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The Effect of Nerol on Biochemical and Histological Parameters in Nonalcoholic Fatty Liver Disease in NMRI Mice

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ABSTRACT

Fatty liver contains a range of clinical symptoms, including the accumulation of fat in the liver cells and it varies from a simple steatosis to nonalcoholic steatohepatitis and cirrhosis. Using natural therapies has always been a great concern for such health-related diseases. Herein, nerol, as a natural monoterpene, was applied to treat nonalcoholic fatty liver-induced NMRI (Naval Medical Research Institute) mice. The assessment included histological studies of the liver along with measurement of biochemical parameters, including insulin, glucose, HDL-C (high-density lipoprotein cholesterol), LDL-C (low-density lipoprotein cholesterol), Aspartate transaminase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), SOD (superoxide dismutase) and catalase. The results demonstrated that treatment with nerol (90 mg kg^{-1}) modified the fatty liver indices by significantly reducing the levels of triglyceride, cholesterol, LDL-C, glucose, and insulin ($p < 0.001$) whereas this treatment notably increased the levels of liver antioxidant enzymes, and HDL-C ($p < 0.001$). Nerol administration also improved the status of the liver tissue of the fatty liver condition. Therefore, nerol, in a dose-dependent mode, showed capability of improving nonalcoholic fatty liver and could offer a reliable remedy.

Keywords: Nerol, Nonalcoholic fatty liver, Lipid profiles, Antioxidant and liver enzymes, Glucose

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is one of the most common chronic liver illnesses in the world and one of the main reasons for adults to refer to hepatology clinics [1]. This disease begins with a wide range of simple liver steatoses and can turn into nonalcoholic steatohepatitis, fibrosis, cirrhosis, hepatic insufficiency and even liver cancer [2,3]. NAFLD is a multi-dimensional illness and a range of factors such as genetics and lifestyle, including diet and the amount of physical activity play roles in its incidence [4]. Moreover, it is a reversible disease which is created by the excess build-up of fat in liver cells and it is defined by increased levels of triglycerides, liver enzymes, some inflammatory biomarkers, and the level of liver steatosis [5]. In this illness, typically more than 5% of liver weight consists of fat in the absence of alcohol consumption [6].

Essential oils have been in use for thousands of years due to their healing and detoxifying effects on the body. These oils have always retained high value among ancient physicians and pharmacists, who utilized them for aromatherapy and treating cutaneous conditions. Essential oils contain powerful antioxidant concentrates obtained from herbal flowers, leaves, wood, skin, fruits, roots, and seeds [7]. The essential oil obtained from the orange blossoms is known as neroli oil. Nerol (*cis*-3,7-Dimethyl-2,6-octadien-1-ol) is a colorless monoterpene originally isolated from neroli oil. It is obtained from both the blossoms of the bitter orange tree, *Citrus aurantium* and rose oil [8]. Bitter orange, as the source of nerol, as well as neroli oil have been found with anti-cancer and antianxiety characteristics while being therapeutically effective in aiding digestion, relieving flatulence, and providing cardiovascular health, and stroke treatment [9]. The monoterpene compounds also exhibit various pharmacological properties [10]. Observing the pharmacological and remedial properties of other monoterpenes, this study aimed to evaluate the therapeutic effects of nerol, as a natural compound, in improving the biochemical parameters and the relevant tissue damages in nonalcoholic fatty liver mice models.

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MATERIAL AND METHODS

Compounds

Nerol was obtained from Sigma-Aldrich, St. Louis, MO, USA. For biochemical analyses, diagnostic kits were purchased from ZistChimi Company, Iran, for the measurements of high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), total cholesterol, triglycerides (TG), glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphate (ALP). Insulin levels of serum was evaluated using ELISA assay kit (Cat. No. YII-YK052-EX) from Yanaihara Institute Inc., Japan.

Fatty Liver Induction Method

A high-fat diet was provided in order to induce fatty liver with the following components: Cholesterol (> 95% [CG] Sigma), Tween80, sucrose, sodium deoxycholate and propylene glycol (Sigma-Aldrich), total milk powder (Aptamil), multivitamin, and carbohydrates [11]. In this diet, total milk powder provided proteins. Carbohydrates source was sucrose, and fat was obtained by corn oil. Meanwhile, the diet was supplemented with multi vitamin and mineral mixture. The compounds (Table 1) were fed to mice via gavage for four weeks. This emulsion was stored at 4 °C and then heated in water bath at 42 °C and fully mixed before use [11]. Animals were weighed every week. The induction of fatty liver in animals was confirmed at the end of four weeks by biochemical and histological studies.

Animals and Treatment

A total of 56 male NMRI (Naval Medical Research Institute) mice weighing 25-30 grams were purchased from the Razi Vaccine and Serum Institute, Karaj, Iran. The mice were kept at Razi Laboratory in Tehran, Science and Research Branch, under standard conditions with 12 h of light/dark cycle and a relative humidity of 50-70% at 21 ± 2 ºC [11]. They were given *ad libitum* access to standard pellet and water. After one week, the animals received high fat diet for induction of fatty liver for a total of four weeks except for the control group receiving regular water and food. After confirming the induction of fatty liver in animals by biochemical and histological studies, the treatment started and continued for another four weeks. 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 7 following groups (n = 8 per group):

- Control group: receiving regular diet and water.
- Fatty-liver group: receiving high fat diet (Table 1) for four weeks to induce fatty liver [11]
- Positive control group: fatty liver-induced mice receiving regular diet and silymarin (90 mg kg^{-1}) *via* oral gavage for four weeks
- Sham group: fatty liver-induced mice receiving regular diet and the solvent of nerol (tween 80) *via* oral gavage for four weeks.
- Experimental group 1 (Exp1): fatty liver-induced mice receiving regular diet and nerol (30 mg kg^{-1}) by oral gavage for four weeks.
- Experimental group 2 (Exp2): fatty liver-induced mice receiving regular diet and nerol (60 mg kg^{-1}) by oral gavage for four weeks.
- Experimental group 3 (Exp3): fatty liver-induced mice receiving regular diet and nerol (90 mg kg^{-1}) by oral gavage for four weeks.

Histological and Biochemical Assessment

At the end of the treatment, the animals' body weights were measured. NMRI mice were anesthetized by inhalation of diethyl ether. Blood samples were then withdrawn from cardiac ventricles with the help of 2.5-ml syringes. The blood serum was isolated and stored at -20 ºC until use for biochemical tests. The liver tissue was removed and divided into two parts: one part was subjected to liquid nitrogen for biochemical tests, and the other part was fixed in 10% formalin buffer solution for histopathological assessment. After embedding the tissues in paraffin, cross sections of 5 µm were prepared and the liver sections were stained with hematoxylin and eosin (H&E) staining [12]. The slides were examined by light microscopy. Liver tissue samples were also used to measure antioxidant enzyme activities and other biochemical parameters. Tissue samples were removed, weighed, and then homogenized (1:3 w/v) in phosphate

Table 1. Composition of the Fatty Diet Fed to Mice for Four Weeks

Corn oil (g)	178
Sucrose (g)	13.37
Full milk powder (g)	7.13
Cholesterol (g)	8.92
Sodium deoxycholate (g)	0.89
Tween 80 (g)	3.25
Propylene glycol (g)	2.78
Multi vitamin (g)	0.22
Salt (NaCl) (g)	0.89
Distilled water (ml)	40.1

saline buffer (pH 7.4). Homogenate samples were then sonicated for 1.5 min and bursts for 30 s. All the procedures were performed at 0-4 °C. Homogenate samples were finally centrifuged at 22,000 g for 17 min at 4 °C. The supernatant was frozen at -40 °C until the time of assay.

In order to use liver tissue for biochemical level determination of hepatic cholesterol, triglycerides, and antioxidant enzymes, including superoxide dismutase (SOD) and catalase, liver (10%, w/v) was homogenized in 50 mM phosphate buffer (pH 7.0) and then centrifuged at 9000 g 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 other biochemical parameters, including HDL, LDL, triglyceride, glucose, insulin, SOD, CAT, 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 and were then centrifuged at 2500 g at 37 ºC for 10 min.

The liver weight index (%) was obtained by the following formula [13]:

Liver weight index (%) = liver weight/body weight × 100

Statistical Analysis

SPSS software 21 was used with ANOVA and Tukey test of variance in order to investigate the significant differences between the groups. The results were reported as Mean ± SEM. The levels of statistical significance was set at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

RESULTS

In this study, nonalcoholic fatty liver was induced in NMRI male mice using a high-fat diet for four weeks. After, fatty liver induction was confirmed by biochemical tests and histological examinations of the liver. Subsequently, animals were treated with nerol at 30, 60 and 90 mg kg^{-1} doses for four weeks. After treatment, evaluation of biochemical parameters, including lipid profiles, antioxidant enzymes, glucose, insulin, and liver enzymes as well as histopathologic studies were performed on the liver.

Effect of Nerol on Liver Lipid Profile

The levels of triglyceride, total cholesterol, and LDL-C showed a considerable increase in the fatty liver-induced group compared to the control group ($p < 0.001$) whereas HDL-C level decreased significantly ($p < 0.001$) in the fatty liver-induced group compared to the control group. Positive control group (receiving silymarin 90 mg kg^{-1}) as well as the groups receiving nerol 60 and 90 mg kg^{-1} showed the most

reduction ($p < 0.001$) in triglyceride, cholesterol and LDL-C levels compared to the fatty liver-induced group after four weeks of treatment. In contrast, HDL-C level increased significantly in the positive control group ($p < 0.001$) and in both experimental groups 2 and 3 ($p < 0.01$) compared to the fatty liver-induced group (Fig. 1).

Effect of Nerol on Fasting Blood Glucose and Insulin

After receiving high-fat diet for four weeks, the levels of fasting blood glucose and insulin showed a significant increase ($p < 0.001$) in the fatty liver-induced group compared with the control group (Fig. 2). In contrast, administration of silymarin (positive control group) and nerol 90 mg kg^{-1} for four weeks decreased the level of these parameters considerably in the treated groups in comparison with the fatty liver-induced group. Meanwhile, experimental group 2 (receiving nerol 60 mg kg^{-1}) showed considerable capability of decreasing the level of glucose compared with the fatty liver-induced group ($p < 0.001$) (Fig. 2).

Effect of Nerol on Biochemical Parameters

Before treatment with nerol, the amount of hepatic enzymes, including AST, ALT and ALP were considerably high in the sham and fatty liver-induced groups compared with the control group ($p < 0.001$). In the cases of treating with 90 mg kg^{-1} silymarin (positive control group) and high dose of nerol, the levels of these parameters decreased notably in the treated group in comparison with the fatty liver-induced group ($p < 0.001$) (Table 2).

The levels of antioxidant enzymes, including SOD and catalase were also measured before and after treatment with nerol. Before treatment, the amount of these enzymes were much lower in the fatty liver-induced group than the control group ($p < 0.001$). After treating with silymarin and nerol, the level of SOD increased significantly in the positive control group and in experimental groups 2 and 3 receiving nerol 60 and 90 mg kg^{-1}, respectively, compared with the fatty liver-induced group ($p < 0.001$) (Table 3).

Effect of Nerol on Body Weight Changes and Liver Weight Index%

Body weight was monitored in all groups throughout the period of the study. Initial and final weights measured at the first and fourth weeks of the treatment are shown in Fig. 3. At the beginning of the experiment, body weights were not significantly different among the groups. At the end of the study period (after receiving treatment), only the body weight of the fatty liver-induced group was increased compared to control group ($p < 0.05$).

At the end of the treatment period, the liver weight index% of the sham, fatty liver-induced and Exp1 groups were significantly higher than the control group ($p < 0.001$). After treating with silymarin and nerol (60 and 90 mg kg^{-1}), the liver weight index % reduced in these groups compared with the fatty liver-induced group ($p < 0.01$) (Fig. 3).

Histological Analysis

As it is demonstrated in Fig. 4, 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 (Fig. 4A). After being treated with fatty diet for four weeks, the liver tissue of the fatty liver-induced group was fattened, and the deposited lipid droplets in the liver tissue were observed (Fig. 4B). Liver tissue examination of the positive control group showed that receiving silymarin at 90 mg kg^{-1} for four weeks could well recover the tissue lesions in this group as there was no lipid droplets left (Fig. 4C). Microscopic observations of the liver tissue of experimental groups 1 and 2 receiving nerol at 30 and 60 mg kg^{-1}, respectively, showed that the liver tissue in these groups were partially repaired as there was a minor accumulation of fat droplets inside the hepatocytes (Figs. 4E and F). Histological examination of liver tissue in experimental group 3 (receiving nerol at 90 mg kg^{-1}) indicated an improvement in the status of the liver tissue compared to the fatty liver-induced group as the accumulation of lipid deposition in hepatocytes were significantly reduced in this group. The tissue images of experimental group 3 was similar to the control group (Fig. 4G).

DISCUSSION

In this study, a high-fat diet was used to create fatty liver in mice. After 4 weeks of receiving high-fat diet, biochemical examinations from the blood serum samples and tissues of these animals showed that fatty liver-related

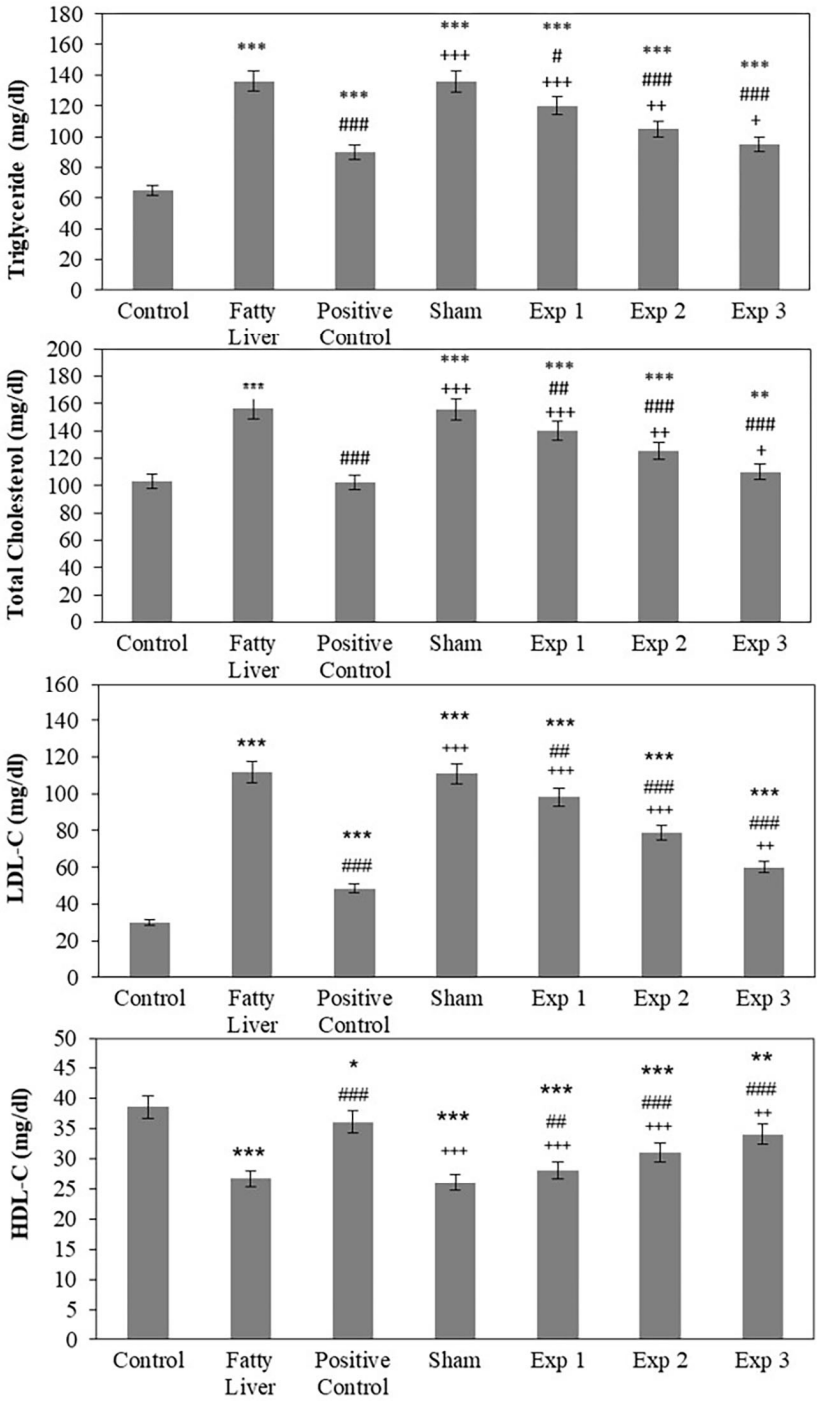

Fig. 1. Overall serum levels of triglyceride, total cholesterol, LDL-C, and HDL-C in different groups. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ are compared to the control group. #$p < 0.05$, ##$p < 0.01$, and ###$p < 0.001$ are compared to the fatty liver group. +$p < 0.05$, ++$p < 0.01$, and +++$p < 0.001$ are compared to the positive control group. Please refer to the methods section for groups' definition.

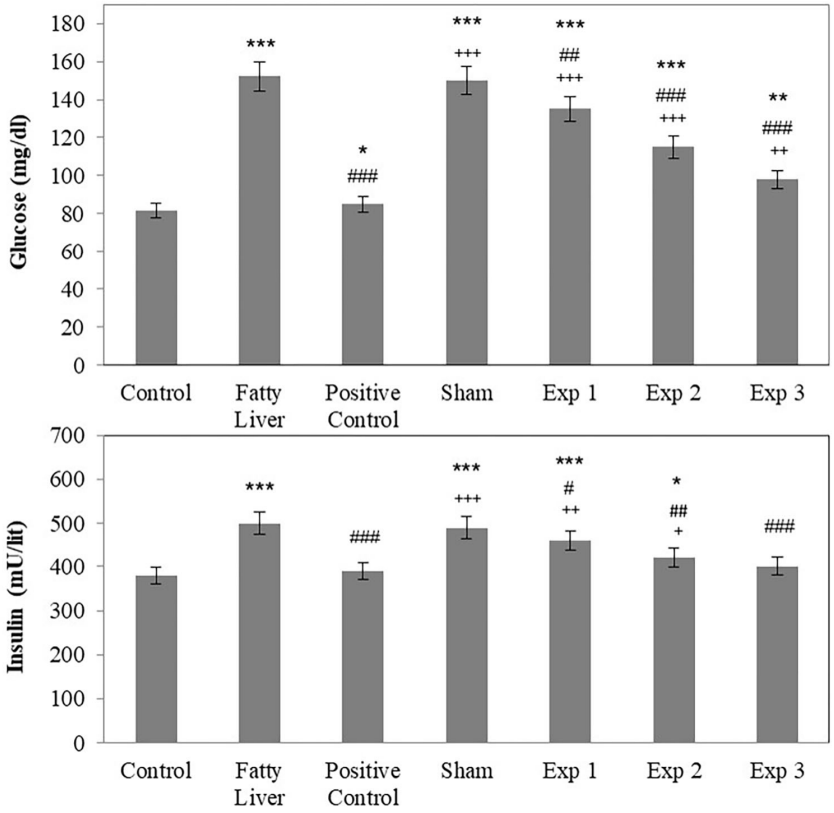

Fig. 2. Overall serum levels of glucose and insulin in different groups. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ are compared to the control group. #$p < 0.05$, ##$p < 0.01$, and ###$p < 0.001$ are compared to the fatty liver group. +$p < 0.05$, ++$p < 0.01$, and +++$p < 0.001$ are compared to the positive control group. Please refer to the methods section for groups' definition.

parameters, including cholesterol, triglyceride, LDL, liver enzymes (ALP, ALT, AST), glucose, and insulin were increased while HDL and antioxidant enzymes were decreased. Histological examinations of the liver confirmed the development of steatosis. These changes represented the creation of fatty liver in animals.

Previous studies have shown a correlation between the occurrence of fatty liver disease and the high intake of saturated fats or carbohydrates [14,15]. Hypercholesterolemia, hypertriglyceridemia, low levels of HDL-C and high levels of LDL-C in serum are common disorders that occur in lipid homeostasis of those suffering from liver steatosis [16,17]. Triglycerides and cholesterol are also among important lipids whose over-received amounts lead to hypertriglyceridemia and hypercholesterolemia. Nonalcoholic fatty liver is specified by the accumulation of triglycerides in hepatocytes formed by the esterification of free fatty acids and glycerol [18]. In a different study, Zou *et al.* induced nonalcoholic fatty liver in rats by using a high-fat diet and reported steatosis while the amount of glucose, blood insulin, cholesterol and triglyceride significantly increased in the fatty liver-induced rats [19]. In the present study, treatment with nerol, in the form of dose-dependent, significantly decreased TG and LDL-C levels and improved HDL-C level in the treated groups. Consequently, another study has also shown that nerol has hypolipidemic effects [20]. In accordance with our study, it has also been shown that treatment with farnesol,

Table 2. Changes in Liver Enzymes in Mice Receiving Control Diet, High Fat Diet and Nerol. n = 8/Group

Parameters / Groups	AST (U/lit)	ALT (U/lit)	ALP (U/lit)
Control	62.64 ± 1.80	83.68 ± 1.03	119.80 ± 1.22
Fatty liver	135.97 ± 1.59 ***	102.71 ± 2.07 ***	226.02 ± 1.14 ***
Positive control (Fatty liver + silymarin 90 mg kg^{-1})	89.41 ± 1.22 * ###	83.00 ± 1.02 ###	139.71 ± 2.31 * ###
Sham (Fatty liver + nerol solvent)	135.00 ± 1.50 *** +++	102.56 ± 1.12 *** +++	226.02 ± 1.46 *** +++
Exp1 (Fatty liver + nerol 30 mg kg^{-1})	125.01 ± 2.00 *** ## +++	95.25 ± 2.31 *** # +++	200.12 ± 2.01 *** ## +++
Exp2 (Fatty liver + nerol 60 mg kg^{-1})	110.02 ± 1.87 *** ### +++	90.50 ± 1.08 ** ## ++	175.23 ± 1.28 *** ### ++
Exp3 (Fatty liver + nerol 90 mg kg^{-1})	95.00 ± 1.20 ** ### ++	88.00 ± 0.43 * ### +	150.07 ± 3.06 ** ### +

Note: Data are expressed as Mean ± SEM. *$p < 0.05$, ** $p < 0.01$, and ***$p < 0.001$ are compared to the control group. #$p < 0.05$, ##$p < 0.01$, and ###$p < 0.001$ are compared to the fatty liver group. +$p < 0.05$, ++$p < 0.01$, and +++ $p < 0.001$ are compared to the positive control group.

maintaining similar structure to nerol, greatly ameliorated dyslipidemia, including cholesterol and triacylglycerol [21]. Moreover, it has been found that the use of thymol and carvacrol, containing similar properties as nerol, demonstrated significant effects on serum levels of TG and cholesterol over a period of 29 days [22]. Furthermore, it has been demonstrated that treatment with nerol in mice consuming a high-fat diet decreased cholesterol and LDL concentrations in plasma which was associated with decreased levels of liver fat [23]. It has been shown that reduction in total cholesterol levels by nerol occurs through the regulation of SREBP2 gene which has a role in cholesterol synthesis [24]. With this regard, the powerful antioxidant activity of nerol controls LDL oxidation leading to increase in the absorption of cholesterol through dispersed macrophage receptors and ultimately results in reducing LDL levels [25].

In the present study, an increase in the serum level of ALP, AST and ALT enzymes of mice fed with a high-fat diet was observed signifying the occurrence of damage in liver cells [26,27]. AST and ALT are the two aminotransferases of great clinical significance as the serum elevations of these two aminotransaminases may occur with a variety of disorders; thus their assessment could be

Table 3. Changes in Superoxide Dismutase and Catalse in Mice Receiving Control Diet, High Fat Diet, and Nerol. n = 8/Group

Parameters / Groups	SOD (U/mg-protein)	CAT (U/mg-protein)
Control	19.60 ± 1.42	22.00 ± 1.02
Fatty liver	14.76 ± 0.65 ***	***
Positive control (Fatty liver + silymarin 90 mg kg^{-1})	18.08 ± 1.30 * ###	* ###
Sham (Fatty liver + nerol solvent)	14.00 ± 0.95 *** +++	*** +++
Exp1 (Fatty liver + nerol 30 mg kg^{-1})	15.20 ± 0.88 *** ## +++	*** ## +++
Exp2 (Fatty liver + nerol 60 mg kg^{-1})	17.05 ± 1.02 ** ### ++	*** ### ++
Exp3 (Fatty liver + nerol 90 mg kg^{-1})	17.88 ± 1.21 * ### +	** ### +

Note: Data are expressed as Mean ± SEM. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ are compared to the control group. #$p < 0.05$, ##$p < 0.01$, and ###$p < 0.001$ are compared to the fatty liver group. +$p < 0.05$, ++$p < 0.01$, and +++$p < 0.001$ are compared to the positive control group.

considered as a disease biomarker [28-30]. It is generally thought that aminotransferase elevations are due to cell damage with plasma membrane disruption [30]. Nerol could improve the levels of AST, ALT and ALP enzymes whose levels become affected in disease conditions [31]. It was demonstrated that geraniol, a monoterpene with a similar structure to nerol, could effectively improve the levels of AST, ALT and ALP [32] and has improved liver damage [33]. Moreover, another study showed that liver damage increased the levels of liver enzymes, including alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase in rats' serum, and limonene (a monoterpene) rescued the liver from damages due to its antioxidant and anti-inflammatory effects [34].

Previous investigations have shown that high-fat diets increased the production of free radicals resulting in oxidative stress. In fact, hypercholesterolemia has shown to decrease the activity of antioxidant enzymes, ultimately leading to cellular damage [35]. 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 [36]. Several studies have also shown that the use of high-fat diet in animals caused a significant

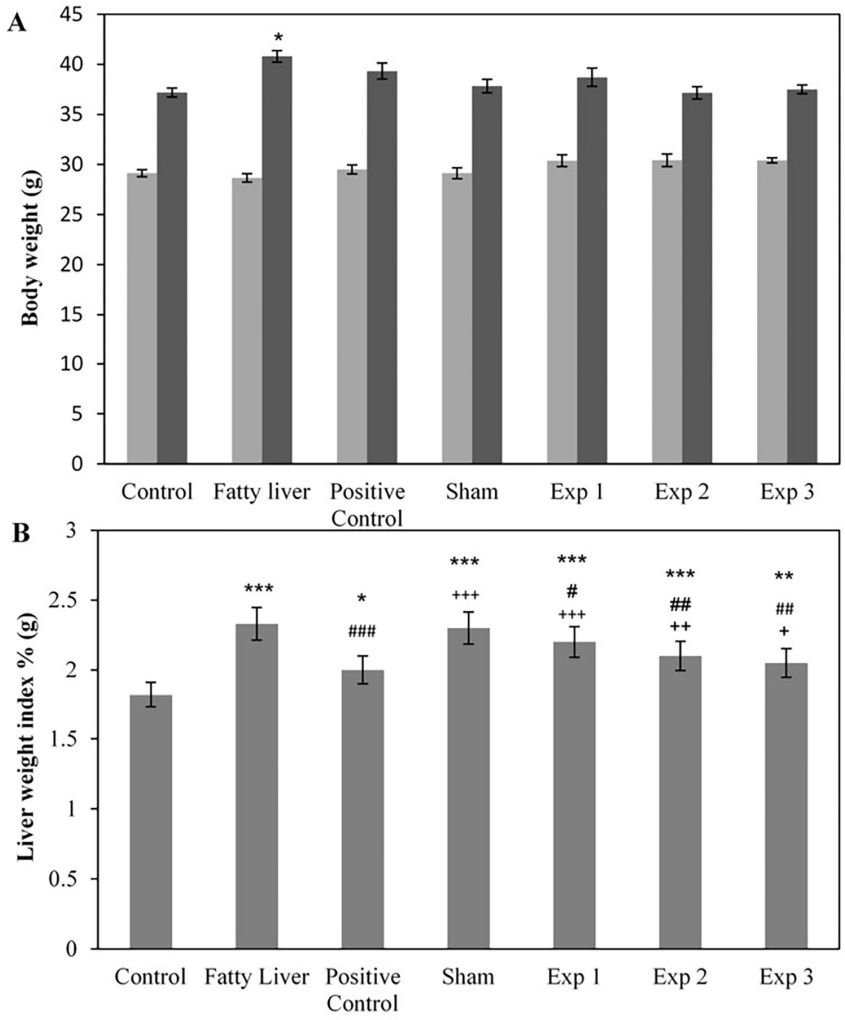

Fig. 3. The body weight (g) of mice in the first week (light gray) and the fourth week (dark gray) of treatment (A). The liver weight index% (g) of mice after four weeks of treatment (B). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ are compared to the control group. #$p < 0.05$, ##$p < 0.01$, and ###$p < 0.001$ are compared to the fatty liver group. +$p < 0.05$, ++$p < 0.01$, and +++$p < 0.001$ are compared to the positive control group. Please refer to the methods section for groups' definition.

reduction in the serum level of antioxidants, including SOD and catalase [37]. SOD is considered to be one of the most active enzymes whose activity is sufficient for inactivation of superoxide anions produced during oxidative stress in cells [38]. Herein, SOD and catalase levels were decreased significantly by consumption of high-fat diet, whereas prescribing nerol significantly increased the amount of enzymes. Linalool, a monoterpene with similar structure to nerol, has also shown the capability of improving the level of the antioxidant (SOD) in mice [39]. Accordingly, myrtenol, a phenolic monoterpene, has shown to significantly increase the levels of SOD [40]. Moreover, phenolic monoterpenes, such as thymol could increase the antioxidant potential [41]. Terpenese such as carvacrol, γ-

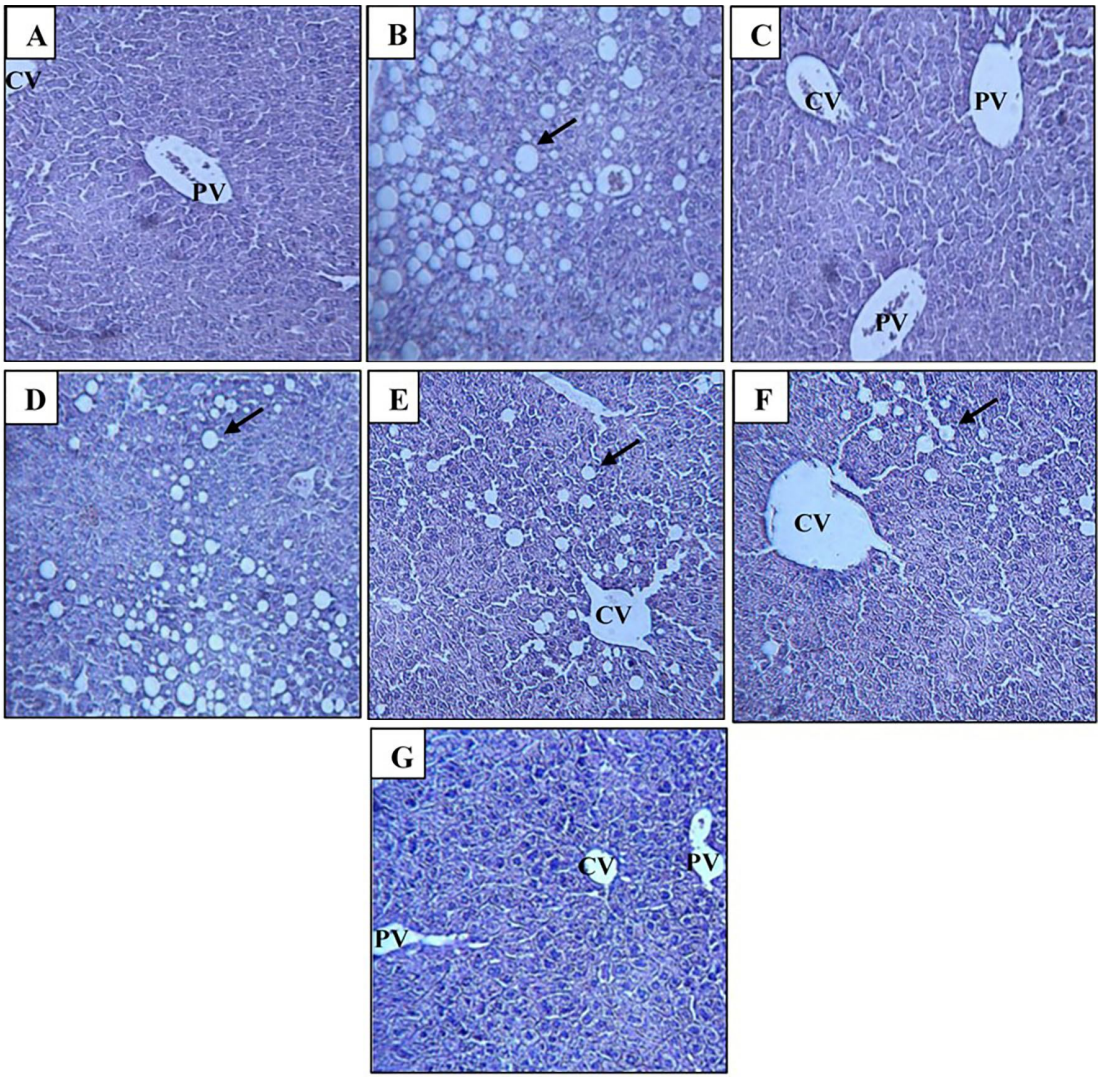

Fig. 4. H&E-stained sections of liver from the following groups: control (A), fatty liver (B), positive control (fatty liver + silymarin) (C), sham (fatty liver + nerol solvent) (D), Exp1 (fatty liver + nerol 30 mg kg^{-1}) (E), Exp2 (fatty liver + nerol 60 mg kg^{-1}) (F), and Exp3 (fatty liver + nerol 90 mg kg^{-1}) (G). Abbreviations: CV: central vein; PV: portal vein. Arrow: lipid droplets sediment. Magnification is 100X.

terpinene, *p*-cymene, and thymol have also demonstarted antioxidant effect *in vitro* [42]. The antioxidant of carvacrol has been further confirmed by another study [43]. Moreover, antioxidant activity of monoterpenoids α- and β-pinene, and 1,8-cineole haven been shown in the work of Perry and colleagues [43].

In the present case, induction of fatty liver in mice caused a significant increase in the levels of glucose and insulin. Higher glucose and insulin levels have been reported in liver disease [44]. Accordingly, it has been demonstrated that using a high-fat diet to induce fatty liver could lead to higher levels of fasting blood sugar [45]. On the other hand, increase in glucose and insulin levels have shown to activate two transcription factors in the liver that

promote *de novo* lipogenesis [46]. In the present study, nerol, in a dose-dependent manner, was capable of decreasing both glucose and insulin levels. Similar monoterpenes, such as farnesol and citral, have also shown antihyperglycemic activities [47].

According to various studies, high-fat diet could increase the size, the weight as well as the percentage of lipid accumulation in the liver [19]. 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 nerol showed no significant changes, whereas the liver weight index (%) was significantly reduced. Monoterpenes, have demonstrated hepatoprotective and therapeutics impacts on liver disorders [48].

Other studies have also investigated different properties of nerol whiles using similar doses. Khodabakhsh *et al.* assessed the analgesic and anti-inflammatory properties of neroli (40-80 mg kg^{-1}) in mice and rats. They demonstrated a significant impact for neroli, especially at 40 mg kg^{-1}, against acute and chronic inflammation [49]. Moreover, doses of 30, 60 and 90 mg kg^{-1} of nerol have been applied in Alzheimer's-induced rats, and therapeutic and protective potential for nerol were shown in counteracting memory and neuronal cell loss associated with Alzheimer's disease [50]. Neuropharmacological properties of nerol (30, 60 and 90 mg kg^{-1}) have also been evaluated in mice and a possible anxiolytic effect of nerol was demonstrated [51]. According to these studies, doses 30, 60 and 90 mg kg^{-1} of nerol were selected for the present study.

Many previous studies have used natural compounds to protect liver injury [52]. In the present study, silymarin was selected as the positive control as it has shown efficacy in the treatment of nonalcoholic steatohepatitis [53]. Herein, treatment with silymarin significantly improved the lipid profiles, liver enzymes as well as the levels of insulin and glucose. In accordance with previous studies, it has been demonstrated that silymarin could well improve the levels of antioxidant enzymes, and hepatic steatosis [54,55]. Along with sylimarin, monoterpene nerol, in a dose-dependent mode, showed capability of improving nonalcoholic fatty liver by increasing the antioxidant enzymes (SOD and catalase) and also decreasing the lipid profiles as well as liver enzymes.

CONCLUSIONS

Use of the simple monoterpene compound nerol, in a dose-dependent manner, has shown a significant effect on blood glucose and insulin levels in fatty liver-induced animals, as well as on ALT, AST and ALP levels. Significant effects were also detected toward lipid profiles which may be indicative of a potential of this compound in this regard. In this case, valuable therapeutic potential for nerol has been shown in nonalcoholic fatty liver disease and the compound seems interesting enough to be further investigated.

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