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Effect of hydro-alcoholic extract of *Ziziphus spina-christi* against scopolamine-induced anxiety in rats

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Abstract

The aim of this study was to observe the effect of *Ziziphus spina-christi* extract against anxiety related behavior induced by scopolamine. Rats were randomly divided into six groups, each group consists of eight rats. Vehicle group received distilled water, negative control received scopolamine (1 mg/kg) and positive control received diazepam (1 mg/mL). Experimental groups received *Z. spina-christi* extract (50, 100 and 200 mg/kg IP) 30 min after scopolamine injection. Anxiety related behaviors were assessed using the elevated plus maze. The rotarod test was used to evaluate motor coordination. Administration of *Z. spina-christi* extract (200 mg/kg) significantly increased the time spent in the open arm of elevated plus maze. The extract also reduced the percentage of closed arms entries and time spent in the closed arms. Different concentration of *Z. spina-christi* extract didn't affect motor coordination and balance. Hydro-alcoholic extract of *Z. spina-christi* significantly ameliorate scopolamine-induced anxiety.

Introduction

Anxiety disorders are the most common mental illness in the general population. Studies have indicated that about one in four people experience an anxiety disorder sometime in their life (Rector et al., 2008). Everyone feels anxiety but severe and chronic anxiety are abnormal and problematic. Prolonged anxiety can lead to sleep disturbance, fatigue, insomnia, and physical problems (Khodami et al., 2011). It accelerates premature aging and contributes to the development of metabolic and cardiovascular diseases (Shawa et al., 2007).

Anxiety disorders can be treated with certain medication or cognitive-behavioral therapy. Benzodiazepines are among the most commonly prescribed medications to treat anxiety. However, the use of benzodiazepines has been limited due to their possible adverse effects such as drowsiness, dizziness, confusion, disorientation, amnesia, breathing difficulties and loss of balance (Rector et al., 2008). They can also cause physical dependence and the cost of treatment with them is considerable (Kheirkhah et al., 2014). Thus, researcher's attention has been shifted toward new drugs without adverse effect. Some medicinal plants and their components have been shown to possess sedative and anxiolytic activity (Bhandari and Kabra, 2014; Dhawan et al., 2001).

Ziziphus spina-christi locally known as sedr in Iran belongs to the botanical family *Rhamnaceae*. It is a deciduous tree, with spiny branches and small, orange-yellow fruits that widely grows in the south of Iran (Yossef et al., 2011). *Z. spina-christi* leaves are traditionally used to treat ulcers, wounds, eye diseases, bronchitis and as an anti-inflammatory agent to treat skin disease. The seeds are sedative and are used to halt nausea, vomiting and abdominal pain associated with pregnancy (Ghafoor et al., 2012). Experimental studies have shown that *Z. spina-christi* has various therapeutic effects such as antibacterial (Dangoggo et al., 2012; Alhakmani et al., 2014), anti-diarrheal (Adzu et al., 2001), anti-cancer (Abdel-Wahhab et al., 2007), anti-fungal (Hadizadeh et al., 2009), anti-hyperglycemic

(Glombitza et al., 1994), anti-oxidant (Dahiru and Obidoa, 2008), anti-allergic (Naika et al., 2013), anti-inflammatory (Cardozoa et al., 2011) and anti-influenza activity (Hong et al., 2015). The effects of *Z. spina-christi* extract on the nervous system such as analgesic and anti-depressant activities have also been reported in previous studies (Adzu et al., 2003, Dahiru and Obidoa, 2008). There was no study on the anxiolytic activity of *Z. spina-christi*. Therefore, this study aimed to evaluate the anxiolytic effect of hydro-alcoholic extract of *Z. spina christi* against scopolamine-induced anxiety.

Materials and Methods

Preparation of hydroalcoholic extract

The leaves of *Z. spina-christi* were dried under shade at room temperature and finely powdered using an electric mill. The powdered sample was extracted with ethanol 70% by using maceration method. After continues shaking for 7 days, the mixture was filtered. The filtrate was concentrated to dryness using a rotary evaporator (Abdel-Zaher et al., 2005).

Experimental animal

Fifty adult male rats weighing 200 to 250 g were used in this study. Rats were housed under standard laboratory conditions (12–12 hours light/dark cycle at 21 ± 2°C) with free access to food and water.

Experimental group

Rats were randomly divided into six groups with 8 in each. Vehicle group received i.p. injections of distilled water instead of both *Z. spina-christi* extract and scopolamine. Negative control group received i.p. injections of scopolamine (1 mg/kg) for 21 days. Positive control group received i.p. injections of diazepam (1 mg/kg i.p) 30 min after scopolamine injection. Extract treated groups received i.p. injections of *Z. spina-christi* extract (50, 100 and 200 mg/kg) 30 min after scopolamine injection for 21 days.

Elevated plus maze test

The elevated plus maze (EPM) test was used to assess the anxiety-related behavior in rats. The apparatus consists of two open arms (5 × 50 cm), two enclosed arms (5 × 50 × 40 cm) and a central platform (5 × 5 cm).

Two arms of each type are opposite to each other. The apparatus was elevated 50 cm above the floor. This animal model of anxiety was unconditional and did not require animal training. Immediately after injection, animals were individually placed in a plexiglass box (40 × 40 × 30 cm) and allowed to explore for 5 min. Each animal was then placed in the central platform facing one open arm. During a 5 min observation period, the number of entries and the time spent in each arm were recorded by observer. Anxiety reduction was indicated by an increase in the percentage of time spent and the number of entries in the open arm (Fernandez Guasti and Picazo, 1999).

Motor coordination and balance

Motor coordination and balance were evaluated using the rotarod task. This device had a rotating rod bar with adjustable speed from 0 to 40 rpm. The rotational speed could be adjusted by changing the position of the movable masses on the bar. In this study the rotational speed was 10 rpm and the acceleration was 7 rpm^2 which was approximately 10-11 rotations per sec. Rats were first trained to walk on the rotating rod. Two hours after injection, animals were placed individually on the rotating bar and the length of time that each animal was able to maintain its balance was recorded as resistance time. Rats were given three trials using maximum time of 60 sec per trial (Rabiei et al., 2014).

Determination of anti-oxidant activity

Stock solutions of extract and BHT (1 mg/mL in ethanol) were diluted to final concentrations of 10, 20, 40, 60, 80 and 100 µg/mL. Then, 2 mL of DPPH solution (0.1 mM, in ethanol) was added to 2 mL of extract and BHT solutions at different concentrations. After 15 min at room temperature, the absorbance values were measured at 517 nm. The mixture of ethanol (2 mL) and DPPH solution (2 mL) served as control. The scavenging activity percentage was determined by the following equation:

$$(AA\%) = 100 \times (A_{control} - A_{sample})/A_{control}$$

The anti-oxidant activity was expressed as IC_{50} (the concentration of extract required to inhibit the formation of DPPH radical by 50%). The IC_{50} values were determined by plotting the graph with concentrations in x axis and percentage of inhibition in y axis (Rabiei et al., 2014).

Determination of total phenolic content

Briefly, 0.1 mL of diluted extract (0.01 g in 10 mL of 60° C methanol) was mixed with 0.5 mL of Folin-Ciocalteu reagent. After 3-5 min 0.4 mL of 7.5% sodium carbonate solution was added to the mixture and allowed to stand at room temperature for 30 min. The absorbance of reaction mixture was measured at 750 nm against distillated water blank. A standard calibration curve was plotted using different concentrations of Gallic acid. The phenolic content was expressed as "mg of Gallic acid equivalents per g of sample (Rabiei et al., 2014).

Determination of total flavonoids content

The amount of total flavonoids in the *Z. spina-christi* extract was determined using the colorimetric method. The diluted extract (0.5 mL) was mixed with 0.5 mL of 2% aluminum chloride and 3 mL of 5% potassium acetate. After 40 min incubation at room temperature, the absorbance of the reaction mixture was measured at 415 nm. A standard curve was prepared using different concentrations of Rutin solution. Total flavonoid content was expressed in mg of Rutin equivalents per gram of dried extract by using a standard curve of Rutin (Rabiei et al., 2014).

Statistical analysis

Statistical analysis was performed using SPSS $_{16}$(SPSS Inc. Chicago, IL, USA). Significant differences between means were estimated using one-way ANOVA followed by Tukey for homogeneous data or Dunnett T3 for non-homogeneous data. Data were presented as the mean ± SE. $p<0.05$ was considered significantly different.

Results

Effect of extract on the behavior of rats in the elevated plus-maze

As shown in Figure 1A, scopolamine-injection caused a significant reduction in the number of entries into the open arms. Rats in the scopolamine group made significantly fewer entries into the open arms than rats in the control group. Injection of diazepam at a dose of 1 mg/kg into scopolamine-treated rats significantly increased the number of entries in the open arms ($p<0.01$). *Z. spina-christi* extract at different doses did not contribute to increase open arms exploration and open arm entry. *Z. spina-christi* extract at doses of 100 and 200 mg/kg significantly reduced the number of open arm entries compared to the control groups ($p<0.01$). The amount of time spent in the open arms of the maze is shown in Figure 1B. Scopolamine-treated group spent significantly less time in the open arms of elevated plus maze compared to the control group ($p<0.05$). Administration of diazepam into scopolamine -treated rats induced an increase in the amount of time spent in the open arms of elevated plus maze. Injection of *Z. spina-christi* extract at doses of 50 and 100 mg/kg into scopolamine-treated rat increased the time spent in open arms, however not significantly. The rats receiving *Z. spina-christi* extract at a dose of 200 mg/kg spent significantly more time in the open arms of elevated plus maze compared to the scopolamine group ($p<0.05$). The effect of different concentrations of *Z. spina-christi* extract on the number of entries and the amount of time spent in the closed arms of elevated plus maze are shown in Figure 1C and D.

According to the results, scopolamine-treated group made significantly more entries in the closed arms compared to the control group. Intraperitoneal injection of diazepam after scopolamine-treatment significantly reduced the closed arm entries ($p<0.05$). *Z. spina-christi* at doses of 50, 100 and 200 mg/kg decreased the number of closed arm entries. However, these decreases were not significant. As shown in Figure 1D, diazepam-treated and control rats spent significantly less time in the closed arm than scopolamine-treated rats. *Z. spina-christi* extract at a dose of 200 mg/kg caused a significant reduction in the time spent in the close arm ($p<0.05$). The extract at doses of 50 and 100 had ability to reduce closed arms entries however not significantly.

Effect of extract on the behavior of rats in the rotarod test

As shown in Figure 2, scopolamine-treated rats had shorter latency to fall from the rotating bar than control rats. Administration of hydro-alcoholic extract of *Z. spina-christi* (50, 100 and 200 mg/kg) into scopolamine-treated rats slightly increased the time spent on the rotating bar.

Anti-oxidant capacity of the extract

Table I shows the anti-oxidant capacity of *Z. spina-christi* extract in DPPH method. The IC_{50} of *Z. spina-christi* extract was 75.8 µg/mL.

Total phenol and flavonoids contents of the extract

Total phenolic, flavonoid and flavonol contents of the *Z. spina-christi* extracts were 800.7, 334 and 488 mg respectively

Discussion

The results of present study showed that the hydro-alcoholic extract of *Z. spina-christi* has some anxiolytic activity. *Z. spina-christi* extract at doses of 50, 100 and 200 mg/kg increased the time spent in the open arms but did not contribute to increase open arm entries. *Z. spina-christi* extract also reduced the number of entries and the time spent in the closed arms.

Anxiolytic activity observed for *Z. spina-christi* extract in our study may be related to the modulation of oxidative stress. Scopolamine has been previously shown to induce oxidative stress in rat brain (Karimi et al., 2015). Oxidative stress is a dominant risk factor associated with the development of neurodegenerative diseases and may accelerate aging and anxiety-related behavior (El-Nekeety et al., 2011). It has been reported that plants with high anti-oxidant activity have ability to attenuate oxidative stress (Kumarappan et al., 2012). In the present study, hydro alcoholic extract of *Z. spina-christi* showed good inhibitory potential against DPPH free radicals. Anti-oxidant activity of *Z. spina-christi* extract has been reported in previous studies. Alhakmani et al. (2014) showed that *Z. spina-christi*

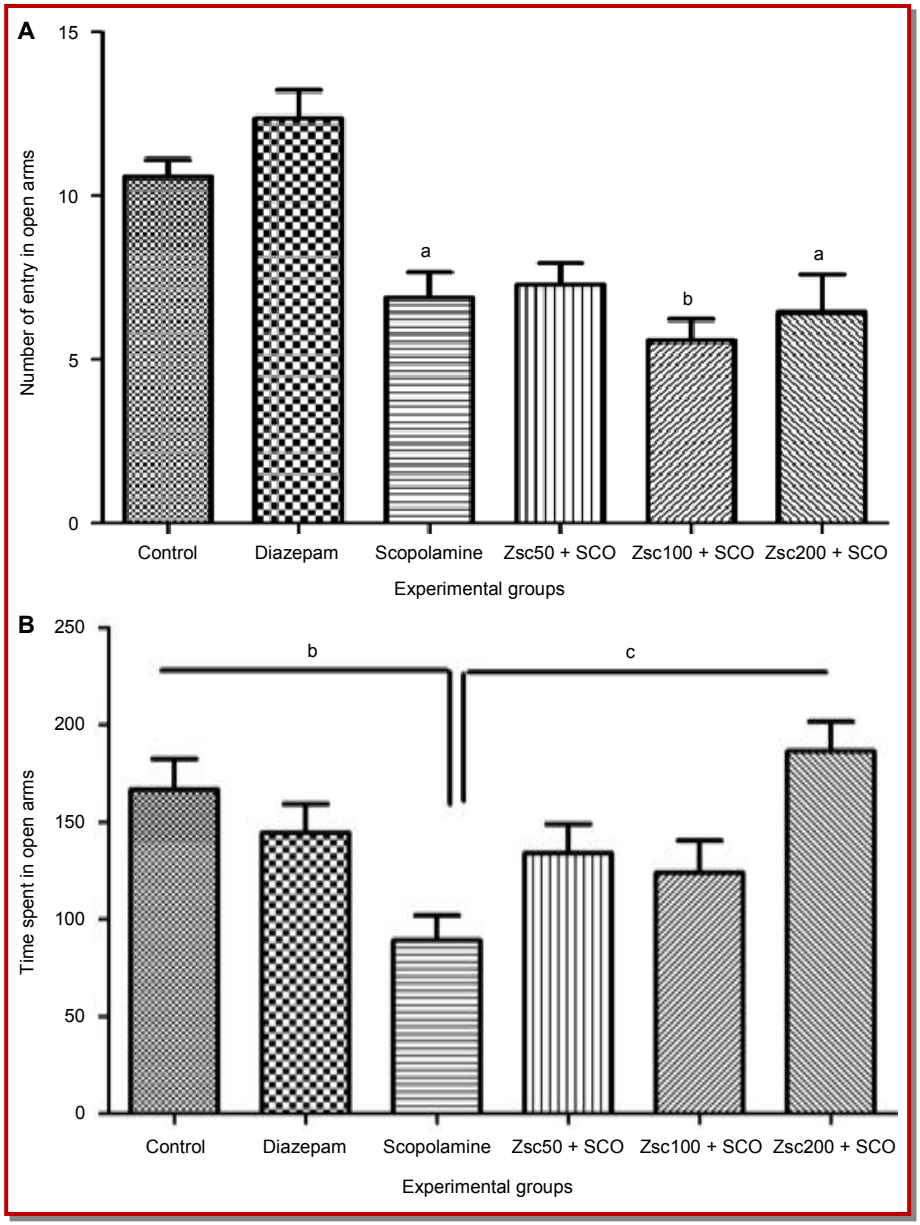

Figure 1: Effects of *Z. spina-christi* extract (50, 100 and 200 mg/kg) on: A) the number of entries in the open arms, B) the amount of time spent in the open arms; C) the number of entries in the closed arms; D) the amount of time spent in the closed arms. Data are presented as mean values (M ± SE)

extract has significant anti-oxidant activity and can be used to prevent oxidative stress and related diseases. In the study conducted by Parsaeyan and Rezvani, (2014) *Z. spina-christi* injection into diabetic rats markedly reduced lipid per-oxidation and serum malondialdehyde level.

It has been reported that flavonoids and polyphenols show protective effects against oxidative stress-related diseases (Kumarappan et al., 2012). We observed that *Z. spina-christi* extract contains high amounts of flavonoids and polyphenols. The presence of these components in the *Z. spina-christi* extract might be responsible for anxiolytic activity (Kumarappan et al., 2012). Adzu et al. (2001) reported that anti-nociceptive activity of *Z. spina-christi* extract may be related to the high amount of saponin.

The results also showed that, IP injection of hydro-alcoholic extract of *Z. spina-christi* (50, 100 and 200 mg/kg) don't affect motor coordination and balance. In the study conducted by Adzu et al. (2002), aqueous extract of *Z. spina-christi* did not exhibit significant effect on the motor performance of the mice. This finding was in harmony with our finding (Adzu et al., 2002).

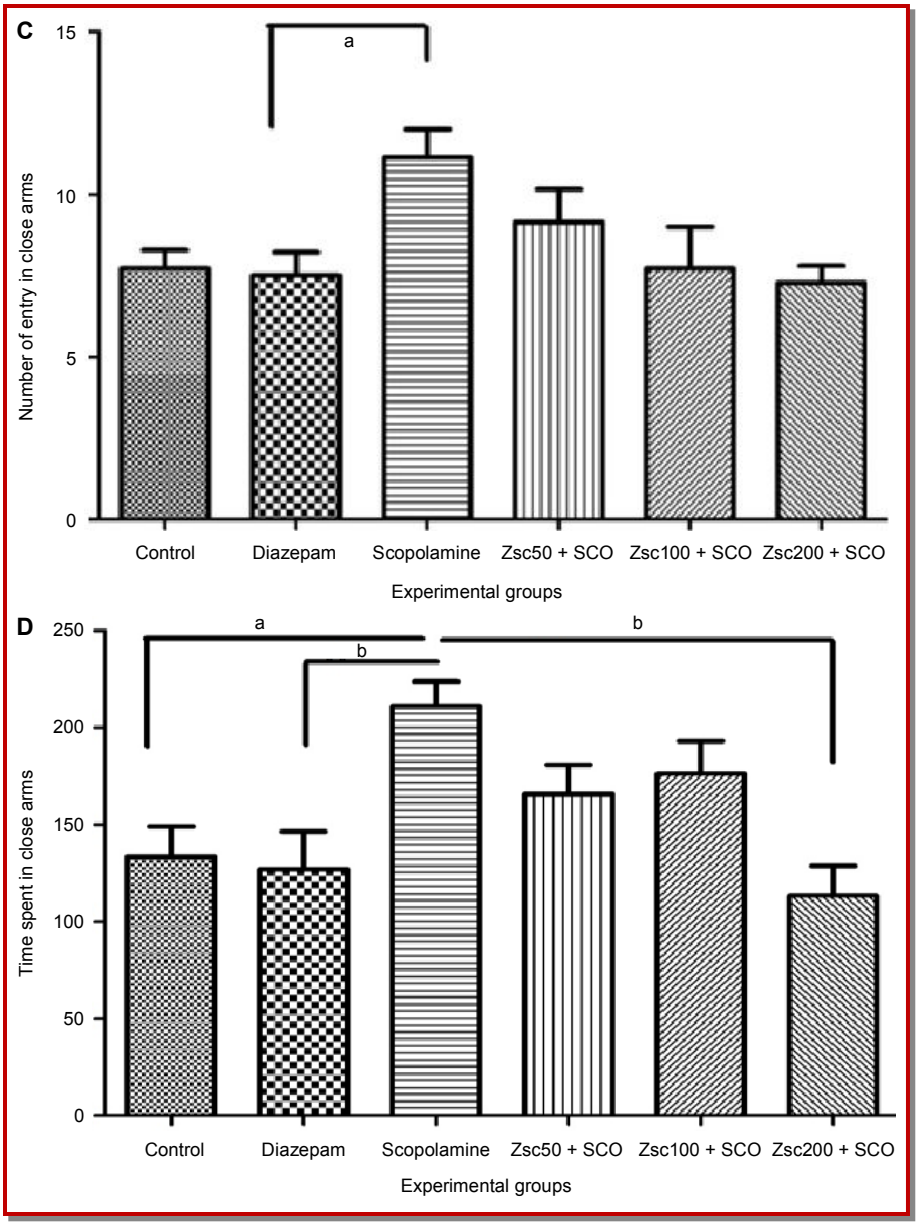

Figure 1: Effects of *Z. spina-christi* extract (50, 100 and 200 mg/kg) on: A) the number of entries in the open arms, B) the amount of time spent in the open arms; C) the number of entries in the closed arms; D) the amount of time spent in the closed arms. Data are presented as mean values (M ± SE) (Cont.)

Conclusion

Z. *spina-christi* might act to attenuate scopolamine-induced anxiety. The anxiolytic activity observed in this study may be related to anti-oxidant effects of extract.

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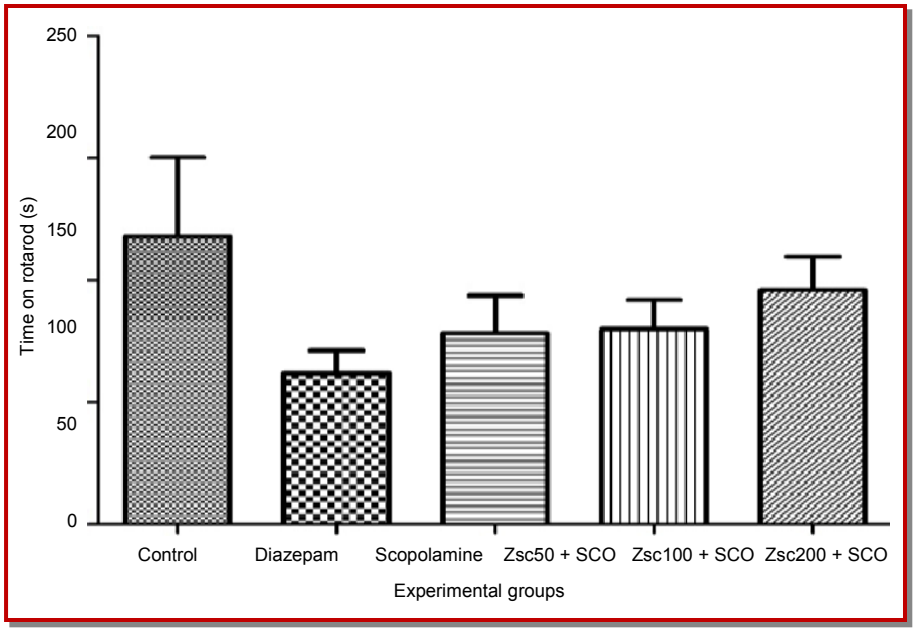

Figure 2: Means ± SEM of rod descent latency following rotarod test

Table I

Anti-oxidant capacity of *Zizyphus spina-christi* extract

DPPH radical scavenging activity inhibition (%) IC_{50} (µg/mL)	*Z. spina-christi* extract (µg/mL)
70	96.1
60	83.4
50	75.8 (IC_{50})
40	63.5
30	47.5
20	35.2
10	14.3

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