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ORIGINAL PAPER

Biochemical and cellular (liver and kidney) restorative properties of garlic (*Allium sativum*) aqueous extract in cow brain-induced hypercholesterolemic model Swiss albino mice

Swarup Kumar Kundu [1,2], Md. Abu Hadi Noor Ali Khan [3], Shonkor Kumar Das [2]

[1] Department of Anatomy and Histology, Faculty of Veterinary, Animal and Biomedical Sciences, Khulna Agricultural University, Khulna, Bangladesh
[2] Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh
[3] Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh

ABSTRACT

Introduction and aim. Garlic is one of the most popular traditional medicinal herbs which has a number of desirable health benefits. The study was designed to depict the improvement of serum biochemical parameters as well as the histomorphological recovery potential of garlic aqueous extract in hypercholesterolemic mice.

Material and methods. A total of thirty Swiss albino mice weighing 24±5g and aged 5 weeks were randomly divided into three groups. Group A: supplied standard mice pellet and water; Group B: standard mice pellet + hypercholesterolemic diet (cow brain: 2 g/kg b.w.t.); and Group C: standard mice pellet + hypercholesterolemic diet (cow brain: 2 g/kg b.w.t.) + garlic extract (25 ml/kg b.w.t.). After four weeks of experimental tenure, samples (blood, liver, and kidney) were collected from each group of mice for serum biochemical analysis and histomorphological study.

Results. Compared with hypercholesterolemic mice, total cholesterol (TC), triglyceride (TG) concentration, and low-density lipoprotein (LDL) levels significantly decreased respectively by 7%, 20% and 48% along with high-density lipoprotein (HDL) levels significantly increased by 47% in garlic extract supplemented group. Based on the histological evaluation in the liver sample of group C, both portal and central veins were normal, and fat droplets were not found in the hepatocytes which were found in the liver of group B. On the other hand, unchanged renal cortex, glomerulus, Bowman's space, and kidney tubules were seen in group C.

Conclusion. Therefore, the above findings of the present research would assist to provide affirmation about the cholesterol-decreasing and cellular restoration potentiality of garlic aqueous extract.

Keywords. cellular restoration, garlic extract, health beneficial, lipid profile, Swiss albino mice

The list of abbreviations:

CHD – coronary heart disease, **PART** – peri–renal adipose tissue, **TC** – total cholesterol, **TG** – triglyceride, **HDL** – high–density lipoprotein, **LDL** – low–density lipoprotein, **WHO** – World Health Organization

Introduction

Liver and kidney are the most essential organs in the body for performing multiple functions like excretion of waste products, hormonal regulation, digestion, and detoxification of harmful drugs.[1] According to the latest World Health Organization (WHO) records released in

Corresponding author: Swarup Kumar Kundu, e-mail: swarupkundu95@gmail.com

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2020, about 1.51% of total deaths were found due to kidney disease as well as 2.94% of total deaths due to liver disease in Bangladesh.[2] Diets rich in highly saturated fat have been identified as one of the major risk factors for developing visceral adiposity like cardiovascular problems, fatty liver syndrome, obesity-related glomerulopathy, etc. Obesity is a burden issue nowadays and the liver and kidneys frequently develop obesity-related different disorders.[3] Cow brain is a high-cholesterol meal that is rich in saturated fat.[4-7] People in Bangladesh, in particular, can purchase this commodity from local marketplaces as well as various online retailers. It has been recorded that consumption of the cow brain is a potential means of transmission of prion disease to individuals in different countries who consume cow brain products in various food preparations.[5] Apart from this, people of Bangladesh are involved in various types of fast food culture like beef pizza, chicken patties, beef brain patties, all sweets, chicken or beef burgers, etc. which are considered a source of excess cholesterol, and saturated fat. People are suffering from various coronary artery diseases, obesity problems, fatty liver syndrome, kidney complications, etc. by consuming these food items.[6] Cholesterol-rich food is the key factor in changing liver pathology. Because large amounts of triglyceride accumulate in the hepatocytes for a high-fat diet.[8] In the present research, the potential author focused more on pathological alteration rather than hepatosteatosis. Accumulation of adipocytes and mononuclear cell infiltration in the renal cortices have been seen previously in the high-fat diet rat, but the present author also revealed a few more pathological alterations in the current study.[9] Different studies revealed that garlic bulbs contain a substance called alliin (S-allyl cysteine sulfoxide) which is converted into sulfur-based antioxidant compound allicin when in contact with air. This allicin is the key product of recovering cholesterol levels. Garlic is also commonly used for diabetes mellitus, hypercholesterolemia, and cancer treatment.[10] Some medicinal plants are rich in antioxidants and garlic belongs to this group.[11,12] Garlic extract has the capacity of restoring mesangial cell proliferation in the glomerulus and basement membrane thickening.[11] In addition, garlic extract has a protective effect against cardiac ischemia, diet-induced oxidative damage in hypercholesterolemia, dilated sinusoids, hepatic steatosis, cellular infiltration, etc.[13]

Aim

Therefore, on the basis of the above description main objectives of the present research were to determine:

I. The negative effects of high-cholesterol food (cow brain) on liver and kidney cellular levels and serum biochemical markers.

II. The restorative properties of garlic extract on biochemical profiles as well as the pathological changes of the liver and kidney in diet-induced hypercholesterolemic mice.

Material and methods

Participants and ethical approval

The current research activity was conducted in the Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202. The Ethical Committee of Bangladesh Agricultural University approved the study protocol (AWEEC/BAU/2019-53). Experimental Swiss albino mice at the age of 5 weeks old were collected from International Center for Diarrheal Disease Research (ICDDR'B), Mohakhali, Dhaka, Bangladesh. The initial weight of the collected mice was recorded at about 24±5 g.

Preparation of garlic aqueous extract

Allium sativum, a plant, is the scientific name for garlic. Its aqueous extract is the source of phenolic compounds which are antioxidant and biologically active.[14] A total of 1 kg of fresh garlic (Variety: BARI Roshun 3) was collected from the Bangladesh Agricultural University horticulture center. Then all the papery skin from the outside of the head of garlic was removed. All the cloves of garlic were separated from each other. Then 250 g of garlic cloves were weighed and crushed in mortar and pestle for 1 min together with 500 ml of distilled water and allowed them standing for 1 h at room temperature. Filtration was done through Whitman no. 4 filter paper to remove the impurities and get up to 100 ml of aqueous garlic extract. Finally, the prepared aqueous garlic extract was taken in a glass container, sealed at its mouth, and stored in the refrigerator for future use.

Study design

Before the commencement of the research, a total of healthy 30 collected Swiss albino mice of either sex were reared in 20x30x10 cm plastic box cages at a standard room temperature (23±2°C), 52±5% relative humidity on a 12 h light and 12 h dark cycle were maintained for 7 days. The rearing boxes were filled with sawdust and changed regularly to maintain hygiene and comfort. At that time mice pellet and fresh drinking water ad libitum were supplied to each mouse. After 7 days of acclimatization, collected Swiss albino mice were randomly categorized into three groups and each group consisted of ten (10) mice.

Group A: Considered as the control group, and given normal mice pellets, and fresh water.

Group B: Cow brain at a dose of 2 g/kg b.w.t as hypercholesterolemic food+ mice pellets+ fresh water.

Group C: Hypercholesterolemic food (cow brain at a dose of 2g/kg b.w.t) +garlic extract at a dose of 25ml/kg b.w.t orally + mice pellets +fresh water (Fig. 1)

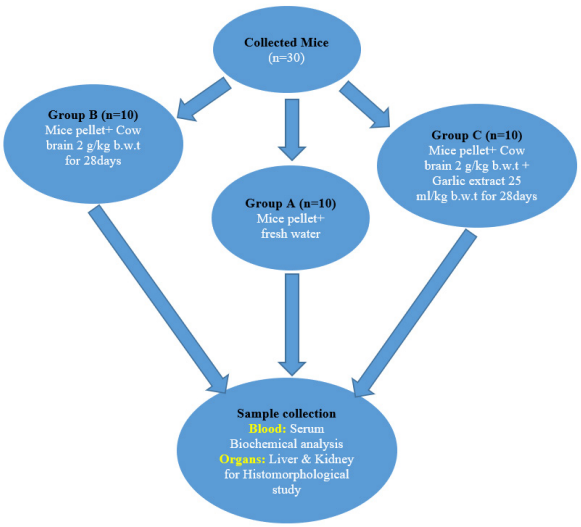

Fig. 1. Graphical distribution of collected Swiss albino mice into different groups

For the 28 days of research tenure, mice of the control group were supplied standard mice pellets (collected from ICDDR'B) and fresh water. In contrast, cow brain at a dose of 2 g/kg b.w.t was given to both groups B and C of mice as hypercholesterolemic food. Cow brain is a definite source of cholesterol, approximately 100g of cow brain contains 3100mg of cholesterol.[4] Eating cow brains in the daily diet is a natural habit of Asian people, especially Bangladeshi that causes pathological changes in the liver and kidney and enhance total cholesterol (TC) and triglyceride (TG) level.[4,8] In addition to hypercholesterolemic feed, mice of group C were also given garlic extract at a dose of 25 ml/kg. Garlic has the potential beneficial effects against hypercholesterolemia. About 8-10% of cholesterol can be reduced by the consumption of 1-2 cloves of garlic in a day.[15] Weight of each group of mice was measured on the 1st, 7th, 14th, 21st, and 28th days of the consecutive days of the study period.

Blood collection, sample processing, and staining

After the experimental tenure (28 days), the mice of each group were sacrificed ethically by anesthesia with isoflurane (2%) and placed on the autopsy plate. The blood sample was taken from the ventricle of the heart and kept in a common blood collection tube with EDTA for preventing coagulation. Then the Liver and Kidney samples were collected from each group of mice and preserved in 10% formalin. Color and weight of the samples were carefully taken into consideration for the gross study. After washing with 0.9% saline the samples were dehydrated in ascending grades of ethanol (70%, 80%, 95%, 100%, 100%, and 100%). The incubation period for ethanol was 2hr. Xylene was used for clearing the tissues. 5-µm thick slices of tissue were taken using an American Optical Spencer model 820 microtome. Finally, hematoxylin and eosin (H&E) staining was done for histopathological analysis of the collected organs. Necessary photographs were randomly taken at 10X and 40X focuses to get better illustration and pictures were captured by photomicroscope (Model: SKU: B120C-E520200610, AMSCOPE LOS ANGLES USA).

Biochemical analysis

Determination of total cholesterol

The TC was determined after enzymatic hydrolysis and oxidation (CHOD-POD method). The indicator quinoneimine was formed from hydrogen peroxide and 4-Aminophenazone in the presence of phenol and peroxidase. Both reagent and sample were kept at room temperature and mixed 1 ml reagent with 10 µl samples in the test tube. Waited for 10 minutes and placed the mixture in the cuvette. The cuvette was placed in a spectrophotometer at 550 nm and recorded the reading. The reading was calculated by comparing it with the standard value and multiplying it by 200 mg/dL. So the result was expressed as mg/dL (catalogue no. of the used reagent: MO-165218).

Determination of triglyceride

The TG was determined after enzymatic hydrolysis with lipase (GPO-POD method). The indicator is a quinoneimine formed from hydrogen peroxide, 4-Aminophenazone, and 4-chlorophenol under the catalytic influence of peroxide. The fatty acid can be hydrolyzed by lipoprotein lipase of an experimental reagent and ultimately produced quinoneimine proportional to triglyceride. This quinoneimine absorbs light of 500 nm. 10 µl samples were mixed with 1ml reagent and kept in a standing position for 15 minutes at room temperature. The mixture was placed in a cuvette of the spectrophotometer and the reading was recorded. This reading was divided by the standard reading and multiplied by 200 mg/dL (catalogue no. of the used reagent: MO-165221).

Determination of LDL-cholesterol

This was assayed in each sample by using RANDOX kit (UK). The reagent and sample were kept at room temperature and mixed 0.4 ml precipitating reagent with 0.2 ml sample. After 10 minutes, this was centrifuged at 12000 rpm for 2 minutes. Then the supernatant was separated. This supernatant was used for LDL determination. 50 **µl** supernatant was mixed with 1 ml cholesterol reagent and stand for 10 minutes at room temperature. This mixture was set in a spectrophotometer by using a cuvette at 500 nm wavelength. The reading was recorded and calculated by comparing standard readings and the fraction was multiplied by 50 mg/dL.

Determination of HDL-cholesterol

The HDL cholesterol was assayed in each sample by using RANDOX kit (UK). Low-density lipoproteins (LDL

and VLDL) and chylomicron fractions were precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol in the HDL fraction, which remains in the supernatant, was determined. The HDL-cholesterol was determined on the principle of estimation of TC (catalogue no. of the used reagent: 1133010).

Statistical analysis

All the collected data were analyzed by using Statistical Package for the Social Sciences (SPSS: version 25, IBM, Armonk, NY, USA) software and revealing the results in tabular form. Statistical analysis was performed using one-way analysis of variance (ANOVA). Results were expressed as mean±SE. Differences between groups were considered significant at $^{**}p<0.001$ and $^{*}p<0.05$ levels.

Results

Body weight

The mean body weight of the hypercholesterolemic group (39.9 g) of mice was significantly higher than the control group (31.4 g) of mice. Garlic extract supplementation successfully maintained the average normal body weight in group C (33.1 g) of mice compared to group B.

Gross morphometric study

Liver assessment

In the current study, no irregular surface, red-brownish color, no detectable pathological lesions, and normal shape of lobes were found in the control group (Group A) of the liver (Fig. 2). Liver become slightly enlarged, and pale yellowish discoloration of the surface was found in the hypercholesterolemic (Group B) group of mice (Fig. 2). In contrast, garlic extract supplementation fully restored the morphological changes in the liver. No detectable abnormalities were seen on the liver surface of group C of mice (Fig. 2). On the other hand, liver weight of the garlic extract-supplemented group was seen as normal compared to the control group of mice but the liver weight of group B of mice was found considerably high (Fig. 3).

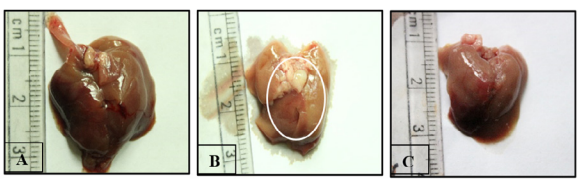

Fig. 2. Gross view of liver of A: control (group A) B: hypercholesterolemic (group B) and C: garlic (group C) extract-supplemented mice. No considerable lesions were observed in the photograph of A and C of the liver. B: Pale and slight hepatomegaly (white oval) seen in the hypercholesterolemic liver

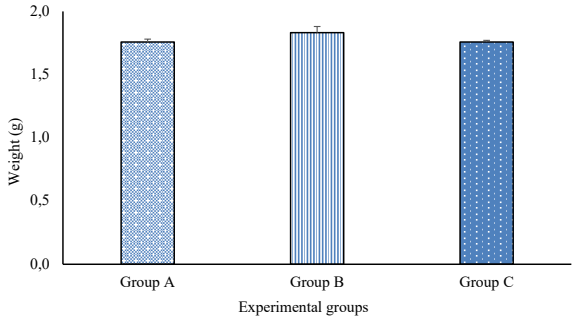

Fig. 3. Diagram represents the liver weight of control (group A), hypercholesterolemic (group B), and garlic extract-supplemented (group C) mice. Liver weight of the garlic extract-supplemented group was shown normal as the control group of mice (mean ± standard error)

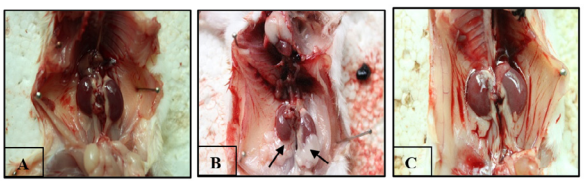

Fig. 4. Gross view of kidney of A: control (group A) B: hypercholesterolemic (group B) and C: garlic (group C) extract-supplemented mice. Surface architecture was observed normal in the photograph of A and C of the kidney. B: Profuse perirenal adipose tissue (black arrow) seen in the hypercholesterolemic kidney

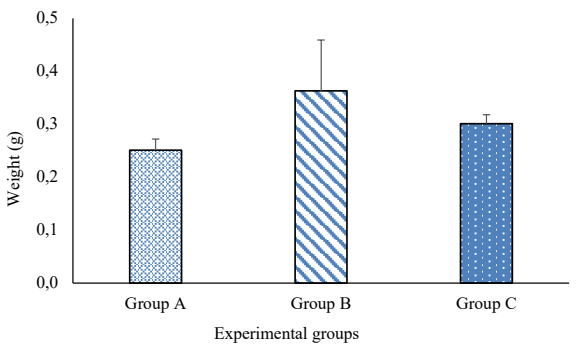

Fig. 5. Diagram represents the kidney weight of control (group A), hypercholesterolemic (group B), and garlic extract-supplemented (group C) mice. Kidney weight of the garlic extract-supplemented group was shown normal as the control group of mice (mean ± standard error)

Kidney assessment

Kidney of the control and garlic extract-treated group of mice showed a normal appearance, brownish-red color, and normal position and shape. No detectable pathological lesions were seen (Fig. 4). Excess peri-renal adipose tissue (PART) accumulated around the lower end of the kidney and the size of the kidney slightly increased compared to other groups of mice (Fig. 4). The weight of the kidney in group C (garlic extract supplemented) of mice

showed normal but hypercholesterolemic feed increased the kidney weight in group B compared to group A of mice (Fig. 5).

Histopathological study

Histological analysis was performed by H&E staining for the confirmation of hepatoprotective and renoprotective effects of garlic aqueous extract.

Hepatoprotective properties of garlic extract

Histological study of the section of the liver of hypercholesterolemic (Group B) mice exhibited narrowing of the portal vein, hypertrophied hepatocytes, radiated hepatocytes from the central vein and formed coherent groups, merging of the central and portal vein which made it difficult to separate them, Kupffer cells in the hepatic sinusoids, mild to moderate congestion in both central and portal vein, dilated central vein, fat droplets in the hepatocytes (Fig. 6). Surprisingly, following the supplementation of garlic extract to group C of mice liver section showed normal central and portal veins, normal hepatocytes (no fat droplets) as well as congestion was not found in the central and portal veins (Fig. 6). Bi-nucleated hepatocytes were found in every section of the liver.

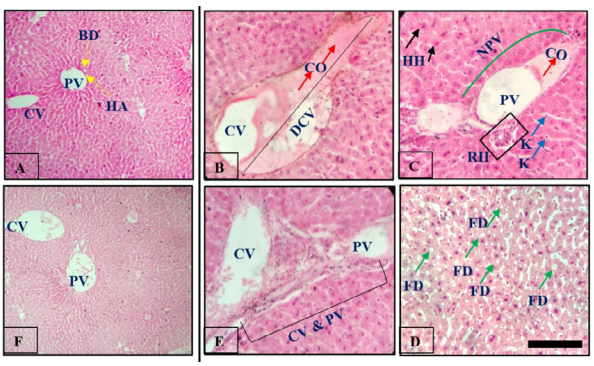

Fig. 6. Transverse section of representative photomicrographs of liver of A: control (group A), B-E: hypercholesterolemic (group B), and F: garlic extract supplemented (group C) mice (H&E stained) at 40X magnification. CV – central vein; PV – portal vein; BD – bile duct; HA – hepatic artery; (B-E): NPV – narrowing portal vein (green arch); HH – hypertrophied hepatocytes (black arrow); RH – radiated hepatocytes (rectangle); CV&PV – merges of the central and portal vein (bracket); K – Kupffer cells (blue arrow); CO – congestion (red arrow); DCV – dilated central vein (line); FD – fat droplets (green arrow); (A, F): Normal CV and PV in the liver of control and garlic extract-supplemented mice. The scale bar stands for 100 µm

Reno-protective properties of garlic extract

Normal architecture of the cortex and medulla was found in the control group of mice. Due to the garlic extract supplementation, glomerular and tubular fatty infiltration, degenerated renal tubules, and hypertensive glomerulosclerosis was successfully restored in group C of mice (Fig. 7). Glomerulus, Bowman's space was also seen as normal like the control group of mice (Fig. 7).

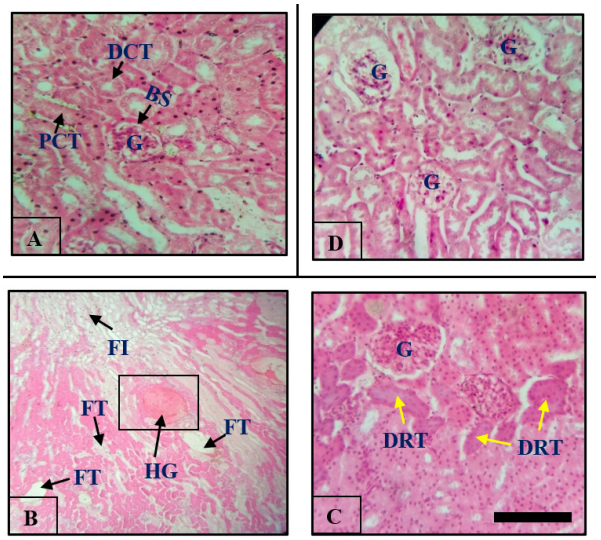

Fig. 7. Transverse section of representative photomicrographs of kidney of A: control (group A), B, C: hypercholesterolemic (group B), and D: garlic extract supplemented (group C) mice (H&E stained) at 40X magnification. DCT – distal convoluted tubule; PCT – proximal convoluted tubule; G – glomerulus; BS – Bowman's space; (B&C): FT – fatty infiltration (black arrow); HG – hypertensive glomerulosclerosis (rectangle); DRT – degenerated renal tubules (yellow arrow); (A, D): Glomerulus, renal tubules, and Bowman's space were found normal. Fatty infiltration and degenerated tubules were also restored by garlic extract supplementation in the C group of mice. The scale bar stands for 100 µm

Study of lipid profile

Improvement of serum lipid profile by garlic extract supplementation

In the present study, the mean value of TC concentration increased by 27% in group (B) (179.044±0.412 mg/dL) (Table 1) compared to group (A) (141.296±0.318 mg/dL) (Table 1) of mice. There was a reduction of TC concentration by 7% in group (C) (166.396±0.249 mg/dL) (Table 1) compared to group (B) of mice. TG concentration increased by 72% in group (B) (231.294±0.258 mg/dL) (Table 1) compared to group (A) (115.108±1.067 mg/dL) (Table 1) of mice. However, group (C) (162.312±1.596 mg/dL) (Table 1) showed a decreased value of TG concentration by 20% compared to group (B) of mice. HDL level (78.906±0.417 mg/dL) (Table 1) decreased by 31% and LDL level (46.544±0.203 mg/dL) (Table 1) increased by 197% in group (B) mice compared to group (A) (94.481±0.395 mg/dL & 24.892±0.273 mg/dL) (Table 1) of mice. Surprisingly, group (C) showed a higher

level of HDL (96.06±0.221 mg/dL) (Table 1) at 47% and a decreased level of LDL (33.02±0.465 mg/dL) (Table 1) at 48% compared to group (B) of mice. Statistically, TC, TG, and LDL levels showed a significant **p<0.001 increase in group B (Fig. 8) compare to control group A, as well as a significant **p<0.001 reductions seen in HDL level of group (B) of mice (Fig. 8). In contrast, garlic extract supplementation (group C) significantly **p<0.001 reduced TC, TG, and LDL concentration and significantly **p<0.001 increased HDL concentration compare to group B (Fig. 8). In addition, the concentration difference of TC, TG, and LDL between the control (group A) and group C was significant at the level of *p<0.05 and LDL concentration was almost similar in both the A and C groups of mice (Fig. 8).

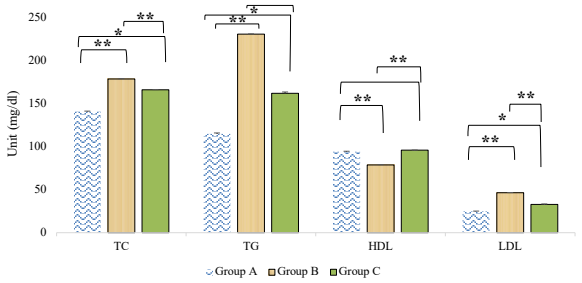

Fig. 8. Diagram represents the analysis of the protective effects of garlic extract in lowering the serum lipid profile. TC – total cholesterol; TG – triglyceride; HDL – high-density lipoprotein; LDL – low-density lipoprotein. Results were expressed as mean ± standard error. (**) indicates a statistically significant difference at the level of **p<0.001; (*) indicates a statistically significant difference at the level of *p<0.05 from the control (group A) of mice

Table 1. TC, TG, HDL, and LDL values in control, diet-induced hypercholesterolemic, and garlic extract-supplemented groups[a]

Parameters (mg/dl)	Group A	Group B	Group C
TC, mg/dL	141.296±0.318	179.044±0.412**	166.396±0.249*
TG, mg/dL	115.108±1.067	231.294±0.258**	162.312±1.596*
HDL, mg/dL	94.481±0.395	78.906±0.417**	96.06±0.221
LDL, mg/dL	24.892± 0.273	46.544±0.203**	33.02±0.465*

[a] (**) and (*) denotes a statistically significant difference at the level of **p<0.001 and *p<0.05 respectively

Discussion

The present study showed that consumption of a cholesterol-rich diet (cow brain) alters the gross and histoarchitecture of the liver, which is in complete agreement with the results of Korish and Arafah. It was reported that regular high-cholesterol feed consumption causes excess energy intake, growing obesity, and a tendency to inactivity. Liver weight was significantly increased compared to the control group. Liver become pale and showed slight hepatomegaly, degenerated hepatocytes, and fatty droplets in the hepatocytes.[16] Kundu et al. reported similar results in their study where cow brain was used as a cholesterol-rich diet.[4,7] Our study exhibited that garlic extract feeding surprisingly restored gross and cellular changes in the hypercholesterolemic liver. Garlic extract supplementation eliminates fat droplet accumulation in hepatocytes and reduces organ weight. The study conducted by Zhang et al. also agrees with our statement. They found a significant reduction in N-nitrosodiethylamine-induced liver weight, surface nodule, and liver enlargement, and improvement in the hepatocellular architecture from hyperplastic nodules and destroyed hepatocytes after garlic oil application during the study period.[17] Tran et al. also speculated in their research, the aqueous extract of garlic reduced the weight and restore the texture of the CCl4-intoxicated liver, and reduced the proliferation of mononuclear cells around the hepatic veins.[18] In addition to this, our current research showed an accumulation of PART, a slight increase in kidney weight, and several fatty changes in the cow brain-induced hypercholesterolemic kidney tubules and glomerulus. In the previous study conducted by Salim et al. stated that a high-fat diet leads to hypercholesterolemia and disturbance of lipid profile, impaired kidney function, and accumulation of fatty droplets in the glomerulus and kidney tubules. There is a close relationship between TG concentration and fat deposition in organs. A higher level of triglyceride is the key factor for the deposition of fat in organs.[19] Increased hypercholesterolemia alters the cellular structure of the tubules and deposits fatty droplets in the tubules that ultimately inhibit cell metabolism and impede filtration.[20] Alansari et al. were also in agreement with our current finding. Tubular deformities, atrophied glomerular capillaries, and vacuolar degeneration in tubules frequently appear due to a high-fat diet.[21] But garlic extract supplementation mitigated the cellular changes induced by a high-fat diet and completely restored fatty infiltration in both tubules and glomerulus and ultimately improved filtration capacity in the current findings. El-Shenawy and Hassan also revealed a statement in a study that garlic extract improved the cellular alteration of kidneys induced by a hypercholesterolemic diet as well as recovered the damages from fatty infiltration in renal tubules.[22]

From the biochemical analysis of the present study, it has been stated that TC and triglyceride TG concentration was significantly reduced by garlic extract consumption. However, this statement was in agreement with Ali and Thomson who mentioned that administration of garlic to hypercholesterolemic rats, humans, and cell cultures is effective in decreasing TC and TG concentration.[23] Sher et al. also observed similar findings. They described in the study, levels of TC (32.8±0.7 mg/dL) and TG (44.0±0.9 mg/dL) concentration significant-

ly ($p<0.001$) decreased after 4 hours of garlic extract administration in rabbits.[24] Adler and Holub also reported in support of the reduction of TC-level and LDL-cholesterol levels respectively by 11.5% and 14.2% by using garlic aqueous extract.[25] Serum TC, LDL, and TG levels were found to be significantly lowered but HDL cholesterol levels increased after regular feeding of garlic extract in the study conducted by Sun et al. and Durak et al.[15,26] Rahman and Lowe also showed that garlic administration suppressed LDL oxidation and increased HDL which may be one of the beneficial effects of garlic in cardiovascular diseases.[27] In our present study, garlic extract supplementation significantly reduced LDL levels which were also revealed in the study of Shela et al., Merat and Fallahzadeh, Aslani et al, and Yeh et al. They observed that garlic administration in rats suffering from hypercholesterolemia induced by a high-cholesterol diet significantly reduced LDL levels.[28-31] In the current study, feeding garlic extract to an induced hypercholesterolemic rat caused a significant rise in HDL levels. This was in agreement with the statement of Aouadi et al.[32] The protective mechanisms of the beneficial effects of garlic in CVDs may be achieved by suppressing LDL oxidation, increasing HDL as well as decreasing TC and TG reported by Katsuki et al. and Gardner et al.[33,34] Therefore, according to the above description, it can be suggested that garlic extract has a protective function to restore serum biochemical parameters and to restore the ability of gross and histomorphological changes of organs (liver and kidney) in hypercholesterolemia induced by a cholesterol-rich diet.

Conclusion

Cholesterol-rich food intake has several adverse health effects including changes in lipid profile, changes in organ weight, fat accumulation in various organs, especially the liver and kidney, various pathological lesions in gross and microscopic aspects, etc. In our society, people are not aware of the tremendous beneficial effects of medicinal herbs like garlic. In the present study, garlic extract supplementation restored the lipid profile, excess organ weight, and histopathological lesions of the liver and kidney, specifically removing fat deposits in the cells. However, further studies on a molecular basis are needed to investigate the pathophysiological mechanism of garlic extract in restoring pathological changes as well as elevated lipid profiles in hypercholesterolemic mice.

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Declarations

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Author contributions

Conceptualization, S.K.K. and S.K.D.; Methodology, S.K.K.; Software, S.K.K.; Validation, S.K.K., S.K.D. and M.A.H.N.A.K.; Formal Analysis, S.K.K.; Investigation, S.K.K., S.K.D. and M.A.H.N.A.K.; Resources, S.K.K. and S.K.D.; Data Curation, S.K.K.; Writing – Original Draft Preparation, S.K.K.; Writing – Review & Editing, S.K.K., S.K.D. and M.A.H.N.A.K.; Visualization, S.K.K.; Supervision, S.K.K., S.K.D. and M.A.H.N.A.K.; Project Administration, S.K.K. and S.K.D.; Funding Acquisition, S.K.K.

Conflicts of interest

Nothing to disclose.

Data availability

Datasets analyzed during the present study and/or are available from the corresponding author upon reasonable request.

Ethics approval

The Ethical Committee of Bangladesh Agricultural University approved the study protocol (AWEEC/BAU/2019-53).

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