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The comparative effects of *Artemisia deserti extract* and diazinon on rat liver

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Abstract

Artemisia is a diverse genus of Asteraceae family that has pharmacological effects and it used for treat diseases, including antioxidant effects against oxidative stress. This study investigated the comparative effects of *Artemisia deserti* extract and diazinon. *A. deserti* was collected from Isfahan, Iran then 20 g of flower powder was extracted with 150 mL 80% ethanol and the 100, 200 mg/kg concentrations of ethanolic extract were prepared. The animals were divided in to 6 groups: 1. control, 2. diazinon, 3 and 4. diazinon and extract (100, 200 mg/kg), 5 and 6. extract (100, 200 mg/kg). The blood samples were collected and the rate of AST, ALT, ALP, HDL, LDL, serum total antioxidant and MDA were assayed. Also, the liver tissue was isolated for histopathological examination. Finally, the statistical comparisons were done with ANOVA test. The rate of ALT and MDA were changed significantly in some groups. Moreover, results indicated tissue disorders in all groups compared to control, including the accumulation of blood and Kupffer cells in central veins and sinusoidal spaces, necrosis of hepatocytes and damage of central vein. Diazinon cause oxidative stress and liver disorders, similar to the effect of artemisinin on liver; therefore, simultaneous use of these compounds could enhance the toxic effects.

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Introduction

Herbal drugs are used for years in daily life to prevent and treat diseases over the world. Moreover, the herbal drugs are known as the oldest form of human health care and the World Health Organization estimates that 80% of the world populations use these drugs and the herbal drugs are usually without side effects (Karakoca *et al*., 2013). The genus *Artemisia* is the largest of family Asteraceae that comprises around 500 species. The genus *Artemisia* is the largest and most widely Asteraceae family genera. Chemical investigation has shown that *Artemisia* species contain acetylenic compounds, flavonoids, coumarins and terpenoids, specifically sesquiterpene lactones and other constituents. *Artemisia deserti* Krasch is a traditional medicinal herb of China, so that, it is cultivating on a commercial scale in China and Vietnam and this is probably due to sesquiterpene lactones compounds (Noori *et al*., 2014). Moreover, 16 components were recognized in the oil of aerial parts from *A. deserti* such as camphor, 1,8-cineole, piperiton, β-pinene and isoborneol that are the major components in the oil of *A. deserti*. Also, the leaf and flower oils of *A. deserti* were observed to be rich in oxygenated monoterpenes while oxygenated monoterpenes and sesquiterpenes were the major in stem (Kazemi *et al*., 2011).

Liver is an important body organ which it has a wide range of functions, including detoxification, protein synthesis and production of biochemicals necessary for digestion, glycogen storage, decomposition of red blood cells, hormone production (Thapa and Walia, 2007). Liver transaminases [AST/ALT (SGOT/SGPT)] are biomarkers of liver damage in a patient. AST and ALT are liver enzymes that are involved in amino acid metabolism (Lee *et al*., 2012). Both AST and ALT are normally present in serum at low levels and these are released into the blood in greater amounts when hepatocytes are damaged (Aragon *et al*., 2010). The liver is exposed to many oxidative agents, thus antioxidant compounds are useful for liver health (Rezaei *et al*., 2013).

The medicinal plants are as a source of phytochemicals that are known as biologically active antioxidants and these compounds inhibit free radicals (Lateef Molan *et al*., 2012). Free radicals are highly reactive molecules that are produced by biochemical redox reactions occurring in natural process of cell metabolism and oxidative stress occurs when the free radicals are produced in large amounts or the antioxidant levels are low, as a result, the free radicals may cause lipid peroxidation and damage to cellular structures, nucleic acids, proteins and lipids. The level of lipid peroxidation is specified as Malondialdehyde (MDA) (Moujerloo, 2010; Ugusman *et al*., 2012).

Diazinon is a organophosphorous pesticides. Organophosphate compounds are useful for pesticides due to their ability to inhibit acetylcholinesterase, also, these compounds can damage to different organs such as liver in human and animals (Razavi *et al*., 2013). Therefore, the aim of this study was comparison the effects of *Artemisia deserti extract* and diazinon on liver.

Materials and methods

Collection of plant

The flowering tops of *A. deserti* were collected in west of Isfahan area (Golpaygan heights), Isfahan of province, Iran, in September 2012. The voucher specimen was deposited at the herbarium of the Research-Institute of Isfahan Forests and Rangelands.

Preparation of extract

The flowering tops of *A. deserti* were air-dried under shade and ground in to coarse powder using electric blender, then, 20 g of flower powder were extracted with 150 mL 80% ethanol by Soxhlet extraction for 8 h. The residue was evaporated by using a rotary evaporator. The dried extracts were stored at 4°C until used. The extract was dissolved in saline at concentrations of 100, 200 mg/kg body weight.

Preparation of diazinon

Diazinon was procured from Sabzavar Pardis Chemical Company. Then, it was dissolved in distilled water at concentration of 100 mg/kg body weight.

Animals

Adult male wistar rats (200-250 g) were obtained from Iran Pastor Institute and divided into 6 groups of eight animals each (48 rats). They were maintained under controlled temperature, 12 h light/12 h dark conditions for 1 week before the start of the experiments for adaptation to laboratory conditions. The procedures in this study were carried out in accordance with the institution's scientific procedures for animals and was approved by the Institutional Animal Care. The animals were randomly divided into the groups that were injected intraperitoneally. These groups include the 1.control group that received normal saline, 2.diazinon treated group (100 mg/kg), 3.diazinon (100 mg/kg)+extract (100 mg/kg), 4.diazinon (100 mg/kg)+extract (200 mg/kg), 5 and 6.extract treated groups (100 and 200 mg/kg) respectively. The animals were treated with diazinon (groups 2, 3, 4) once daily for 4 days then, 2 days after treatment with diazinon they were treated with extract (groups 3, 4, 5, 6) once daily for 6 days.

The animals were anesthetized by injection with Ketamine (0.07 ml/100 g body weight) and the blood samples were collected 2 days after the last injection of extract. The biochemical parameters such as AST, ALT, ALP, HDL and LDL were assayed using autoanalyzer (902 Hitachi Automatic Analyzer, Roche, India) and the rate of MDA and serum total antioxidant were assayed respectively using TBA (*Thiobarbituric Acid)* and FRAP (Ferric Reducing Ability of Plasma) methods (Esterabeur and Cheeseman, 1990; Benzi and Strain, 1999). Then, the animals were killed and liver tissue was fixed in 10% formalin, dehydrated in ethanol, cleared in xylene and embedded in paraffin. Sections were prepared and then stained with Hematoxylin-Eosin (H&E) for photomicroscopic (Olympus, Japan) observation.

Statistical analysis

All data were presented as Mean ± SD. The statistical comparisons were done with ANOVA test by SPSS 18 software.

Results

According to the results, no significant changes were observed in the mean value of AST, ALP, HDL, LDL and serum total antioxidant. Although the rate of these parameters changed between groups but these changes were not significantly. Whereas, the rate of ALT decreased significantly in the group 3, 4 (diazinon and extract with concentration of 100, 200 mg/kg) when compared with other groups ($p< 0.05$) (Table 1) (Figure 1). Moreover, the rate of MDA increased significantly in the group 6 (extract with concentration of 200 mg/kg) ($p< 0.05$) (Table 2) (Figure 2).

Table 1. Comparison of blood factors tested in 6 groups (One-Way ANOVA).

Groups	AST (U/L) Mean ± SD	ALT (U/L) Mean ± SD	ALP (U/L) Mean ± SD	HDL (mg/dl) Mean ± SD	LDL (mg/dl) Mean ± SD
Control	162.37 ± 16.12	57 ± 12.68	447.12 ± 92.09	67.25 ± 8.18	4.75 ± 3.55
Diazinon (100mg/kg)	134.12± 24.21	48.25 ± 13.51	404.50 ± 93.28	69.50 ± 6.86	4.32 ± 2.85
Diazinon+Extract (100mg/kg)	138.87 ± 30.17	*44.87 ± 9.80	363.87 ± 104.03	64.75 ± 5.36	4.27 ± 2.58
Diazinon+Extract (200mg/kg)	137 ± 25.89	*44.25 ± 6.29	356.50 ± 68.65	356.50 ± 68.65	4.67 ± 3.21
Extract (100mg/kg)	135.25 ± 12.54	55.87 ± 16.94	354.62 ± 94.32	65.87 ± 11.78	4.72 ± 3.91
Extract (200mg/kg)	146 ± 27.91	60.12 ± 10.62	418.12 ± 106.10	60.25 ± 9.25	4.97 ± 3.62

Histological studies showed that the liver tissue was normal in the control group. But, the treated animals with *A. deserti* extract and diazinon showed significantly histopathological alterations. These alterations include congestion of blood in the central veins, portal veins, sinusoidal spaces and necrosis of hepatocytes. Also, the kupffer cells were activated and signs of fatty degeneration were observed. Moreover,

the central vein´s wall damaged and the neutrophils and lymphocytes infiltration caused the accumulation of these cells around the portal vein and bile duct (Figure 3). These changes were more in the groups treated with the extract (groups 3, 4, 5, 6) as compare with diazinon (group 2). Moreover, these changes were increased in the group 6 (concentration of 200 mg/kg) in compare with group 5 (concentration of 100 mg/kg). Therefore, the extract of this plant has not antioxidant activity at 100 and 200 mg/kg.

Table 2. Comparison of MDA and serum total antioxidant tested in 6 groups (One-Way ANOVA).

Groups	MDA (nmol/mg protein) Mean ± SD	Antioxidant (u/mg protein) Mean ± SD
Control	0.87 ± 0.703	0.217 ± 0.308
Diazinon (100mg/kg)	0.96 ± 0.462	0.098 ± 0.015
Diazinon+Extract (100mg/kg)	0.68 ± 0.388	0.113 ± 0.040
Diazinon+Extract (200mg/kg)	0.60 ± 0.375	0.146 ± 0.120
Extract (100mg/kg)	0.97 ± 1.60	0.110 ± 0.027
Extract (200mg/kg)	*1.97 ± 0.511	0.106 ± 0.022

***:** Significant increase (p< 0.05) of MDA in extract group (200 mg/kg) (ANOVA, Duncan).

Discussion

Results of this study showed that, the rate of ALT was decreased significantly in groups 3 and 4 (diazinon and extract with concentrations of 100, 200 mg/kg) as compared with other groups. In fact, the *A. deserti* extract + diazinon (groups 3 and 4) reduced the rate of ALT in these groups. Whereas, diazinon and extract alone (groups 2, 5 and 6) did not change the blood factors.

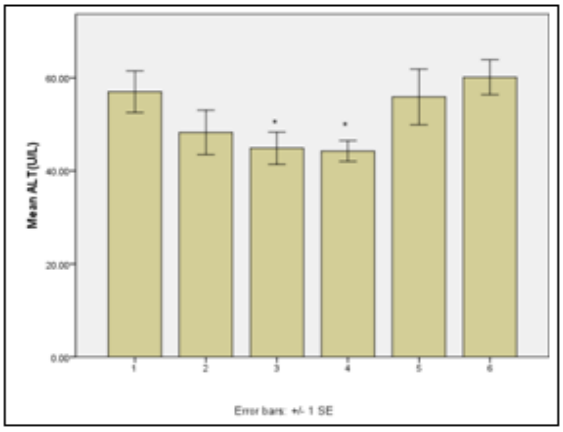

Fig. 1. Significant reduction (p< 0.05) of ALT in groups 3 and 4 (diazinon+ extract 100, 200 mg/kg).

Rezaei *et al.* (2013) reported the significant increases in AST, ALT and ALP with thioacetamide (50 mg/kg) so that, the *A. aucheri* alcoholic extract decreased significantly these parameters at concentrations of 100, 200, and 300 mg/kg. The protective effects of this extract may be due to its ability to block the bioactivation of thioacetamide. Also, the acetaminophen (640 mg/kg) increased *the rate of AST and ALT,* whereas, the methanolic extract of *A. scoparia* (150 mg/kg) decreased these factors; thus, the extract of this plant contains hepatoprotective constituents (Gilani and Janbaz, 1993). Kim and Lee (1996) reported that aminotransferase increased with ethanol (5 ml/kg). Whereas, *A. selengensis* methanol extract (200 mg/kg) decreased significantly the rate of aminotransferase. Therefore, the extract have a possible protective effect on the ethanol-induced hepatotoxicity. In another study, *the rate of AST and ALT increased* with diazinon (13.5 mg/kg) for 3 weeks (Ahmad, 2011). Moreover, Kalender *et al.* (2005), reported that diazinon (10 mg/kg) *increased* the aminotransferase and alkaline phosphatase in rats for 7 weeks. Whereas, in present study the blood parameters did not change after treatment with diazinon (group 2) and extract (groups 5, 6). While *A. macivera* chloroform extract (50, 100 and 200 mg/kg) *increased the rate of* AST, ALT and ALP for 60 days. These changes were returned to normal after treatment. They concluded that long-term exposure to this extract is relatively safe but high dose exposure may cause liver damage (Atawodi *et al.*, 2011). In another study, no significant alterations were observed in the AST and ALT in 2% *A. abyssinica* leaves diet. Whereas, these factors were increased in 10% diet. These results indicated that the sensitivity

of the animals to plant materials was dependent to the active ingredient and concentration added to the diet (Adam *et al*., 2000).

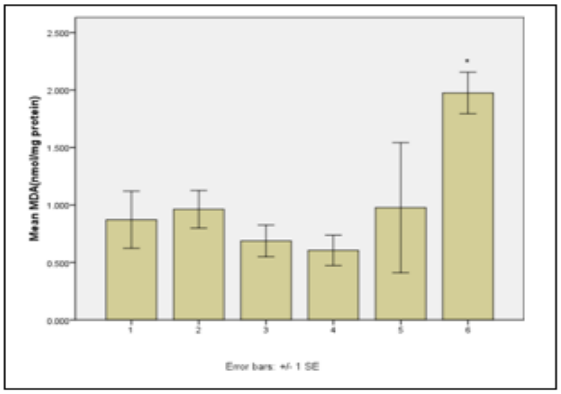

Fig. 2. Significant increase (p< 0.05) of MDA in group 6 (200 mg/kg extract).

In present study the rate of MDA increased significantly in the group 6 (200 mg/kg) which indicate the extract of *A. deserti* caused oxidative stress in this group. While the rate of antioxidant enzyms were not changed. This result was not similar to the results of Temraz and Tantawy (2008), that *A. vulgaris* aqueous extract (100 mg/kg) increased the antioxidant enzymes in rats. These findings indicated this extract is a potential source of natural antioxidants. In the present study, the MDA did not change after treatment with diazinon. Whereas, in research of Amirkabirian *et al.* (2007), diazinon (60 mg/kg) increased significantly the rate of MDA. Jia *et al.* (2010), reported the galactose (1.2 g/kg) increased the rate of MDA in mice. Whereas, the *A. selengensis* water extract (3, 6, 12 g/kg) especially, 12 g/kg concentration, decreased significantly the MDA. In another study, the aqueous extract of *A. absinthium* (50, 100, 200 mg/kg) was investigated against CCl4 (10 ml/kg). The extract decreased the MDA and the antioxidant enzymes returned to normal. This indicated the protective effects of extract against acute liver injury may be attributed to its antioxidant and immunomodulatory activity (Amat *et al*., 2010).

The *A. deserti* extract and diazinon also, caused significant histopathological changes in the liver tissue. Similar to our results, Sarhan and Sahhaf (2011) reported the diazinon (20 mg/kg) induced blood vessel congestion, leucocytic infiltrations in the liver parenchyma and fatty degeneration. In another study, diazinon only at concentrations of 32.5 mg/kg caused liver tissue damage that these changes were dose-dependent (El-Shenawy *et al*., 2009). Whereas, *A. sieberi* methanol extract (1, 10, 100 mg/ml) did not cause any disorder in the liver, that probably related to the type of extract, plant species and concentration of extract (Nahrevanian *et al*., 2012). Other researchers did not observe any significant alterations in rat liver in 2% *A. abyssinica* diet whereas reduction in cytoplasmic basophilia with small fatty vacuoles in the centrilobular hepatocytes was observed in 10% diet (Adam *et al*., 2000). Kalantari *et al. (*2013*)* were observed pathological changes in liver tissue with A. dracunculus extract. This is possibly due to the presence of genotoxic and mutagenic compounds in the extract. Moreover, *A. aucheri* extract prevented liver damage induced by thioacetamide and this is probably due to the antioxidant properties of this plant (Rezaei *et al*., 2013). In study of Gilani and Janbaz (1993) also, acetaminophen caused damage to liver tissue but, *A. scoparia* extract reduced this damage.

According to the research, diazinon may cause oxidative stress in the liver tissue. Moreover, the *A. deserti* flowering tops extract has toxic effects on liver and this is probably due to the presence of Artemisinin (a toxic compound) that it is a sesquiterpene lactone that exist in *Artemisia* genus (Chaturvadi, 2011). So that, these effects were increased with increasing of concentration of extract. On the other hand, *A. deserti* extract have probably the antioxidant effect but, it seems that, the lower concentrations and more treatment time with extract are close us to my intended results. Ferreira *et al.* (2010) reported that artemisinin was metabolized by the liver CYP450 enzyme. But the pharmacological levels of artemisinin in the blood would decrease significantly after 5-7 days of treatment with the extract. This is due to induction CYP450 enzyme. Therefore, more research needs to be done about the sampling time, number of injection and different concentrations of extract and diazinon.

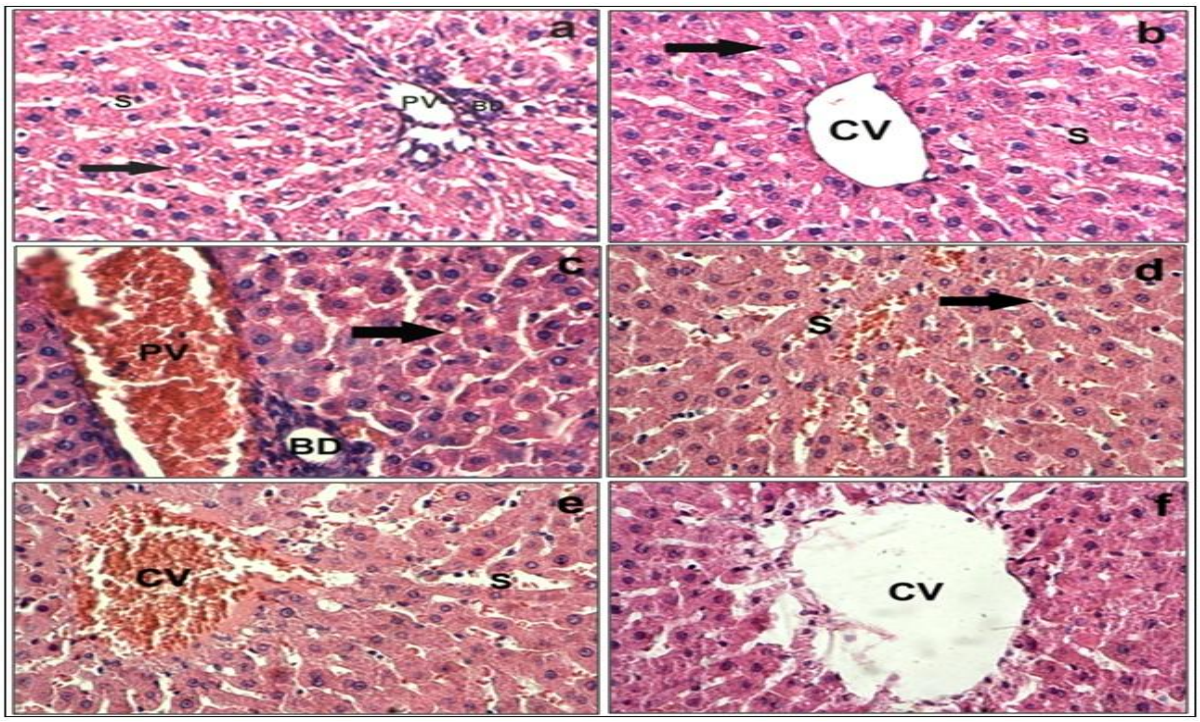

Fig 3. Sections of the liver in the control and treated groups. (a, b) the group 1 (control) show the hepatocytes (arrow), sinusoidal spaces, central vein and portal vein were normal, (c) the groups treated with extract and diazinon show the congestion of blood in the portal vein and infiltration of neutrophils and lymphocytes (thin arrow) around the portal vein and bile duct and fatty degeneration (thick arrow), (d) the groups treated with extract and diazinon show the congestion of blood in the sinusoidal spaces and activated kupffer cells (arrow) and necrosis of hepatocytes, (e) the groups treated with extract and diazinon show the congestion of blood in the central vein, damage of central vein ،s wall and disarrangement of sinusoidal spaces, (f) the groups treated with extract and diazinon show the damage of central vein ،s wall and disarrangement of sinusoidal spaces (S, Sinusoid; CV, Central Vein; PV, Portal Vein; BD, Bile Duct) (×400).

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