Original Article

Eugenol Administration Improves Liver Damage Induced by a Fructose-Rich Diet

Abstract

Backgrounds: The prevalence of metabolic syndrome (MetS) is increasing in developing countries that affects the liver in a variety of ways. This study was designed to investigate the protective role of eugenol on liver damage caused by fructose-induced MetS. **Materials and Methods:** Thirty male Wistar rats were randomly divided into five groups: 1: tap water (control), 2: fructose, 3: fructose + eugenol solvent, 4: fructose + eugenol 50 mg/kg, and 5: fructose + eugenol 100 mg/kg. At the end of the experiment, blood samples were taken for measurement fast blood glucose (FBG), serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), low-density lipoprotein, high-density lipoprotein, cholesterol, and triglyceride. **Results:** FBG significantly increased in Group 2 compared to Group 1 ($P < 0.001$); however, it significantly decreased in Groups 4 and 5 compared to Group 2 ($P < 0.05$). SGOT and SGPT levels significantly increased in Group 2 compared to the control group ($P < 0.001$). However, SGOT and SGPT levels significantly decreased in Groups 4 and 5. Malondialdehyde (MDA) and liver tissue damage score (LTDS) significantly increased in Group 2 compared with the control group ($P < 0.01$), whereas MDA and LTDS decreased in Groups 4 and 5 compared to Group 2 ($P < 0.05$). **Conclusion:** Eugenol may ameliorate liver damage in a rat model of fructose-induced MetS, and these protective effects may in part be mediated by improving antioxidant status and reducing oxidative stress and lipid peroxidation. It may also reduce hepatic inflammation and fat accumulation as well as fibrosis of liver cells.

Keywords: *Eugenol, fatty liver, fructose, metabolic syndrome*

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Introduction

The prevalence of metabolic syndrome (MetS) is increasing in developing countries. This disorder is accompanied with an increased risk of heart disease. It is also associated with obesity and insulin resistance. This disease is not only associated with an increased risk of Type 2 diabetes but also affects the liver in a variety of ways. Nonalcoholic fatty liver disease (NAFLD), a hepatic manifestation of MetS, is characterized by the accumulation of triglycerides (TGs) in the hepatocytes, inflammation, and varying degrees of liver damage.[1]

Despite significant advances in understanding the pathogenesis of MetS and related liver damage, many aspects of it are still unknown. Various theories have been proposed on the mechanism of induction of liver damage, including obesity, high-fat diet, and insulin resistance are responsible for the deposition of TGs in hepatocytes a prerequisite for hepatocyte injury.[2,3] Signaling mechanisms, including oxidative stress, free radicals, inflammatory cytokines, and adipokines may stimulating inflammatory responses and fibrogenesis.[2,3]

The importance of oxidative stress in inducing liver damage has been proven by extensive studies. These patients show increased levels of reactive oxygen species, lipid peroxidation products, and decreased concentrations of antioxidants, such as glutathione.[3,4]

It has been shown that consumption of high fructose along with other sugars, such as sucrose, increase the risk of NAFLD and nonalcoholic steatohepatitis. It is documented that people who have consumed sugary drinks containing high fructose for more than 6 months have increased liver fat at the end of the study. Increased fat accumulation in the liver could be the result of hepatic fructose

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metabolism, which impairs fatty acid beta-oxidation and increases *de novo* lipogenesis.[5-7]

Eugenol (4-allyl-2-methoxyphenol) is a phenolic compound of (Eugenia caryophyllata) with several biological activities.[8] It is a powerful antioxidant compound, fivefold higher than that observed for alpha-tocophero,[9] which reduces lipid peroxidation to about 96.7%. Eugenol acts as an effective free radical scavenger, and pharmacological studies have shown that it has anticonvulsant, local anesthetic, anti-stress, bactericidal, and antifungal properties.[8]

Abd El Motteleb *et al*. have shown that low doses of eugenol have a protective effect on ischemic damage/hepatic reperfusion by reducing lipid peroxidation and inflammatory factors and ultimately reducing apoptosis, while high doses exacerbate it and cause liver damage. The usage of low doses of eugenol reduced the level of lipid and malondialdehyde (MDA) oxidation and also increased the plasma antioxidant activity.[10]

Based on the description above, this study was designed to investigate the protective role of eugenol administration in reducing liver damage caused by MetS.

Materials and Methods

For the present study, thirty male Wistar rats (200 to250 g) were provided from the animal center under controlled (12 h light and 12 h dark) situations, temperature (23°C–25°C) and humidity (40%–45%), they were held in the cages with free access to the normal rat chow and drinking water (before the onset of experiment protocols). The experiments were carried out based on the committee for the purpose of control and supervision of animal experiments. The Animals Ethics Committee of Zahedan University of Medical Sciences, Iran, approved the experiments (No#8997, Ethical Committee Approval ID: IR.ZAUMS.REC.1399.257).

The animals in the control group received drinking water (tap water), Group 2 fructose+water (F) received water containing fructose (10% weight/volume), and in Groups 3, 4, and 5 animals supplied with water containing fructose plus IP injection of sesame oil (as solvent for eugenol) (F + V),[11] Eugenol 50 mg/kg (F + E50), and Eugenol 100 mg/kg (F + E100), respectively.[11]

Fructose regimens have been continued for 8 weeks to induce MetS.[12] On day 31, peritoneal injection of eugenol was started in the treated groups. At the end of the experiment, after 12 h fasting and under anesthesia with ketamine 75 mg/kg and xylazine 10 mg/kg (IP),[13] blood samples were taken from the heart for the measurement of serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), low-density lipoprotein (LDL), high-density lipoprotein (HDL), cholesterol (Chol) fast blood glucose (FBG), and triglyceride(TG). The liver was removed and weighed, divided into two sections, one for biochemical measurements (MDA and nitrite) and the other for tissue damage surveying in 10% formalin. After preparation and hematoxylin staining, the tissues were examined by two blind pathologists. Liver tissue damage score (LTDS) was determined based on the protocol of Veteläinen *et al*. [Table 1].[14]

The level of nitrite (stable NO metabolite) in supernatant was measured using a colorimetric assay kit (Zellbio, Germany) that involves the Griess reaction. MDA in the supernatant of homogenized liver tissue was measured based on the manual methodology.[15-17]

Eugenol (cat #E-91791) and fructose (D-fructose >99%) were obtained from Sigma and Syarikat System Malaysia Company, respectively.

Statistical analysis

The data are reported as mean ± standard error of the mean. Data were analyzed using one-way analysis of variance (ANOVA) followed by the LSD *post hoc* tests. Histopathological results were evaluated by the Kruskal–Wallis and Mann-Whitney U tests. The results were considered statistically significant if $P < 0.05$.

Results

The comparison of mean weight of animals in different groups showed a statistically significant difference between the group receiving fructose and the tap water group ($P < 0.05$). Treatment with eugenol (50,100 mg/kg/day) significantly reduces body weight compared to fructose group ($P < 0.05$), as shown in Table 2. However, liver weight (g/100 g BW) showed

Table 1: Histopathology score of liver damage

Histological criteria	Severity	Description	Score
Steatosis	Absent	<10%	0
	Mild	10%-30%	1
	Marked	31%-60%	2
	Severe	>60%	3
Inflammation	None		0
	Moderate	Scattered[a]	1
	Marked	Foci[a]	2
	Severe	Diffuse[a]	3
Necrosis	Absent	0%	0
	Mild	<10%	1
	Marked	10%-50%	2
	Severe	>50%	3
Fibrosis[b]	Absent		0
	Mild		1
	Marked		2
	Severe		3

[a]Amount of inflammatory cells, [b]Mild: Moderately thickened CLV, marked: Markedly thickened CLV, Severe: Cirrhosis, CLV: Centrolobular vein

no significant difference between the two treatment groups received different doses (50 and 100 mg/kg/day) of eugenol [Table 2].

As shown in Table 2, significant differences in fast blood glucose (FBG) were observed among the treatment groups. FBG significantly increased in Group 2. (fructose + water) compared to tap water group ($P < 0.001$). However, FBG significantly decreased in F + E50 and F + E100 groups compared to Group 2, fructose + water, ($P < 0.05$ and $P < 0.001$) respectively. Furthermore, significant difference in FBG ($P < 0.05$) was observed between F + E50 and F + E100 groups.

The levels of SGOT and SGPT in the experimental groups are illustrated in Table 3. SGOT and SGPT levels significantly increased in Groups 2 (F) and 3 (F + V) compared to the tap water group of rats ($P < 0.001$). However, treatment with two different doses of eugenol (50 and 100 mg/kg) significantly decreased SGOT and SGPT levels compared to that in Group 2 (F) ($P < 0.05$). There was no significant difference in SGOT and SGPT levels between Groups 4 and 5 (eugenol 50 and 100 mg/kg).

As shown in Table 3, Chol, LDL, and TG levels significantly increased in Group 2 (fructose + water) compared to that in the tap water group ($P < 0.01$). Although treatment with both doses (50 and 100 mg/kg) of eugenol did not changed the increase in Chol and TG, just low dose (50 mg/kg) of eugenol declined LDL compared to that in Group 2 (fructose + water).

Table 2: The Body weight (g), liver weight (g/100g BW) and fast blood glucose, FBG (mg/dl) in each group

Groups	Body weight (g) Mean±SEM	Liver weight (g/100g BW) Mean±SEM	FBG (mg/dl) Mean±SEM
Tap water	268±8.40	3.07±0.28	112.8±2.3
F	292±9.61	3.02±0.11	157.66±10.52*
F + V	301±7.32	3.21±0.06	161.42±2.16*
F + E 50	290±8.20	2.85±0.07	133.75±1.73$^{\$,\#}$
F + E100	290±9.51	2.77±0.12	115.15±2.46$^{\#}$

The symbols indicate a significant difference, *from tap water group, $^{\$}$from fructose + eugenol 100 mg/kg group, $^{\#}$from fructose group ($P<0.05$). Fructose, F; Fructose + vehicle, F + V; Fructose + eugenol 50 mg/kg, F + E50; Fructose+eugenol 100 mg/kg, F + E100

LDL levels had marked difference between Groups 4 and 5 compared to Groups 2 (fructose + water) ($P < 0.001$).

Figure 1a represents nitrite and MDA levels in different experimental groups. MDA significantly increased in Group 2 (fructose + water) compared to the tap water group ($P < 0.01$), whereas it significantly decreased in F + E50 and F + E100 groups compared to Group 2 (fructose + water) ($P < 0.05$). Furthermore, significant difference in MDA ($P < 0.05$) was observed both low and high treatment eugenol groups. On the other hand, the mean level of tissue nitrite showed a decrease following induction of MetS (fructose + water) compared with tap water rats ($P < 0.05$). Treatment with both doses of eugenol (fructose + eugenol 50 and 100 mg/kg groups) increased nitrite levels significantly ($P < 0.05$), although no difference in nitrite levels was seen between the two eugenol-treated groups (F + E50 and F + E100) [Figure 1b].

The histopathology score showed a significant increase in tissue damage, including steatosis, inflammation, necrosis, and fibrosis in Group 2 (F) compared to those in tap water rats. ($P < 0.05$). On the other hand, the results show that treatment with eugenol 50 and 100 mg/kg/day reduced LTDS compared to the fructose group ($P < 0.001$). However, there was no significant difference in the amount of tissue damage between the two groups receiving eugenol (50 and 100 mg/kg/day), [Figure 1c]. These findings were compatible with the liver pathological changes [Figure 2]

Discussion

This study showed that the use of eugenol at doses of 50 and 100 mg/kg improves lipid profile and liver function in the experimental model of MetS. The effects of long-term fructose consumption on the liver in the present study were similar to the study results of Pardhe *et al*., who reported that high fructose diet increased liver enzymes and lipid profile according to the NAFLD grade.

High fructose intake has been shown to cause fatty liver, hepatotoxicity, and increased inflammatory cytokines, which have played an important role in the development of liver cell damage and NAFLD.[18] Therefore, most previous studies were consistent with our study.

Table 3: The SGOT (mg/dl), SGPT (mg/dl), LDL (mg/dl), Chol (mg/dl), TG (md/dl) and HDL (mg/dl) in each group

Groups	SGOTmg/dl Mean±SEM	SGPT mg/dl Mean±SEM	LDLmg/dl Mean±SEM	Chol mg/dl Mean±SEM	TG mg/dl Mean±SEM	HDL mg/dl Mean±SEM
Tap water	159±9.49	79.40±1.28	106.00±1.26	153.41±1.07	138.00±0.70	34.60±1.40
F	249.51±18.61*	111.02±3.86*	117.17±0.70*	172.50±1.25*	156.83±2.54*	40.33±0.84
F + V	223.42±15.64*	126.02±2.24*	116.14±1.43*	168.43±3.47*	157.43±3.09*	37.71±1.71
F + E 50	129.37±6.55$^{\&}$	69.50±4.65$^{\&}$	110.75±1.32$^{\&}$	173.50±2.75	166.5±5.47	40.75±1.91
F + E100	124.93±11.33$^{\&}$	71.25±10.93$^{\&}$	120.62±2.76	168.50±4.29	168.75±4.83	39.56±1.42

The symbols indicate a significant difference,*from tap water group, $^{\&}$from fructose and fructose + vehicle groups. Tap Water, TW; Fructose, F; Fructose + vehicle, F + V; Fructose + eugenol 50 mg/kg, F + E50; Fructose + eugenol 100 mg/kg, F + E100

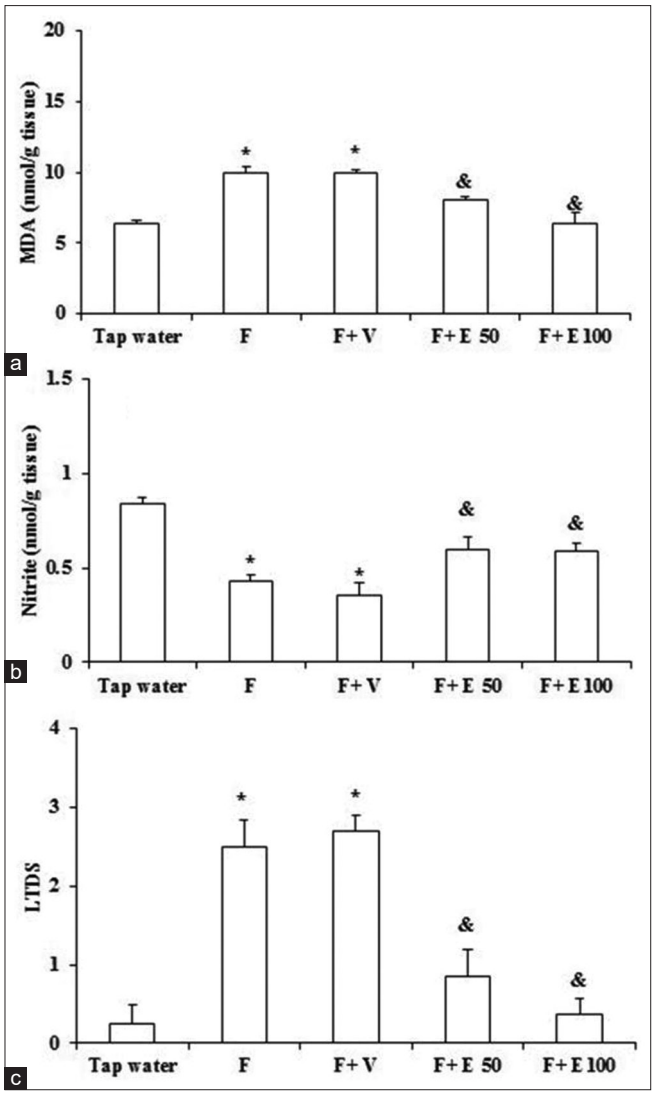

Figure 1: Evaluation of MDA (a) and nitrite (b) levels of liver and liver tissue damage score LTDS (c).* Indicates significant differences compared to Tap water group, & Indicates significant differences compared to fructose and fructose + vehicle group, (P < 0.05). Tap Water, Fructose, F; Fructose + vehicle, F + V; Fructose + eugenol 50 mg/kg, F+E 50; Fructose + eugenol 100 mg/kg, F+E 100

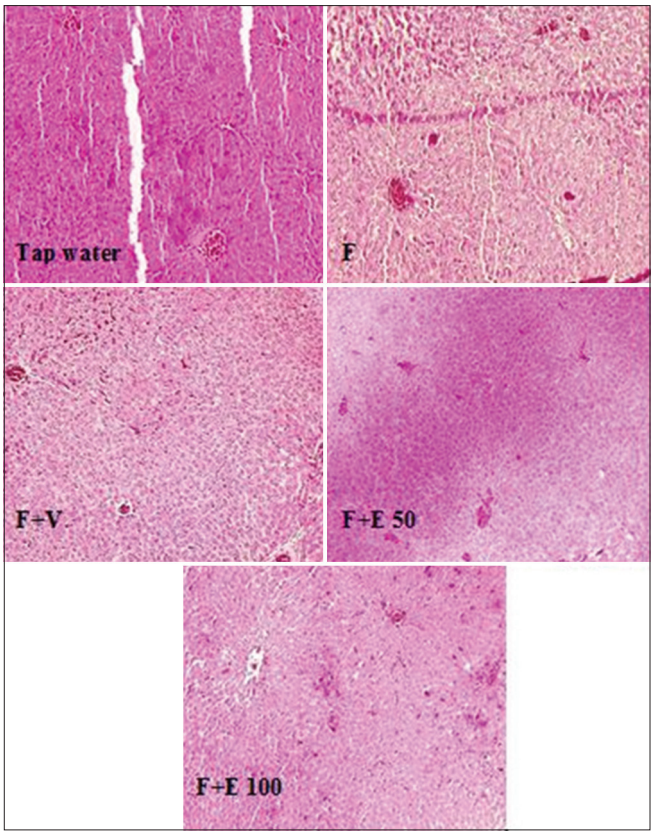

Figure 2: The pathology images (magnification × 100) of liver tissue in experimental groups. The groups received tap water Fructose, F; Fructose + vehicle, F + V; Fructose + eugenol 50 mg/kg, F + E50; Fructose + eugenol 100 mg/kg, F + E100

The development of steatosis, inflammation of the lobules, and liver damage by fructose has been reported in other studies, and concomitant treatment with eugenol by reducing liver enzymes has improved its function, which is confirmed by histological results.[19,20]

Another study reported that doses of 50 and 200 mmol of eugenol for 48 h reduced fat accumulation by affecting the AMP-activated protein kinase-Sterol regulatory element binding protein (AMPK-SREBP) signaling pathway, suggesting that eugenol acts as an anti-fatty liver agent. They have suggested that eugenol and the herbal compounds it contains as a dietary supplement may have beneficial effects on the treatment of fatty liver and hepatic fibrosis in the early stages.[21] In this regard, Harb, Amani. *et al*. reported that eugenol has reduced Chol, LDL, and the atherogenic index, whereas it has no effect on HDL and TG. In addition, it reduced steatosis, liver inflammation, and hepatomegaly, as well as liver enzyme levels and improved superoxide dismutase and catalase activity(CAT). Their study showed that the hypercholesterolemic effects of eugenol are not mediated by the inhibition of cholesterol synthesis. According to this study, TRPV1 channel, a nonselective cation channel that is highly permeable to calcium, may play an important role in hyperlipidemia. This channel is also increased in inflammatory and lipidemic conditions. In fact, activation TRPV1 channel causes the accumulation of fat in liver cells, whereas inhibition of this channel can prevent the fat accumulation in the hepatocytes.

Associated studies have shown that eugenol prevents the build-up of hepatic fat and improves its function by reducing the TRPV1 channel. They have suggested that chronic use of eugenol has a protective effect against hypercholesterolemia.[22]

Our study showed that eugenol has protective effects on the liver damage induced by a fructose-rich diet. Eugenol may improve hepatic injury by reducing the fat accumulation in liver and lowering hypercholesterolemia. These observations are supported by the finding that eugenol has anti-fatty effects on the liver and as a dietary supplement improves

hepatic steatosis and liver fibrosis. In this study, we showed that eugenol improves liver enzymes. Our results are in agreement with other studies that have reported eugenol lowers liver enzyme levels in thioacetamid-induced hepatotoxicity,[23] stereptozocin-induced diabetes,[24] high-fat diet-induced fatty liver disease,[21] and triton-induced hypercholesterolemia.[25] The results also revealed that eugenol has anti-inflammatory effects. Indeed, eugenol has antioxidant properties that are partially mediated by lowering the MDA and increasing the level of hepatic nitrite. In confirmation of this result, it has been reported that nitrite attenuates oxidative stress and maintains AMPK activity in the mouse model. In addition, nitrite has been shown to protect the liver against diet-induced steatosis.[26]

Conclusion

Eugenol may ameliorate liver damage in a rat model of fructose-induced MetS, and these protective effects may in part be mediated by improving antioxidant status and reducing oxidative stress and lipid peroxidation. It may also reduce hepatic inflammation and fat accumulation (steatosis), as well as fibrosis of liver cells.

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Conflicts of interest

There are no conflicts of interest.

References

1. Marchesini G, Bianchi G. Metabolic syndrome: Relevant for all types of chronic liver diseases? Gut 2010;59:1314-5.
2. Zhang X, Ji X, Wang Q, Li JZ. New insight into inter-organ crosstalk contributing to the pathogenesis of non-alcoholic fatty liver disease (NAFLD). Protein Cell 2018;9:164-77.
3. Mann JP, Valenti L, Scorletti E, Byrne CD, Nobili V. Nonalcoholic fatty liver disease in children. Semin Liver Dis 2018;38:1-3.
4. Hatton G, Alterio T, Nobili V, Mann JP. Unmet needs in pediatric NAFLD research: What do we need to prioritize for the future? Expert Rev Gastroenterol Hepatol 2018;12:961-7.
5. Jegatheesan P, De Bandt JP. Fructose and NAFLD: The multifaceted aspects of fructose metabolism. Nutrients 2017;9:230.
6. Jensen T, Abdelmalek MF, Sullivan S, Nadeau KJ, Green M, Roncal C, *et al*. Fructose and sugar: A major mediator of non-alcoholic fatty liver disease. J Hepatol 2018;68:1063-75.
7. Mai BH, Yan LJ. The negative and detrimental effects of high fructose on the liver, with special reference to metabolic disorders. Diabetes Metab Syndr Obes 2019;12:821-6.
8. Gülçin İ. Antioxidant activity of eugenol: A structure-activity relationship study. J Med Food 2011;14:975-85.
9. Nagababu E, Rifkind JM, Boindala S, Nakka L. Assessment of antioxidant activity of eugenol *in vitro* and *in vivo*. In: Free Radicals and Antioxidant Protocols. Methods Mol Biol 2010. p. 165-80.
10. Abd El Motteleb DM, Selim SA, Mohamed AM. Differential effects of eugenol against hepatic inflammation and overall damage induced by ischemia/re-perfusion injury. J Immunotoxicol 2014;11:238-45.
11. Daniel AN, Sartoretto SM, Schmidt G, Caparroz-Assef SM, Bersani-Amado CA, Cuman RK. Anti-inflammatory and antinociceptive activities A of eugenol essential oil in experimental animal models. Rev Bras Farmacogn 2009;19:212-7.
12. Bantle JP. Dietary fructose and metabolic syndrome and diabetes. J Nutr 2009;139:1263S-8.
13. Lakzaei H, Safari T, Komeili GR. Interaction of sex hormones and the renin-angiotensin system in ovariectomized rats subjected to ischemia-reperfusion induction. Adv Biomed Res 2019;8:64.
14. Veteläinen RL, Bennink RJ, de Bruin K, van Vliet A, van Gulik TM. Hepatobiliary function assessed by 99m Tc-mebrofenin cholescintigraphy in the evaluation of severity of steatosis in a rat model. Eur J Nucl Med Mol Imaging 2006;33:1107-14.
15. Nematbakhsh M, Ebrahimian S, Tooyserkani M, Eshraghi-Jazi F, Talebi A, Ashrafi F, *et al*. Gender difference in cisplatin-induced nephrotoxicity in a rat model: Greater intensity of damage in male than female. Nephrourol Mon 2013;5:818-21.
16. Sadeghi F, Nematbakhsh M, Noori-Diziche A, Eshraghi-Jazi F, Talebi A, Nasri H, *et al*. Protective effect of pomegranate flower extract against gentamicin-induced renal toxicity in male rats. J Renal Inj Prev 2015;4:45-50.
17. Botsoglou Nickos A, Fletouris Dimitrios J, Papageorgiou Georgios E, Vassilopoulos Vassilios N, Mantis Antonios J, Trakatellis Antonios G. Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. J Agricultur Food Chem 1994;42:1931-7.
18. Pardhe BD, Shakya S, Bhetwal A, Mathias J, Khanal PR, Pandit R, *et al*. Metabolic syndrome and biochemical changes among non-alcoholic fatty liver disease patients attending a tertiary care hospital of Nepal. BMC Gastroenterol 2018;18:109.
19. Azab M. Eugenol ameliorates pathogenic features in experimental model of NAFLD in rats. Al Azhar J Pharm Sci 2017;56:115-22.
20. Al-Okbi SY, Mohamed DA, Hamed TE, Edris AE. Protective effect of clove oil and eugenol microemulsions on fatty liver and dyslipidemia as components of metabolic syndrome. J Med Food 2014;17:764-71.
21. Jo HK, Kim GW, Jeong KJ, Kim DY, Chung SH. Eugenol ameliorates hepatic steatosis and fibrosis by down-regulating SREBP1 gene expression via AMPK-mTOR-p70S6K signaling pathway. Biol Pharm Bull 2014;37:1341-51.
22. Harb AA, Bustanji YK, Almasri IM, Abdalla SS. Eugenol reduces LDL cholesterol and hepatic steatosis in hypercholesterolemic rats by modulating TRPV1 receptor. Sci Rep 2019;9:1-10.
23. Yogalakshmi B, Viswanathan P, Anuradha CV. Investigation of antioxidant, anti-inflammatory and DNA-protective properties of eugenol in thioacetamide-induced liver injury in rats. Toxicology 2010;268:204-12.
24. Srinivasan S, Sathish G, Jayanthi M, Muthukumaran J, Muruganathan U, Ramachandran V. Ameliorating effect of eugenol on hyperglycemia by attenuating the key enzymes of glucose metabolism in streptozotocin-induced diabetic rats. Mol Cell Biochem 2014;385:159-68.
25. Venkadeswaran K, Muralidharan AR, Annadurai T, Ruban VV, Sundararajan M, Anandhi R, *et al*. Antihypercholesterolemic and antioxidative potential of an extract of the plant, piper betle, and its active constituent,

eugenol, in triton WR-1339-induced hypercholesterolemia in experimental rats. Evid Based Complement Alternat Med 2014;2014:11 page.
26. Cordero-Herrera I, Kozyra M, Zhuge Z, McCann Haworth S, Moretti C, Peleli M, *et al*. AMP-activated protein kinase activation and NADPH oxidase inhibition by inorganic nitrate and nitrite prevent liver steatosis. Proc Natl Acad Sci U S A 2019;116:217-26.