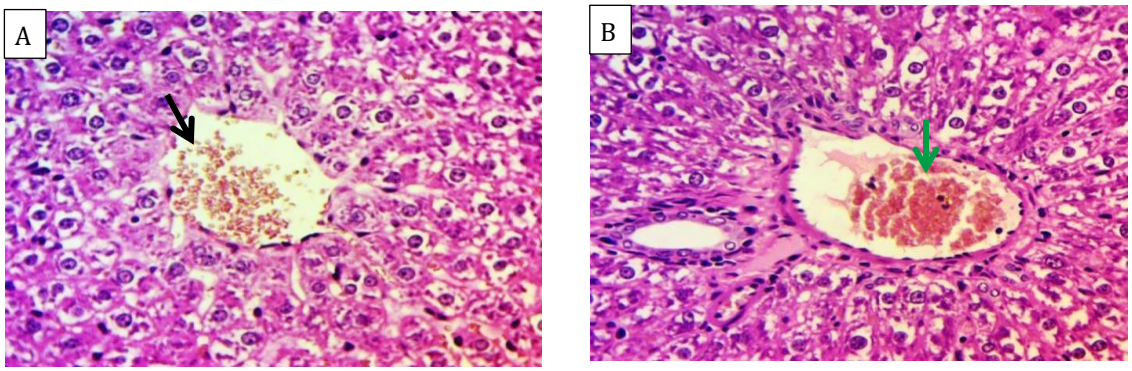

Figure 2. Liver sections of control positive rats (Group 2, fed on 1% cholesterol diet and left without treatment). A: Congestion of the central vein (black arrow) and marked cytoplasmic vacuolation and swelling of the hepatocytes. B: Marked cytoplasmic vacuolation and swelling of the hepatocytes, and congestion of the portal vein (green arrow) associated with focal infiltration of mononuclear inflammatory cells. H&E, 400×

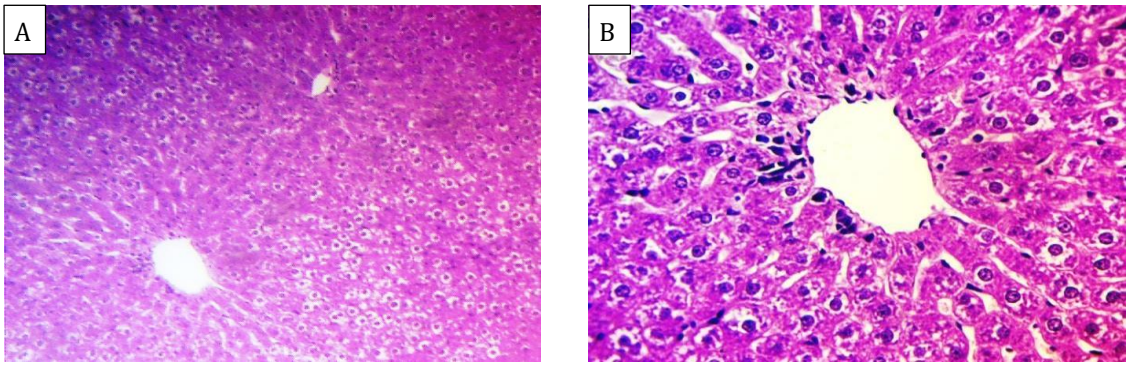

Figure 3. Liver sections of rats fed on 1% cholesterol diet and treated orally with methanolic extract of green walnut husk, 200 mg/kg BW/day (Group 3). A: Marked cellular swelling and severe narrowing of the sinusoidsparticularly in the midzonal and periportal regions of the illustrated hepatic lobule. B: Marked cytoplasmic vacuolation of the hepatocytes and a focal infiltrate of mononuclear inflammatory cells. H&E, A: 100×, B: 400×

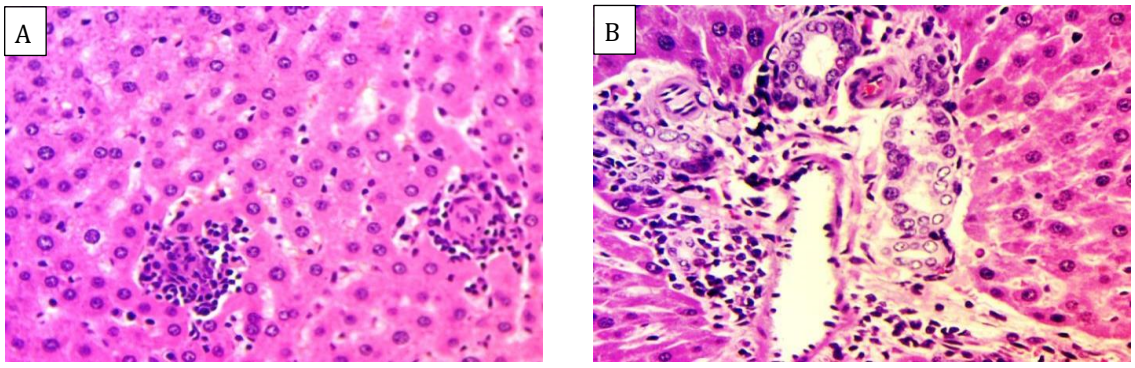

Figure 4. Liver sections of rats fed on 1% cholesterol diet and treated orally with methanolic extract of green walnut husk, 400 mg/kg BW/day (Group 4). A: Mild hepatocellular swelling and focal infiltration with mononuclear inflammatory cells. B. Bile duct hyperplasia and focal infiltration by mononuclear inflammatory cells. H&E, 400×

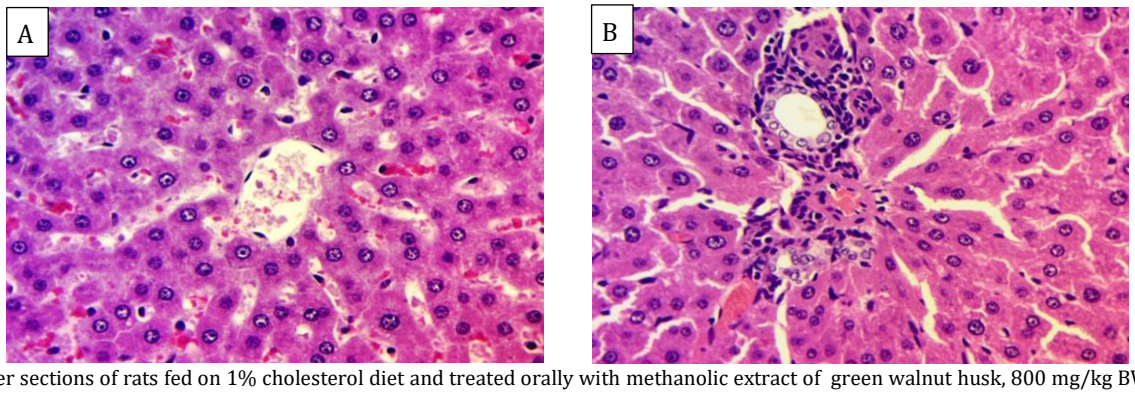

Figure 5. Liver sections of rats fed on 1% cholesterol diet and treated orally with methanolic extract of green walnut husk, 800 mg/kg BW/day (Group 5). A: Mild congestion of the central vein and hepatic sinusoids. B. Focal infiltration of the portal area by mononuclear inflammatory cells and slight congestion of the hepatic artery and portal vein branches. H&E, 400×

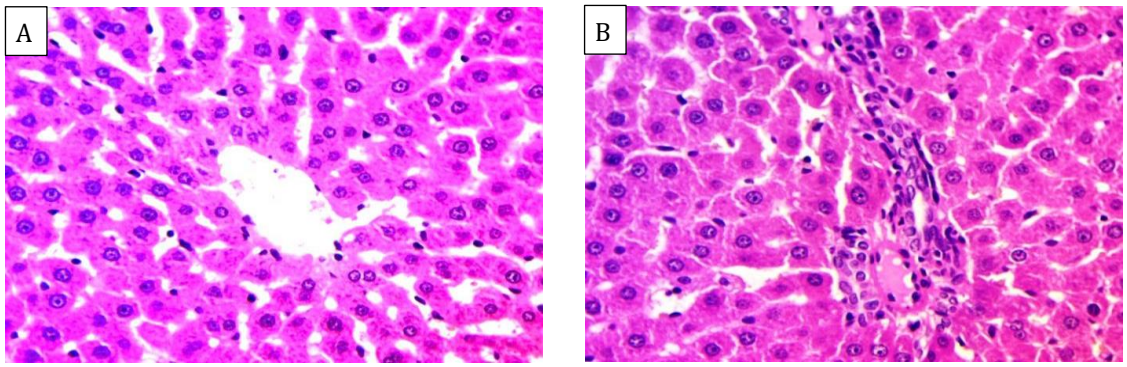

Figure 6. Liver sections of rats fed on 1% cholesterol diet and treated intraperitoneally with 0.8 mg/kg BW/day atorvastatin (treatment control, Group 6). A: Normal hepatolobular structure. B: Slight congestion of the portal veins associated with slight, focal infiltration with mononuclear inflammatory cells. H&E, 400×

DISCUSSION

Different compounds such as phenolic acids, flavonoids, fiber, micronutrients, (such as vitamins C, E, and B9, minerals, such as Cu, Ca, K, and Mg), plant protein, plant sterols, and carotene are available in the green walnut husk and can be potential mechanisms through which green walnut husk may enhance lipid profiles (17). Green walnut husk fiber can assist with lipid and cholesterol intake and metabolism, and hence, it has a beneficial effect. Current findings confirm the beneficial effect of green walnut husk on blood lipid levels in hypercholesterolemic rats. Triglycerides and LDL-C levels decreased in rats treated with walnut leaf extracts. They have also shown that HDL-C was increased by treatment with walnut leaf (18). Flavonoids can induce glycogenesis, inhibit the K channel in the pancreas' beta cells, and control the intestinal intake of sugar. It can also correctly distribute the lipid profile and prevent lipids and cholesterol accumulation (19).

The green walnut husk is an excellent source of polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs), which have a beneficial impact on blood lipids and lower overall cholesterol and LDL-C levels (17). This study demonstrated that GWH, a natural plant with antioxidant propriety, expeditiously inhibits HFD-induced liver injury and hyperlipidemia by stimulating the lipid clearance and antioxidant defense system and regulating inflammatory cytokines secretion. Many studies have shown that high-cholesterol diets may increase TG, TC, VLDL, and LDL and decrease HDL, known as risk factors for cardiovascular diseases (20).

Our experimental results discovered that HFD-fed rats developed hyperlipidemia. GWH decreased total serum cholesterol, LDL triglycerides, and VLDL and protected the system by increasing HDL levels in hyperlipidemic rats. It is investigated that LDL, AST, ALT, and ALP levels in HFD-fed rats and noticed that these levels were brought down significantly upon GWH supplementation compared to atorvastatin.

Literature shows that antiatherogenic HDL has multiple biological roles, such as stimulating cholesterol efflux and reverse lipid transport, inhibiting LDL oxidation, reducing toxic phospholipids, and showing anti-inflammatory activity (21). Thus, the HDL promoting activity of atorvastatin is exerted by the lowered levels of these markers. Also, oxidation of LDL and their further uptake by macrophages result in foam cell formation, a decisive step in initiating atherosclerotic consequences (22).

BUN may be used to measure renal function, but it is not as precise as creatinine. Since BUN is affected by a high-protein diet, protein synthesis variables, and patient hydration status, it is not the preferred marker for clearance. BUN is not the best marker for GFR on its own (23). Hence, serum creatinine and blood urea parameters slightly decreased during 42 days of GWH administration compared to the positive control group.

In this study, the liver's histopathological examinations helped identify alternations in the liver's histological sections, such as the features of the hepatocytes, portal area, hepatic artery or vein, and bile ducts. Congestion of the central and portal vein occurs due to any factor, such as right ventricular heart failure, leading to hepatic congestion and injury by three mechanisms: decreased liver blood flow, reduced blood oxygen saturation, and elevated hepatic venous pressure (24).

Raised central venous pressure affects the hepatic veins and sinusoids, causing decreased portal venous inflow. Elevated hepatic venous pressure leads to sinusoidal congestion, dilation of sinusoidal fenestrae, and protein and fluid exudation into the extravascular space. Accumulation of this exudate leads to impairment of oxygen and nutrient diffusion into the hepatocyte (25).

Congestion of central and portal veins was observed in all rats with fat diets but in various levels, while administration of GWH, especially 800 mg/kg BW, reduced the congestion. There were also inflammatory cell infiltrations inside the liver cells, a sign of free radical agents infiltrating the liver cells. These inflammatory cells seem to have been activated to help the already damaged liver tissue. Additionally, minor, and subacute liver damage was diffusely scattered among the hepatocytes, which was shown to be swelling with non-lipid cytoplasmic

vacuolation. Several research lines suggest that this transition may be a positive cellular adaptation rather than a degenerative change (26). According to this study, the GWH extract partially reversed the liver pathology by regulating hepatocellular necrosis, preventing cellular infiltrations, and preventing vacuolation.

Administration of GWH at oral dosages of 200, 400, and 800 mg/kg BW as a preventive measure and as a supplementary therapy with atorvastatin following the formation, growth, and establishment of atheromatous plaques had significant antiatherogenic reducing the lesion's severeness in a time- and dose-dependent manner. GWH also suppressed lipid peroxidation and oxidative stress injury, resulting in a substantial reduction in serum lipid profile. Supplementation of GWH to a high cholesterol diet for 42 days as a prophylactic step resulted in reduced hyperlipidemia and comparatively lowered the atherosclerotic lesions' grades in a dose-dependent manner. The safety profile of GWH warrants further analysis to establish the beneficial effect of this herbal product against the production of early hypercholesterolemia when used at a higher dose regimen for a more extended period.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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دور قشور الجوز الأخضر في تخفيف الآثار السامة التي يسببها النظام الغذائي عالي الدهون في الجرذان

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الخلاصة

يُعد فرط كوليسترول الدم أحد العوامل الخطرة والمهمة للإصابة بأمراض القلب والأوعية الدموية.تعد مضادات الأكسدة من العلاجات الأكثر فعالية في مواجهة فرط كوليسترول الدم. أظهرت العديد من الدراسات أن قشور الجوز الأخضر له خصائص مضادة للأكسدة، لذلك تم تصميم البحث الحالي لاختبار هذا التأثير في الجرذان المصابة بفرط كوليسترول الدم. تم استخدام ثلاثين من ذكور الجرذان المهقاء *Rattus norvegicus* (*domestica* بعمر ١٠-١٢ أسبوعًا ووزن ١٥٠-١٨٠ جم وتم تقسيمها بالتساوي إلى ست مجموعات. كانت المجموعة الأولى هي المجموعة الضابطة غير المعالجة، حيث كانت تتبع نظامًا غذائيًا قياسيًا للجرذان. تناولت المجموعات من ٢ إلى ٦ نظامًا غذائيًا عالي الدهون (١٪ كوليسترول في نظام غذائي قياسي). أعطيت الجرذان المصابة بفرط كوليسترول الدم (المجموعات ٣-٥) وجبات منتظمة تحتوي على ٢٠٠ و ٤٠٠ و ٨٠٠ ملغم/كغم من قشور الجوز الأخضرعن طريق الفم. أعطيت المجموعة ٦ أتورفاستاتين داخل الصفاق بمعدل جرعة 0.8 ملغم/كغم/يوم. استمر العلاج لمدة ٨٤ يومًا. تم قياس المعلمات الكيميائية الحيوية مثل الدهون الثلاثية والكوليسترول وكوليسترول البروتين الدهني منخفض الكثافة (LDL-C) وكوليسترول البروتين الدهني عالي الكثافة (HDL-C) في عينات الدم المأخوذة من الكيس حول الحجاج (بعد ٤٢ يومًا من التجربة) والوريد الأجوف الخلفي (بعد ٨٤ يومًا من التجربة). أظهرت النتائج أن تناول قشور الجوز الأخضر يقلل من نسبة الكوليسترول، LDL-C ، والدهون الثلاثية مع زيادة HDL-C، ويمكن الاستدلال على أن أوراق الجوز لها آثار مفيدة على مستويات الكوليسترول و الدهون في مصل الجرذان المصابة بفرط كوليسترول الدم ، ويرجع ذلك على الأرجح إلى خصائصه المضادة للأكسدة ، وأنه يمكن استخدامه لتقليل مخاطر الأمراض القلبية الوعائية.

الكلمات المفتاحية: قشر الجوز الأخضر، فرط شحميات الدم، الكولسترول، الجرذان