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HYPNOTIC EFFECT OF *SOLANUM LYCOPERSICUM* AND *SOLANUM NIGRUM* ON PENTOBARBITAL-INDUCED SLEEP IN MICE

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Abstract

Solanumly copersicum and *Solanum nigrum* have been recommended for its sedative property. However, no pharmacological studies have yet evaluated the effect of these plants on sleep. Given the side effects of sedative drugs, this study aimed to investigate the effects of these herbs on mice.

***Key words**: Solanum lycopersicum*, *Solanum nigrum*, Sleep, Diazepam

Introduction

Sleep disorders are among the foremost health issues associated with physical, mental, social, and emotional dysfunction. According to statistics, approximately 10% of every population suffers from clinically significant sleep disorders. Some of the most common diagnoses in patients with sleep disorders are insomnia, sleep apnea, and restless leg syndrome, which are related to factors such as age, gender, and race in different populations (Ram, Seirawan, Kumar, and Clark, 2010).

Prevalence rate of sleep disorders has been reported to be on the rise in the United States, and 75% of Americans aged 20-59 years regularly experience sleep difficulties (Ram *et al.*, 2010). Benzodiazepines are the most common hypnotic medications widely used in recent years. Use of these tranquilizers is associated with severe complications, such as drug dependence, amnesia, psychomotor disorders, and interference with other nervous system agents (Roth, 2009). As such, introduction of effective medicines to prevent sleep disorders is of paramount importance.

Tomato plant (*Solanum lycopersicum*) belongs to the Solanacea family, which originally grows in the central and southern America. Tomato is a perennial plant in its native habitat, while it grows annually in temperate climates. Height of this plant is 1-3 meters, and it's fruits weight approximately 100 grams. Tomato plant has a weak stem that often overspreads on the ground (Cook, 2003). Black nightshade (*Solanum nigrum*) is generally considered a toxic plant; however, the hypnotic properties of this plant are widely applied, especially for children in Europe. Furthermore, black nightshade is used in the treatment of cardiac diseases, allergies, dropsy, arthritis, and restoration of systemic homeostasis (Juvin and Desmonts, 2000). In the references of the Iranian traditional medicine, such as Makhzan-ul-Advia, which was written by Seyyed Mohammad Hossein Aghili Alavi Khorasani Shirazi in 18th century A.D., nightshade was

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mentioned to be of a cold nature and used in the treatment of sleep disorders. This reference book is the largest traditional Persian pharmacopeias on natural medicines (Aghili M, 2008). Tomato and black nightshade belong to a large, diverse genus of flowering plants called Solanum, which includes 1,500-2,000 plant species. Nightshade crops have a perceptible aroma and hypnotic properties (Ramoutsaki, Askitopoulou, and Konsolaki, 2002). Several studies have confirmed the hypnotic effects of the nightshade family (Juvin and Desmonts, 2000; Rashed, 2002). Black nightshade is a common ingredient in the production of hypnotic drugs (Rashed, 2002). Also, tomato vinegar was reported to positively influence sleep quality (Saputo and Faass, 2010). Despite the elaboration on the hypnotic effects of tomato in the literature, experimental evidence is scarce in this regard. Melatonin (N-acetyl-5-methoxytryptamine) abundantly exists in various plant species and plays a pivotal role in circadian adjustments, nocturnal behaviors, and sleep modulation (Okazaki and Ezura, 2009; Tan *et al.*, 2003). Melatonin is found in the leaves, stems, roots, flowers, and fruits of tomato, while the highest concentration of melatonin is commonly found in the seeds of this plant (Huang and Mazza, 2011; Okazaki and Ezura, 2009; Riga, Medina, García-Flores, and Gil-Izquierdo, 2014). This compound exists in black nightshade as well (Korkmaz, Deđer, and Cuci, 2014).

Previous studies have investigated the hypnotic effects of different plants, such as lettuce (Rakhshandeh, Dolati, Hamid, and Ghorbani, 2011; Yakoot, Helmy, and Fawal, 2011), *Ziziphus jujube* (Jiang, Huang, Chen, and Lin, 2007), *Pinus eldarica* (Forouzanfar, Ghorbani, Hosseini, and Rakhshandeh, 2016), *Perovskia abrotanoides* (Forouzanfar, Hosseini, Amiri, and Rakhshandeh, 2017) and Crocus sativus (Hosseinzadeh and Noraei, 2009). However, the hypnotic effects of tomato and black nightshade have not been assessed so far. Therefore, the present work was designed to evaluate sleep-prolonging action of *Solanum lycopersicum* and black nightshade and its fractions. Also, the safety of this plant was evaluated by determination of LD 50 and testing its effect on the viability of neural cells.

Materials and Methods

Drugs and chemicals

Pentobarbital sodium, penicillin-streptomycin, flumazenil and 3- (4,5-dimethyl thiazole-2yl) - 2,5-diphenyl tetrazolium bromide (MTT) were obtained from Sigma (USA). Diazepam was purchased from Chemidarou Company (Iran). Tween 80 was bought from Merck (Germany). Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were bought from GIBCO (USA). Flumazenil, pentobarbital and diazepam were dissolved in saline to make a 30 mg/mL and 3 mg/mL solutions, respectively.

Preparation of extract

The *Solanum lycopersicum* fruit was bought from local market in Mashhad, Iran and black nightshade fruits were collected from Pardis of Ferdowsi University of Mashhad.

Identifications of the *Solanum lycopersicum* was confirmed and for future reference a voucher specimen (39321) was deposited at the herbarium of school of Pharmacy (Mashhad University of Medical Sciences, Iran and black nightshade's voucher specimen was 38416 at the herbarium of school of Pharmacy (Mashhad University of Medical Sciences, Iran) too. The first step, each of The fruits were separately dried, powdered and subjected to extraction with 70% ethanol in a Soxhlet apparatus for 48 h. then The *Solanum lycopersicum* HAE and black nightshade (HAE were dried on a water bath) their yields were 25% w/w and 28%w/w respectively (and dissolved in saline containing 1% (v/v) of tween 80. For preparation of fractions, 10 g of HAE was suspended in distilled water and transferred to a separator funnel. With solvent-solvent extraction, they were fractionated with ethyl acetate and N- hexane. The ethyl acetate fraction (EAF) and N-hexane fraction (NHF) were separated to obtain water fraction (WF). These fractions were dried on a water bath and working solutions made up in saline, saline containing 1% tween for WF, EAF and NHF (Forouzanfar, Ghorbani, *et al.*, 2016).

Animals

Male albino mice weighting 20-30 g and male Wistar rats weighing 200-250 g were maintained at a controlled temperature (22±1^{0}C) with a 12 h light/dark cycle and free access to water and food. The study was executed in accordance with ethical guidelines of Mashhad University of Medical Sciences.

Sleep Induction Protocol

The animals were given intra peritoneal (i.p) a single dose of vehicle, diazepam, and extracts. After 30 minutes, pentobarbital (30 mg/kg body weight (i.p) was injected to induce sleep. For evaluation of sleep duration, the mice were considered asleep if stayed immobile and lost its righting reflex when positioned on its back. The time interval between pentobarbital injection and onset of sleep was noted as sleep latency (Hosseini, Forouzanfar, and Rakhshandeh, 2016).

Animals were randomly divided into 18 groups of seven. Initially, to determine the sleep-prolonging effects

of HAE for each extract, the animals received saline (control group), diazepam (positive control) and different doses of HAE (25,50 and 100 mg/kg). In the second experiment, to determine the most effective fraction of HAE for each extract, the animals were treated with WF, EAF and NHF.

Furthermore, to assess the mechanism of sleep, animals were treated with 2 mg/kg flumazenil as a diazepam antagonist before receiving diazepam, HAE or NHF. All the test compounds were administered intra peritoneally (ip).

LD50 determination

18 groups, each containing 2 mice were used for determination of LD50 of *Solanum nigrum* and *Solanum lycopersicum* of samples. Groups 1-8 were injected intraperitoneally with 25, 50, 100, 200, 400, 800, 1600 and 3200 mg/kg of *Solanum nigrum* and *Solanum lycopersicum* of samples and group 9 received normal saline as vehicle, the treated animals were observed for mortality for 24 hr. period. The highest dose which did not kill any mice and the lowest dose which led to death of one animal were recorded. The mean of these two doses was noted as the median lethal dose (Akhila, Shyamjith, and Alwar, 2007).

Rotarod test

For measurement of motor resistance and coordination we used rotarod test. The experimental procedure for learning and adaptation was done for 3 consecutive days. On the next day, rats were placed on a rotating rod that accelerated smoothly from 4 to 40 rpm over a period of 5 min. The length of time they could maintain their balance on the turntable against the movement's strength was recorded. Then, the extract or vehicle was ip injected and after 30 min, the animals were placed on rotarod again (Vafaee *et al.*, 2014).

Cytotoxicity and Neurotoxicity Assessment

The rat pheochromocytoma-derived (PC12) cells were seeded (5000 cell/well) in 96-well plates for overnight in DMEM supplemented with 10% FBS, penicillin (100 units/ml) and streptomycin (100 µg/ml) at 37^0C with 5% CO_2 (Forouzanfar, Ghorbani, *et al.*, 2016).

Then, the medium was changed to a fresh one containing saline, HAE (100, 200, 400 and 800 µg/ml) or the fractions (800 µg/ml). Then, the cells were further incubated for 24 hr at 37^0C in an atmosphere of 5% CO_2. After that, cell proliferation was evaluated using MTT assay (Forouzanfar, Afkhami Goli, Asadpour, Ghorbani, and Sadeghnia, 2013). At the end of incubation with HAE or its fractions, 10 µl of MTT solution (5 mg/ml) was added to cell culture medium of the each well. Then, the culture medium was incubated for 2 hr at 37^0C with 5% CO_2. Then, the resulting formazan of each well was dissolved in DMSO. The optical density of formazan dye was read at 545 nm using microplate reader. Cell proliferation which reflects cell viability was calculated as percentage of untreated cells (Forouzanfar, Torabi, Askari, Asadpour, and Sadeghnia, 2016).

Statistics

All values are expressed as mean ± SEM. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Tukey-Kramer post hoc test by Graph pad Prism® (version 6.01, Graph Pad Software Inc., La Jolla, CA, USA) software package. Values were considered significant if $p<0.05$.

Results

Toxicity assessments

For both of plants the highest dose which did not kill any mice and the lowest dose which led to death of one mouse were 1.6 and 3.2 g/kg, respectively. Mean of these two doses (2.4 g/kg) was calculated as LD50. It was found that up to 24 h none of HAE concentrations of both plant extracts decreased proliferation of PC12 cells. In the presence of 100, 200, 400 and 800 µg/ml of the *Solanum nigrum* extract, the percent of viable cells were 99±3.7, 93.5±4, 98±4.8, and 102±6.9 respectively, as compared to vehicle (100 ± 0.81%) (fig 1). For *Solanum lycopersicum* extract, the cell viability was 100.3±4, 94.25±3.5, 99.25±7.4, and 101.3±6.5 5% in the presence of 100, 200, 400 and 800 mg/ml, respectively. Again, there was no significant difference in the viability of PC12 cells when compared to vehicle (100±1.6%) (fig 2).

In the rotarod performance, the statistical analysis revealed that the latency of fall did not diminish in receiving mice at dose of 100mg/kg of HAE ($p>0.05$ data not shown).

Effect of *Solanum nigrum* on sleep duration

The reference drug diazepam significantly increased the duration of sleep (49.20±1.6 min, $P<0.001$ vs. control). HAE could significantly increase the duration of sleep to 31.80 ± 4.6 min ($p<0.05$), 51.80±3.8 min ($p<0.001$) at doses of 50 and 100 mg/Kg, pretreatment of mice with flumazenil could restore the hypnotic effect of both diazepam (23±1.5, $p<0.001$) and 100 mg/kg HAE (19.4±1, $p<0.001$) (Fig 3). As shown in fig. 2, NHF was the only fraction that significantly increased the sleep duration from 18±1.5 min (saline) to 46.40±0.9 (100 mg/kg) ($P<0.001$) (fig 4).

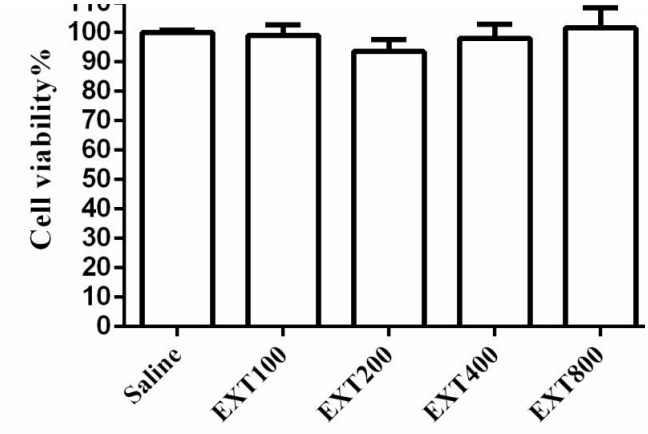

Fig. 1: Effect of Hydoalcoholic Extract and its Fractions of *Solanum nigrum* on PC12 Cell Viability. Cell viability was quantitated by MTT assay. PC12 cells exposed to extract for 24 hours. Values are expressed as mean ± SEM (EXT: extract).

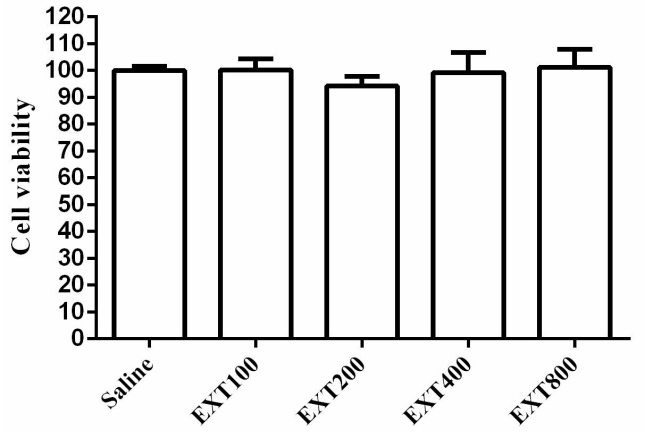

Fig 2: Effect of Hydoalcoholic Extract and its Fractions of *Solanum lycopersicum* on PC12 Cell Viability. Cell viability was quantitated by MTT assay. PC12 cells exposed to extract for 24 hours. Values are expressed as mean ± SEM (EXT: extract).

Effect of *Solanum nigrum* on sleep latency

Diazepam (2.8±0.4 min, $p<0.01$) and HAE at doses of 50 (3.4±0.5, $p<0.01$) and 100 (2±0.3, $p<0.001$ mg/kg) significantly decreased the latency to sleep in comparison to the saline (8±0. 6 min). As can be seen in Figure 5, flumazenil (2 mg/kg, i.p.) blocked the effects of diazepam (7.6±0.8 min vs. diazepam, $P<0.001$) and HAE (100 mg/kg) (7.8±0.3 min vs. HAE, $P<0.001$).

Among the three fractions, only NHF significantly decreased the sleep latency from 8 ± 0.6 min (saline) to 4.200±3.7 min (100 mg/kg, $p<0.05$). As shown in Figure 6, flumazenil could block this effect of NHF (9.4±0.7 min, $P<0.001$).

Effect of *Solanum lycopersicum* on sleep duration

As shown in Figure 7, sleeping duration in the negative control group receiving normal saline before pentobarbital

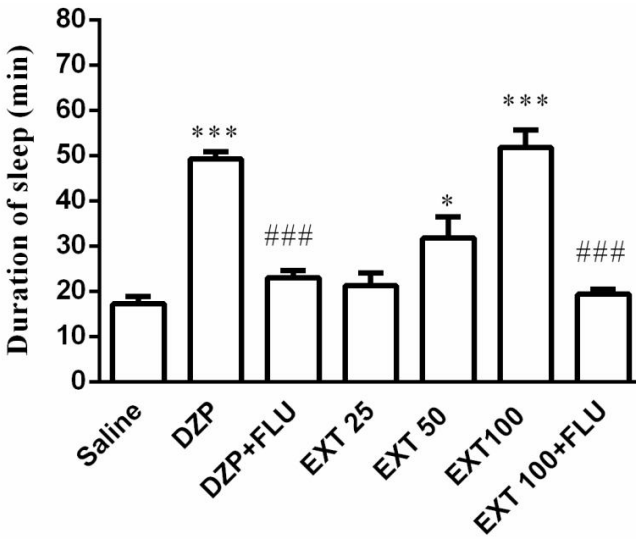

Fig. 3: Effects of *Solanum nigrum* Hydro-Alcoholic Extract on Sleeping Time in Pentobarbital-Induced Hypnotic Test. DZP, diazepam; Ext, extract; Flu, flumazenil; Solvent, diazepam (3 mg/kg) and different doses (25, 50 and 100 mg/kg) of the extract were intra-peritoneal administered 30 minutes before challenging animals with pentobarbital (30 mg/kg, i.p.). Flumazenil (2 mg/Kg) was used 15 minutes before the extract or diazepam. Data are mean ± SEM of 6 - 8 animals in each group. $P<0.05$ (*); $P < 0.001$ (***) significantly different from control; $P < 0.001$ (###) significantly different from the same group plus flumazenil (2mg/Kg).

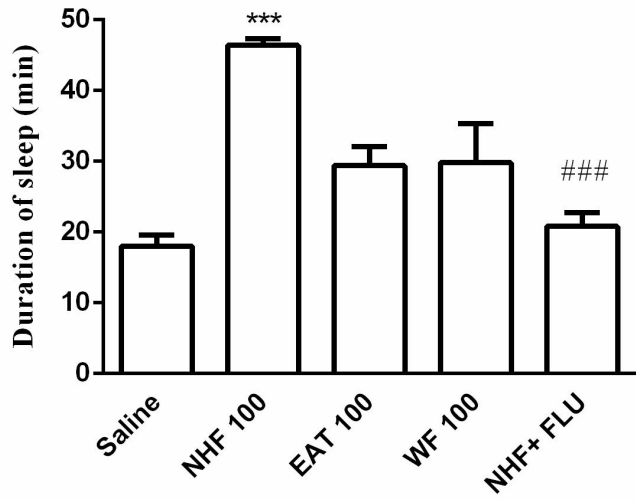

Fig. 4: Effects of Hydro Alcoholic Extract Fractions of *Solanum nigrum* on Sleeping Time in Pentobarbital-Induced Hypnotic Test. The WF, EAF and NBF were intraperitoneally administered 30 minutes before challenging animals with pentobarbital (30 mg/kg, i.p.). Values are expressed as mean ± SEM. N = 6 - 8 animals in each group; $P < 0.001$ (***) significantly different from control; $P< 0.001$ (###) significantly different from the same group plus flumazenil (2 mg/Kg).

was 18.2±1.6 min. The reference drug diazepam significantly increased the duration of sleep (47.67±1.2

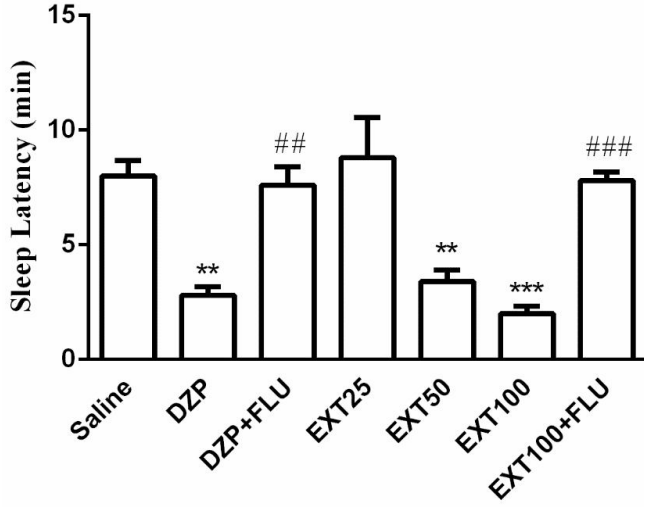

Fig. 5: Effects of *Solanum nigrum* Hydro-Alcoholic Extract on Sleeping Latency in Pentobarbital-Induced Hypnotic Test. DZP, diazepam; Ext, extract; Flu, flumazenil, Solvent, diazepam (3 mg/kg) and different doses (25, 50 and 100 mg/kg) of the extract were intraperitoneal administered 30 minutes before challenging animals with pentobarbital (30 mg/kg, i.p.). Flumazenil (2 mg/Kg) was used 15 minutes before the extract or diazepam. Data are mean ± SEM of 6 - 8 animals in each group. P < 0.01 (**); P < 0.001 (***) significantly different from control; P < 0.01 (##); P < 0.001 (###) significantly different from the same group plus flumazenil (2mg/Kg).

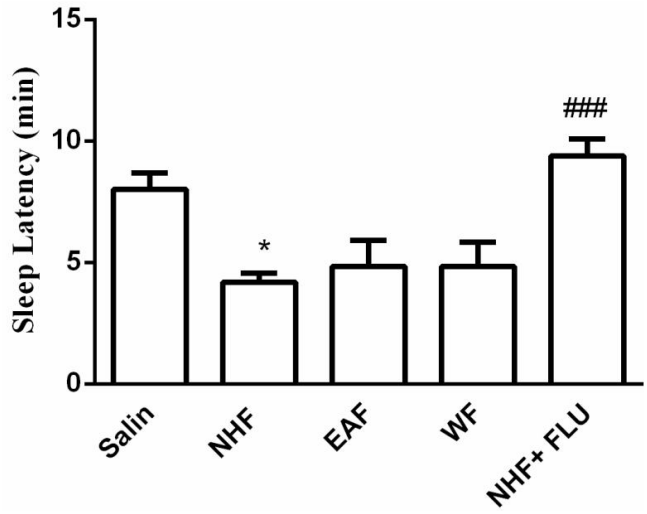

Fig. 6: Effects of Hydro Alcoholic Extract Fractions of *Solanum nigrum* on Sleeping Time in Pentobarbital-Induced Hypnotic Test. The WF, EAF and NBF were intraperitoneally administered 30 minutes before challenging animals with pentobarbital (30 mg/kg, i.p.). Values are expressed as mean ± SEM. N = 6 - 8 animals in each group; P < 0. 05 (*) significantly different from control; P< 0.001 (###) significantly different from the same group plus flumazenil (2 mg/Kg).

min, P<0.001 vs. control). HAE could significantly increase the duration of sleep to 32.40 ± 5.2 min (p<0.05), 43.20 ± 4.8 min (p<0.001) at doses of 50 and 100 mg/Kg, pretreatment of mice with flumazenil could restore the hypnotic effect of both diazepam (21.6 ±1.2, p<0.001)

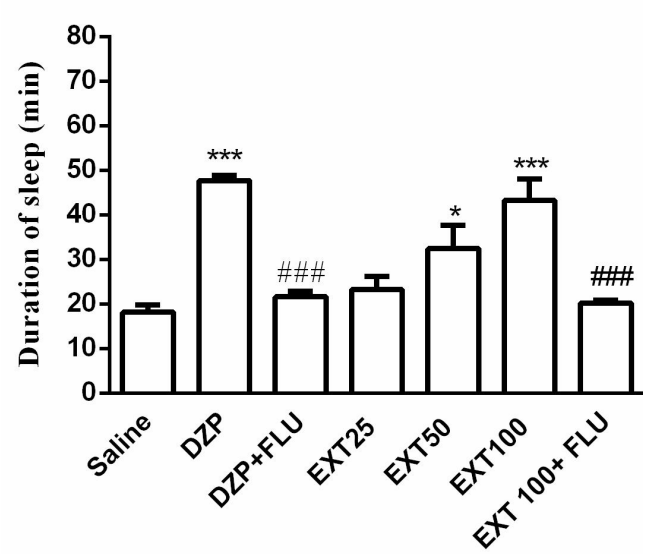

Fig.7: Effects of *Solanum lycopersicum* Hydro-Alcoholic Extract on Sleeping Time in Pentobarbital-Induced Hypnotic Test. DZP, diazepam; Ext, extract; Flu, flumazenil; Solvent, diazepam (3 mg/kg) and different doses (25, 50 and 100 mg/kg) of the extract were intra-peritoneal administered 30 minutes before challenging animals with pentobarbital (30 mg/kg, i.p.). Flumazenil (2 mg/Kg) was used 15 minutes before the extract or diazepam. Data are mean ± SEM of 6 - 8 animals in each group. P < 0.05 (*); P < 0.001 (***) significantly different from control; P < 0.001 (###) significantly different from the same group plus flumazenil (2mg/Kg).

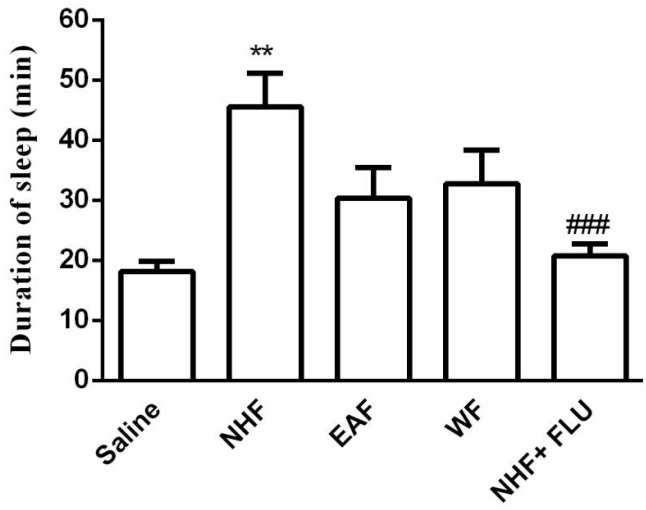

Fig. 8: Effects of Hydro Alcoholic Extract Fractions of *Solanum lycopersicum* on Sleeping Time in Pentobarbital-Induced Hypnotic Test. The WF, EAF and NBF were intraperitoneally administered 30 minutes before challenging animals with pentobarbital (30 mg/kg, i.p.). Values are expressed as mean ± SEM. N = 6 - 8 animals in each group; P < 0.01 (**) significantly different from control; P< 0.001 (###) significantly different from the same group plus flumazenil (2 mg/Kg).

and 100 mg/kg HAE (20.2±0.6, p<0.001). As shown in Figure 8, NHF was the only fraction that significantly increased the sleep duration from 18.17±1.6 min (saline) to 45.5±5.6 (25 mg/kg, P<0.01).

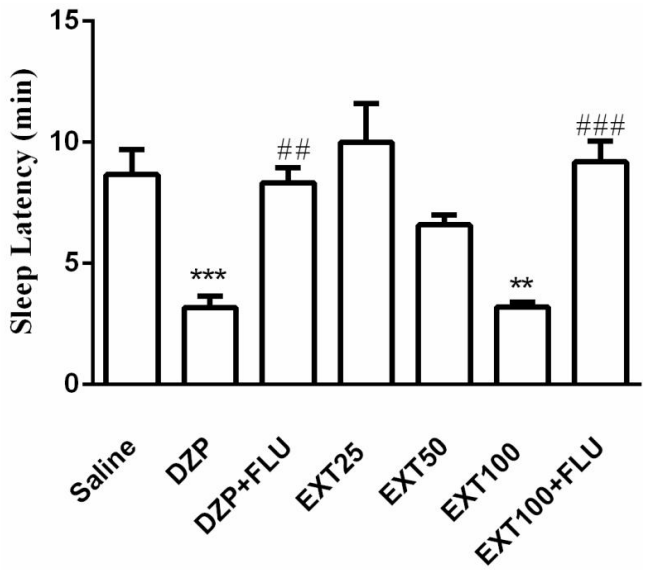

Fig.9: Effects of *Solanum lycopersicum* Hydro-Alcoholic Extract on Sleeping Latency in Pentobarbital-Induced Hypnotic Test. DZP, diazepam; Ext, extract; Flu, flumazenil, Solvent, diazepam (3 mg/kg) and different doses (25, 50 and 100 mg/kg) of the extract were intraperitoneal administered 30 minutes before challenging animals with pentobarbital (30 mg/kg, i.p.). Flumazenil (2 mg/Kg) was used 15 minutes before the extract or diazepam. Data are mean ± SEM of 6 - 8 animals in each group. $P < 0.01$ (**); $P < 0.001$ (***) significantly different from control; $P < 0.01$ (##); $P < 0.001$ (###) significantly different from the same group plus flumazenil (2mg/Kg).

Effect of *Solanum lycopersicum* on sleep latency

Diazepam (3.1±0.4 min, $p<0.01$) and HAE at dose 100 (3.2±0.2, $p<0.01$ mg/kg) significantly decresed the latency to sleep in comparison to the saline (8.6 ±1 min). As can be seen in Figure 9, flumazenil (2 mg/kg, i.p.) blocked the effects of diazepam (8.3±0.6 min vs. diazepam, $P<0.001$) and HAE (100 mg/kg) (9.2±0.9 min vs. HAE, $P<0.001$). Among the three fractions, only NHF significantly decreased the sleep latency from 8 ± 0.6 min (saline) to 4 ± 0.5 min (100 mg/kg, $p< 0.01$). As shown in Figure 10, flumazenil could block this effect (10.4 ± 0.5 min, $P<0.001$).

Discussion

This is the first study of *Solanum nigrum* and *Solanum lycopersicum* extracts' effects on mice sleep. According to the obtained results, sleep duration increased after the administration of *Solanum nigrum* and *Solanum lycopersicum* extracts. Moreover, Hypnotic effects of flumazenil were restored before the use of diazepam and 100 mg/kg of *Solanum nigrum* and *Solanum lycopersicum* HAE. NHF was the only fraction to significantly increase sleep duration. In addition, our findings indicated that use of HAE decreased sleep latency more significantly compared to normal saline. Among the three fractions, only NHF was found to reduce sleep latency. As is known, N-acetyl-5-methoxytryptamine plays a pivotal role in circadian adjustments, nocturnal behaviors, and sleep modulation (Tan *et al.*, 2003). According to a study by Okazaki *et al.*, *Solanum lycopersicum* contains melatonin, which is mostly found in Upper and lower parts such as seeds, flowers, leaves, stems and roots. Melatonin concentration varies in the fruits and leaves of *Solanum lycopersicum* depending on the developmental stage of the plant (Okazaki and Ezura, 2009). Regulation of sleep-wake cycle is influenced by multiple neurotransmitters and endogenous molecules. gamma-aminobutyric acid) GABA (is released from sleep - inducing neurons and inhibits the wake-inducing action in brain (Datta, 2010).

Pentobarbital-induced sleep has been shown to be caused by GABA dependency for benzodiazepine receptor agonists (Gottesmann, 2002). Most sedative plants belong to the large botanical family of *Solanaceae* (Carter, 1996). Hypnotic effects of other species of Solanum have been investigated in previous studies, the results of which have confirmed the effects of genus Solanum on sleep cycles (Kiranmai, Hemamalini, and Vasireddy, 2013). Flavonoids, terpenes and saponins compounds in these plants can cause hypnotic effect. Hypnotic activity of these herbal species has been attributed to phytochemical compounds, such as flavonoids, terpenes, and saponins (de Moura Linck *et al.*, 2009; Jiang *et al.*, 2007), which are found in the herbal extracts of Solanum as well (Oyeyemi *et al.*, 2015). Hypothetically, hypnotic effects of this medicinal herb might be associated with the presence of flavonoids in the methanolic extract of Solanum (Kiranmai *et al.*, 2013). These compounds could bind to the benzodiazepine site of GABAA receptor with high dependency (Wasowski and Marder, 2012). Since *Solanum nigrum* is a species of the genus Solanum (Mohy-Ud-Din, Khan, Ahmad, and Kashmiri, 2010). Investigation of the other species of this genus could be beneficial in analysing sleep processes. According to a study by Kiranmai *et al.*, *Solanum pubescens* exerts brain sedation has been attributed to GABAA receptors and its positive allosteric modulation sedative effects on the brain, possibly through the positive allosteric modulation of GABAA receptor complex. In the mentioned research, use of the extract and fractions of *Solanum pubescens* was reported to reduce sleep latency and increase sleep duration, which signifies central inhibition via the stimulation of CNS inhibitory pathways (Kiranmai *et al.*, 2013). Similar to our study, findings of another research showed that the extract of *Solanum nigrum* effectively shortened the duration of

thiopental-induced sleep in the mice poisoned with carbon tetrachloride (Momin, 1987). Previous studies have assessed the hypnotic effects of different plants (Ghorbani, Rakhshandeh, and Sadeghnia, 2013; Ghorbani, Youssofabad, and Rakhsh, 2012). As expected, procedures used in the present study confirmed that diazepam administration leads to the enhancement of pentobarbital-induced sleeping time, which was optimized based on the other study. In the current research, three fractions were prepared in order to elucidate the nature of compounds involved in the hypnotic activities of HAE, including N-Hexan fraction (NHF), ethyl acetate fraction (EAF) and water fraction (WF). water fraction (WF), ethyl acetate fraction (EAF) and N-Hexan fraction (NHF). NHF was the most effective fraction which NHF was the only fraction to significantly increase sleep duration and decrease sleep latency, while WF and EAF caused no significant reduction in sleep latency or increase in sleep duration. Since sleep parameters are influenced by NHF only, non-polar agents could be responsible for the effects of herbal extracts on sleep parameters. As a non-polar and fat-soluble substance, melatonin might be able to enhance the hypnotic effects of NHF. Moreover, evidence supports the possible role of terpenoids in this regard. It is noteworthy that terpenoids are essential components in traditional herbal remedies (Ghorbani *et al*., 2013). On the other hand, findings of the current study indicated that herbal extracts of *Solanum nigrum* and *Solanum lycopersicum* increased sleep duration and decreased sleep latency. This discrepancy could be due to the different types of plants used in the studies. In general, hypnotic effects of *Lactuca sativa*, *Solanum nigrum* and *Solanum lycopersicum* have been attributed to the flavonoid content of these herbs.

Evaluation of the toxicity of *Solanum lycopersicum* and *Solanum nigrum* showed that LD_{50} value for HAE is 2.4 g/kg. This value was significantly higher that hypnotic doses of *Solanum lycopersicum* and *Solanum nigrum*. Similarly, HAE and its fractions did not reduce the viability of neuronal cells even at high concentrations. Therefore, it seems that sleep prolonging effect of *Solanum lycopersicum* and *Solanum nigrum* is not accompanied by a neurotoxic effect.

Conclusion

According to the results of this study, use of *Solanum lycopersicum* and *Solanum nigrum* extracts is a safe therapeutic approach without neurotoxicity for altering sleep processes.

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Conflict of Interests

The authors declare that they have no conflict of interests.

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