Resveratrol prevents liver fibrosis via two possible pathways: Modulation of alpha fetoprotein transcriptional levels and normalization of protein kinase C responses

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Abstract:

OBJECTIVE: Liver fibrosis is a global health problem that causes approximately 1.4 million deaths per year. It is associated with inflammation, oxidative stress, necrosis and ends with cirrhosis, liver cancer, or liver failure. Therefore, the present study was constructed to investigate the protective effect of resveratrol (RVT) on liver fibrosis, focusing on the possible involvement of alpha 1-fetoprotein and protein kinase C signaling.

MATERIALS AND METHODS: Rats received thioacetamide (TAA) (200 mg/kg, intraperitoneal) twice weekly, for 4 successive weeks to induce liver fibrosis. RVT (30 mg/kg, per os) and vehicle were administered orally for 1 month before and another month during TAA intoxication. Body weights and mortality rate were assessed during the experiment. Liver functions and protein concentration were determined in serum, while liver tissues were analyzed for oxidative and fibrotic biomarkers. Moreover, histological examinations were performed to liver biopsies.

RESULTS: RVT prevented the debility of TAA; liver functions including alanine aminotransferase, aspartate aminotransferase, bilirubin, and albumin were also protected. RVT prevented TAA oxidative stress, and normal liver contents of malondialdehyde and reduced glutathione were markedly preserved. In addition, RVT abolished the stimulant effect of TAA to fibrosis markers and conserved normal liver contents of nuclear factor kappa B, hydroxyproline, and alpha fetoprotein. Histological examinations indicated normal liver architecture in RVT-administered rats as compared to their TAA-administered peers.

CONCLUSION: RVT was able to enhance liver functions, prevent oxidative stress, and eliminate liver fibrosis. Hence, the present data highlight the therapeutic potential of RVT as a protective agent against liver fibrosis.

Keywords: Alpha 1-fetoprotein, liver fibrosis, protein kinase C signaling, rats, thioacetamide

Introduction

Liver fibrosis is a considerable health problem, associated with significant morbidity and mortality worldwide.[1] The principal causative factors of liver fibrosis in the developing countries are viral or parasitic infection, while in the developed countries, the frequent cause is excessive alcohol consumption.[2] Other stimuli for hepatic fibrosis are autoimmune disorders, metabolic disorders, drug-induced diseases, and genetic diseases.[3] Despite various etiologies, fibrosis represents a hallmark of...
Liver fibrosis results from iterative hepatic injury which proceeds to cellular damage and activation of resident inflammatory cells and myofibroblasts. Persistent inflammation makes the normal wound healing response aberrant, and excessive generation of reactive oxygen species (ROS) starts to occur. In turn, ROS induce massive production of inflammatory mediators, including cytokines, chemokines, and growth factors. Chemokines recruit more inflammatory and immune cells; then, cytokines and growth factors bind to their corresponding receptors on these cells and activate the expression of several transcriptional regulators and genes. In turn, an array of common regulatory pathways will be altered, and all of them promote cell growth, proliferation, and differentiation. Extracellular matrix components (ECMs) including collagen, elastin, and glycoproteins will be extensively synthesized and deposited in the perisinusoidal region. Matrix remodeling starts to take place due to abnormal upregulation of matrix degrading enzymes called matrix metalloproteinases (MMPs) and their respective inhibitors tissue inhibitor of metalloproteinases. In addition, the expression of genes controlling interleukins and growth factors is continuously enhanced. This forms a vicious circle of liver damage, which leads to perpetual disruption of the normal liver functions, and eventually liver fibrosis.

Resveratrol (RVT) is a natural product with certain qualities that enable it to be used against liver fibrosis. It was found primarily in the roots of Polygonum cuspidatum in 1963 and in many dietary sources such as red grapes, peanuts, rhubarb, pistachios, and wine. RVT has various pharmacological effects including cardioprotective, neuroprotective, and hepatoprotective effects. It also possesses antiaging, antioxidant, anti-inflammatory, and anticancer properties. These characteristics qualify it to be a possible antifibrotic drug; hence, the present study was conducted to find out the protective effect of RVT against liver fibrosis and in turn to develop prevention to this devastating disease.

Materials and Methods

Animals

Adult male albino Wistar rats, weighing 200–250 g, were obtained from animal house colony of the National Research Center (NRC) of Egypt. The animals were fed standard diet ad libitum, allowed free access to water, and acclimated for 14 days before starting the experiment. They were housed six per cage in controlled room temperature (25°C ± 1°C), humidity (50% ± 10%), and alternating 12 h cycles of light and dark. Animals were treated throughout the experiment according to the following ethical guidelines, and an ethical approval (# 12033) was obtained from NRC committee.

- Squeezing, pressure, and unnecessary disturbance were avoided
- Instruments used for drug preparation and animal injection were previously cleaned, and doses were accurately calculated
- Animals were anesthetized with ether before blood samples collection and before scarification
- Animals’ cadavers and parts of tissues were handled with care and frozen at −20°C till being incinerated in the NRC incinerator.

Drugs and chemicals

RVT is buff fine powder that obtained from GO Healthy Nutritional Company in New Zealand. It is hardly soluble in water and was freshly prepared as suspension in 1% (v/v) Tween 80 in distilled water for oral administration (per os [p.o.]). Thioacetamide (TAA) is creamy white crystals obtained from Loba Chemie in India. It is freely soluble in water and was freshly prepared as solution in saline (0.9% NaCl) for intraperitoneal (i.p.) injection.

Experimental design

TAA model was used to induce liver fibrosis in rats. TAA is specific for liver and results in histological and biochemical changes similar to that in human liver fibrosis. It was injected twice weekly for 4 successive weeks in a dose (200 mg/kg/biweekly, i.p.).

Fifty-four rats were randomly allocated into three groups (n = 18) according to the following scheme, and large number of rats were used due to TAA expected mortality. Negative control group received 1% (v/v) Tween 80 in distilled water p.o. daily and saline i.p. biweekly; positive control group received 1% (v/v) Tween 80 in distilled water p.o. daily and TAA (200 mg/kg/biweekly, i.p.); and RVT group received RVT (30 mg/kg/day, p.o.) and TAA (200 mg/kg/biweekly, i.p.). Liver fibrosis was induced in positive control and RVT; orally administered drugs of negative control, positive control, and RVT groups were received for 1 month before TAA injection and during the month of TAA intoxication.

Determination of growth rate

Body weights of rats in each group were measured weekly during the month of TAA intoxication to monitor their weight change. Moreover, the number of rats in each group was recorded at the beginning and at the end of the experiment, and the mortality percent was calculated.
Mortality percent = (number of dead rats in a group/ total number of rats in the same group) × 100.

Sample collection and preparation
Twenty-four hours after the last TAA injection, blood samples were withdrawn and allowed to coagulate and then centrifuged at 4000 rpm 4°C for 15 min. The obtained serum was then used to measure the chosen parameters. Immediately after blood sampling, animals were euthanized by cervical dislocation, and livers were rapidly removed then washed in saline. A weighed part of each liver was homogenized (MPW-120 homogenizer), with ice-cooled saline to prepare 30% w/v homogenate. The homogenate was then centrifuged at 4000 rpm for 10 min. The aliquot was divided into five parts for assessment of the selected parameters.

Liver functions and protein estimation
Liver function biomarkers including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined colorimetrically using Biodiagnostic Kits and UV Spectrophotometer (Helios Gamma UVG111520). Likewise, serum albumin level was measured colorimetrically as an estimate of protein concentration.

Determination of oxidative stress
The magnitude of oxidative stress was quantified in liver homogenate in terms of lipid peroxidation byproduct malondialdehyde (MDA) and reduced glutathione (GSH) contents via Biodiagnostic Kits.

Liver fibrosis biomarkers
The extent of fibrosis was determined by measuring the contents of nuclear factor kappa B (NF-kB) and hydroxyproline (HP) in liver homogenate. Contents of NF-kB were determined using Glory Kit. The kit employs the quantitative sandwich enzyme-linked immunosorbent assay (ELISA), and the developed color with respect to NF-kB amount is measured spectrophotometrically at 450 nm wavelength. Total liver collagen was determined as HP, the method resides on the acid digestion of collagen, and the produced HP was left to react with Ehrlich’s reagent to form a reddish-brown color that was measured spectrophotometrically at wavelength 550 nm.

Determination of alpha 1-fetoprotein
Alpha 1-fetoprotein (AFP) was measured in liver homogenate by ELISA kit from Glory, which performs the same previously mentioned technique. The developed color, in proportion to AFP amount, is measured spectrophotometrically at wavelength of 450 nm.

Histological examinations
Immediately after scarification, specimens of the liver were dissected from the three major lobes, washed thoroughly with saline, and fixed in 10% neutral-buffered formal saline for 72 h. Sections of 6 µm thick were cut and stained with hematoxylin and eosin (HE) and Masson trichrome (MT) stains. All sections were scanned and analyzed, and images were captured and processed using Adobe Photoshop, version 8.0 (Adobe System, San Jose, CA, USA).

Statistical methods
All data were subjected to statistical analysis using Statgraphics Centurion XV Version 15.2.06. (StatPoint Inc., Herndon, Washington, D.C, USA). Results of body weights were analyzed using repeated measures two-way ANOVA to test for interaction between drug, time, and weight followed by Tukey’s multiple comparison test. However, other the rest of the data were statistically analyzed using one-way ANOVA followed by Tukey’s multiple comparison test. For all data, results were expressed as mean value ± standard error of the mean, n = 18, and a P ≤ 0.05 was used as criterion for statistical significance. Exact P ≤ 0.05 is mentioned; however, P < 0.001 are presented as P < 0.001. The * sign was used to show significant difference from normal group while the @ sign was used to show significant difference from control group.

Results

Determination of growth rate
Rats of the negative control group showed regular growth in their body weights and reached 54% increase, when compared to positive control group in the 4th week of TAA intoxication. RVT treatment prevented the significant decrease of body weight that happened in positive control group and restored the body weight by 25% increase [Figure 1a]. No mortality was observed in normal group; however, 67% and 32% of rats were found dead in control and RVT groups, respectively [Figure 1b].

Liver functions and protein estimation
Administration of TAA in positive control group reduced ALT and AST activities to subnormal levels while pretreatment of RVT markedly increased serum ALT and AST when compared to both negative and positive control rats. Correspondingly, serum bilirubin level showed a remarkable increase in positive control and RVT groups when related to the negative control rats. Furthermore, protein synthesis significantly inhibited in positive control group, and serum albumin level decreased by 20% from the negative control values. On the contrary, RVT-treated rats preserved normal level and increased albumin 37% in comparison with the positive control rats [Table 1].
Determination of oxidative stress
TAA intoxication exerted considerable oxidative stress in positive control rats; MDA accumulated in liver up to 40%, and GSH stores reduced to 70% less than the negative control group. Pretreatment of RVT kept the oxidant-antioxidant balance in the liver, and conserved normal contents of MDA and GSH in RVT rats [Table 2].

Liver fibrosis biomarkers
Rats of positive control group revealed respectable quantities of liver fibrosis markers NF-κB and HP; they reached 98% and 102% increase than the negative control rats. Contrariwise, RVT rats maintained normal liver contents of NF-κB and HP and abolished the effect of TAA [Table 3].

Determination of alpha 1-fetoprotein
Alpha 1-fetoprotein was found in significant amounts in the livers of positive control rats; 53% increase more than the negative control group was estimated. However, RVT rats were able to prevent this effect and showed normal AFP content in liver [Figure 2].

Histological examinations
Liver tissue of negative control rats demonstrated regular hepatic architecture, preserved lobular structures, normal hepatocytes, ordinarily arranged hepatic cord [Figure 3a], and normal portal areas with no evidence of fibrosis or necrosis [Figure 3b]. In contrast, liver sections of positive control group exhibited portal fibrosis, hepatocellular necrosis, intense mononuclear cell infiltration [Figure 3c], extensive blue-stained collagen deposition around the portal triad and distortion of the normal architecture [Figure 3d]. Examination of RVT liver tissue showed great similarity to normal liver sections. Normal lobular pattern, regenerating nodules, apoptotic hepatocytes [Figure 3e], minimal fibroblastic proliferation, and few collagen bundles surrounding hepatic nodules could be identified [Figure 3f].

Table 1: The effect of resveratrol on liver functions and protein synthesis of thioacetamide-induced liver fibrosis in rats

| Groups    | ALT (IU/L) | AST (IU/L) | Bilirubin (mg/dL) | Albumin (g/dL) |
|-----------|------------|------------|-------------------|---------------|
| Negative control | 54.40±1.54 | 45.68±1.23 | 0.70±0.07         | 3.82±0.24     |
| TAA (P)   | 50.05±1.98 | 44.14±1.99 | 1.01±0.02         | 3.09±0.16     |
| RVT (P)   | 63.67*±1.47 (<0.001) | 49.33±1.26 | 1.14*±0.10 (<0.001) | 4.23*±0.27 (0.002) |

Statistical analysis was carried out by one-way ANOVA followed by Tukey’s test. Data expressed as mean±SEM (n=18). *Significant from normal group at respective time at P≤0.05. **Significant from control group at respective time at P≤0.05. Exact P≤0.05 is mentioned; however, P<0.001 are presented as P<0.001. SEM=Standard error of mean, ALT=Alanine aminotransferase, AST=Aspartate aminotransferase, TAA=Thioacetamide, RVT=Resveratrol

Figure 1: (a) The protective effect of resveratrol on rats’ body weights in TAA-induced liver fibrosis model. Statistical analysis was carried out by two-way ANOVA followed by Tukey’s test. Data expressed as mean ± standard error of the mean, n=18. *Significant from normal group at respective time at P≤0.05. **Significant from control group at respective time at P≤0.05. Exact P≤0.05 is mentioned; however, P<0.001 are presented as P<0.001. (b) Resveratrol decreased the percent of mortality in TAA-induced liver fibrosis rats. Numbers of rats were checked daily, and from which the mortality percent was calculated by using the formula (number of dead rats in a group/total number of rats in the same group) × 100. TAA = Thioacetamide, RVT = Resveratrol

Figure 2: The effect of resveratrol on alpha 1-fetoprotein in thioacetamide-induced liver fibrosis model in rats. Statistical analysis was carried out by one-way ANOVA followed by Tukey’s test. Data expressed as mean ± standard error of the mean, n=18. *Significant from normal group at respective time at P≤0.05. **Significant from control group at respective time at P≤0.05. Exact P≤0.05 is mentioned; however, P<0.001 are presented as P<0.001. RVT = Resveratrol
Table 2: Restored effect of resveratrol on the oxidant-antioxidant balance in thioacetamide-induced liver fibrosis in rats

| Groups   | Parameters          | MDA (mmol/g) | GSH (mmol/g) |
|----------|---------------------|--------------|--------------|
| Negative control | 149.36±10.48 | 8.13±0.43 |
| TAA (P)  | 210.20±11.13 (<0.001) | 2.43±0.20 (<0.001) |
| RVT (P)  | 147.11±11.64 (<0.001) | 7.28±0.39 (<0.001) |

Statistical analysis was carried out by one-way ANOVA followed by Tukey’s test. Data expressed as means±SEM (n=18). *Significant from normal group at respective time at P≤0.05. **Significant from control group at respective time at P<0.05. Exact P<0.001 are presented as P<0.001. SEM=Standard error of mean, MDA=Malondialdehyde, GSH=Glutathione, TAA=Thioacetamide, RVT=Resveratrol

Table 3: The inhibitory actions of resveratrol on fibrosis biomarkers in thioacetamide-induced liver fibrosis in rats

| Groups   | Parameters          | NF-κB (ng/g) | HP (µg/g) |
|----------|---------------------|--------------|-----------|
| Negative control | 7.01±0.54 | 25.52±1.09 |
| TAA (P)  | 13.88±1.19 (<0.001) | 51.59±3.78 (<0.001) |
| RVT (P)  | 7.01±0.96 (<0.001) | 31.35±2.59 (<0.001) |

Statistical analysis was carried out by one-way ANOVA followed by Tukey’s test. Data expressed as means±SEM (n=18). *Significant from normal group at respective time at P≤0.05. **Significant from control group at respective time at P<0.05. Exact P≤0.05 is mentioned; however, P<0.001 are presented as P<0.001. SEM=Standard error of mean, TAA=Thioacetamide, RVT=Resveratrol, HP=Hydroxyproline, NF-κB=Nuclear factor kappa B

Discussion

TAA-induced liver fibrosis is a reliable animal model to test RVT against liver fibrosis. Although the induction time is long, TAA resulted in biochemical and histological changes like that of human liver fibrosis and caused lower mortality rates when compared to other models.[14]

Positive control rats were not able to gain body weight; this is due to lower levels of nutrient absorption, energy utilization, and metabolic inefficiency caused by TAA.[20] RVT is a sirtuin 1 mimetic; it deacetylates proteins that contribute to cell proliferation and longevity.[21] Subsequently, after RVT administration, weight gain was comparable to those of the negative control group, and the mortality rate was greatly reduced. It could be suggested that RVT had practically no adverse effect on the growth rate; instead, it can enhance the growth and survival rate in liver fibrosis.

ALT and AST are cytoplasmic in origin, and abnormal serum amounts indicate cell membrane damage and hepatocytes death.[24] In advanced stage of liver fibrosis, ALT and AST serum activities return to normal or subnormal levels, due to exhaustion of hepatocytes and complete depletion of ALT and AST from them.[22] This explains our data; induction of liver fibrosis by TAA did not alter the serum activity of ALT and AST enzymes when compared to the normal activities; in fact, it slightly inhibited them. Administration of RVT exerted a cytoprotective effect on hepatocytes and conserved the ALT and AST levels. RVT is a free radical scavenger; it acts as a hydrogen-electron donor through its phenolic hydroxyl groups.[14] In addition, it activates the antioxidant cellular defensive mechanisms by inducing mitochondrial superoxide dismutase.[9,23] This was further confirmed by the normal liver contents of GSH and MDA in RVT-administered rats. TAA biotransformation, as well as liver fibrosis condition, causes extensive production of free radicals and ROS which are responsible for oxidative stress.[24] MDA is the end product of many cellular biomolecules, which are denatured under the influence of ROS.[15] GSH has a prominent role in detoxification of free radicals; however, generation of a great deal of them exceeds the capacity of GSH and depletes GSH endogenous stores.[9] Our results prove that RVT could efficiently control the oxidative stress; in turn, it could prevent further stimulation of inflammatory cells and alleviate liver fibrosis.

Bilirubin is conjugated and excreted by liver,[25] therefore, its serum levels give a rise to the capacity of RVT in restoring the normal functions of hepatocytes. Despite the persistent hyperbilirubinemia in our data, another study[24] that used double of the protection time indicated that RVT can maintain normal bilirubin serum levels. Former research also mentioned that prolonged time is needed to restore normal serum bilirubin level.[27]

Protein kinase C (PKC) is a family of proteins that normally regulate numerous cellular responses including gene expression, cell proliferation, and inflammatory response. In chronic inflammation, as well as in liver fibrosis, PKC signaling alters several pathways that...
lead to exacerbation of the condition.⁴⁹ Among these pathways is the NF-κB; a transcriptional regulator that has been involved in hepatic fibrosis via upregulation of the inflammatory cytokines.⁵⁰ The liver contents of NF-κB in TAA-administered rats were significantly increased, reflecting aberrant stimulation of PKC signaling. RVT normalized the PKC response and abolished the driving action of NF-κB on inflammatory cells.⁵¹ Moreover, the histological examinations emphasized that RVT can prevent TAA-induced hepatocyte necrosis, portal fibrosis, and hyperplasia of biliary epithelium. RVT preserved hepatocytes with normal lobular pattern and no evidence of fibrosis.⁵²

Activator protein 1 (AP-1) is another transcription factor activated by PKC; it controls cellular differentiation and proliferation through gene expression of cytokines and growth factors. In liver fibrosis, AP-1 overstimulates MMPs activity, which in turn results in ECM accumulation.⁵³ HP is the major component of ECM proteins; it thus, its level is strongly related to MMPs activity. Our results, in harmony with other studies, showed a significant increase in the liver content of HP in TAA group. Pretreatment with RVT maintains a normal content of HP; this reflects the normal MMPs activity and the normal upstream PKC signaling in RVT-treated rats.⁵⁴ Histological examinations, in line with others, fortifies the findings; TAA group showed a marked increase in ECM deposition, while minimal collagen deposition was observed in RVT group and the normal architecture of liver was preserved. Like other protein kinases, PKC contains a catalytic domain and a regulatory region; when inactive, the regulatory region binds to the catalytic domain and prevents its activity.⁵⁵ A possible explanation is that RVT either may act as a pseudosubstrate and binds the substrate binding cavity of the catalytic domain keeping the PKC inactive or may bind to the C1 domain of regulatory region and prevents its dissociation from catalytic domain and which is essential for the conformational structural changes of PKC and activation of subsequent substrates.

Studies have postulated that AFP prevalence can be used in diagnosis and assessment of liver fibrosis without the presence of hepatocellular carcinoma. AFP is an oncofetal protein, synthesized solely by hepatocytes and normally repressed after birth.⁵⁶ Elevated levels of AFP during adult life indicates the presence of chronic liver diseases and germ cell tumors. In accordance, our data showed a marked increase in the liver content of AFP after TAA administration. RVT prevented the overproduction of AFP and maintained its normal transcription levels in liver.⁵⁷ We thought that RVT could bind to the enhancer region of AFP gene and prevent its abnormal expression. To further investigate this, we measured the serum albumin level. Serum albumin does not only correlate with the hepatocellular synthesis capacity of the liver⁵⁸ but also indicate the normal AFP expression in liver. AFP and albumin genes are present on the same chromosome in all mammals, studies have proved that both genes share common regulatory elements; and that AFP enhancer region can modulate albumin gene expression in both directions.⁵⁹ In the current study, RVT protected the rats against hypoalbuminemia and high AFP contents. This proves that RVT can retain the normal hepatocytes synthetic mechanisms; in addition, it gives a strong possibility that RVT can interfere with AFP enhancer region to prevent AFP abnormal overexpression and abolish the inhibitory effect on albumin expression. Further research should be conducted to decipher the RVT/AFP/albumin relationship.

Conclusions

The above-discussed results conclude that the hepatoprotective effect of RVT against liver fibrosis can be attributed to multiple actions, which are presented in Figure 4. RVT exerts its effect through receptors or through direct interaction with intracellular components. RVT has a positive growth effect and strong antioxidant effect. It can protect hepatocytes and preserve its normal functions. Moreover, it prevents the aberrant PKC signaling in chronic inflammation and maintains AFP normal expression. Therefore, the present findings enable RVT to be of therapeutic potential in the prevention of liver fibrosis to patients in risk.

![Figure 4](image-url)

**Figure 4**: This has been proposed by our group as a possible explanation for RVT effects against liver fibrosis. RVT is a lipophilic compound; it can readily cross the cell membrane and bind to intracellular components. It can also exert its action through binding to specific receptors. RVT act as a free radical scavenger; thus, it prevents the detrimental effect of ROS on hepatocytes. It may also bind to PKC domains and AFP enhancer region and returns them back to their normal activities.

Legend

- **RVT**
- **ROS**
- **GSH**
- **ALT**
- **AST**

TAA = Thioacetamide, RVT = Resveratrol, ROS = Reactive oxygen species, GSH = reduced glutathione, ALT = Alanine aminotransferase, AST = aspartate aminotransferase, PKC = protein kinase C, AP-1 = Activator protein 1, MMP = Matrix metalloproteinase, AFP = Alpha 1-fetoprotein, ECM = Extracellular matrix component, NF-κB = Nuclear factor kappa B
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Conflicts of interest
There are no conflicts of interest.

References
1. Pellicer A, Ramachandran P, Iredale JP, Fallowfield JA. Liver fibrosis and repair: Immune regulation of wound healing in a solid organ. Nat Rev Immunol 2014;14:181-94.
2. Seki E, Brenner DA. Recent advancement of molecular mechanisms of liver fibrosis. J Hepatobiliary Pancreat Sci 2015;22:512-8.
3. Ramachandran P, Iredale JP, Fallowfield JA. Resolution of liver fibrosis: Basic mechanisms and clinical relevance. Semin Liver Dis 2015;35:119-31.
4. Czaja AJ. Hepatic inflammation and progressive liver fibrosis in chronic liver disease. World J Gastroenterol 2014;20:2515-32.
5. Pinzani M. Pathophysiology of liver fibrosis. Dig Dis 2015;33:492-7.
6. Koyama Y, Wang P, Brenner DA, Kisseleva T. Stellate cells, portal myofibroblasts and epithelial-to-mesenchymal transition. In Stellate cells in health and disease. Elsevier Inc 2015.
7. Ahmad A, Ahmad R. Understanding the mechanism of hepatic fibrosis and potential therapeutic approaches. Saudi J Gastroenterol 2012;18:155-67.
8. Zhang H, Sun Q, Xu T, Hong L, Fu R, Wu J, et al. Resveratrol attenuates the progress of liver fibrosis via the akt/nuclear factor-kB pathways. Mol Med Rep 2016;13:224-30.
9. Tanriverdi G, Kaya-Dagistanli F, Ayla S, Demirci S, Eser M, Unal ZS, et al. Resveratrol can prevent CCl4-induced liver injury by inhibiting notch signaling pathway. Histol Histopathol 2016;31:769-84.
10. Friedman SL. Hepatic fibrosis: Emerging therapies. Dig Dis 2015;33:504-7.
11. Ahmed RF, Abdel-Rahman RF, Abdallah H, Saleh DO, Farid OA, Hessin AF. Antidepressant-like effect of resveratrol in a subchronic model of depression. J Arab Soc Med Res 2014;9:48.
12. Crossan C, Tschantzis EA, Longworth L, Gurusamy K, Davidson B, Rodriguez-Perálvarez M, et al. Cost-effectiveness of non-invasive methods for assessment and monitoring of liver fibrosis and cirrhosis in patients with chronic liver disease: Systematic review and economic evaluation. Health Technol Assess 2015;19:1-409, v-vi.
13. Smoliga JM, Bost J, Maroon JC. Potential benefits of resveratrol supplementation for optimizing health and preventing chronic disease. Antiaging Ther 1997;11.
14. Tan L, Wang W, He G, Kuick RD, Gossner G, Kueck AS, et al. Resveratrol inhibits ovarian tumor growth in an in vivo mouse model. Cancer 2016;122:722-9.
15. Abramovitch S, Dahan-Bachar L, Sharvit E, Weisman Y, Ben Tow A, Brazowski E, et al. Vitamin D inhibits proliferation and profibrotic marker expression in hepatic stellate cells and decreases thioacetamide-induced liver fibrosis in rats. Gut 2011;60:1728-37.
16. Hessin A, Hegazy R, Hassan A, Yassin N, Kenawy S. Lactoferrin enhanced apoptosis and protected against thioacetamide-induced liver fibrosis in rats. Open Access Maced J Med Sci 2015;3:195-201.
17. Bruck R, Genina O, Aeed H, Alexiev R, Nagler A, Avni Y, et al. Halofuginone to prevent and treat thioacetamide-induced liver fibrosis in rats. Hepatology 2001;33:379-86.
18. Bland J. Resveratrol opportunism: What is the science behind the claims? Integr Med 2009;7:50-51.
19. Woesnsen JR. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Arch Biochem Biophys 1961;93:440-7.
20. Kadir FA, Kassim NM, Abdulla MA, Yehye WA. Hepatoprotective role of ethanol extract of vitex negundo in thioacetamide-induced liver fibrosis in male rats. Evid Based Complement Alternat Med 2013;2013:739850.
21. Ahmed A, Ahmad R. Resveratrol mitigate structural changes and proinflammatory factors of liver cirrhosis in rats. World J Gastroenterol 2013;19:241-8.
22. Alshawsh MA, Abdulla MA, Ismail S, Amin ZA. Hepatoprotective effects of orthosiphon stamineus extract on thioacetamide-induced liver cirrhosis in rats. Evid Based Complement Alternat Med 2011;2011:10309.
23. Park BH, Kim YS, Lee TI, Kim SJ, Lee WR, Kim BI, et al. Melittin attenuates liver injury in thioacetamide-treated mice through modulating inflammation and fibrogenesis. Exp Biol Med (Maywood) 2011;236:1306-13.
24. Ahmed A, Ahmad R. Resveratrol mitigate structural changes and hepatic stellate cell activation in N’-nitrosodimethylamine-induced liver fibrosis via restraining oxidative damage. Chem Biol Interact 2014;221:1-2.
25. Brenner DA. Fra, fra away: The complex role of activator protein 1 in liver injury. Hepatology 2014;59:19-20.
26. Woo JH, Lim JH, Kim YH, Suh SI, Min DS, Chang JS, et al. Resveratrol inhibits phorbol myristate acetate-induced matrix metalloproteinase-9 expression by inhibiting JNK and PKC delta signal transduction. Oncogene 2004;23:1845-53.
27. Pterostilbene inhibits dimethylnitrosamine-induced liver fibrosis in rats. World J Gastroenterol 2013;19:241-8.
28. McCleary JD, editor. Clinical Laboratory Medicine. Philadelphia: Lippincott Williams & Wilkins; 2002.
29. Lee MF, Liu ML, Cheng AC, Tsai ML, Ho CT, Liou WS, et al. Pterostilbene inhibits dimethylnitrosamine-induced liver fibrosis in rats. Food Chem 2013;138:802-7.
30. Shirin H, Sharvit E, Aeed H, Gavish D, Bruck R. Atorvastatin and rosvastatin do not prevent thioacetamide induced liver cirrhosis in rats. World J Gastroenterol 2013;19:241-8.
31. Al-Shawsh MA, Abdulla MA, Ismail S, Amin ZA. Hepatoprotective effects of orthosiphon stamineus extract on thioacetamide-induced liver cirrhosis in rats. Evid Based Complement Alternat Med 2011;2011:10309.
32. Roy S, Sannigrahi S, Majumdar S, Ghosh B, Sarkar B. Resveratrol regulates antioxidant status, inhibits cytokine expression and restricts apoptosis in carbon tetrachloride induced rat hepatic injury. Oxid Med Cell Longev 2011;2011:703676.
33. Gopalakrishna R, Jaken S. Protein kinase C signaling and oxidative stress in the pathogenesis of breast cancer. Expert Rev Mol Med 2006;8:1-17.
34. Harn HJ, Lin SZ, Hung SH, Subeq YM, Li YS, Syu WS, et al. Vitamin D inhibits proliferation and protects against thioacetamide-induced liver fibrosis in rats. Evid Based Complement Alternat Med 2012;2012:215678.
35. Roy S, Sannigrahi S, Majumdar S, Ghosh B, Sarkar B. Resveratrol regulates antioxidant status, inhibits cytokine expression and restricts apoptosis in carbon tetrachloride induced rat hepatic injury. Oxid Med Cell Longev 2011;2011:703676.
36. Al-Shawsh MA, Abdulla MA, Ismail S, Amin ZA. Hepatoprotective effects of orthosiphon stamineus extract on thioacetamide-induced liver cirrhosis in rats. Evid Based Complement Alternat Med 2011;2011:10309.
37. Faghihzadeh F, Adibi P, Rafiei R, Hekmatdoost A. Resveratrol supplementation improves inflammatory biomarkers in patients with nonalcoholic fatty liver disease. Nutr Res 2014;34:837-43.
38. Al-Jumaily EF. The effect of chronic liver diseases on some biochemical parameters in patients serum. Curr Res J Biol Sci 2012;4:638-42.
39. Lee YA, Wallace MC, Friedman SL. Pathobiology of liver fibrosis: A translational success story. Gut 2015;64:830-41.
40. Park JH, Kym YS, Lee TI, Kim SJ, Lee WR, Kim BI, et al. Melittin attenuates liver injury in thioacetamide-treated mice through modulating inflammation and fibrogenesis. Exp Biol Med (Maywood) 2011;236:1306-13.
41. Ahmed A, Ahmad R. Resveratrol mitigate structural changes and hepatic stellate cell activation in N’-nitrosodimethylamine-induced liver fibrosis via restraining oxidative damage. Chem Biol Interact 2014;221:1-2.
42. Brenner DA. Fra, fra away: The complex role of activator protein 1 in liver injury. Hepatology 2014;59:19-20.
43. Woo JH, Lim JH, Kim YH, Suh SI, Min DS, Chang JS, et al. Resveratrol inhibits phorbol myristate acetate-induced matrix metalloproteinase-9 expression by inhibiting JNK and PKC delta signal transduction. Oncogene 2004;23:1845-53.
44. Gopalakrishna R, Jaken S. Protein kinase C signaling and oxidative stress. Free Radic Biol Med 2000;28:1349-61.
45. Harn HJ, Lin SZ, Hung SH, Subeq YM, Li YS, Syu WS, et al. Adipose-derived stem cells can abrogate chemical-induced liver fibrosis and facilitate recovery of liver function. Cell Transplant 2012;21:2753-64.
46. Hamza RZ, El-Shenawy NS. Anti-inflammatory and antioxidiant role of resveratrol on nicotine-induced lung changes in male rats. Toxicol Rep 2017;4:399-407.
47. Salama SM, Abdulla MA, Alrashidi AS, Hadi AH. Mechanism of hepatoprotective effect of boesenbergia rotunda in thioacetamide-induced liver damage in rats. Evid Based Complement Alternat Med 2013;2013:157456.
48. García-García AG, Polo-Hernández E, Taberner A, Medina JM.
Alpha-fetoprotein (AFP) modulates the effect of serum albumin on brain development by restraining the neurotrophic effect of oleic acid. Brain Res 2015;1624:45-58.

38. Elmaouhoub A, Dudas J, Ramadori G. Kinetics of albumin- and alpha-fetoprotein-production during rat liver development. Histochem Cell Biol 2007;128:431-43.