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In vivo antianxiety and antidepressant activity of almonds (*P. amygdalus*) and walnuts (*J. regia*)

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Abstract
Almond (*P. amygdalus*) and Walnut (*J. regia*) are one of the frequently used 'dry fruits' and are considered to be a brain tonic. The nuts are a rich source of various phytoconstituents. The present study was designed to evaluate antianxiety and antidepressant potential of the kernels of almond and walnut. Four different extracts viz. petroleum ether, chloroform, ethanol and aqueous of *P. amygdalus* and *J. regia* kernels were prepared by successive Soxhlet extraction, and were evaluated for antianxiety and antidepressant activity in mice using Elevated Plus Maze and Forced Swim Test, respectively. Among all the extracts, ethanol extract of *J. regia* showed significant dose dependant antianxiety and antidepressant activity at 200 and 400 mg/kg, respectively. However, chloroform extract of *P. amygdalus* exhibited only antianxiety activity at 200 mg/kg dose.

Keywords: *P. amygdalus*, *J. regia*, elevated plus maze, forced swim test, antianxiety, antidepressant

Introduction
Affective disorders, especially anxiety and depression are one of the most impairing and prevalent psychiatric disorders. Epidemiological data from World Health Organization show that 3.6 % and 4.4 % of the global population suffers from anxiety and depression, respectively [1]. Pharmacological treatment for anxiety and depression includes synthetic drugs like barbiturates, benzodiazepines and selective serotonin reuptake inhibitors. However, their use is limited because of their unwanted side effects such as psychological and physical dependence, impaired cognition and coordination, withdrawal reactions and suicidal ideation [2]. Therefore, people are inclining towards other form of complementary and alternative medicines (CAM) as these are perceived to be natural and with fewer side effects [3]. Many forms of CAM, including dietary cures, herbalism, homeopathy, acupuncture, etc. have become integral part of health care [4]. As per the survey of National Institute Health in 2012, the most commonly used complementary medicines are the herbal medicines [5, 6]. Nuts are probably the earliest foods consumed by humans and are considered to be important because of their nutritional properties. These have also been used traditionally by different civilizations as drugs to prevent or treat several diseases [7].

P. amygdalus (Rosaceae), commonly known as Almond (English) or Badam (Hindi) is a small deciduous tree which grows to between 4 and 10 meters in height with trunk diameter up to 30 cm [8]. The plant is indigenous to Central and Western Asia, regions around the Mediterranean Sea and Europe. United States, specifically California, is the major producer [9]. In India, it is mainly cultivated in Kashmir where it is one of the chief crops. It has been used in folk medicine for the treatment of various ailments and is regarded as *Medhya rasayana* in Ayurveda, i.e. mental health promoter. The kernels and their oil are used as emollient, lithontropic, aphrodisiac, demulscent, nervine tonic, cerebrotonin, laxative, antitussive and as skin rejuvenator. The nut is also very popular in the Unani system of medicine because of its wide range of pharmacological actions [10].

J. regia (Juglandaceae), commonly known as Persian or English walnut and Akhrot (Hindi), is one of the frequently used 'dry fruits' having high commercial value and is considered to be a brain tonic. The plant has a long history of medicinal use and has been a part of folk medicine to treat wide range of ailments. In Ayurveda, *J. regia*, known as Aksoda or Aksota, has been used by both Charaka and Sushruta for wounds, phthisis and diseases of nervous system. *J. regia* is a large deciduous tree which is 25-35 m in height and 2 m in diameter. It is cultivated commercially in USA, France, Italy, Poland, Germany, Czech Republic, Bulgaria, Turkey, Chile, North India and Australia [11]. Keeping in mind the traditional use of *J. regia* and *P. amygdalus* kernels as nervine tonic/cerebrotonic, these were selected for systematic investigation of anti anxiety and antidepressant activity.

Material and Methods
Plant material
Kernels of *P. amygdalus* and *J. regia* were purchased from the local market, sector-22, Chandigarh, India. These were authenticated by comparing their morphological and microscopical characters with those reported in the literature [12, 13].

Chemicals and reagents
Solvents used include petroleum ether 60-80°C (Merck India Ltd., Mumbai), chloroform (Thermo Fisher Scientific India Pvt. Ltd., Mumbai), ethanol (Panipat Sugar Mill, D-Unit, Panipat), and distilled water prepared in our laboratory. Diazepam (Java Pharmaceuticals, Gurugram) was used as standard antianxiety agent.

Preparation of extracts
Coarsely powdered kernels of *P. amygdalus* and *J. regia* (1 kg each) were exhaustively soxhlet extracted successively with pet ether, chloroform and ethanol. The marc was finally boiled (1 h) with distilled water to prepare the water extract. The extracts were dried using Eyela N 1100 rotary vacuum evaporator, and were preserved in a vacuum desiccator containing anhydrous silica gel blue.

Experimental animals

Lacca mice (either sex), housed at the Central Animal House, Panjab University, were allowed standard pellet diet (Ashirwad, Chandigarh) and water ad libitum. Groups of 6 mice (20-30 g) were used in all sets of experiments. The animals were fasted for 12 h before use. Approval (PU/IAEC/S/16/112) from the Institutional Animal Ethical Committee of Panjab University, Chandigarh was taken before carrying out biological studies.

Preparation of doses

Tween 80 (5 %) in aqueous Carboxymethyl cellulose (CMC 0.5 % w/w) was used as a vehicle for preparing the suspension of extracts/standard drug. Doses of various test substances were prepared by suspending appropriate quantities in the vehicle so as to administer these to mice in volumes ranging between 0.20-0.30 ml per oral route. Doses were administered orally using tuberculin syringe fitted with an oral cannula.

Acute toxicity studies of the extracts

Acute toxicity studies of pet ether, chloroform, ethanol and water extracts of *P. amygdalus* and *J. regia* were carried out on mice as per OECD 423 guidelines [14]. After 12 h of fasting, different groups of mice were administered single oral dose (500, 1000 and 2000 mg/kg) of the four extracts. Immediately after dosing, animals were observed for signs of toxicity during the first 0.5, 1, 2, 4, 8 and 12 h, and at every 24 h for 14 days. Behavioural parameters, tremors, lethargy, death, amount of water and feed taken were observed.

Antianxiety activity evaluation

Elevated plus maze (EPM) model was used for evaluating antianxiety activity. The apparatus consisted of two open arms (16×5 cm) and two closed arms (16×5×12 cm) having an open roof. It was kept elevated (25 cm) from the floor for evaluating the anxiolytic behavior. The dose administration schedule was so adjusted that each mouse was having its turn on the EPM 60 min after the administration of the vehicle, diazepam (2 mg/kg) and the test extracts (100, 200 and 400 mg/kg). Each mouse was placed at the centre of EPM with its head facing towards the open arm. During 5 min duration of the experiment, behavior of the mouse was recorded as (a) the number of entries into the open arms and (b) mean time spent by the mouse in open arms [15, 16].

Antidepressant activity evaluation

Forced swimming test (FST) was used to evaluate antidepressant activity [17, 18]. Mice were forced to swim in vertical glass jar (25×12×25 cm) filled with water (25±2 °C) to a height of 15 cm. Different groups of mice were administered a single dose the standard antidepressant imipramine (10 mg/kg) and the test extracts (100, 200 and 400 mg/kg) 60 min before the evaluation. After an initial period of vigorous activity to escape, the animals assumed a typical immobile posture (ceased to struggle with minimal limb movements just sufficient to keep their head above the level of water). Total immobility period during 6 min test session was noted.

Phytochemical screening

The bioactive chloroform extract of *P. amygdalus* and ethanol extract of *J. regia* were screened for different classes of phytoconstituents using the standard procedures [19].

Statistical analysis

Results have been expressed as mean±standard error mean. The significant difference among the groups was assessed by one-way analysis of variance (ANOVA) followed by Tukey's multiple range test. The results were considered statistically significant at $p<0.001$. Statistical analysis was performed using the Graph Pad Prism 5.

Results

Yield of extracts

Table 1 represents the percentage yield of various extracts of *P. amygdalus* and *J. regia* kernels.

Table 1: Percentage yield of various extracts of *P. amygdalus* and *J. regia* kernels.

Extract	*P. amygdalus* (%w/w)	*J. regia* (%w/w)
Pet ether	52.90	44.94
Chloroform	6.78	21.85
Ethanol	4.60	4.20
Water	3.80	3.10

Acute toxicity studies

All the extracts neither exhibited signs of acute toxicity nor mortality upto the dose of 2000 mg/kg, po.

Antianxiety activity of extracts

Administration of diazepam (2 mg/kg) significantly increased the number of entries and the time spent in the open arms compared to the control group. Among all the extracts, chloroform extract (200 mg/kg) of *P. amygdalus* kernels (Figure 1) and ethanol extract (200 mg/kg) of *J. regia* kernels (Figure 2) showed significant ($p<0.001$) antianxiety activity.

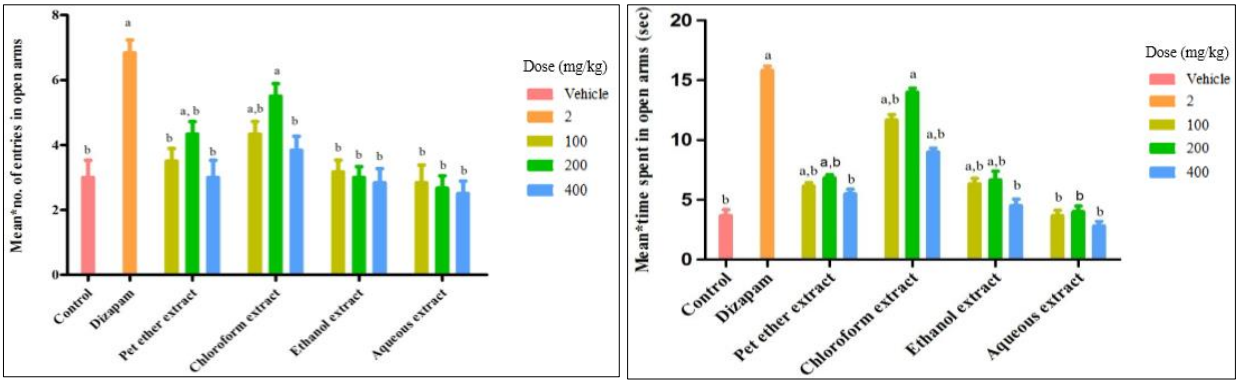

Fig 1: Antianxiety activity profile of extracts of *P. amygdalus* kernels using EPM. n=6; [a]$p<0.001$ *vs* control; [b]$p<0.001$ *vs* diazepam; one way ANOVA followed by Tukey's multiple range test.

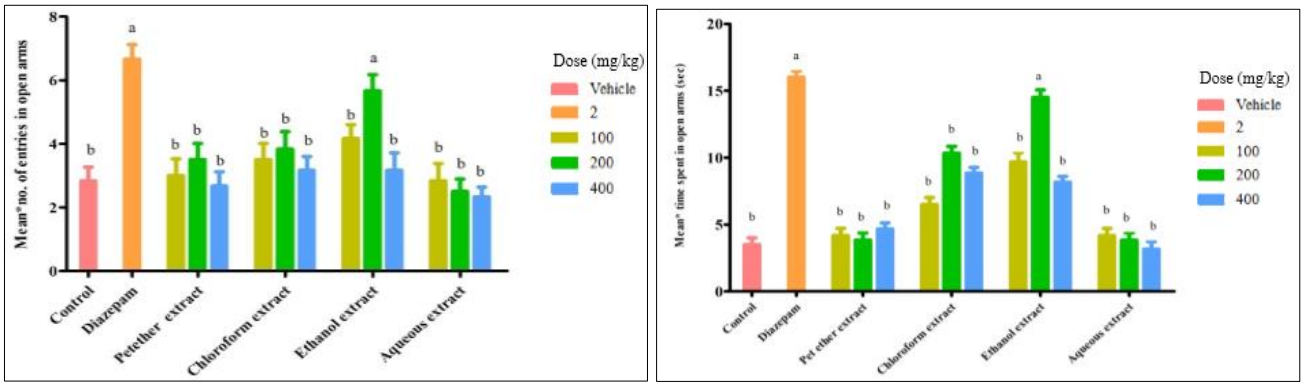

Fig 2: Antianxiety activity profile of various extracts of *J. regia* kernels using EPM. n=6; [a]p < 0.001 *vs* control; [b]p < 0.001 *vs* diazepam; one way ANOVA followed by Tukey's multiple range test.

Antidepressant activity of extracts

Among all the extracts, ethanol extract (400 mg/kg) of *J. regia* (Figure 3) exhibited statistically significant (p<0.001) diminution of immobility time when the animals were subjected to FST. However, none of the extracts of *P. amygdalus* exhibited antidepressant activity (Figure 4).

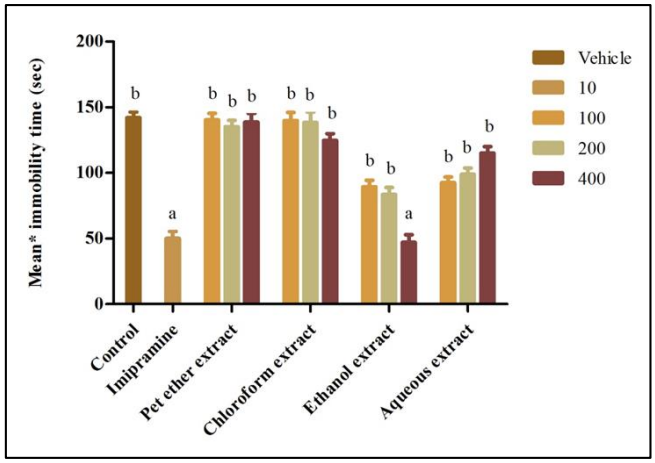

Fig 3: Antidepressant activity profile of various extracts of *J. regia* kernels using FST. n=6; [a]p < 0.001 *vs* control; [b]p < 0.001 *vs* imipramine; one way ANOVA followed by Tukey's multiple range test.

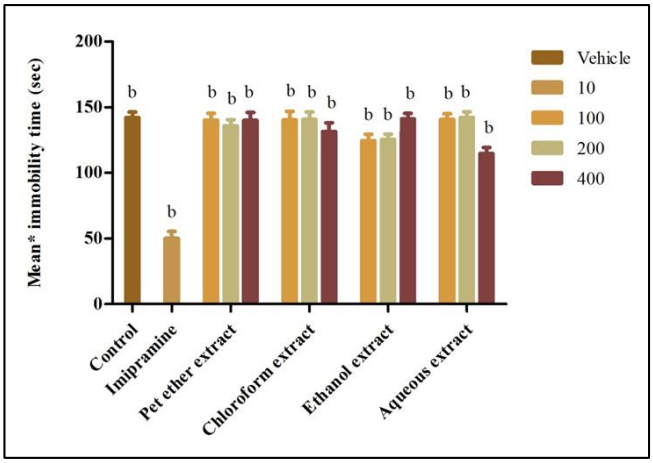

Fig 4: Antidepressant activity profile of various extracts of *P. amygdalus* kernels using FST. n=6; [a]p < 0.001 *vs* control; [b]p < 0.001 *vs* imipramine; one way ANOVA followed by Tukey's multiple range test.

Phytochemical investigation

The bioactive chloroform extract of *P. amygdalus* showed the presence of fixed oils, terpenoids, flavonoids, phenols and tannins while the bioactive ethanol extract of *J. regia* tested positive for triterpenoids, flavonoids, phenols and tannins (Table 2).

Table 2: Results of phytochemical screening of bioactive chloroform extract of *P. amygdalus* and ethanol extract of *J. regia*.

Phytoconstituent	*P. amygdalus*	*J. regia*
Fixed oils and fats	+	-
Terpenoids/steroids	+	+
Flavonoids	+	+
Coumarins	-	-
Alkaloids	-	-
Phenols and tannins	+	+
Saponins	-	-
Glycosides	-	-
Carbohydrates	-	-
Proteins and amino acids	-	-

Discussion

Traditionally, consumption of nuts has been encouraged because of their brain nourishing and numerous nutritional properties. Based on the ethnobotanical uses, the present study was designed to evaluate the antianxiety and antidepressant potential of two widely consumed nuts – *P. amygdalus* (Almond) and *J. regia* (Walnut). Four different extracts were prepared to separate the phytoconstituents based on their polarity. The antianxiety activity was evaluated using well established EPM model which is considered to be etiologically similar to the anxiety observed clinically in human beings. An anxiolytic agent increases both the frequency of entries and the time spent in open arms of the EPM, and is thought to act via the GABA-A (γ-aminobutyric acid type A) receptor complex, justifying the use of diazepam as a positive control in the study. Antianxiety agents increase the number of entries and time spent by the animal in the open arm. Antidepressant activity was evaluated using FST which has been used in preclinical tests to evaluate behavioural despair, i.e., measure of failure to escape from an aversive stimulus. Results indicated that among all the extracts, ethanol extract of *J. regia* showed significant dose dependant antianxiety and antidepressant activity at 200 and 400 mg/kg, respectively, which was statistically comparable to diazepam and imipramine, respectively. However, chloroform extract of *P. amygdalus* exhibited only antianxiety activity at 200 mg/kg dose. The

activity decreased at 400 mg/kg which might be due to mild sedation at higher doses.

Phytochemical investigation of both the bioactive extracts showed the common presence of terpenoids, flavonoids, phenols and tannins indicating that these might be responsible for their antianxiety effect. Also, previous biochemical and pharmacological reports have shown that flavonoids have significant effects on the CNS [20], primarily due to their affinity for the central benzodiazepine receptors [21]. Thus, the antianxiety activity of the two extracts might be due to the flavonoids present in them.

Conclusion

The results of the present study suggest that kernels of both *P. amygdalus* and *J. regia* exhibit significant antianxiety activity. However, only the ethanol extract of *J. regia* shows significant antidepressant activity. Therefore, their use in our daily diet can be very useful for allaying anxiety and depression.

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Conflict of Interest: Nil

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