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Article

The effect of 6-gingerol on biochemical and histological parameters in cholesterol-induced nonalcoholic fatty liver disease in NMRI mice

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Fatty liver contains a range of clinical symptoms, including the accumulation of fat in the liver parenchyma and it varies from a simple steatosis to non-alcoholic steatohepatitis and cirrhosis. Using natural therapies has always been a great concern for such health-related diseases. Herein, 6-gingerol, as a natural compound, was applied to treat non-alcoholic fatty liver induced in NMRI mice. The assessment included histological studies of the liver along with measurement of biochemical parameters, including insulin, glucose, adiponectin, leptin, HDL-C (high-density lipoprotein cholesterol), LDL-C (low-density lipoprotein cholesterol), VLDL-C (very low-density lipoprotein cholesterol), Aspartate transaminase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), SOD (superoxide dismutase), and catalase. The results demonstrated that treatment with 6-gingerol (800 mg/kg) modified the fatty liver indices by significantly reducing (p<0.001) the levels of triglyceride, cholesterol, LDL-C, and VLDL-C, glucose, insulin, insulin resistance, and leptin, whereas this treatment notably increased (p<0.001) the levels of liver antioxidant enzymes, HDL-c, and adiponectin. Therefore, 6-gingerol, in a dose-dependent mode, showed capability of improving non-alcoholic fatty liver and could offer a reliable remedy.

Keywords: 6-gingerol. Non-alcoholic fatty liver (NAFLD). Lipid profiles. Antioxidant enzymes. Insulin resistance. Adiponectin. Leptin.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease and its prevalence has been reported among 20-30% of the population of Western countries (Paschos, Paletas, 2009). NAFLD is a term used to describe a range of related diseases: the earliest stage of the disease is liver steatosis, which is described by triglyceride deposition in the form of lipid droplets in the cytoplasm of hepatocytes associated with enlargement of the liver (hepatomegaly). In steatosis, triglyceride drops contain more than 5% of cytoplasm of hepatocytes (Anderson, Borlak, 2008; Cohen, Horton, Hobbs, 2011). Liver steatosis can be extended to non-alcoholic steatohepatitis (NASH), which is distinguished from simple steatosis by hepatocyte injuries, infiltration of inflammatory factors, and/or collagen deposition (fibrosis). NASH can be eventually developed into cirrhosis. About 10-29% of people with NASH would suffer from cirrhosis within 10 years (Argo, Caldwell, 2009). In cirrhosis, hepatocytes are replaced by scar tissue (cicatrix), which is mainly composed of type1 collagen. Ultimately, cirrhosis can be developed into liver cancer. Studies have shown that 27% of people with cirrhosis, induced by NASH, have been diagnosed with liver cancer (Starley, Calcagno, Harrison, 2010). NAFLD has also been associated with cardiovascular diseases, metabolic syndrome, and insulin resistance (Kim*, et al.*, 2012a; Parker *et al.*, 2012; Paschos, Paletas, 2009). Elevation of a few factors in the liver, including free fatty acids supply from diet, *de novo* lipogenesis, insulin resistance, oxidative stress, and inflammatory markers play roles in inducing liver steatosis (Chen, Varghese, Ruan, 2014; Cohen, *et al.*, 2011; Sumida *et al.*, 2013), and controlling of any of these factors can be effective in the improvement of the fatty liver.

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Ginger is from the root of *Zingiber officinale* and is one of the most used spices. In the traditional Chinese medicine, ginger is used to treat inflammation (Rahimlou *et al*., 2016), and its active ingredients have shown anti-diabetic, anti-cancer, anti-pain, and anti-inflammatory properties (Rahimlou, *et al*., 2016; Young, *et al*., 2005). Moreover, ginger extract has demonstrated antioxidant activity and can decrease blood sugar, insulin, and blood triglyceride (Rahimlou, *et al*., 2016). Ginger retains nutritional value due to having a variety of bioactive compounds, including gingerols, zingerone, and shogaols (Butt, Sultan, 2011). The spicy taste of fresh ginger rhizome is attributed to the presence of gingerols, a group of volatile phenolic compounds, among which 6-gingerol is the major compound that is responsible for the plant's sharpness and spice. Other gingerols, such as 4, 8, 10, and 12-gingerols are also present in lower concentrations which are sensitive to heat and will turn into shogaols at high temperatures creating bitter, spices and sweets aromas (Wohlmuth, *et al*., 2005). The compound 6-gingerol was first isolated from the rhizome of ginger as a volatile yellow oil at room temperature in 1879. After the discovery of 6-gingerol, numerous studies focused on determining its structure (Thresh, 1879). A number of pre-clinical studies have shown the effects of gingerols in the treatment of diabetes, obesity, diarrhea, allergies, pain, fever, rheumatoid arthritis, inflammation, and various forms of cancer. Moreover, ginger and its metabolites, as strong antioxidants, have been known for their abilities to inhibit free radical oxidation and nitric oxide production. Also, numerous studies have shown that gingerol plays protective role in liver, kidney, and cardiopulmonary, and central nervous systems by providing antioxidant, anti-nausea, anti-gastric acid, anti-angiogenesis, and antimicrobial effects (Semwal, *et al*., 2015).

Knowing the proven properties of gingerol holding antioxidant as well as other remedial effects (Dugasani, *et al*., 2010; Semwal, *et al*., 2015; Tripathi, *et al*., 2007), the therapeutic properties of 6-gingerol in improving and treating non-alcoholic fatty liver and the relevant tissue damages were investigated in the present study in mice models.

MARERIAL AND METHODS

Compounds

6-Gingerol was purchased from Sigma Aldrich Company. Diagnostic kits were purchased from Iran's Biochemistry Co. for the measurement of cholesterol, triglyceride, glucose, HDL-C (high-density lipoprotein cholesterol), LDL-C (low-density lipoprotein cholesterol), VLDL-C (very low-density lipoprotein cholesterol), Aspartate transaminase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), SOD (superoxide dismutase), and catalase. The YaK052 rat Elisa kit (Japan's Yanaihara Company) was used to measure leptin. Rat Elisa kit (Otsuka Pharmaceutical of Japan) was used to measure adiponectin, and American ALPCO Diagnostics ultra-sensitive Rat Elisa kit was used to measure insulin.

Animals

A total of 30 male NMRI mice weighing 25 ± 4 grams were purchased from the Pasteur Institute (Iran) and they were kept at Razi Laboratory in Tehran, Science and Research Unit, under standard conditions with 12 hr of light/dark cycle and a relative humidity of 50–70% at 21 ± 2 ºC. After one week, the animals received high-fat diet for induction of fatty liver, except for the control group which received regular water and food, for a total of 30 days. After confirming the induction of fatty liver in the animals by biochemical and histological assessments, the treatment started and continued for another 30 days. All experiments were performed in accordance with the international guidelines set in the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996) and approved by the Research and Ethics Committee of Science and Research Branch, Azad University.

Animals were first weighed and were randomly divided into the 5 following groups (n = 6 per group):

- Control group: receiving regular water and food
- Positive control group: fatty liver-induced mice receiving silymarin (90 mg/kg) for 30 days once every other day via oral gavage
- Negative control group: fatty liver-induced mice receiving the solvent of 6-gingerol (in distilled water) for 30 days once every other day via oral gavage
- Group I: fatty liver-induced mice receiving 6-gingerol (400 mg/kg) for 30 days once every other day via oral gavage
- Group II: fatty liver-induced mice receiving 6-gingerol (800 mg/kg) for 30 days once every other day via oral gavage

Fatty liver induction method

A high-fat diet consisting of two parts was provided in order to induce fatty liver: the first part contained the compounds that were mixed with the animals' fed pellets providing full-fat food, and the second part contained the compounds that were fed to mice via gavage for 30 days (Table I). Animals were weighed every week. The induction of fatty liver in animals was confirmed at the end of 30 days by biochemical and histological examinations.

TABLE I – The first and second parts of the fatty diet fed to mice for 30 days

First part of fatty diet		Second part of fatty diet			
Sunflower oil (liquid)	50 mL	Corn oil	280 mL	Tween 80	5.2 mL
Pastry Oil	150 mL	Sucrose	21.4 g	Propylene glycol	4.5 mL
Hydrogenated vegetable oil	100 mL	Full milk powder	5.8 g	Multivitamin	0.33 mL
Milk powder	100 mL	Cholesterol	14.3 g	Salt (NaCl)	1.45 g
Cholesterol	50 mL	Sodium deoxycholate	1.45 g	Distilled water	65 mL

Histological and biochemical evaluations

At the end of the treatment, the animals' body weights were measured. Animals were then anesthetized by inhalation of diethyl ether, after which blood sample was taken from the cardiac ventricles using 2.5-mL syringes. The blood serum was isolated and stored at -20 ºC until use for biochemical tests. The liver tissue was immediately removed and divided into two parts: one part was subjected to liquid nitrogen for further biochemical tests and the other part was fixed in 10% formalin buffer solution for histopathological evaluation. Fixed tissues were cross-sectioned into 5-mm sections according to routine protocols and were further stained with hematoxylin and eosin (H&E). The slides were examined by light microscopy.

In order to use liver tissue for the level determination of hepatic lipid profiles, hormones, and antioxidant enzymes, liver (10%, w/v) was homogenized in 50 mM phosphate buffer (pH 7.0) and was then centrifuged at 9000 rpm at 4 ºC for 20 min, after which the supernatants were separated and stored in Eppendorf tubes at -70 ºC until analysis. The level of these biochemical parameters were determined by the previously mentioned assay kits according to the manufacturers' protocols.

The level of other biochemical parameters, including insulin, glucose, adiponectin, leptin, HDL, VLDL, LDL, AST, ALT, and ALP were measured in the blood serum using commercially available kits mentioned previously. In order to separate blood serum, blood samples were allowed to clot for 30 min at room temperature (RT) and were then centrifuged at 2500 rpm at RT for 10 min.

The insulin resistance index was evaluated using homeostasis model assessment (HOMA) formula: HOMA = fasting serum insulin (mU/L) x fasting plasma glucose (mM)/22.5 (Kim, *et al*., 2012a). The liver weight index (%) was also obtained by the following formula (Kim, *et al*., 2012b): Liver Weight Index (%) = liver weight/body weight × 100.

Statistical analyses of data

One-way ANOVA and Tukey test were used for statistical examination of data. The results were reported as Mean ± SD. The level of statistical significance was set at $p<0.001$, $p<0.01$, and $p<0.05$. Statistical analyzes were performed by SPSS software and the graphs were drawn using Excel software.

RESULTS

In the present study, non-alcoholic fatty liver was induced in NMRI male mice using a high-fat diet. After 30 days of using a high-fat and high-cholesterol diet, fatty

liver induction was confirmed by the biochemical and histological assessments of the liver. Subsequently, animals were treated with 6-gingerol at 400 and 800 mg/kg doses for 30 days. After treatment, evaluation of biochemical parameters, including lipid profiles, antioxidant enzymes, adiponectin and leptin hormones as well as histopathologic examinations were performed on the liver.

Effect of 6-gingerol on liver lipid profile

The levels of triglyceride, cholesterol, LDL-C and VLDL-C in the negative control group showed a considerable increase (p<0.001) compared with the control group after 30 days of receiving high-fat diet, whereas the level of HDL-C decreased significantly (p<0.001) in the negative control group compared with the control group (Table II). The positive control group as well as groups I and II showed substantial reductions in the levels of triglyceride, cholesterol, LDL-C, and VLDL-C compared with the negative control group after 30 days of treatment. In contrast, HDL-C level increased significantly in the positive control group and in both groups I and II compared with the negative control group (Table II).

TABLE II – Changes in liver lipid profile in mice receiving control diet, high-fat diet, and 6-gingerol. n = 6/group

Groups	Triglyceride (mg/dL)	Total cholesterol (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL (mg/dL)
Control group	60.00 ± 0.22	97.51 ± 2.68	41.04 ± 3.11	44.50 ± 0.67	12.00 ± 1.12
Negative control (fatty liver + solvent)	128.76 ± 2.10 $^{+++}$	225.50 ± 4.45 $^{+++}$	24.76 ± 1.55 $^{+++}$	132.02 ± 7.42 $^{+++}$	24.28 ± 4.48 $^{+++}$
Positive control (fatty liver + silymarin 90 mg/kg)	61.55 ± 0.24 ***	61.49 ± 1.49 $^{+++}$, ***	39.58 ± 1.43 ***	39.81 ± 1.09 ***	12.39 ± 1.08 ***
Group I (Fatty liver + 6-gingerol 400 mg/kg)	75.32 ± 0.42 $^{+++}$, ***	96.00 ± 0.21 ***	36.17 ± 1.54 **	45.15 ± 1.42 ***	14.90 ± 1.96 ***
Group II (Fatty Liver + 6-gingerol 800 mg/kg)	69.46 ± 0.10 ***	73.70 ± 0.51 $^{++}$, ***	36.50 ± 1.34 **	42.30 ± 4.39 ***	13.85 ± 0.52 ***

Note: Data are expressed as mean ± SD. ++ p <0.01 and +++ p <0.001 are significant differences compared to the control group. ** p <0.01 and *** p <0.001 are compared to the negative control group.

Effect of 6-gingerol on fasting blood glucose, insulin and insulin resistance levels

After receiving 30 days of high-fat diet, the levels of fasting blood glucose, insulin, and insulin resistance (HOMA) showed a significant increase (p<0.001) in the negative control group compared with the control group. In contrast, administration of silymarin (positive control group) and both doses of 6-gingerol for 30 days decreased the level of these parameters considerably in the treated groups in comparison with the negative control group (Figure 1A-C).

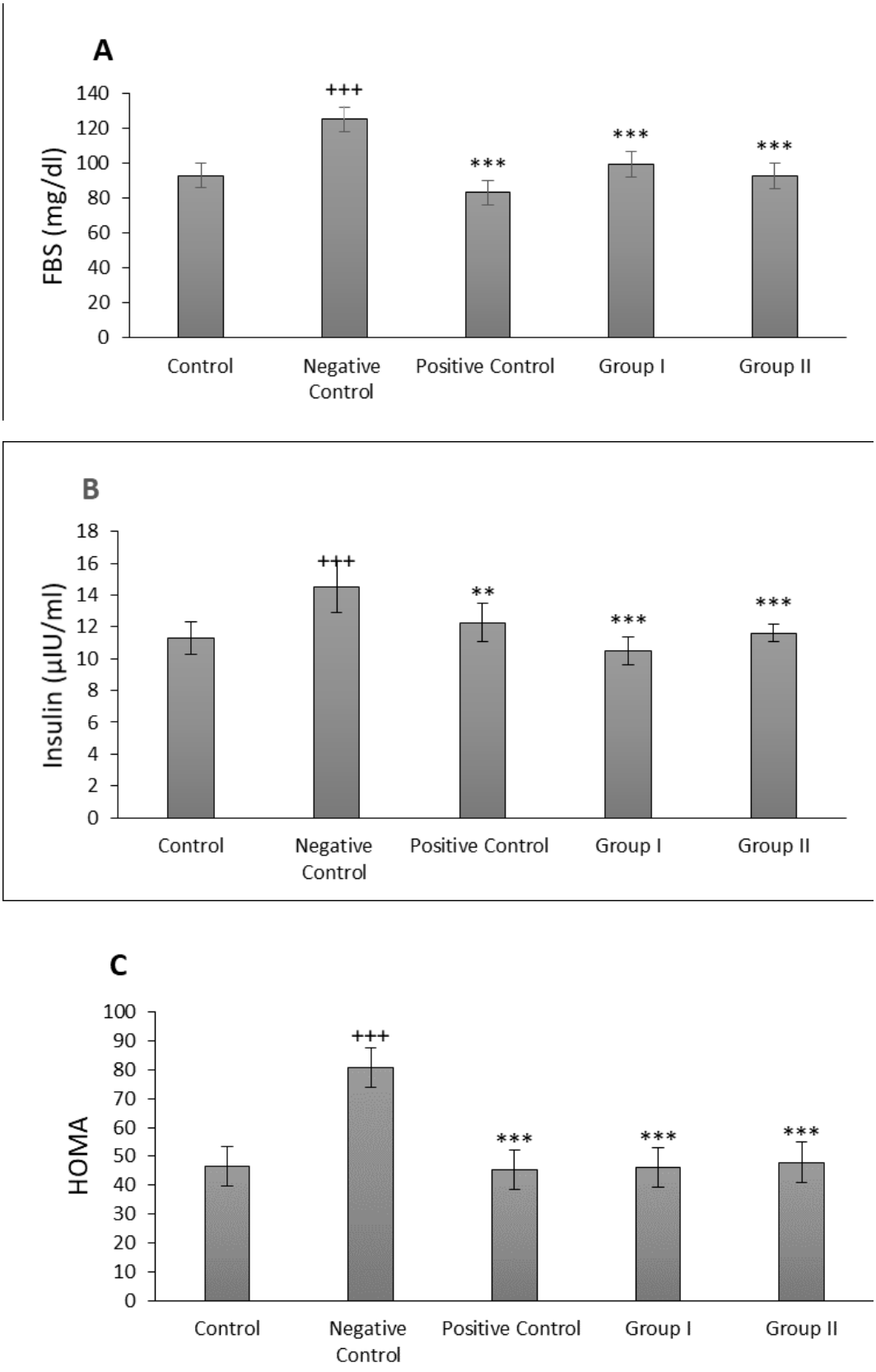

FIGURE 1– Changes in the levels of fasting blood glucose (A), insulin (B), and insulin resistance (HOMA) (C) affected by 6-gingerol in fatty liver-induced mice. Values are reported as mean ± SD. +++ $p<0.001$ as compared with the control group; ** $p<0.01$ and *** $p<0.001$ as compared with the negative control group.

Effect of 6-gingerol on leptin and adiponectin levels

As shown in Figure 2A, the level of leptin increased in the negative control group compared with the control group (p<0.001). Meanwhile, after 30 days of treatment, the leptin level was notably improved in the positive control as well as in groups I and II compared with the negative control group (p<0.001). On the other hand, the level of adiponectin was significantly lowered in the negative control group compared with the control group (p<0.001) while it was elevated in the treated groups I and II compared with the negative control group (p<0.001) (Figure 2B).

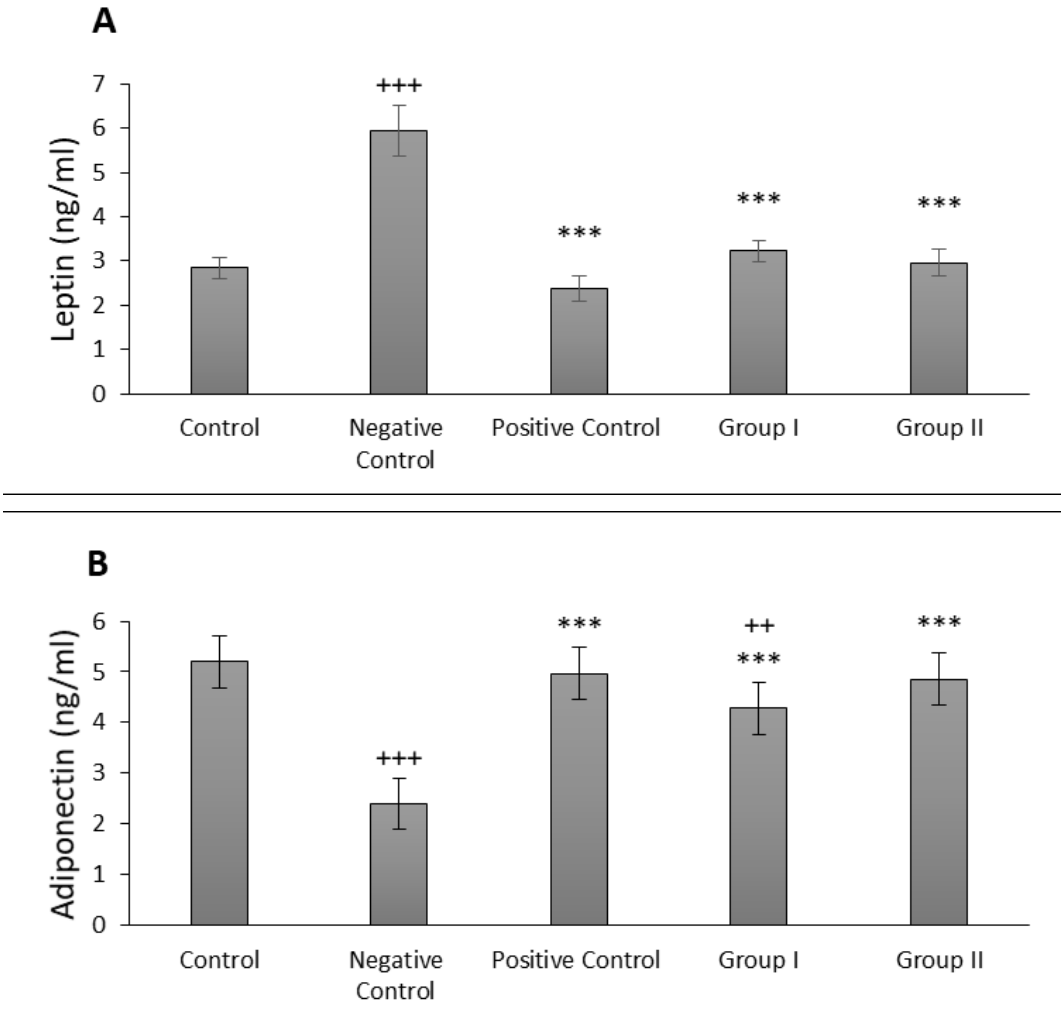

FIGURE 2 – Changes in the levels of leptin (A) and adiponectin (B) affected by 6-gingerol in fatty liver-induced mice. Values are reported as mean ± SD. ++ p<0.01 and +++ p<0.001 as compared with the control group. *** p<0.001 as compared with the negative control group.

Effect of 6-gingerol on the hepatic and antioxidant enzymes

Before treatment with 6-gingerol, the amount of hepatic enzymes, including AST, ALT, and ALP were considerably high in the negative control group compared with the control group (p<0.001). In the cases of treating with 90 mg/kg silymarin (positive control group) and both doses of 6-gingerol, the levels of these enzymes decreased notably in the treated groups in comparison with the negative group (p<0.001) (Table III).

The levels of antioxidant enzymes, including SOD and catalase were also measured before and after treatment with 6-gingerol. Before treatment, the amount of these enzymes were much lower in the negative control group than the control group (p<0.001). After treating with silymarin and 6-gingerol, the level of SOD increased significantly in the positive control group and in group II compared with the negative control group (p<0.001). However, the increase in the level of SOD in group I was not as notable as group II. Regarding the catalase enzyme, its level was higher in the positive control group as well as in groups I and II compared with the negative control group (p<0.001) (Table III).

TABLE III – Levels of hepatic and antioxidant enzymes in mice receiving control diet, high-fat diet, and 6-gingerol. n = 6/group

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	SOD (u/mg-protein)	Catalase (u/mg-protein)
Control group	67.60 ± 0.27	69.31 ± 2.60	130.25 ± 0.34	13.90 ± 0.04	64.79 ± 0.32
Negative control (fatty liver + solvent)	90.42 ± 2.25 $^{+++}$	107.75 ± 5.89 $^{+++}$	158.93 ± 1.84 $^{+++}$	12.27 ± 0.17 $^{+++}$	40.37 ± 2.37 $^{+++}$
Positive control (fatty liver + silymarin 90 mg/kg)	67.39 ± 1.33 ***	65.16 ± 1.85 ***	130.91 ± 1.65 ***	14.30 ± 0.22 ***	65.28 ± 0.29 ***
Group I (Fatty liver + 6-gingerol 400 mg/kg)	80.32 ± 1.19 $^{+++}$, ***	86.42 ± 1.56 $^{++}$, ***	143.74 ± 1.20 $^{+++}$, ***	12.74 ± 0.20 $^{++}$	63.80 ± 0.31 ***
Group II (Fatty Liver + 6-gingerol 800 mg/kg)	74.53 ± 1.68 $^{+}$, ***	76.40 ± 2.36 ***	138.58 ± 1.34 $^{++}$, ***	13.64 ± 0.23 ***	64.77± 0.20 ***

Effect of 6-gingerol on body weight changes and liver weight index

The body weights of mice were monitored in all groups throughout the period of the study. The initial weights, measured at day 30, and the final weights of all groups are shown in Table IV. At the beginning of the experiment, the body weights were not significantly different between the groups. Also, after receiving a high-fat diet (the 30th day of the experiment) and also at the end of the study period (after receiving treatment), no significant difference was observed in the body weights between different groups.

After receiving high-fat diet, the liver weight and the index of liver weight were significantly increased in the negative control group compared with the control group (p<0.001). However, after being treated with silymarin and 6-gingerol, the values of these two parameters were notably reduced in the positive control group and groups I and II, respectively, compared with the negative control group (p<0.001) (Table IV).

TABLE IV – The body weight and index of liver weight of mice receiving control diet, high-fat diet, and 6-gingerol. n = 6/group

Groups	The starting weight (g)	The weight of 30th day (g)	The final weight (g)	The liver weight (g)	Index of liver weight% (g)
Control group	28.17 ± 1.851	37.17 ± 1.352	36.82 ± 0.603	1.87 ± 0.035	5.10 ± 0.144
Negative control (fatty liver + solvent)	28.17 ± 1.078	37.33 ± 0.881	36.33 ± 0.882	2.55 ± 0.141 +++	8.14 ± 0.533 +++
Positive control (fatty liver + silymarin 90 mg/kg)	28.50 ± 0.563	38.83 ± 0.946	38.75 ± 0.920	1.83 ± 0.100 ***	4.72 ± 0.243 ***
Group I (Fatty liver + 6-gingerol 400 mg/kg)	27.67 ± 1.229	38.00 ± 1.155	38.70 ± 0.959	1.80 ± 0.032 ***	4.66 ± 0.146 ***
Group II (Fatty Liver + 6-gingerol 800 mg/kg)	27.33 ± 0.494	38.17 ± 0.946	39.48 ± 0.501	1.81 ± 0.028 ***	4.58 ± 0.097 ***

Note: Data are expressed as mean ± SD. +++ p <0.00 significant difference compared with the control group. *** p <0.001 significant difference compared with the negative control group.

Histological examinations

As it is demonstrated in Figure 3, the liver section of the control group was healthy with no lesion, and the hepatocytes, sinusoidal spaces, and central and portal veins could be seen in intact forms (Figure 3A). After receiving a high-fat diet for 30 days, the liver tissue of the negative control group was fattened and the deposited lipid droplets could be seen in the liver tissue. Also, the accumulation of the inflammatory cells was clearly visible in the tissue sections of the negative control group (Figure 3B). Liver tissue examination of the positive control group showed that receiving silymarin at dose of 90 mg/kg for 30 days could well recover the tissue lesions in this group as neither lipid droplets nor accumulation of the inflammatory cells were observed (Figure 3C). Microscopic observations of the liver tissue of group I (receiving 6-gingerol at 400 mg/kg) showed partial improvement as there was a minor accumulation of both inflammatory cells and fat droplets inside the hepatocytes (Figure 3D). Histological examination of the liver tissue of group II (receiving 6-gingerol at 800 mg/kg) indicated an improvement in the status of the liver tissue compared with the negative control group as there was a significant reduction in the accumulation of both inflammatory cells and lipid droplets in hepatocytes. Meanwhile, the tissue images of group II were similar to the control group (Figure 3E).

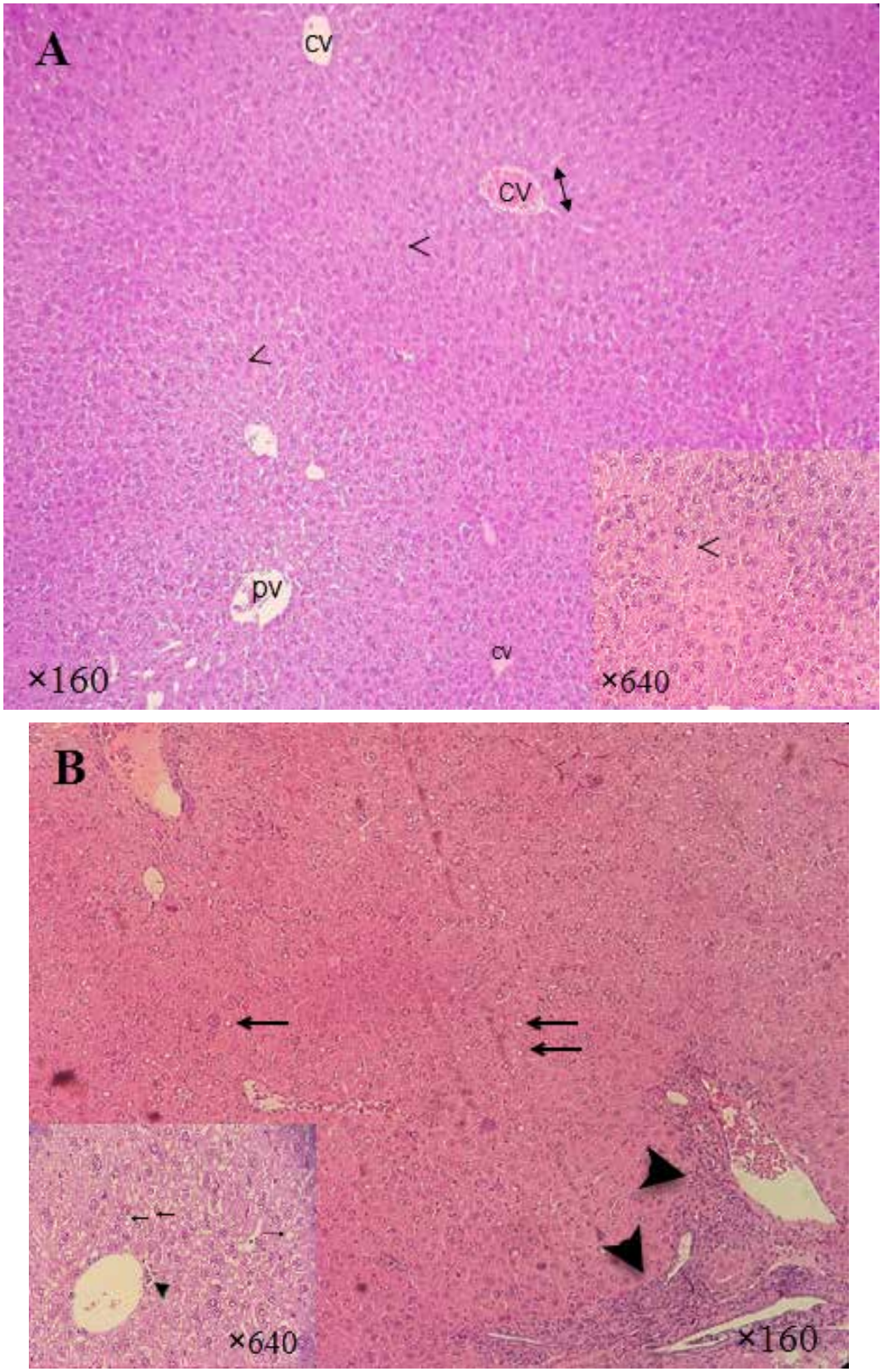

FIGURE 3 – H&E-stained sections of liver from the following groups: control (A), negative control (fatty liver + solvent) (B), and positive control (fatty liver + silymarin) (C), group I (fatty liver + 6-gingerol 400 mg/kg) (D), group II (fatty liver + 6-gingerol 800 mg/kg) (E).
Abbreviations: CV: central vein; PV: portal vein. Symptoms: lipid droplets sediment (one-pointed arrow); accumulation of inflammatory cells (thick arrow tip); sinusoidal spaces (two-pointed arrow), hepatocytes (thin arrow tip).

DISCUSSION

In this study, a high-fat diet was used to create fatty liver in mice. After 30 days of receiving high-fat diet, the results demonstrated that the fatty liver-related parameters, including triglyceride, cholesterol, LDL, leptin, and liver enzymes were increased while adiponectin, HDL, and antioxidant enzymes were decreased. Meanwhile, histological examinations of the fatty liver confirmed the development of steatosis. These changes confirmed the creation of fatty liver in the animals.

Triglycerides and cholesterol are among important lipids whose over-received amounts lead to hypertriglyceridemia and hypercholesterolemia. Non-alcoholic fatty liver is specified by the accumulation of triglycerides in hepatocytes formed by the esterification of free fatty acids and glycerol (Magesh, *et al*., 2006; Shen, Qian, 2006; Xiang, *et al*., 2006). In numerous studies, researchers have been able to induce fatty liver in animals using high-cholesterol and high-calorie diets. A group of researchers showed that fatty liver can be induced in animals in a short period of time by using high-calorie diet containing fructose, cholesterol and fat (Clapper, *et al*., 2013). In another study, researchers evaluated the effect of high-fat diet on the induction of fatty liver over various times. They found that changes related to the fatty liver appeared only within two weeks after receiving a high-fat diet in animals (Gauthier, Favier, Lavoie, 2006). The fatty diet used in the present study could effectively induce fatty liver in mice after 30 days. Consumption of the present high-fat diet caused changes of dyslipidemia, which was identified by increasing the serum levels of total cholesterol, triglyceride, and LDL and also by the reduction in the HDL level. These changes were also previously reported in non-alcoholic fatty liver (Noeman, Hamooda, Baalash, 2011).

According to various studies, high-fat diet could increase the size, the weight as well as the percentage of lipid accumulation in the liver (VanSaun, *et al*., 2009; Zou, *et al*., 2006). However, the diet used in the present study, despite the induction of non-alcoholic fatty liver, did not significantly increase the body weights in mice. Furthermore, the body weight in the group receiving 6-gingerol showed no significant changes, whereas the liver weight index (%) was significantly reduced. Accordingly, gingerol has shown to cause reduction in the body weight of obese rats (Saravanan, *et al*., 2014).

In the present study, treatment with 6-gingerol, in a dose-dependent mode, significantly decreased TG, LDL-C and VLDL levels and improved HDL-C level in the treated groups. In accordance with our study, it has been shown that gingerol could reduce the levels of the lipid profiles in obese rats and also decrease the absorption of lipids, fat, and cholesterol by inhibiting pancreatic lipase activity (Saravanan, *et al*., 2014). Another study has demonstrated that 6-gingerol, by regulating key genes associated with inflammation and lipid metabolism, could offer its protective effect against non-alcoholic steatohepatitis (Tzeng, *et al*., 2015). 6-gingerol is the active constituent of fresh ginger, which has shown to improve lipid profiles efficiently (Mazidi, *et al*., 2016). Another study has shown that ginger extract had hypoglycemic effects and could lower cholesterol and LDL in diabetic rats (Al-Noory, Amreen, Hymoor, 2013).

In the present case, induction of fatty liver in mice caused a significant increase in the levels of glucose, insulin, and insulin resistance (HOMA). Mazidi *et al*. have reported that ginger could effectively reduce the levels of glucose, insulin, and insulin resistance in obese rats (Mazidi, *et al*., 2016). This hypoglycemic effects of ginger could be attributed to its contents of phenols, polyphenols, and flavonoids (Shanmugam, *et al*., 2011). As one of the active phenolic compounds of ginger, 6-gingerol has shown to increase the cellular glucose adsorption by increasing the expression of type 4 glucose carrier gene (Li, *et al*., 2012). Meanwhile, gingerol has been reported to decrease the pancreatic amylase secretion, resulting in the reduction of the intestinal absorption of carbohydrates, and also increase insulin sensitivity (Ali, Amreen, Hymoor, 2006). These phenomena could be associated with the anti-obesity and anti-hyperglycemic effects of gingerol (Saravanan, *et al*., 2014). Also, a group of researchers examined the therapeutic potential of 6-gingerol in improving hyperglycemia in diabetic rats. According to their observations, 6-gingerol increased the insulin secretion in response to glucose and improved glucose tolerance. They suggested that 6-gingerol could increase insulin secretion by facilitating the exocytosis of insulin-containing vesicles (Samad, *et al*., 2017). Accordingly, in the present study, insulin level was also higher in the group receiving 800 mg/kg of 6-gingerol.

In 2015, it was reported that 6-gingerol regulated glucose metabolism through the AMPK pathway (Lee, *et al*., 2015). It was also shown that 6-gingerol could offer anti-hyperglycemic effects by increasing muscle

glycogen sediments via adjusting the synthesis and the activity of glycogen (Samad, *et al*., 2017). Consequently, the plasma glucose level in the present study was lower in the group treated with 800 mg/kg of 6-gingerol than the group receiving 400 mg/kg of this compound, indicating higher uptake of glucose.

In the present study, the levels of leptin and adiponectin showed significant increase in the negative control group compared with the control group. Treatment with 6-gingerol and silymarin resulted in a significant decrease in leptin level and a significant increase in adiponectin level. Accordingly, researchers have demonstrated that 6-gingerol improved insulin sensitivity following an increase in adiponectin levels (Isa, *et al*., 2008). The hypoglycemic and hypolipidemic effects of ginger extract have been shown in rats fed with high-fat diet by improving the levels of lipid profiles and decreasing the insulin and glucose levels. It was demonstrated that the relationship between the expression of leptin, adiponectin, PPARα and PPARγ in the liver could be the key mechanism for providing hypolipidemic effects by ginger (de las Heras, *et al*., 2016). The researchers have stated that 6-gingerol can reverse the regulation of adiponectin expression in adipocytes with its anti-inflammatory effects (Isa, *et al*., 2008). Several studies have also confirmed the effects of ginger and 6-gingerol on the reduction of serum leptin levels in treated animals (Saravanan, *et al*., 2014; Wadikar, Premavalli, 2011).

In the present study, high-fat diet significantly increased the levels of AST, ALT and ALP enzymes in the negative control group compared with the control group, and prescription of 6-gingerol significantly reduced the levels of these enzymes. Accordingly, it has been shown that 6-gingerol could significantly reduce the levels of AST and ALT in non-alcoholic steatohepatitis (Tzeng, *et al*., 2015). Several studies have also shown that hydroalcoholic, aqueous, and ethanolic extracts of ginger could significantly decrease the level of all the mentioned liver enzymes (Al-Naqeeb, *et al*., 2003; Bhandari, *et al*., 2003; Poorrostami, Farokhi, Heidari, 2014). Interestingly, another study has shown that ginger extract was capable of reducing the activity of these enzymes in the rats poisoned with carbon tetrachloride and acetaminophen (Yemitan, Izegbu, 2006).

Previous investigations have shown that high-fat diets increased the production of free radicals and induced oxidative stress. In fact, hypercholesterolemia was associated with lipid peroxidation and it decreased the activity of antioxidant enzymes, ultimately leading to cellular damage (Amirkhizi, *et al*., 2010). More studies have demonstrated that increasing the oxidative stress and production of free radicals, due to fat accumulation in the liver, would lead to the development of liver steatosis, fibrosis, and cirrhosis (Mohajeri, 2013). Several studies have also shown that the use of high-fat diet in animals caused a significant reduction in the serum level of antioxidants, including SOD and catalase (Liu, Lloyd, 2013; Lu, Chiang, 2001; Zou, *et al*., 2006). Herein, SOD and catalase levels were decreased significantly by consumption of high-fat diet, whereas prescribing 6-gingerol significantly increased the amount of these enzymes. Ginger has shown to present high antioxidant effects due to containing active compounds, in particular gingerol (Chang, *et al*., 1994). Increasing the level of antioxidant enzymes, such as SOD and catalase, by ginger extract has been furthermore confirmed by other researches (Amin, Hamza, 2006; Motawi, *et al*., 2011; Poorrostami, Farokhi, Heidari, 2014). It has been reported that phenolic compounds in ginger, including 6-gingerol inhibit free radicals and lipid peroxidation, protect liver, and increase the antioxidants (Siddaraju, Dharmesh, 2007; Aeschbach, *et al*., 1994; Chung, Yow, Benzie, 2003).

The most important characteristic of steatosis is fat accumulation in the liver cells, resulting in both insulin resistance and inflammation in the liver. (Janczyk, Socha, 2012). In the development of steatosis, cellular ballooning and inflammation are visible (Nalbantoglu, Blunt, 2014). In the present study, accumulation of fat and inflammatory cells were observed, confirming the production of fatty liver by applying fatty diet. In the hepatic tissue sections of the treated groups with 6-gingerol, the improvement in the liver tissue status was clearly visible. It has been shown that 6-gingerol could inhibit the adipogenesis (differentiation of adipocytes) and cytoplasmic accumulation of lipid droplets in the 3T3-L1 cell line (Tzeng, Liu, 2013).

Many previous studies have used natural compounds to protect liver injury (Al-Rasheed, *et al*., 2018). In the present study, silymarin was selected as the positive control as it has shown efficacy in the treatment of NASH (Solhi, *et al*, 2014). Herein, treatment with silymarin significantly improved the lipid profiles and the levels of insulin, glucose, and HOMA. Silybin, forming 50-60% of the silymarin, has shown to significantly reduce the levels of triglyceride, total cholesterol, LDL, and VLDL and increase the HDL level (Gobalakrishnan, Asirvatham, Janarthanam, 2016;

Yao, Zhi, Minhu, 2011). It has also been demonstrated that hydroalcoholic extract of silymarin improved lipid disorders created in diabetic rats (Sajedianfard, Behroozi, Nazifi, 2014), reduced blood glucose levels in type 2 diabetes (Sajedianfard, Behroozi, Nazifi, 2014; Voroneanu, *et al*., 2016), and decreased both plasma lipid and insulin levels in obese rats (Guo, *et al*., 2016). In accordance with previous studies, we demonstrated that silymarin could well improve the levels of leptin, adiponectin, antioxidant enzymes, and hepatic steatosis (Abdel-Moneim, *et al*., 2015; Guo, *et al*., 2016; Cacciapuoti, *et al*., 2013).

Along with sylimarin, polyphenolic 6-gingerol, in a dose-dependent mode, showed capability of improving non-alcoholic fatty liver by increasing the antioxidant enzymes (SOD and catalase) and adiponectin levels and also decreasing the insulin resistance, serum leptin level, and lipid profiles.

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