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Effect of Lycopene and Genistein on Hepatic Inflammation and Fibrosis in Thioacetamide Induced Liver Injury in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Lycopene, a red crystalline carotenoid, and genistein, a phytoestrogen, have shown considerable promise as effective agents for chronic diseases prevention by reducing oxidative stress. The objective of this experiment was to elucidate the protective effect of lycopene, genistein and their combination against thioacetamide-induced chronic liver injury. Thirty-five rats were randomized into five groups: one untreated group (Control) and four groups treated with the hepatotoxicant thioacetamide (TAA) 200 mg/kg b.w. i.p., for 8 weeks. Concomitantly, the rats received a standard diet (control and TAA), lycopene 6 mg/kg, p.o. (TAA+L), genistein 1 mg/kg, p.o. (TAA+G) or lycopene and genistein (TAA+L+G). After 8 weeks of treatment, the rats were killed and blood and

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liver samples were collected. TAA administration elevated liver malondialdehyde (MDA), collagen type 1, tumor necrosis factor-α (TNF-α), transforming growth factor β-1 (TGF-β1), nuclear factor kappa B (NF-κB); depleted heme oxygenase-1 (HO-1), nuclear factor-E2-related factor-2 (Nrf2) and glutathione peroxidase (GPx) activity. Lycopene and genistein supplementation reduced liver MDA concentration, fibrosis/inflammation scores (P <0.001), alpha-smooth muscle actin (α-SMA) scores, TGF-β1, TNF-α, NF-κB (P <0.001) and collagen type 1 (P<0.01) levels in TAA-treated rats. Lycopene and genistein intake increased HO-1 (P <0.01), Nrf2 (P <0.001) and GPx activity (P<0.05) in the liver of TAA-treated groups. Lycopene combined with genistein significantly reduced fibrosis/inflammation, α-SMA scores (P <0.001), compared to the lycopene treated group. In conclusion, our results demonstrated that lycopene and genistein supplementation protected the rat liver from TAA-caused fibrogenesis by suppressing hepatic inflammation and inhibiting HSC activation, possibly through downregulation of NF-κB and activation of Nrf2 pathways. Lycopene combined with genistein intake presented greater beneficial effects than lycopene against liver injury induced by TAA in rats.

Keywords: Liver fibrosis; thioacetamide; genistein; lycopene.

1. INTRODUCTION

Liver fibrosis is mainly characterized by the accumulation of extracellular matrix proteins such as collagen in relation with alcohol, viruses, and toxic chemicals. Hepatic stellate cell (HSC)'s play a key role in the development of liver fibrosis [1,2]. Thioacetamide (TAA) leads to centrilobular necrosis and hepatitis in acute applications [3,4] and to liver cirrhosis in chronic applications [5,6]. It causes membrane damage, oxidative stress and accumulation of lipid droplets in the hepatocyte cytoplasm to enhance inflammation and liver injury in rodents. It can be translated to human disease and therapy [7].

Several studies suggest that some micronutrient including antioxidants can prevent or reverse the liver cirrhosis because of the role of oxidative stress in this chronic disease [8-10]. Lycopene, a non-provitamin A carotenoid, was reported to be the most common carotenoid in the human diet and to display a strong antioxidant effect [11,12]. Various epidemiological and experimental studies have shown that lycopene had a protective effect on various types of cancer, including prostate cancer, gastric cancer, breast cancer, and lung cancer [13-15]. Genistein, a phytoestrogen from the flavonoid group, is defined as a compound widely found in plants and having a high antioxidant activity [16]. Known to have antitumoral, anti-inflammatory and antioxidant effects, genistein is asserted to be able to prevent cell growth in a great number of cellular systems by regulating the transforming growth factor-beta-1 (TGF-β1) [17]. In two recent *in vitro* studies, genistein was reported to inhibit the proliferation and activation of stellate cells responsible for hepatic fibrogenesis, as well as having potential preventive effects on liver fibrosis [18,19]. Genistein is considered to exert potent antitumor effect partially through its anti-angiogenesis property [20]. However, the role of combination of lycopene and genistein supplementation on TAA-induced chronic liver injury not reported. In the present study, we compared the antifibrotic effects of lycopene and genistein, both strong antioxidants, on TAA-induced chronic liver injury. We also examined whether their co-administration increases their effects.

2. MATERIALS AND METHODS

2.1 Animals and Experimental Design

The study was conducted in the Experimental Research Center at Firat University (FÜDAM; approval number: 2012/12) in compliance with standard ethical rules for experimental animal studies, upon the receipt of approval from the Ethics Committee for Animal Experiments at Firat University (FÜHADEK). Thirty five wistar albino male rats were used with an average weight of 220 grams. The rats were kept in an environment where a temperature of 22 ±1°C and a 12-h light-darkness cycle were ensured according to ethical rules. The animals were fed standard diet and tap water. The rats were weighed and recorded weekly throughout the experiment. The rats were divided into 5 groups of 7 animals each:

Group I (Control group) (n=7): Rats received a standard diet + saline solution intraperitoneally for 8 weeks.

Group II (TAA) (n=7): Rats received a standard diet + 200 mg/kg TAA intraperitoneally (i.p.) twice a week for 8 weeks + saline solution i.p.

Group III (Lycopene group) (TAA+L) (n=7): Rats received a standard diet + 200 mg/kg TAA i.p. twice a week for 8 weeks + 6 mg lycopene/kg/day, p.o.

Group IV (Genistein group) (TAA+G) (n=7): Rats received a standard diet + 200 mg/kg thiocetamide i.p. twice a week for 8 weeks + 1 mg genistein/kg/day, p.o.

Group V (Lycopene + Genistein group) (TAA+L+G) (n=7): Rats received a standard diet + 200 mg/kg TAA i.p. twice a week for 8 weeks + 1 mg genistein/kg/day, p.o. + 6 mg lycopene/kg/day, p.o.

Genistein (Bonistein, DSM, Switzerland) was dissolved in 0.5 ml dimethyl sulfoxide 30% and administered to rats by gavage. Lycopene (Redivivo, DSM, Switzerland) was dissolved in distilled water and administered by gavage. The selection of doses for lycopene, genistein and TAA were based on previously Sahin K [21,22] and Jamal MH et al. [23] studies which have demonstrated in rodents, respectively. TAA (Sigma-Aldrich Chemical Co., USA) was prepared in 200 mg/kg doses to be given twice a week for 8 weeks and administered i.p. Distilled water was used as a solvent.

Eight weeks later, the rats were decapitated while under anesthesia following overnight fasting and blood samples were collected. The blood samples were centrifuged at 5,000 rpm for 5 minutes and the resulting serum samples were stored at -20°C until the analysis. The abdomens of the rats were then opened and their livers were removed, without impairing the integrity of the tissue, to be weighed and recorded. Tissue samples were collected from different sections of the liver and fixed in formalin 10% solution. Paraffin blocks were prepared.

2.2 Laboratory Analyses

Serum aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transpeptidase (GGT) levels were examined by Olympus AU 600 autoanalyzer using Olympus kits. Serum glutathione peroxidase (GPx) was examined by ELISA method using a commercial kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. Lipid peroxidation was measured in terms of Malonyldialdehyde (MDA) formation, which is the major product of membrane lipid peroxidation done by a previously described method [24] with slight modification. The liver MDA content was measured by high performance liquid chromatography (HPLC, Shimadzu, Tokyo, Japan) using a Shimadzu UV–vis SPD-10 AVP detector and a CTO-10 AS VP column in a mobile phase consisting of 30 mM KH2PO4 and methanol (82.5+17.5, v/v; pH 3.6) at a flow rate of 1.2 ml/min. Column effluents were monitored at 250 nm and the volume was 20 µl. The liver homogenate (10%, w/v) was prepared in 10 mM phosphate buffer (pH 7.4), centrifuged at 13,000 ×g for 10 min at 4°C, and the supernatant was collected and stored at -80°C for MDA analysis.

Tissue GPx (anti-glutathione peroxidase 1 antibody, Abcam, Cambridge, UK), tumor necrosis factor (TNF-α; Anti-TNF alpha antibody, Abcam, Cambridge, UK), transforming growth factor beta (TGF-β; Anti-TGF beta antibody, Abcam, Cambridge, UK), nuclear factor-kappa B (NF-κB; Anti-NFκB p65 antibody, Abcam, Cambridge, UK), and collagen type 1 (anti-collagene type I antibody, Abcam Cambridge, UK), heme oxygenase-1 (HO-1, Abcam Cambridge, UK) and nuclear factor erythroid 2-related factor 2 (Nrf2; Abcam Cambridge, UK) were examined by Western Blot method using the related kits.

2.3 Histopathological Assessment

Liver slices were collected from blocks fixed in paraffin and stained with hematoxylin and eosine (H&E) for histopathological examination. Masson Trichrome staining was used for fibrosis assessment. The stained histological section were examined by the specialist pathologist in a blind manner, using Olympus BX-50 light microscope with 40x, 100x, 200 xs, and 400x magnification.

Histological section stained with H&E and Masson's Trichrome were thoroughly examined under a light microscope with 400x magnification. In the histopathological assessment, the tissue fibrosis was evaluated according to the Metavir scoring system [25].

Score 0: No fibrosis.
Score 1: Dilatation in portal areas, no septa formation.
Score 2: Dilatation in portal areas, rare septa formation.

Score 3: Significant septa formation, no cirrhosis.
Score 4: Cirrhosis.

Inflammation was averaged by counting the inflammatory cells per square millimeter in 10 randomly selected areas under 400 x magnifications to determine the number of inflammatory cells per square millimeter. Necrosis was determined as the number of necrotic foci per square millimeter [26].

2.4 Immunohistochemical Examination

Liver tissue was stained immunohistochemically by α-SMA (α-smooth muscle actin; Actin, Smoooth Muscle Ab-1, Thermo Scientific, UK.) to show the activation of HSC. The presence of α-SMA-reactive HSC in the liver tissue was scored semi-quantitatively [27].

Score 0: No staining or staining in very few cells.
Score 1: Staining in less than 30% of HSCs in sinusoidal areas.
Score 2: Staining in 31% to 60% of HSCs in sinusoidal areas.
Score 3: Staining in 61% to 90% of HSCs in sinusoidal areas.
Score 4: Staining in more than 90% of HSCs in sinusoidal areas.

2.5 Statistical Analyses

Data from groups were presented as average ± standard deviation. SPSS 13.0 package program was used for the preparation of statistics. In the evaluation of parameters, data from groups were assessed using Kruskal-Wallis variance analysis and Mann-Whitney U-test. $p<0.05$ was accepted as a significant value.

3. RESULTS

3.1 Body and Liver Weights

As shown in Table 1, the TAA control group weighed significantly less than all other groups ($P<0.05$). The highest liver weight was observed in the TAA control group. Administration of lycopene significantly lowered the liver weight, an effect comparable to that in the genistein administered group ($P<0.05$).

3.2 Biochemical Analyses

As shown in Table 2, in TAA group, serum levels of ALT (74 vs 101), AST (160 vs 324) and GGT (2.83 vs 0.43) activities were significantly increased compared with controls ($P < 0.001$), whereas the levels of ALT ($P < 0.05$, $P <0.001$ and $P < 0.05$), AST ($P < 0.05$), and GGT ($P < 0.01$, <0.05 and $P < 0.01$, respectively) in TAA+L, TAA+G and TAA+L+G group were much lower (Table 1). No significant difference was detected among TAA+L, TAA+G, and TAA+L+G groups ($P >0.05$) (Table 1). Antioxidant enzymatic activity of GPx in liver homogenates is shown in Table 2. GPx activity was decreased in TAA controls compared with normal controls (from 1472 to 920). However, there was no statistically significant difference in serum GPx levels among groups ($P > 0.05$) (Table 2).

Liver MDA levels increased significantly in TAA-treated rats compared with normal control rats (Fig. 1; $P <0.001$). Compared to TAA-treated animals, administration of lycopene, genistein and particularly with a combination of the supplements significantly lowered the MDA level, restoring a level comparable to normal controls. The effect of combination of lycopene and genistein was more pronounced on these parameters.

Table 1. Body and liver weight of rats*

Groups	Inisial body We (gr)	Final body We (gr)	Liver weight (gr)
Control	218.29±7.73	$280.57±18.18^{a}$	9.51±0.48
TAA	219.71±14.50	$221.29±6.69^{b}$	10.12±0.94
TAA+Lyc	218.14±7.40	$238.67±3.05^{b}$	10.92±0.27
TAA+Gen	219±5.85	$254±8.18^{a,b}$	9.82±0.69
TAA+Lyc+Gen	219.29±8.73	$249±16.62^{a,b}$	10.68±1.1

**Data are presented as average ± standard deviation. Control, no treatment; TAA, Rats treated with TAA; TAA+L, Rats treated with TAA and lycopene; TAA+G; Rats treated with TAA and genistein. TAA+L+G, Rats treated with TAA and lycopene and genistein. We: Weight. Different superscripts (a,b) indicate group mean differences. The TAA control group weighed significantly less than all other groups (P<0.05)*

Table 2. Effects of lycopene and genistein on the serum biochemical parameters in rats

	Control	TAA	TAA+L	TAA+ G	TAA+L+G
ALT (U/L)	74.14±3.27	101.83±8.20	82.75±9.23$^{\#}$	74.71±5.23$^{\#\#\#}$	88.33±11.78$^{\#}$
AST (U/L)	160.5±21.3	324.17±20.7	182.75±55.79$^{\#}$	178.29±12.21$^{\#}$	184.67±43.70$^{\#}$
GGT (U/L)	0.43±0.30	2.83±1.512	0.25±0.25$^{\#\#}$	0.57±0.30$^{\#\#}$	0.33±0.33$^{\#\#}$
GSH-Px (U/L)	1472±262	920±171	1156±97	1032±82	1099±140

Data are presented as average ± standard deviation. Control, no treatment; TAA, Rats treated with TAA; TAA+L, Rats treated with TAA and lycopene; TAA+G; Rats treated with TAA and genistein. TAA+L+G, Rats treated with TAA and lycopene and genistein. ALT: Aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma glutamyl transpeptidase; GSH-Px: Glutathione peroxidase.
**Compared with the TAA group: $^{\#}$ p<0.05; $^{\#\#}$ p<0.01; $^{\#\#\#}$ p<0.001*

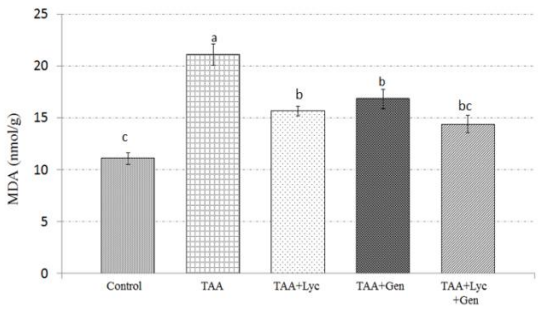


Fig. 1. Comparison of malondialdehyde concentration between the group
Different superscripts (a–c) indicate group mean differences (P < 0.05). Compared to TAA-treated animals, administration of lycopene, genistein and particularly with a combination of the supplements significantly lowered the MDA level (p<0.05). By Fisher's multiple comparison test

3.3 Western Blot Analyses

As shown in Fig. 2, the levels of TNF-α, TGF-β, NF-κB, type 1 collagen (Fig. 2C-F) in liver were significantly elevated in TAA rats as compared to that of the control group (*P*<0.001). Expression of GSH-Px levels in liver was lower in rats treated with TTA as compared to control rats (*P* < 0.01) (Fig. 2A and B). Lycopene or genistein each alone similarly decreased the TNF-α, TGF-β, NF-κB, and type 1 collagen levels, but the magnitude of the decrease was more in case of the combination of supplements (*P* < 0.05). However, lycopene or genistein each alone or their combination increased (*P* <0.05) GSH-Px levels in this tissue (*P* <0.05). Expressions of Nrf2 and HO-1 in liver were lower in rats treated with TAA as compared to control (Fig. 2H; P<0.001 for both). Supplementing lycopene or genistein or a combination of them resulted in a increase in expression of Nrf2 and HO-1 in the TAA treated rats (*P* < 0.05).

3.4 Histopathological Results

To determine the effects of lycopene, genistein and their combination on hepatocellular inflammation, histological changes in liver were observed by hematoxylin-eosin (H&E) staining (Fig. 3). Liver sections from the TTA group demonstrated significant changes in liver structure with severe infiltration of inflammatory cells around the central vein and centrilobular regions. However, treatment with lycopene, genistein and their combination and TAA demonstrated only moderate inflammatory cell infiltration involving the liver interstitial areas and maintained a rather normal morphology. These data clearly indicated that lycopene and genistein and their combination significantly diminished the degree of liver injury. A significant increase in fibrosis, inflammation and necrosis was observed in the TAA group compared to the control group (*P* <0.001 for each). Fibrosis and inflammation were observed to be significantly lower in the TAA+L, TAA+G, and TAA+L+G groups compared to the TAA group (*P* <0.001). The development of fibrosis was detected to be lower in the TAA+G and TAA+L+G groups compared to the TAA+L group (*P* <0.05 and *P* <0.001). There was no significant difference in the fibrosis score between the TAA+G and TAA+L+G groups (*P* >0.05).

The immunohistochemical examination revealed a significant increase in the number of α-SMA-reactive HSCs compared to the control group (*P* <0.001). Actin staining was significantly lower in the TAA+L, TAA+G, and TAA+L+G groups compared to the TAA group (*P* <0.001). Immunohistochemical staining was shown in Fig. 4. There was no significant difference between the TAA+L and TAA+G groups while actin staining was significantly lower in the TAA+L+G group compared to the TAA+L group (*P* <0.001) (Table 3).

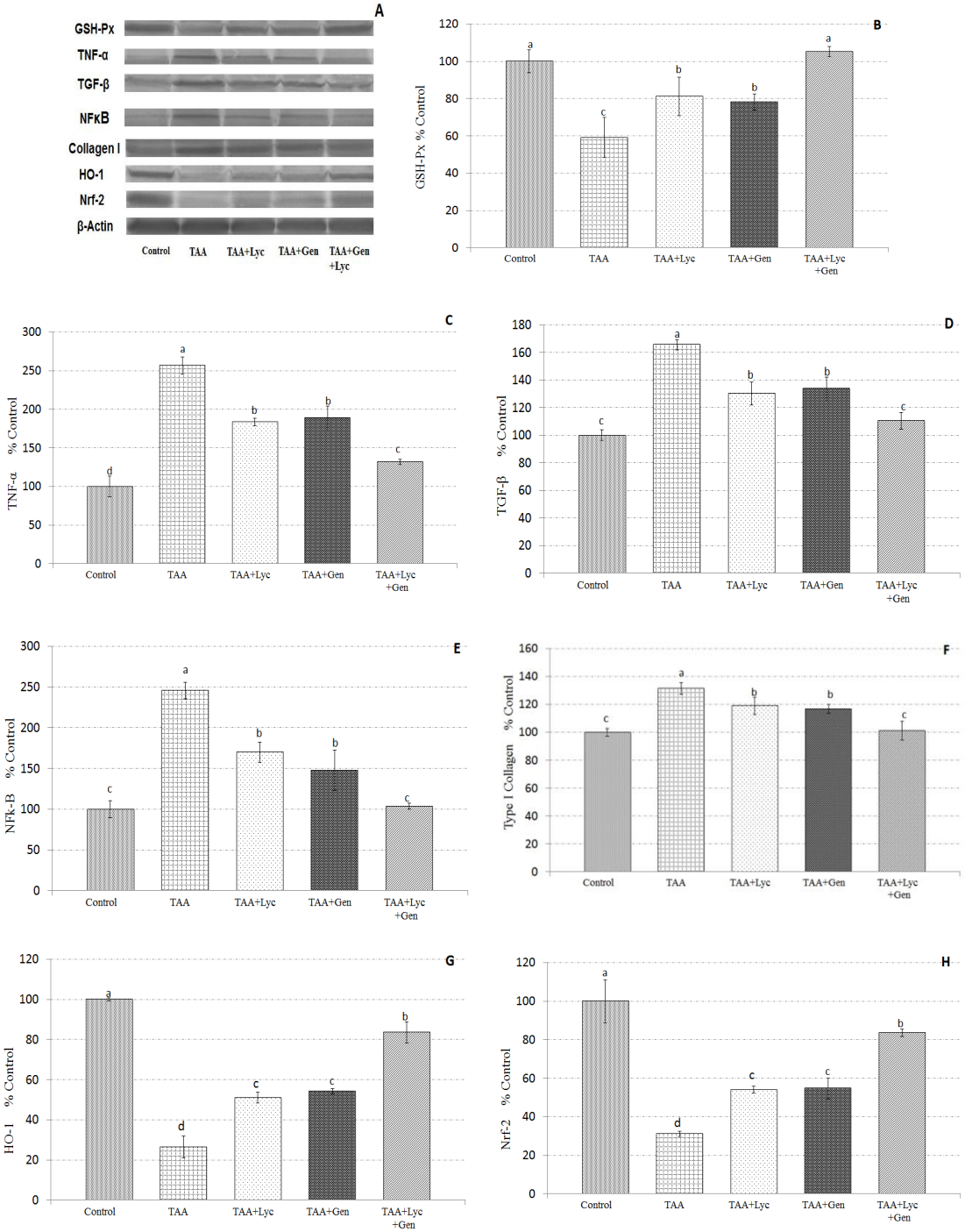


Fig. 2. Liver tissue GSH-Px, TNF-α, TGF-β, NFκB, Type I Collagen, HO-1 and Nrf-2 expressing levels (Panel A) western blot strips, Comparison of GSH-Px (Panel B), TNF-α (Panel C), TGF-β (Panel D), NFκB (Panel E), Type I Collagen (Panel F) and HO-1 (Panel G) and Nrf-2 (Panel H) protein expression of levels between the groups

The intensity of the bands was quantified by densitometric analysis. Data are expressed as a ratio of normal control value (set to 100%). The bar represents the standard error of the mean. Blots were repeated at least 3 times (n=3) and a representative blot is shown. Different superscripts (a–c) indicate group mean differences ($P < 0.05$)

Table 3. Results of histopathological examination by group

	Control	TAA	TAA+L	TAA+ G	TAA+L+G
Fibrosis	0	3.29±0.18	1.58±0.20$^{###}$	0,72±0.184$^{###}$	0.43±0.20$^{###}$
Inflammation	1.97±0.24	7.64±0.8	4.23±0.40$^{###}$	3.43±0.35$^{###}$	2.84±0.38$^{###}$
Necrosis	0.043±0.029	0.94±0.25	0,80±0.164	0.23±0.05$^{##}$	0.17±0.04$^{##}$
α-SMA	0	3.43±0.20	1.86±0.14$^{###}$	0.57±0.20$^{###}$	0.43±0.20$^{###}$

Data are presented as average ± standard deviation. Control, no treatment; TAA, rats treated with TAA; TAA+L, rats treated with TAA and lycopene; TAA+G; rats treated with TAA and genistein. TAA+L+G, rats treated with TAA and lycopene and genistein.
** Compared with the TAA group: $^{#}$ p<0.05; $^{##}$ p<0.01; $^{###}$ p<0.001*

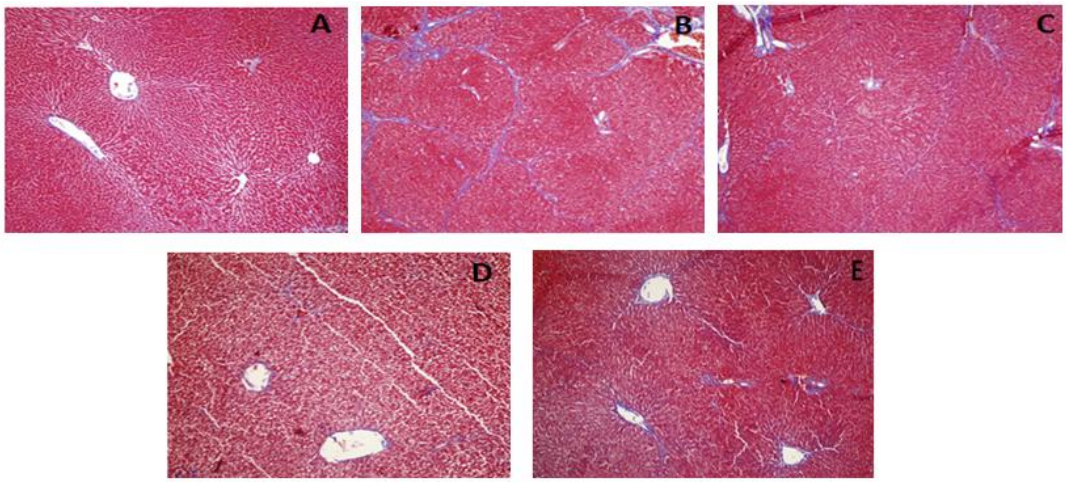
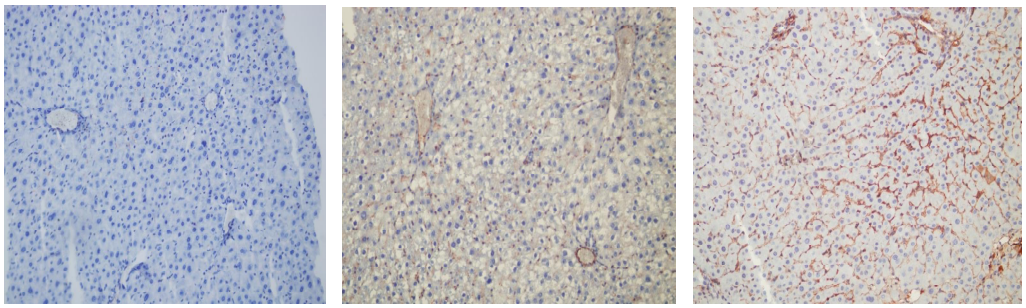


Fig. 3. Histopathological appearance of the liver tissue from control group rats (Masson Trichrom, x200). B: Histopathological appearance of the liver tissue from TAA group rats (Masson Trichrom, x200). Significant formation of fibrosis and regeneration nodules is observed. C: Histopathological appearance of the liver tissue from TAA+L group rats (Masson Trichrom, x200). It can be observed that lycopene treatment led to a significant decrease in fibrosis and disappearance of regeneration nodules. D: Histopathological appearance of the liver tissue from TAA+G group rats (Masson Trichrom, x200). A significant decrease in fibrosis and disappearance of regeneration nodules can be observed. E: Histopathological appearance of the liver tissue from TAA+L+G group rats (Masson Trichrom, x200).
A significant decrease in fibrosis and disappearance of regeneration nodules can be observed



(A) **(B)** **(C)**

Fig. 4. Immunohistochemical staining of the liver tissue (Actin staining, x200). A: Control group B: α-SMA expression was increased from TAA group rats. C: α-SMA expression was significantly decreased from TAA+L group rats

4. DISCUSSION

This study detected the significant therapeutic effects of separate and combined administration of genistein and lycopene on fibrosis and inflammation in TAA-induced chronic liver injury in rats. The effect was greater in combination of lycopene and genistein group. We detected that the antifibrotic effect of genistein was stronger compared to lycopene. Thioacetamide (TAA) is well-established model of experimental liver fibrosis especially causes membrane damage, oxidative stress and accumulation of lipid droplets in the hepatocyte cytoplasm to enhance inflammation and liver injury in rodents. It can be can be translated to human disease and therapy [7].

A significant decrease was detected in liver GSH-Px, TNF-α, TGF-β, NF-κB, and Type I collagen levels in the group receiving combination of lycopene and genistein. We think that the superiority of genistein in preventing fibrosis may result from its ability to inhibit tyrosine kinase activity [28]. As is known, thyrosine kinase is one of the essential factors in HSC activation and proliferation, which underlie liver fibrosis. This pathway may be more dominant than other mechanisms. It well reported that tyrosine kinases regulate a broad variety of physiological cell processes such as metabolism, growth and differentiation. Abnormal tyrosine kinase activity disturbs the physiological cell homeostasis and can lead to fibrosis. Additionally, different tyrosine kinases have been identified as determinants of fibrosis progression and potential targets for anti-fibrotic therapies. This includes both receptor tyrosine kinases (e.g., PDGF receptor, VEGF receptor, EGF receptor, and JAK kinases) as well as non-receptor tyrosine kinases (e.g., c-Abl, c-Kit, and Src kinases) [29]. However, there are no previous studies investigating the effects of combination of lycopene and genistein supplementation on on these parameters in humans or animals with which to compare this study.

Cirrhosis develops as a result of the activation of HSC and their transformation from passive cells to contractile myofibroblasts, in other words, to a proliferative and fibrogenic state [30]. Oxidative stress plays a significant role in HSC activation. Studies suggest that the oxidative stress causes this effect by increasing c-myb expression and NF-κB activation [31]. The resulting free oxygen radicals react with polyunsaturated fatty acids, nucleotides, and sulfhydryl groups on the cell wall, leading to tissue injury. The extent of the tissue injury was observed to be related to antioxidants such as tocopherol, ascorbic acid, beta carotene, glutathione, selenium, and superoxide dismutase [32].

Genistein belongs to the isoflavone group in the phytoestrogen family. It generally undergoes biotransformation in the liver and small intestine. It was detected to decrease the lipid peroxidation in the liver and to increase the total antioxidant capacity when administered orally to hamsters [33]. In addition, TGF was detected to prevent tumor growth by inhibiting thyrosine kinase, which accelerates topoisomerase I-II, and preventing new capillary formation [34]. In a study examining the effects on carbon tetrachloride (CCl4)-induced acute liver injury in rats, administered genistein at a dose of 1 mg/kg/day subcutaneously for 4 days to one group and for 8 days to another group [35]. Compared to two other groups, which received only CCl4 for the same period, AST and ALT levels were significantly lower while MDA levels were significantly higher. In an experimental study investigating the protective role of genistein in a non-alcoholic fatty liver model, Yalniz and his colleagues [36] detected that genistein reduces inflammation and provides protection against hepatosteatose by decreasing plasma TNF-α levels.

Lycopene was found to be the most potent antioxidant carotenoid [37] and to prevent cell proliferation in some studies examining the structure of thymidine [38]. Lycopene was thought to have this effect by stimulating or inhibiting cell growth via intercellular gap junctions [38]. However, as this mechanism has not been fully clarified, it is rather stressed that it provides protection by interacting with oxygen radicals [39]. In groups receiving lycopene, a decrease was observed in lipid peroxidation (MDA), oxidative stress parameters and fibrosis indicators (type I collagen, SMA) while liver glutathione levels increased. Glutathione displays a protective effect by binding free radicals causing lipid peroxidation [40]. Lycopene blocks the HSC activation by preventing lipid peroxidation resulting from inflammation [41].

TNF-α is a proinflammatory cytokine. It plays a role in apoptosis and hepatocyte injury. It also stimulates the activation of NF-κB, a transcription factor present in most of the TNF-α organisms and playing a role especially in the expression of

various inflammatory cytokines. Ganai AA et al. [42] found that genistein significantly suppressed the production of proinflammatory cytokines, including tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β. These inhibitory effects were associated with the suppression of nuclear factor-kappa B (NF-κB) activation, In a study examining the effects of lycopene and tomato extract on hepatocarcinogenesis in an experimental non-alcoholic fatty liver basis, Wang et al. [43] detected that lycopene reduces the inflammatory foci in the liver by inhibiting TNF-α and NF-κB activation. In this study, both TNF-α and NF-κB levels were lower in rats receiving lycopene compared to the TAA group.

Oxidative stress and inflammation are two of the most critical factors implicated in TAA-induced liver injury. The Nrf2 protein is redox-sensitive transcription factor that plays a role in induction of phase II detoxifying/antioxidant defense mechanism to cope with oxidative stress through enhancing the expression of a number of enzymes [44]. The literature on the Nrf2 pathway activity in the liver in response to dietary supplementation with combination of lycopene and genistein in TAA-induced liver injury in rats is limited. TAA treatment showed an inverse correlation between the the Nrf2, which is in agreement with previous studies. Lycopene, genistein and their combination supplementation increased Nrf2, accompanied by an increase in HO-1, and GPx. This is in agreement with earlier reports demonstrating the anti-inflammatory and antioxidant effect of lycopene and genistein [21,45].

5. CONCLUSION

In conclusion, genistein and lycopene play a preventive role in TAA-induced liver fibrosis. Genistein provides more powerful protection compared with lycopene. Genistein+lycopene combination has a greater antifibrotic effect. Lycopene and genistein display an anti-inflammatory and antifibrotic effect by inhibiting TNF-α and NF-κB, two important proinflammatory cytokines, as well as TGF-β, a profibrogenetic cytokine. In addition, these two substances reduce oxidative stress by preventing lipid peroxidation and increasing antioxidant enzyme levels and activating NRf2 pathway. The protective effects of genistein and lycopene against chronic liver injury are most likely associated with their ability to prevent oxidative stress and inflammation.

CONSENT

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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