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POTENTIATING EFFECTS OF ALCOHOLIC EXTRACT OF THE AERIAL PARTS OF *JASMINUM OFFICINALLE* ON PENTOBARBITAL-INDUCED SLEEP

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Abstract

Jasminum officinalle has been recommended to be used as a hypnotic plant in the folk medicine for long time and to demonstrate such effect we planned to study and investigate the sleep prolongation effect of the plant. Administration of the aerial parts of the plant as alcoholic extract in different doses together with the extract fractions which include:- the water fragment (WF), the ethyl acetate fragment (EAF), and the n-butanol fragment (NBF) to mice intra peritoneal 30minutes prior to pentobarbital injection. The study also indicate the extract toxicity both *in-vivo* and *in-vitro*. Qualitative studies was also applied to evaluate the alcoholic extract and NBF of the plant using fingerprint HPLC. The alcoholic extract of *Jasminum officinalle* aerial part potentiate and prolong the sleeping time duration (min) in a dose of 800mg/kg, NBF only prolong the sleep duration time and decrease the latency time for sleeping and such effect is similar to the action possessed by diazepam as positive standard drug that act as control. The calculated LD_{50} was found to be 2.65g/kg, and there was no neurotoxic effect shown by both alcoholic extract of the aerial part or its fraction on PC12 cultured cell like neurons. The findings in this study demonstrates the potentiating of hypnotic effect of pentobarbital with no toxic effect which could be mediated through the components that is likely non-polar one which presents in the NBF of aerial parts of the plant.

Key words : *Jasminu officinalle*, alcoholic extract, sleeping time, pentobarbital, PC12, animal.

Introduction

Sleep is a physiologic recuperative state that can be disturbed by many factors such as illness, stress and noise. Chronic sleep disorder led to some health repercussions such as slower reactions, poor memorizing, emotional disturbances, and changes in the immune response (Orzel-Gryglewska, 2010; Zaharna and Guilleminault, 2014). Today, sleep disorders have a relatively high prevalence and are a growing public health problem. It is estimated that more than 27% of people worldwide suffer from sleep disorders with difficulty in initiating or maintaining sleep. In addition, it is expected that by the middle of the 21st century, about 3-10% of all people will be chronic and frequent users of sleep medications (Roth, 2009; Quera-Salva and Orluc, 1999; Weyerer and Dilling, 1998). Currently, the most widely used medications for sleep disorders are the benzodiazepines. However, the clinical uses of benzodiazepines are accompanied with unpleasant side effects such as drug dependence, tolerance, rebound insomnia, amnesia, psychomotor impairment and potentiating of other central depressant drugs (Uzun *et al.*, 2010). Therefore, the search for new hypnotic agents with lesser side effects has continued. Medicinal plants have always been a good source to find new remedies for human health problems. The secondary metabolites are considered as the most active constituents in plant medicinal therapy ,and contribute for industry fields, since lots of physiological and medical studies are applied all over the world, providing a great source for the developing of new agents for treating different diseases with less toxicity. Meanwhile, it is interesting to explore and record the folk uses of medicinal plants and to indicate the active constituents that possess the pharmacological activity. *Jasminum officinalle* Linn. Family Oleaceae is cultivated plant in Asia, that the most chemical constituents is alkaloid (Jasmine), alcohol, salicylic acid, indol and resin (Al-Rawi and Chakravarty, 1964). Studies indicates that the plant is used traditionally as antiseptic, expectorant, analgesic, anti-inflammatory, uterine tonics antidepressant, aphrodisiac and sedation activities. Essential oil extracted from *Jasminum* species are mostly used in cosmetics

for skin care products, while Jasmine oil as fixed oil decreases the inflammation of skin, nourishes the skin, and improve the mood (Fatouna *et al*., 2010). The major chemical compounds extracted from the leaves of *Jasminum officinale* plant was glycoside oleuropein (Montinee *et al*., 2012) and applied as pharmacological tonic agent, with anesthetic properties, associated with sedation activity and CNS suppressions effect (Al-Rawi and Chakravarty, 1964; Elisha *et al*., 1986; Al-Maliki *et al*., 1988). In aromatherapy, jasmine plant was expressed to show calming activity, soothing effect with suppression of stress, with pulmonary depression, aphrodisiac and have been prescribed for sexual problems (Ody, 2012). The other therapeutic activities of Jasmine leaves juice is for the relieve of mouth ulcer, gastric and deudinal ulcer, also as anti-infective properties specially in typhus fever and other *Stapyllococcus* infection that causes ear discharges and shows action on ring warms parasitic infections (Ody, 2012; Awadh-Ali *et al*., 2013). Therefore, the present study was planned to evaluate the sleep-prolonging effect of alcoholic extract of *Jasminum officinalle* plant and its fractions. Moreover, the possible neurotoxicity of the plant was assessed using PC12 neuron cells, a rat pheochromocytoma-derived cell line, to ensure that the effect accompanied with no negative impact on neurons.

Materials and Methods

Drugs and chemicals

Dimethyl sulfoxide (DMSO), pentobarbital sodium, penicillin-streptomycin were purchased from Sigma (Sigma, USA). Diazepam was obtained from Chemidarou Company (Iran). Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were bought from GIBCO (GIBCO, USA). For high performance liquid chromatography (HPLC), all solvents used were of HPLC-grade and purchased from Caledon (Caledon, Canada). Pentobarbital and diazepam were dissolved in saline to make a 3mg/ml and 0.3mg/ml solution, respectively. Pentobarbital and diazepam were dissolved in saline to make a 3mg/ml and 0.3mg/ml solution, respectively.

Preparation of the extract

Fresh aerial parts of *Jasminum officinalle* were collected, shade-dried and powdered mechanically. About 60g of the aerial parts powder were extracted with 600ml of 70% ethanol by maceration at room temperature for 4 h using a mechanical shaker. The extract was dried at 40ºC under vacuum and the yield of the extract was (37% w/w). The alcoholic extract was then dried on a water bath and the yield (37% w/w), dissolved in saline containing 1% (v/v) of Tween 80. For preparation of fractions, a part of dried alcoholic extract was suspended in distilled water and transferred to a separator funnel. Using solvent-solvent extraction, it was sequentially fractionated with ethyl acetate and *n*-butanol. The ethyl acetate fraction (EAF) and *n*-butanol fraction (NBF) were separated to obtain water fraction (WF). The resulting fractions were dried on a water bath and working solutions made up in saline, saline containing 1% Tween, and 10% DMSO for WF, EAF and NBF, respectively (Khandelwal, 2004).

Animals

Male albino mice weighting 22-32g were used in each experiment. The animals were maintained at a controlled temperature with a 12h light/dark cycle with free access to food and water. The study was conducted in accordance with ethical guidelines approved by the Animal Care used in the College of Sciences at Baghdad University. The animals were randomly divided into ten groups consisting of 10 mice each. In the first experiment, to determine if the alcoholic extract of the aerial parts of *Jasminum officinalle* has hypnotic effect, the following solutions were injected (IP) to six groups: saline as vehicle, diazepam (3mg/Kg) as positive control and the extract (100, 200, 400, 800mg/Kg).

Acute oral toxicity and LD50 determination test

To determine the acute toxicity of *Jasminum officinalle* alcoholic extract, in different doses ranging from 10-5000mg/kg given orally. The signs appears when toxicity occurs such a convulsions, ataxia, hypoactivity, breathing disorders, and death finally was recognized in every hour in the day one of the experiment, then the animals model were observed every two days for two weeks after feeding with *Jasminum officinalle* extract, and then euthanized. Calculation of *Jasminum officinalle* alcoholic extract LD50 was exhibited according to the method created by Lorke (1983). Based on the results, the doses of further pharmacological studies were fixed to be 100, 200, 400 and 800mg/kg orally.

Sleep induction

The procedure that demonstrate the sedation and hypnotic action mainly based upon the induction of sleeping time possessed by pentobarbital drug (Rakhshandah *et al*, 2006, 2007; Akhila *et al*., 2007). In such method, the experimental animal model were injected intraperitoneal (IP) with negative control or vehicle, positive control diazepam and the different doses of the alcoholic extract of the aerial parts of the plant. Then, thirty minutes prior, pentobarbital was given as 30mg/kg (IP) for sleeping induction. Immobility and losing

of right reflex when set the mice on its back are the signs that recommended as sleep state. The latency of sleep is calculated as a period time between administration of pentobarbital and sleep onset.

Neurotoxicity assessment

Plates with 96 well were filled and cultured with Dulbecco's Modified Eagle's Medium (DMEM) supplemented by FBS as10%, streptomycin (microgram/ml) and penicillin (100 IU/ml) for 2 days at 37°C and 5% carbon dioxide. Then after the media were changed to fresh media that contain the negative control, different doses of alcoholic extract of the aerial part of the plant ranging from 100-800mg/kg and the therapeutic active dose fraction (800mg/ml). Choosing the concentration was performed according to the value used *in-vitro* and on the principles that the volume of extracellular fluids in rodents about 25% of body weight (Barratt and Walser, 1969). However, further incubation were applied for another day. Cell proliferation were measured and calculated using MTT assay to show the activity of the alcoholic extract of the plant after finishing of treatments as assessed by Rakhshandah *et al*. (2007) and Tavakkol Afshari *et al*. (2013). For confirmation of the results, the assay was applied three times and repeated twice. Cytotoxicity was assessed as the survive cells percentage.

Characterization of the extracts by HPLC

The quality of alcoholic extract and NBF of *Jasminum officinalle* was characterized by HPLC-UV fingerprint. Application was done by reverse-phase Waters C18 analytical column (250 × 4.6 millimeter, 5 micrometer particle size), using an isocratic acetonitrile/water /H_3PO_4 (80:20:0.3% volume/volume) as the rate of flow 1 milliliter /minute, as isocratic mobile phase. The ultraviolet detector wavelength was 330 nanometer. The samples of alcoholic extract and NBF were dissolved in distilled water and acetonitrile, respectively, and passed through 0.45 µm membrane filter. Then, 20µl of samples (400 µg/l) was injected to the HPLC column.

Statistics

The data obtained were statistically checked by the application of analysis of variance with one way classification for its statistical significances. The means differences were estimated at $p < 0.05$ using (ANOVA), then complete the analysis by the test of Tamhane's T2 post-hoc.

Results and Discussion

The sleep action of *Jasminum officinalle* extract

The observed duration of sleep in the animals receiving saline before pentobarbital injection was 25.5±1.91min (fig. 1). As expected, diazepam could increase the pentobarbital-induced sleeping time (43.7±4.24minutes, $p<0.05$ compared to saline). Likewise, 800mg/kg dose from alcoholic extract was significantly increased the sleep duration (38.9 ± 2.92 minutes, $p < 0.05$ compared to saline). In fig. 3, the different doses of alcoholic extract of *Jasminum officinalle* has no significant effect on the latency of sleep compared to control. Elucidation on the nature of the chemical constituents that are responsible for the activity of alcoholic extract, three fragments were applied: 1- WF 2 : EAF and NBF. As shown in figs. 2 and 4, the NBF shows significant activity for increasing the time and duration of sleeping period and minimizing the latency of sleep. When NBF was administrated, the sleep period was increased to 44.7±4.65min ($p < 0.01$ *vs* DMSO) and the latency time was decreased from 7.8±0.96 (vehicle) to 3.8±0.93min ($p < 0.01$). Neither the alcoholic extract doses (100, 200 and 400 mg/kg) nor the WF and EAF could cause a significant reduction in the sleep latency.

Acute oral toxicity

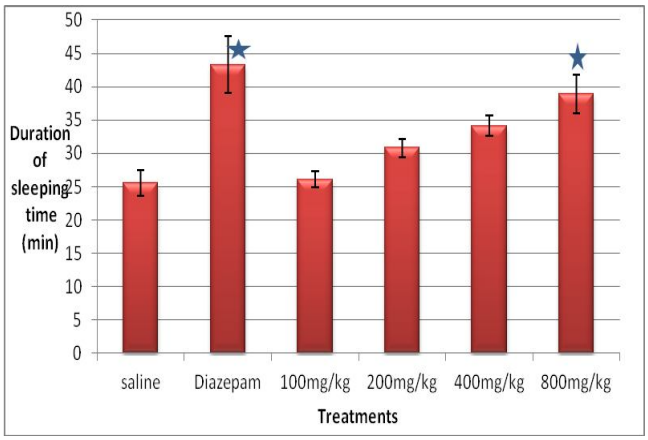
It was observed that the alcoholic extract of 2900-5000 mg/kg, p. o. induced hypoactivity, mild depression and ataxia seen in animals (both sexes of mice) for the 1st half hour until a period of 6hrs after oral feeding of alcoholic extract of *Jasminum officinalle*. Meanwhile, the animals treated with dose lower than 1600mg/kg shows no signs of toxicity or death. No differences in water and food consumption, and with no changes in the weight of the experimental animals were recorded during the first two weeks of the experiment. The calculated LD50 is about 2650 mg/kg when administered orally in mice.

Toxicity assessments

The value calculated for the alcoholic extract is 2.65g/Kg. This value is so far from the effective dose of alcoholic extract, 800mg/Kg. All the alcoholic extract in different concentrations ranging from 100-800mg/ml of the aerial parts of the plant possess no activity on PC12 cells proliferation, surviving of the cells was 113 ± 2.4, 111 ± 2.3, 115 ± 1.8 and 112 ± 1.3%, respectively, as compared to untreated cells. The alcoholic extract fragments shows no cyto-toxic effects. The number of surviving cells was 113 ± 2, 117 ± 1.4 and 102 ± 9 for WF, EAF and NBF, respectively.

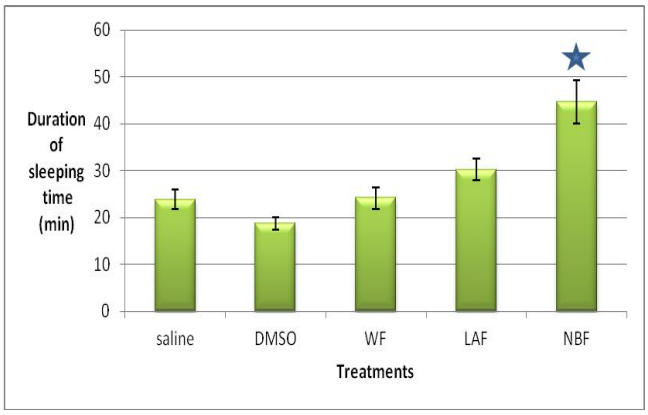
Standard fingerprints

The HPLC fingerprint is a fast method to evaluate the quality of extracts and to provide information about the proportion of the main constituents. In the fingerprints



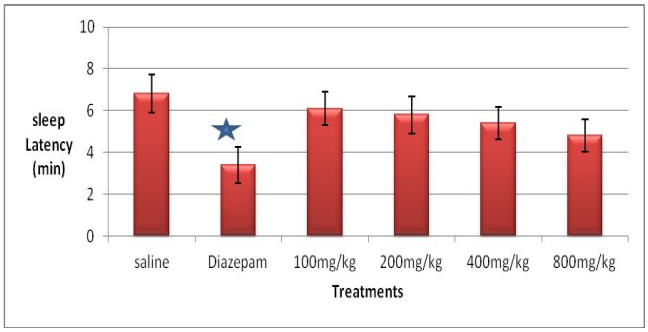
Results expressed values as M ± S.E.M, significant *represent P<0.001 compared to control according to ANOVA test.

Fig. 1 : Effect of different doses of *Jasminum officinalle* extract, negative control saline and positive control diazepam on the duration of sleeping time (min).



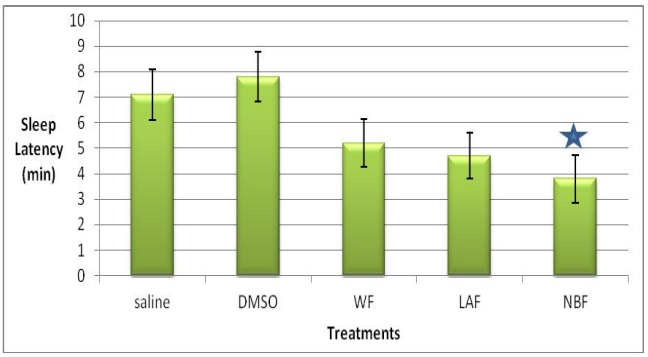
Results Expressed values as M ± S.E.M, significant * represent P<0.01 compared to DMSO according to ANOVA test.

Fig. 2 : Effect of 800mg/kg of *Jasminum officinalle* extract or the water fragment (WF), ethyl acetate fragment (EAF), and n-butanol fragment (NBF), saline and DMSO on duration of sleeping time (min).



Results Expressed values as M ± S.E.M, significant * represent P<0.05 compared to control according to ANOVA test

Fig. 3 : Effect of different doses of *Jasminum officinalle* extract, negative control saline and positive control diazepam on the sleep latency time (min).



Results Expressed values as M ± S.E.M, significant * represent P<0.01 compared to DMSO according to ANOVA test.

Fig. 4 : Effect of 800mg/kg of *Jasminum officinalle* extract or water fragment (WF), ethyl acetate fragment (EAF), and n-butanol fragment (NBF), saline and DMSO on latency of sleep time (min).

of alcoholic extract and NBF of *Jasminum officinalle*, there were seven common peaks within retention time range of 2-6min (fig. 5). In both profiles, peak 4 showed the greatest difference and was more prominent in NBF fingerprint. Therefore, its constituent(s) may be responsible for sleep enhancing effect of the extract. In future studies, it would be interesting to elucidate, if the constituent(s) are of terpenoid agents.

Sedative and hypnotic experimental procedure mostly depends on the increment or prolong the period of sleep produced by pentobarbital, which is almost applied for the discovering of sedative and hypnotic drugs. The present study showed that *Jasminum officinalle* alcoholic extract of the aerial part, further enhances sleep behavior, confirming that this plant has a hypnotic action as claimed in traditional medicine. To our knowledge, this is the first pharmacological study showing the effects of the alcoholic extract of *Jasminum officinalle* by increment and prolong the period of sleeping time and the time required to achieve sleep (latency of sleep). Fractionation of alcoholic extract of *Jasminum officinalle* in to three types: 1- the water fragment that dissolve the polar components with plant soluble chemical constituents in water such as quaternary and tertiary alkaloids, glycosides, tannins, 2- the ethyl acetate fragment that solubilize intermediate polarity components, 3- the n-butanol fragment that solubilize or extract the non-polar components such as terpenoids, sterols and alkane (Seidel, 2014; Mortazavian *et al*., 2012). The fact that only NBF potentiates the hypnotic boundary indicates that the components that is responsible for this action is the non-polar one in the alcoholic extract of *Jasminum officinalle*. Among the components showing hypnotic activity is terpenoids: Lactucin, which is a *sesquiterpene lactone* of *Jasminum* species, that possess a sedation hypnotic activity

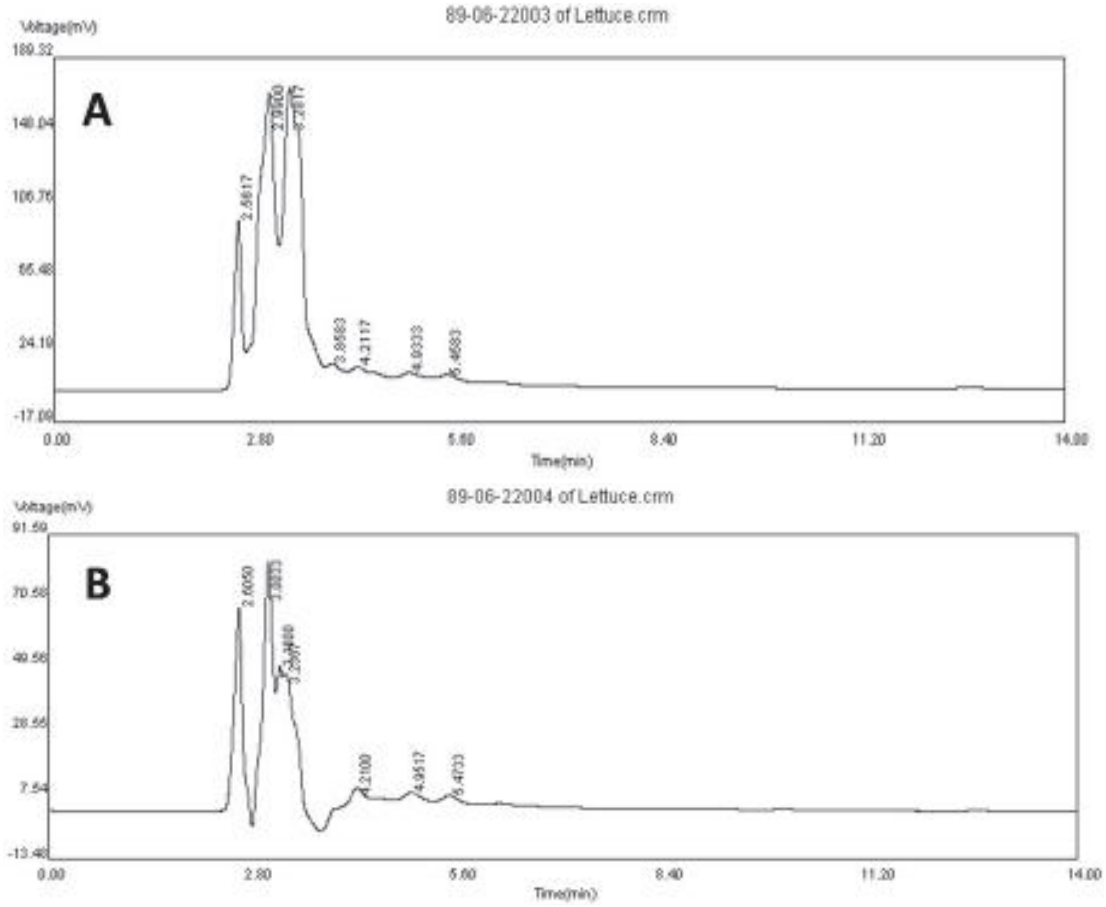


Fig. 5 : HPLC fingerprints of alcoholic extract. (A) and *n*-butanol fraction (B) of *Jasminum officinalle*. Chromatogram detected by UV at 330 nm.

elucidated in the locomotor function test (Wesolowska *et al*., 2016; (2) Phytol, a diterpenoid isolated from the ethanolic fraction of *Jasminum officinalle* was found to raise the levels of gamma-aminobutyric acid, a sleep-promoting neurotransmitter, in the brain (Bang *et al*., 2012). The calculated LD50 and the acute toxicity both *invivo* and *invitro* study indicates that the plant is safe and have a wild range of therapeutic dose. Therefore, the hypnotic effect of *Jasminum officinalle* accompanied with no neurotoxicity, leading to further support of its safety. In conclusion, results obtained in the study is achieved or set up for the first time to demonstrate the jasmine prolongation of the induction in the sleeping period behavior caused by pentobarbital in experimental animals model. The sleep prolonging effect was comparable to that of induced by diazepam and accompanied with no neuron toxicity. The most chemical constituents shows the hypnotic activity is non-polar agents found in NBF. Isolation of the exact component that produce and yield a new sedative–hypnotic drug is required in future.

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