



# Potential effect and mechanism of action of *Zataria multiflora* in liver disorders

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## Abstract

**Introduction and Objective.** *Zataria multiflora* Boiss or Iranian thyme (Lamiaceae family) is traditionally used for the management of liver disorders. Due to the traditional importance of *Z. multiflora* in liver diseases, the aim of the review is to evaluate the preventive and therapeutic effects of *Z. multiflora* in liver diseases.

**Review methods.** Information was obtained from original peer reviewed articles published in scientific sources up to September 2021. Google Scholar, PubMed, Scopus, AMED, Cochrane Library electronic literature databases were investigated by the key words of '*Zataria multiflora*', 'liver', 'hepato-protective', 'ROS', 'oxidative stress', and 'clinical trial'. The collected data are summarized, categorized, analyzed, and discussed.

**Brief description of the state of knowledge.** The result of one clinical trial showed the efficacy and safety of *Z. multiflora* powder in non-alcoholic fatty liver diseases. *Z. multiflora* powder and its extracts reduced the liver oxidative stress induced by drugs, environmental toxins, cyst hydatid, and endogenous factors. The antioxidant activity of *Z. multiflora* essential oil is higher than its extracts or powder, due to the high content of thymol and carvacrol in its oil. Reactive oxygen species and oxidative stress play important roles in liver diseases; therefore, *Z. multiflora* and its extracts are regarded as natural antioxidants protecting the liver against free radicals.

**Conclusion.** Although there is one clinical study on *Z. multiflora* in non-alcoholic fatty liver diseases, large clinical trials are required to evaluate the efficacy and safety of *Z. multiflora* in liver diseases.

## Key words

oxidative stress, NAFLD, liver, free radicals, plant, thyme

## Abbreviation

**IBS** – Irritable bowel syndrome; **IBD** – Inflammatory bowel disease; **ROS** – Reactive oxygen species; **CAT** – catalase; **SOD** – super oxide dismutase; **GPx** – Glutathione peroxidase; **GSH** – Glutathione; **RNS** – nitrogen species; **ALT** – alanine transaminase; **AST** – aspartate transaminase; **ALP** – alkaline phosphatase; **HepG2** – human liver cancer cell line; **MDA** – malondialdehyde; **NAC** – N-acetyl cysteine; **LDH** – lactate dehydrogenase; **GST** – Glutathione S-transferase; **FRAP** – ferric reducing ability of plasma; **GGT** – Gamma-glutamyl transferase; **OH<sup>•</sup>** – reactive hydroxyl radicals; **hs-CRP** – high-sensitivity C-reactive protein; **FVC** – Forced vital capacity; **PEF** – peak expiratory flow; **HOMA-IR** – Homeostatic Model Assessment of Insulin Resistance

## INTRODUCTION

*Z. multiflora* Boiss ('Avishan Shirazi', 'Zaatar' or 'Iranian Thyme') is a flowering plant from the Lamiaceae family with limited distribution in Iran, Pakistan, and Afghanistan [1]. In the traditional medicine of Iran, *Z. multiflora* is used as a diuretic, an anti-parasite, appetizer, anti-flatulence, anti-diarrhea, analgesic (teeth, joint, neuralgic, premature labour), and for the treatment of dysmenorrhea, sore throat, whooping cough, fever, catarrh, hysteria, infantile enuresis, dyspepsia, tonsillitis, and wounds [2, 3]. Rosmarinic acid, caffeic acid [4], multiflorol, multiflorol, luteolin, p-hydroxy benzoic acid, dihydroxy aromadendrane, and essential oil have been isolated from the aerial parts of *Z. multiflora*. The yield of essential oil from *Z. multiflora* aerial parts is between 1-4%. Phenolics (thymol and carvacrol) are the prominent compounds in *Z. multiflora* essential oil (Fig. 1)

[5]. The potency of *Z. multiflora* in gastrointestinal disorders (stomatitis, intra-oral, gastric and duodenal ulcers, Irritable Bowel Syndrome (IBS), Inflammatory Bowel Disease (IBD), colon cancer) [6, 7], Alzheimer [8], diabetes [9], pain [10], cough [11], infections [12, 13], and aphthous stomatitis have been reported (Fig. 2) [14].

Free radicals, reactive oxygen species (ROS) and oxidative stress play a crucial role in liver diseases [15]. Failure of the antioxidant defence system, and lipid peroxidation can change the membrane structures, and leads to tissue damage and the production of free radicals [16]. Due to the antioxidant activity of *Z. multiflora* [17, 18], it can be a good candidate for the management of liver disorders. Therefore, the aim of this review was to evaluate the potential effects of *Z. multiflora* and its mechanism of action in the treatment of liver diseases.

Information for the review was obtained from original peer reviewed articles published in scientific resources up to September 2021. Google Scholar, PubMed, Scopus, AMED, Cochrane Library electronic literature databases were investigated by the key words '*Zataria multiflora*', 'liver', 'hepato-protective', 'ROS', 'oxidative stress', and 'clinical

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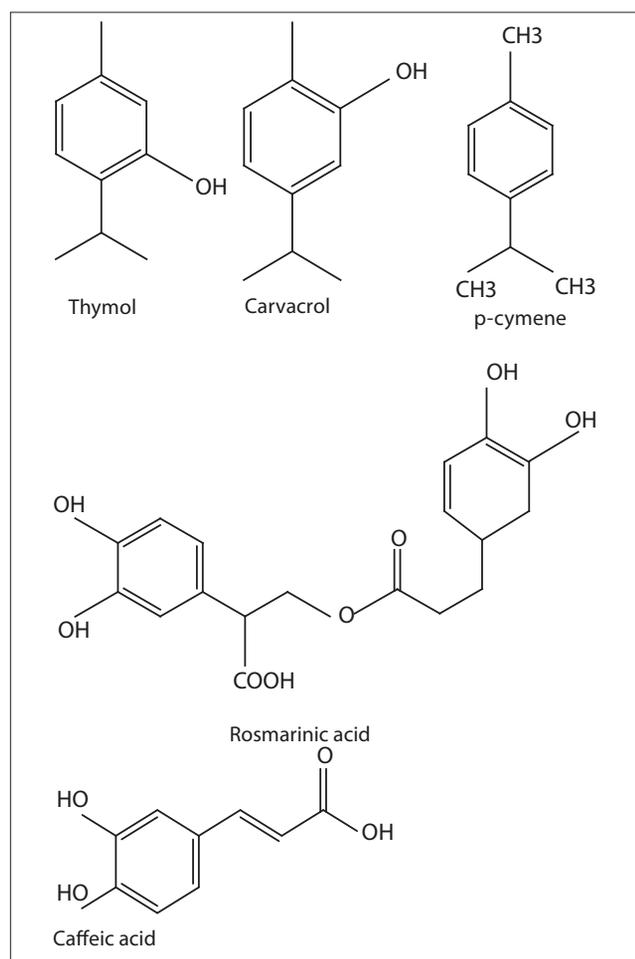


Figure 1. Structures of main components of *Z. multiflora*

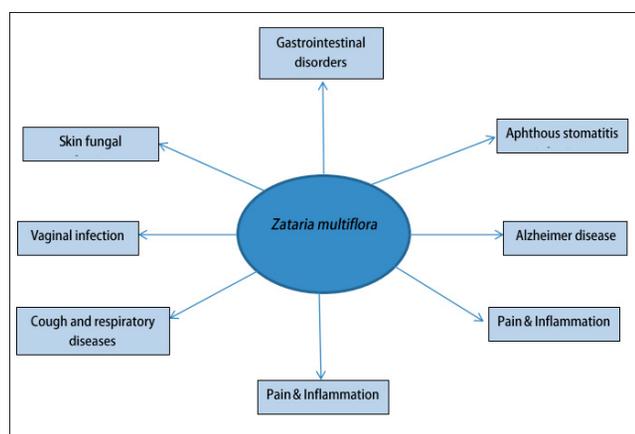


Figure 2. *Z. multiflora* and its efficacy in different ailments

trial'. The collected data are summarized, categorized, analyzed, and discussed.

**The role of ROS in liver diseases.** Exogenous (alcohol, drug, environmental toxins, virus, UV light) and endogenous (obesity, insulin resistance) factors are the major reasons for oxidative stress in liver. Oxidative stress in liver triggers the hepatic stellate, inflammasome, leads to damage the mitochondrial functions, DNA, proteins, and immune cell's infiltration, and the liver diseases (fibrosis/Cirrhosis, chronic hepatitis, hepatocellular carcinoma, and Steatosis)

[19]. Both non-enzymatic and enzymatic antioxidant systems are involved in reducing the oxidative stress. Antioxidant enzymes (catalase (CAT), super oxide dismutase (SOD), Glutathione peroxidase (GPx) and Glutathione (GSH)) are the biomarker of oxidative stress in the body and the use of various antioxidants and anti-oxidative therapies are used to treat the liver diseases.

#### *Z. multiflora* and Oxidative Stress Caused by Drugs.

The liver is the target of drug's toxicity, and the misuse of drugs is associated with production of free radicals (ROS), reactive nitrogen species (RNS) in the liver [20]. The misuse of paracetamol as an analgesic agent and adriamycin as an effective drug for cancers is associated with some toxicity and centrilobular hepatic necrosis [21, 22]. The hepatotoxic effects of these drugs are mediated by activation of ROS and induction of oxidative stress in liver. Adriamycin disrupts the structural integrity of liver cells and increases the activity of liver enzymes (ALT, AST, ALP), and the serum malondialdehyde (MDA) level in the body, while the activity of antioxidant enzymes (SOD and CAT) reduce it [23]. *Z. multiflora* extract protects the liver against paracetamol [24], Adriamycin [25] induced hepatotoxicity in rats, by reducing the inflammatory cells, hepatic lesions, necrotic tissues, MDA in the body and modulation of ALT, AST, ALP, SOD, Cat and GPx [24, 25].

*Z. multiflora* had hepatoprotective effects against drug-induced liver injuries in animal studies, Furthermore, *Z. multiflora* is a complementary alternative treatment, instead of acetaminophen or adriamycin as analgesic [26] and anti-cancer[27] agents to reduce the doses of drugs and their hepatotoxic effects.

#### *Z. multiflora* and oxidative stress caused by environmental toxins.

Exposure of liver to different environmental chemicals and toxins has major effects on human health, and are the main reason for liver diseases, abnormalities, social and emotional injuries [28].

Malathion is an organophosphate insecticide, which significantly induces lesions and degeneration in the periportal area of liver tissues by increasing the plasma level of AST, ALT, and oxidative damages. *Z. multiflora* methanol extract had protective effect against malathion induced oxidative stress in mice by returning the activity of AST, ALT, GSH and antioxidant enzymes to the normal levels. *Z. multiflora* significantly inhibited the lipid peroxidation and improved the activity of SOD and CAT [29].

Halothane as a general anesthetic compound is used for anesthesia and its toxicity effect is associated with hepatitis or acute liver failures. The presence of antibodies against hepatocytes and liver oxidative stress through CYP450 with production of ROS [30], AST, ALT, ALP, LDH and total bilirubin are involved in its toxic effects. Pre-treatment of rats with 800 ppm of *Z. multiflora* ethanol extract for 5 days significantly reduced the ALT, ALP, LDH levels in halothane induced hepatotoxic model, compared with control group [31].

Aflatoxins as secondary metabolites of some certain species of fungi are carcinogen and mutagen which and produce the oxidative stress, and acute liver toxicity [32]. *Z. multiflora* seeds (20 g/kg) ameliorated the aflatoxin induced liver damage in broilers by improving the focal hepatic necrosis, parenchymal and mononuclear cell aggregation [33].

The toxic effects of metallic nanoparticles on liver cells are associated with production of ROS and other free radicals in the body, leading to a reduction in the cell survival rate and the induction of hepatocellular necrosis [34]. Treatment of animals with iron nanoparticles significantly inactivated the CYP450, which is associated with increase in activity of AST, and reduction in activity of Glutathione S-transferase (GST). Thyme essential oil returned the activity of GST, AST to normal levels ( $p < 0.05$ ), with no effect on CYP 450 protein and other liver enzymes. The protective effect of thyme oil was confirmed by intact vacuole, small necrosis and low hepatocellular damage in histopathological samples of animals, compared to control group [35].

GST is responsible for the production of different enzymes, which catalyzes the carcinogen and cytotoxic compounds. Normal activation of GST and modulation of CYP450 by the protective effect of thyme oil against iron nanoparticles is associated with the normal activity of different enzymes involved in catalyzing the carcinogen and cytotoxic compounds.

The hepatoprotective effects of *Z. multiflora* ethanol extract was confirmed against cyclophosphamide induced hepatotoxicity in mice, which was exhibited by improvement in liver enzymes and liver histopathological features [36].

According to the results of pharmacological studies, *Z. multiflora* protects the liver against oxidative stress caused by contact with environmental toxins, such as halothane, metal nanoparticles, aflatoxins, and malathion; this protective effect is mediated by modulation of the liver and antioxidant enzymes, and reduction of produced free radicals.

***Z. multiflora* and endogenous (obesity, insulin resistance) factors.** Non-alcoholic fatty liver disease (NAFLD) as a chronic liver disorder is associated with hypertension, obesity, dyslipidemia and type 2 diabetes mellitus [37]. The anti-diabetic effects of *Z. multiflora* essential oil were confirmed against Streptozotocin-induced diabetic rats. *Z. multiflora* essential oil (orally) significantly increased the total protein and insulin, and reduced high plasma glucose, ALP, AST, and ALT in Streptozotocin-induced diabetic rats. The free radical scavenging effects of *Z. multiflora* essential oil protects the rats against diabetic damage [38]. In addition to the anti-diabetic effect of *Z. multiflora* essential oil, its ethanol extract significantly reduced the blood glucose level in diabetic mice and significantly improved the insulin, ALT, and AST levels in treated animals. The improvement in liver enzymes and blood glucose was associated with significant reduction in hyperglycaemia, inflammation, and oxidative stress. *Z. multiflora* ethanol extract increased the activity of SOD, CAT and reduced the MDA and TNF- $\alpha$  in diabetic mice. In the presence of *Z. multiflora* ethanol extract, a significant improvement in cholesterol, total glyceride, HDL, and LDL levels was observed, while no signs of pathological damage were found in the liver of treated mice [39]. Significant reduction of LDL-cholesterol and activation of triglyceride lipase enzyme reduced the lipid accumulation in the liver of healthy animals, without any effects on serum level of AST, ALT and ALP [40].

Insulin resistance as key feature of type 2 diabetes in the body is associated with hyperglycaemia, hyper-insulinaemia, and dyslipidaemia. *Z. multiflora* aqueous extract (1,000 mg/kg), similar to pioglitazone (10 mg/kg), increased the insulin,

glucose, triglyceride and PPAR $\gamma$  levels in the liver, and reduced adiponectin and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) levels in fructose-fed insulin resistant rats, but with a difference in the mechanism of actions: pioglitazone significantly reduced the total free fatty acids level ( $p < 0.05$ ), while *Z. multiflora* had no effect on total free fatty acid content [41]. The anti-hyperglycaemic activity of *Z. multiflora* is superior to its effect on insulin secretion. Inhibition of liver gluconeogenesis and increase of free fatty acids muscle oxidation by increase of adiponectin and PPAR $\gamma$  protein and translocation of GLUT4 in adipose tissue improved the insulin resistant and insulin sensitivity in insulin resistant animals. Therefore, *Z. multiflora* may have 3 mechanism of anti-hyperglycemic effect, which is mediated by a direct insulin-like effect, increase in the expression of PPAR  $\gamma$  and adiponectin.

***Z. multiflora* and hepatic hydatid cysts.** Hydatid cyst, especially hepatic hydatid infection, is caused by *Echinococcus* sp., especially *E. granulosus*, which is the most common infection [42].

Administration of *Z. multiflora* water (40 ml/liter in drinking water for 8 months) reduced the cyst sizes and weights in infected mice with hydatid cyst, and damaged the morphology and germinal layers of cysts similar to albendazole (150 mg/Kg) [43, 44]. The growth and reproduction of protoscolices are mediated by germinal layers, as an important organ for infectivity of hydatid cysts. *Z. multiflora* can destroy the germinal layers of protoscolices and separate the germinal from laminated layers and produce the vacuolated laminated layers [45]. Furthermore, *Z. multiflora* with the immune-modulatory effects and increase of NO production can protect the Kupffer, fat-storing, and endothelial liver cells, which lead to activation of macrophages, T-cells and production of cytokines in infected animals with hydatid cyst [46].

*Z. multiflora* aqueous extract had protective effects against long-term administration of albendazole-induced hepatic injury in mice Balb/C [47]. Although co-administration of *Z. multiflora* with albendazole significantly reduced the deleterious effects of albendazole on liver, further pharmacological and clinical studies are required on different extracts of *Z. multiflora* against albendazole-induced hepatic injuries in order to reduce the effective dose or length of treatment with albendazole.

**Hepato-protective effects of *Z. multiflora* in clinical studies.** Although, *Z. multiflora* is known as a suitable alternative hepatoprotective agent in animal studies, and its efficacy should be confirmed in human studies. There is one 12-week randomized double blind placebo controlled clinical trial on the efficacy and safety of 700 mg *Z. multiflora* powder ( $n=45$ ), administered twice daily in comparison with placebo ( $n=40$ ) in patients ( $n=85$ ) with NAFLD. *Z. multiflora* powder significantly reduced the serum insulin level ( $p=0.03$ ) and insulin resistance ( $p=0.023$ ) of patients, with no effect on ALT, high-sensitivity C-reactive protein (hs-CRP), TNF- $\alpha$ , lipid profiles and the grade of liver fat, compared with placebo. The treatment was safe and well tolerated [48].

Insulin resistance is known as one important risk factor for pathogenesis of NAFLD. Although, the daily dose of 1,400 mg *Z. multiflora* powder for 12 weeks reduced the insulin resistance, serum insulin with no effects on other

factors, the short length of treatment, and no assessment of daily calorie intake, was the limitation of this clinical study. Thus, the use of *Z. multiflora* essential oil, or its ethanol extract and daily calorie assessment of patients, are recommended for future clinical studies.

#### The antioxidant effects of *Z. multiflora* in clinical studies.

Sulfur mustard is the reason for injuries in different organs of the body, especially in the liver. Forty-seven veterans who were exposed to sulfur mustard, were enrolled in a clinical trial, and randomly divided into 3 groups administered 5 mg/kg *Z. multiflora* (n=17), 10 mg/kg *Z. multiflora* (n=14), and a placebo (n=16). The volunteers were given the treatments 3 times a day for 2 months, together with their previous treatments (inhaled salbutamol, corticosteroid, and theophylline). However, during 2 months of treatments, *Z. multiflora* had no effect on other haematological parameters, placebo had no effects on the level of thiol, MDA, SOD and CAT activity, while *Z. multiflora* significantly increased the level of thiol, CAT, SOD and decreased the MDA level, compared to the baseline. The treatment of patients with *Z. multiflora* ethanol extract significantly decreased the serum level of TNF- $\alpha$ , MCP-1, EGF, and VEGF [49]. The potency of *Z. multiflora* in increase in the activity of antioxidant enzymes and reduction in MDA level was confirmed in the current clinical study.

#### Mechanism of action for hepatoprotective effects of *Zataria multiflora*.

The results of pharmacological studies on the hepatoprotective effects of *Z. multiflora* are related to its ability to scavenge the free radicals, and its antioxidant activities and their effects on inflammatory cytokines in the body. *Z. multiflora* methanol extract significantly reduced the IL-6 expression, which is involved in some inflammatory diseases, such as obesity, diabetes and arthritis rheumatoid [50].

The antioxidant activity of *Z. multiflora* extracts is related to its carvacrol and thymol content: carvacrol had protective effects against d-galactosamine- [51] and thioacetamide-induced hepatotoxicity in rats [52, 53]. The inflammation and oxidative stress of acetaminophen- exposed HepG2 cells were inhibited by thymol and carvacrol in a dose-dependent manner. Thymol and carvacrol increased the activity of anti-oxidant enzymes and GSH level, and reduced the levels of ALT and LDH and pro-inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ ) in acetaminophen- induced HepG2 cell lines [54]. Administration of 20 mg/kg carvacrol for 28 days significantly reduced the MDA level, serum level of ALP, ALT, and AST in adriamycin-exposed Wistar rats [25]. Carvacrol reduced the lipid peroxidation and MDA by activation of anti-oxidant enzymes (SOD, CAT and GPx) in cerulean-induced acute pancreatitis in animals. The levels of 8-OH-dG as a marker of DNA oxidative stress, was lower in carvacrol treated acute pancreatitis than in the acute pancreatitis animal model. An increase in glycogen content of hepatocytes and reduction in fibrosis was observed in carvacrol treated acute pancreatitis animals [55]. The protective effect of carvacrol against MDA production was mediated via post transcriptional action by suppressing the HMG-CoA reductase in the liver [56].

Carvacrol had chemo-preventive effects against diethylnitrosamine (DEN-induced liver cancer in rats). The preventative effects of carvacrol against lipid peroxidation

and its protective effects against the antioxidant system inhibited hepatic cell damage in DEN-induced hepatocellular carcinogenesis [57]. Carvacrol scavenges the free radicals by activation of host antioxidant defence mechanisms and drug metabolizing enzyme, detoxifies the free radicals and reduces the lipid peroxidation [58].

The effects 1.2 mg/kg per day of carvacrol on 10 patients exposed to sulfur mustard in comparison with placebo for two months significantly increased the activity of antioxidant enzymes (SOD, CAT), and thiol level and reduced the MDA level in patients [59]. Reduction in inflammatory cytokines (IL-2, IL-4, IL-6, IL-8) and a significant increase in IL-10, IFN- $\gamma$ , IFN- $\gamma$ /IL-4 ratio, were observed after treatment with carvacrol after two months [60].

Thymol exhibited hepatoprotective effects against carbon tetrachloride (CCL<sub>4</sub>) induced hepatotoxicity in mice [61] and H<sub>2</sub>O<sub>2</sub> induced cytotoxicity in HepG2 cell lines [62]. Thyme aqueous extract completely protected the G6PD against nitroso-arginine and nitroso-cysteine. Thyme extract protected the activity of the G6PD enzyme and inhibited the production of cellular reactive oxygen species to inhibit the RBC hemolysis [63]. Strong radical scavenging activity was reported for thymol with IC<sub>50</sub> 0.169 mg/ml [64]. The hepatoprotective potency of treatment for thymol was better than carvacrol in acetaminophen exposed HepG2 [54]. Therefore, the hepatoprotective effects of *Z. multiflora*, thymol and carvacrol are related to the reduction of oxidative stress, improvement of antioxidant defence, and inhibition of pro-inflammatory cytokines.

## CONCLUSIONS

There is one clinical trial on efficacy and safety of *Z. multiflora* in NAFLD. The investigation on mechanism of action of *Z. multiflora* in liver disorders exhibited that liver as the vital organ in the body is the target of oxidative stress and free radicals and there is a positive correlation between the liver injury and oxidative stress. *Z. multiflora* preparations scavenged the free radicals and acted as antioxidant agent (Table 1). *Z. multiflora* extracts protected the liver against drug (acetaminophen, adriamycin), environmental toxins (malathion, halothane, aflatoxins, etc.), endogenous factors (metabolic disorders, aging), and hydatid cyst (Figure 3). *Z. multiflora* powder significantly reduced the blood GSH, MDA, liver SOD [65], TBARS concentration [66] and glycogen content and improved the liver function by increasing the serum ALT, AST, and ALP enzymes and total antioxidant status in liver [67].

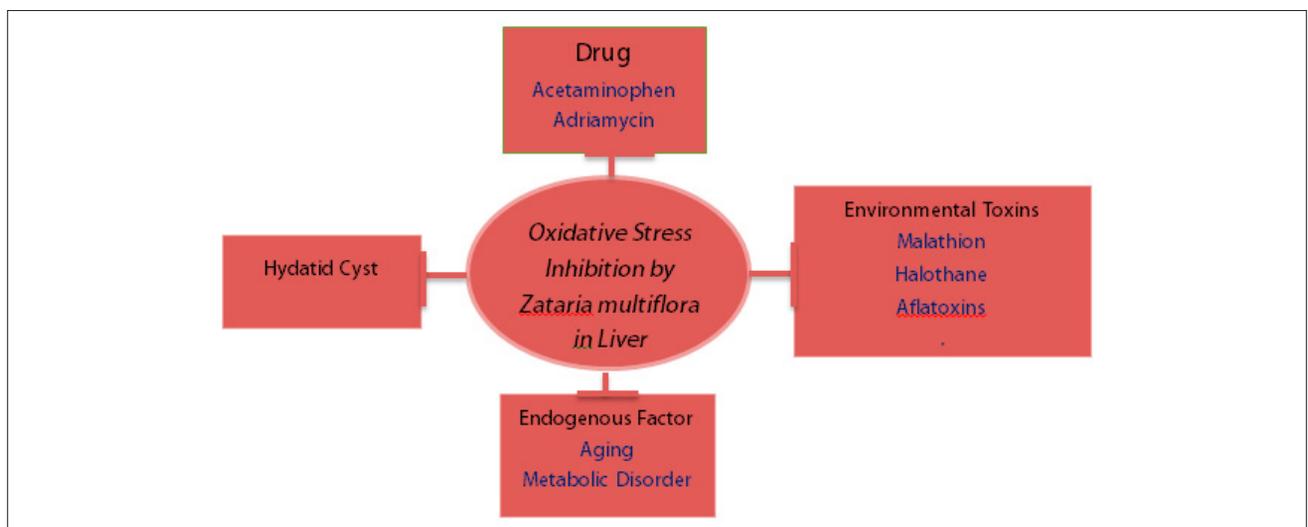
*Z. multiflora* methanol, ethanol or aqueous extracts have been used as hepatoprotective agent in most preclinical studies, and its liver protective effects are related to the thymol and carvacrol content. Among different extracts of *Z. multiflora*, its essential oil is rich of thymol and carvacrol, and has higher antioxidant activities; therefore, *Z. multiflora* essential oil can be used in pharmacological and clinical studies for its liver protective effects.

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**Table 1.** Hepato-protective effects of *Z. multiflora* against endogenous factors, and oxidative stress in animal models

Compound	Dose	Animal model	Results	References
<i>Z. multiflora</i> methanol extract	100, 200, 400 mg/kg	Paracetamol (2 g/kg) induced hepatotoxicity in male Wistar rats	↓ Inflammatory cells ↓ Hepatic lesions ↓ Necrotic tissues ↓ MDA level ↑ SOD, Cat and GPx to normal level ↓ ALT, AST, ALP to the normal values	[24]
<i>Z. multiflora</i> ethanol extract	200 mg/kg	Adriamycin (5 mg/kg) in male Wistar rats	↓ Liver ALT, ALP and MDA levels	[25]
<i>Z. multiflora</i> methanol	200 mg/kg	Malathione induced oxidative stress in mice	↓ AST and ALT to normal level ↓ lipid peroxidation ↑ SOD, CAT	[29]
<i>Z. multiflora</i> ethanol extract	800 ppm for 5 days	Halothane induced hepatotoxicity Wistar albino rat's model.	↓ ALT, ALP, LDH levels	[31]
<i>Z. multiflora</i> seeds	20 g/kg	Anti-aflatoxin B <sub>1</sub> effects in broilers	↓ Liver damage ↓ Focal area of hepatic necrosis ↓ Parenchymal ↓ Mononuclear cell aggregation	[33]
<i>Thymus vulgaris</i> essential oil	100, 200 mg/kg b.w	Iron nanoparticles	↓ CYP 450 protein level activity ↓ GST, AST to normal levels No vacuole destruction	[35]
<i>Z. multiflora</i> ethanol extract	50, 100, 200, or 400 mg/kg for 7 consecutive	cyclophosphamide induce hepatotoxicity in mice	↓ ALP, AST and ALT improvement of liver histopathological features	[36]
<i>Z. multiflora</i> essential oil	50, 100 µl/kg for 28 days	Streptozotocin induced diabetes in rats	↑ Total protein, insulin, ↓ plasma glucose, ALP, AST, ALT Scavenge the oxygen and nitrogen radicals	[38]
<i>Z. multiflora</i> ethanol extract	250, 500, 1000 mg/kg/day for 28 days	Streptozotocin induced diabetes in rats	↓ Blood glucose level ↑ Insulin level ↓ Serum level of ALT, AST ↓ Hyperglycaemia ↓ Inflammation ↓ Oxidative stress. ↑ SOD, CAT ↓ MDA, TNF-α	[39]
<i>Z. multiflora</i> ethanol extract	100, 200, 300, 400 mg/kg for 28 days	Rats	↓ LDL-Cholesterol ↑ Triglyceride lipase enzyme ↑ Plasma level Deletion of lipid from the body without any adverse effects on liver enzymes	[40]
<i>Z. multiflora</i> aqueous extract	1000 mg/kg	Fructose fed insulin resistant rats	↑ Insulin, glucose, triglyceride and PPARγ levels ↓ Level of adiponectin and HOMA.1R No effect on total free fatty acids content Effect of <i>Z. multiflora</i> on PPARγ was on protein level, not on mRNA level, but had no effect on GLUT.4 protein.	[41]

**Figure 3.** Hepato-protective effects of *Z. multiflora* by inhibition of oxidative stress

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