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Evaluation of neuro-pharmacological activities in six homeopathic drugs

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The effects of extracts of *Digitalis purpurea* L., *Sambucus nigra* L., *Thuja occidentalis* L., *Urtica urens* L., *Arctostaphylos uva–ursi* L. and *Apis mellifica* L. were analyzed *in vivo* in mice. The analysis of neuro-pharmacologiacl activity was recorded at different doses such as 100, 300 and 500 mg/kg and compared with control and standard drug, Diazepam. The tested extracts exhibited significant neuro-pharmacological activities in open field, dip cage, light and dark, cage cross and forced swimming activities. Our research supports the safe and effective use of the extracts in low doses.

Key words: Neuro-pharmacological, open field activity, dip cage activity, light and dark activity, cage cross activity, forced swimming test.

INTRODUCTION

This study was carried out on *Apis mellifica* L., *Digitalis purpurea* L., *Sambucus nigra* L., *Thuja occidentalis* L., *Urtica urens* L., *Arctostaphylos uva–ursi* L., with the objective to explore the neuro-pharmacological activities of these drugs which are in current use of cardiac diseases, skin problems (dermatitis), urinary tract infection, gastro-intestinal tract disorders, allergic conditions, maintaining normal blood pressure, in the removal of kidney stone etc. These activities are reported by different researchers (Klass et al., 2002; Navarro et al., 2000; Bisset and Wichtl, 2001; Chang et al., 2000; Chrubasik et al., 2007; Matsuda et al., 1992).

MATERIALS AND METHODS

The homeopathic mother tincture of drugs (sealed packs from William Schwabe, Germany) were purchased from local supplier (*Apis mellifica* L. Lot # 0730707; *Digitalis purpurea* L. Lot # 2010207; *Sambucus nigra* L. Lot # 0012188808; *Thuja occidentalis* L. Lot # 7100710; *Urtica urens* L. Lot # 3030909 and *Arctostaphylos uva–ursi* L. Lot # 0512208827). To obtain dried, solid extract, the ethyl alcohol was evaporated by keeping open bottles in sterilized condition in lab for 1 to 2 days at room temperature. Later the residue (dry extract) of each drug was used for experiment. The doses of extract were prepared in 0.5 ml distilled water that is, 300 and 500 mg/kg/0.5 ml (Farah-Saeed, 2014)

Experimental animals

Wistar male mice (25 to 30 g) were obtained from Animal House, Dow University of Health Sciences, Karachi, Pakistan. Animals were kept in Animal House, DUHS, Karachi. They were kept in a climate and light controlled room at least 7 days before initiating neuro-pharmacological activities on them and were provided

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with food and water *ad libitum*.

Assessment of neuro-pharmacological activity

Neuro-pharmacological activities (field test, head dip test, cage cross, light and dark and forced swimming activities) were carried out in calm and peaceful environment. Each group comprised of 5 animals (n = 5). Diazepam as 2 mg/kg orally was used as standard. The crude drug and the diazepam were diluted in distilled water and administered orally. The control animals were treated orally with same volume of saline as the crude extract. In all the tests, observations were made after 30 to 40 min of oral dose of the test substance.

Open field activity

The open field apparatus designed in the laboratory consists of 76 × 76 cm square area with opaque walls 42 cm high. The floor is divided by lines into 25 equal squares (Ahmad et al., 2013). Mice were placed in the center square of the open field (one at a time). Number of squares crossed with all four paws was counted for 30 min. Activities of control and drug treated mice were monitored in a balanced design to avoid order effect.

Light and dark test

Light and dark test is one of the apparatus designed to test neuro-pharmacological behavior in mice. The apparatus consists of a plastic box with two compartments one of which is made of transparent plastic and the other of black color plastic. Each animal is placed at the center of the transparent compartment and then the number of entries in each space, as well as time spent in each compartment is recorded for 30 min (Crawley and Goodwin, 1980).

Head dip test

Head dip box is used to check exploratory behavior of mice. A specially designed square shaped having several holes at bottom were used in this study. The observation was to count the number of head dips by the animal through these holes in specified time (Durcan and Lister, 1988; Hossain and Uma-Devi, 2001; Sultana and Najam, 2012). The control and drug treated animals were placed individually in the head dip box and the observations were made for 30 min.

Cage crossing movements

The test was performed on mice in a specifically designed box having rectangular shape. Both control and treated mice were placed into the cage and their cage crossing movements were noted in 30 min. The test was performed to evaluate motor activity of mice. This test was performed according to the method described by Najam and Anser (2011).

Forced swimming test

Forced swimming test was performed according to Porsolt (1977, 1978). This test determines the muscle and central nervous system (CNS) activity of the crude extract. Mice were placed individually for six minutes in the specially designed plastic cylinder filled with water at room temperature up to the marked level. Mouse when placed in water suddenly starts to move its front and hind paws. The activity time of animal is determined with the help of stopwatch out of total observation time of six minutes.

Statistical analysis

All values were compared with the control and standard drug reading by taking out mean and standard error of mean. Level of significance was determined by student t-test (Alcaraz et al. 1989).

RESULTS AND DISCUSSION

The neuro-pharmacological activity of six crude extracts were assessed using open field, head dip, light and dark, cage cross and forced swimming activities (Table 1).

Neuro-pharmacological effects of *A. mellifica* L. on mice

The neuro-pharmacological effects were observed at the dose of 100 mg/kg of *A. mellifica* extract as follows; in open field activity (28 ± 2.84) counts in 30 min were observed, while in head dip test, the mice dipped head (13.33 ± 2.61) times. At the dose of 300 mg/kg the pronounced depressed effects were observed in case of light and dark, cage cross and swimming activities. Number of entries in light compartment is 9.33 ± 2.93 times. The readings of cage cross is (23.33 ± 2.44) times. In forced swimming test (FST) the mean forced mobility time was (1.25 ± 0.04) s. Locomotor and exploratory activity was observed to be considerably reduced in comparison to control and standard Diazepam (2 mg kg^{-1}) (Figure 1).

Neuro-pharmacological effects of *D. purpurea* L. on mice

The most significant neuro-pharmacological effect was observed at the dose of 500 mg/kg of *D. purpurea* extract as follows; in head dip test, the mice dipped head (19.16 ± 4.92) times. Number of entries in light compartment is 4 ± 1.35 times. The readings of cage cross is (6.67 ± 1.48) times. In forced swimming test (FST) the mean forced mobility time was (4.14 ± 0.48) s. At the dose of 300 mg/kg, in case of open field activity (44 ± 4.54) counts in 30 min were observed. Locomotor and exploratory activity was observed to be substantially reduced in comparison to control and standard diazepam (2 mg kg^{-1}) (Figure 2).

Neuro-pharmacological effects of *S. nigra* L. on mice

At the dose of 100 mg/kg of *S. nigra,* CNS inhibitory effects were observed in following activities; in open field activity (75.16 ± 3.68) counts in 30 min, in light and dark

Table 1. Neuro-pharmacological activity of some crude extracts in comparison with the control and the standard drug – Diazepam.

Treatment	Concentration mg/kg	Open field activity (Counts in 30 min.)	Dip cage activity (No. of times in 30 min.)	Light and dark activity (No. of entries in light portion in 30 min.)	Cage cross activity (No. of times in 30 min.)	FST (mobility time s)
Control		392.83±11.79	272.5±4.84	21.5±2.90	72.5±4.10	3.69±0.12
Apis mellifica L.	500	220.5±34.10	112.83±21.33	20±3.93	115.83±4.33	8.98±0.52
	300	101.66±4.50	35.33±4.14	9.33±2.93*	23.33±2.44*	1.25±0.04*
	100	28±2.84*	13.33±2.61*	13.33±3.83	32.66±2.85	2.47±0.03
Digitalis purpurae L.	500	283±17.10	19.16±4.92*	4±1.35*	6.67±1.48*	4.14±0.48*
	300	44±4.54*	52.83±3.23	10.33±1.80	54.16±4.46	4.24±0.04
	100	215.5±2.31	40.16±4.05	6.16±1.55	55.33±4.32	5.28±0.04
Sambucus nigra L.	500	103.16±6.68	7.16±0.77*	5±0.63	22±4.69	1.62±0.21
	300	175.83±4.12	9.67±0.96	4.5±0.83	27±3.64	0.24±0.03*
	100	75.16±3.68*	9±1.16	3.83±0.65*	8.33±1.01*	0.508±0.02
Thuja occidentalis L.	500	223.83±29.72	94.83±9.70	15±3.48	50.16±3.92	1.23±0.05
	300	60.33±3.94	3.5±0.96	3±0.93	13.16±1.27	0.43±0.02
	100	10±1.41*	2.83±0.65*	1.16±0.52*	5.67±0.96*	0.29±0.01*
Urtica urens L.	500	253.16±50.43	152.33±23.56	49.83±11.89	101±10.91	2.74±0.38
	300	127.33±6.01	4.67±1.28*	4.83±1.03*	77.16±3.26	1.37±0.02*
	100	85.83±3.53*	32.67±3.47	11.83±1.36	67.5±3.15*	3.46±0.02
Arctostaphylos uva–ursi L.	500	253.5±31.77	18.83±5.11*	10.83±2.30	28.5±6.13*	4.48±0.78
	100	541±21.59	109.83±3.95	18.5±2.52	49.33±3.99	1.22±0.03*
	50	444.83±4.99	83.78±3.82	18.5±2.54	116±3.84	3.17±0.09
	30	177.5±3.17*	33.16±2.52	10±2.11*	59.33±2.72	2.59±0.09
	10	347±6.80	44.67±3.99	13.33±1.48	93.67±3.40	4.18±0.14
Standard-Diazepam 2 mg/kg		12.5±0.83	11.5±0.83	1±0.4	19.5±0.83	1.60±0.06

Significant CNS inhibitory effect observed in each activity amongst different doses of extracts is indicated by*

activity 3.83 ± 0.65 times in cage cross test, 8.33 ± 1.01 times. Whereas, in forced swimming test (FST) the mean force mobility time recorded was 0.24 ± 0.03 s, at 300 mg/kg dose of *S. nigra* extract. At 500 mg/kg of *S. nigra*, the mice dipped its head 7.16 ± 0.77 times. Locomotor and exploratory activity was observed substantively reduced in comparison to control and standard drug, diazepam (2 mg kg^{-1}) (Figure 3).

Neuro-pharmacological effects of *T. occidentalis* L. on mice

The most significant neuro-pharmacological effect was observed at the dose of 100 mg/kg of *T. occidentalis* extract as follows; in open field activity 10 ± 1.41 counts in 30 min were observed. In head dip test, the mice dipped its head 2.83 ± 0.65 times. In light and dark activity, number of entries in light compartment were found 1.16 ± 0.52 times. The observations of cage cross were found 5.67 ± 0.96 times. In forced swimming test (FST) the mean forced mobility time was 0.29 ± 0.01 s. Locomotor and exploratory activities were noticeably reduced in comparison to control and standard drug, diazepam (2 mg kg^{-1}) (Figure 4).

Neuro-pharmacological effects of *U. urens* L. on mice

The neuro-pharmacological effects were observed at the dose of 100 mg/kg of *U. urens* extract in case of open field activity, 85.83 ± 3.53 counts in 30 min and cage cross 67.5 ± 3.15 times activity. At the dose of 300 mg/kg of *U. urens*; maximum CNS depression was observed in head dip cage, the mice dipped its head 4.67 ± 1.28 times. Number of entries in light compartment, in light and dark activity was 4.83 ± 1.03 times. In forced swimming test (FST) the mean forced mobility time was

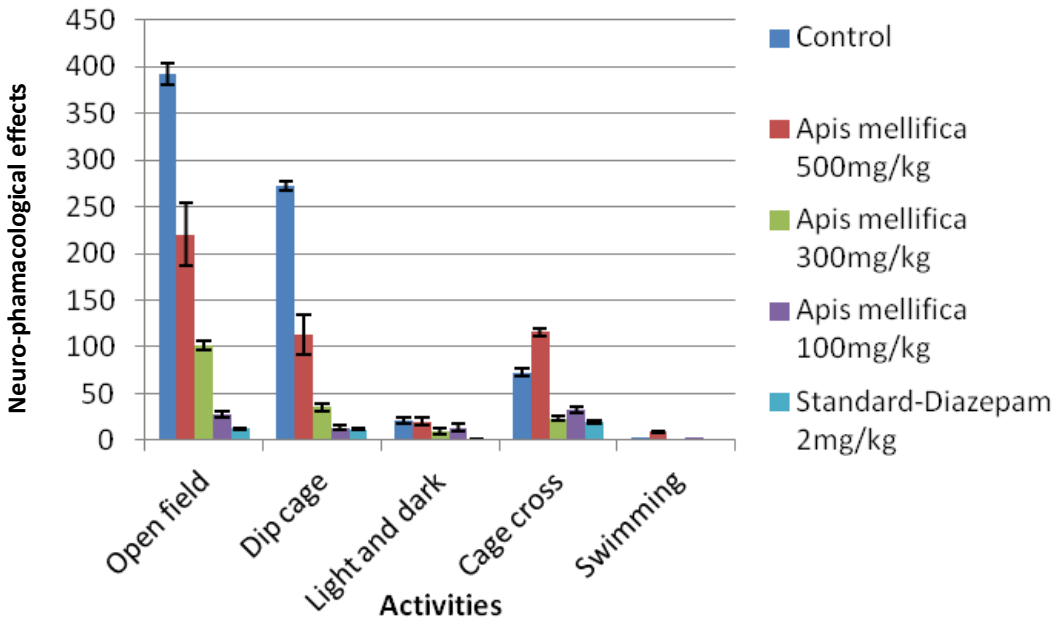

Figure 1. The neuro-pharmacological effects of 100, 300 and 500 mg/ml concentrations of *A. mellifica* extract on mice in comparison with control and standard drug.

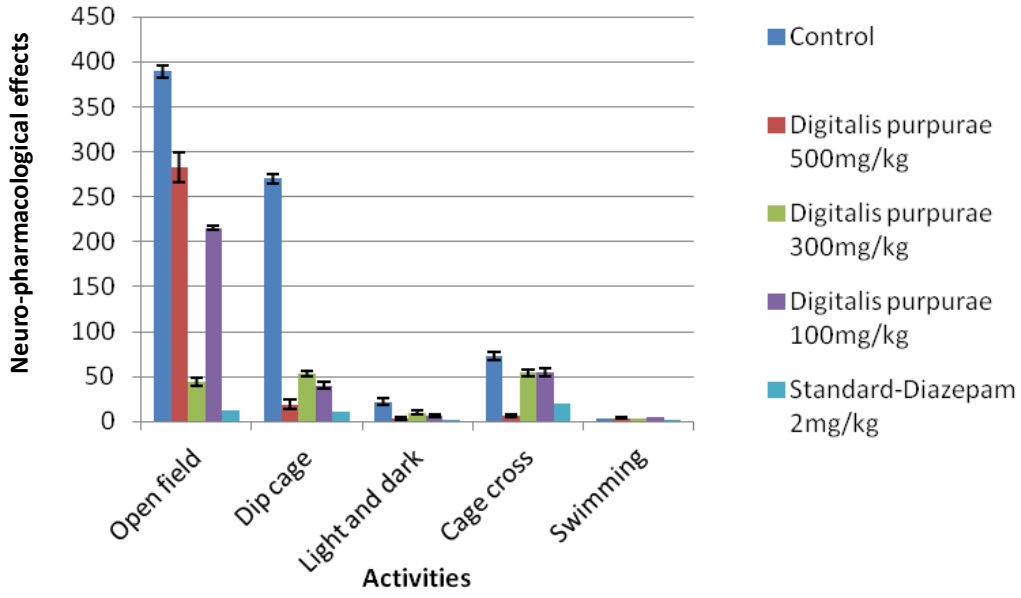

Figure 2. The neuro-pharmacological effects of 100, 300 and 500 mg/ml concentrations of *D.purpurae* extract on mice in comparison with control and standard drug.

1.37 ± 0.02 s. Locomotor and exploratory activity was observed considerably reduced in comparison to control and standard drug, Diazepam (2 mg/kg) (Figure 5).

Neuro-pharmacological effects of *A. uva-ursi* L. on mice

At the dose of 30 mg/kg of *A. uva-ursi* during open field activity 177.5 ± 3.17 counts in 30 min were observed. Whereas, number of entries in light compartment were 10 ± 2.11 times, in light and dark activity. In forced swimming test (FST) the mean forced mobility time was 1.22 ± 0.03 s at the dose of 100 mg/kg. On administration of 500 mg/kg of *A. uva-ursi*, the mice dipped its head 18.83 ± 5.11 times, during head dip activity and the observations on cage cross were found 28.5 ± 6.13. Neuropharmaco-

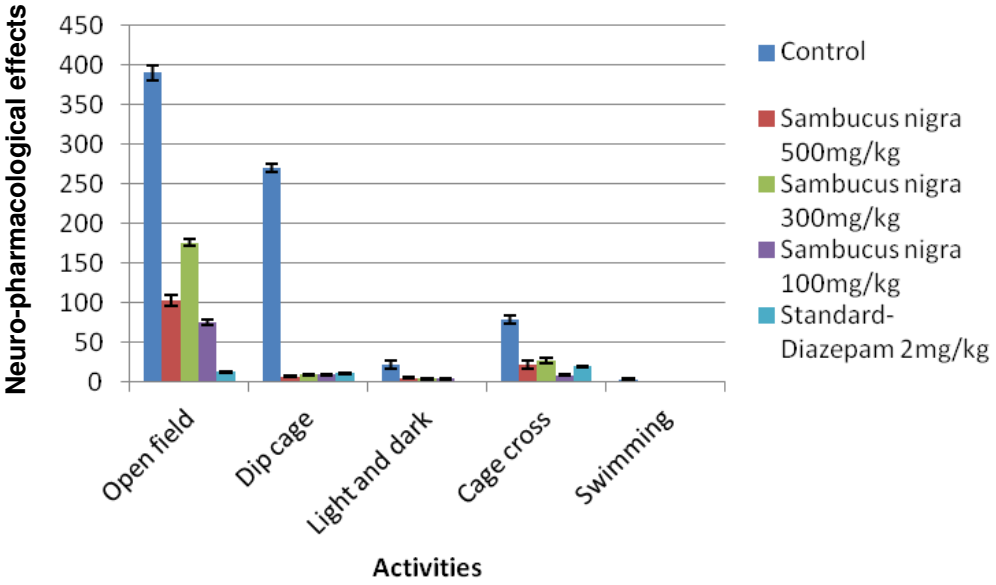

Figure 3. The neuro-pharmacological effects of 100, 300 and 500 mg/ml concentrations of S.*nigra* extract on mice in comparison with control and standard drug.

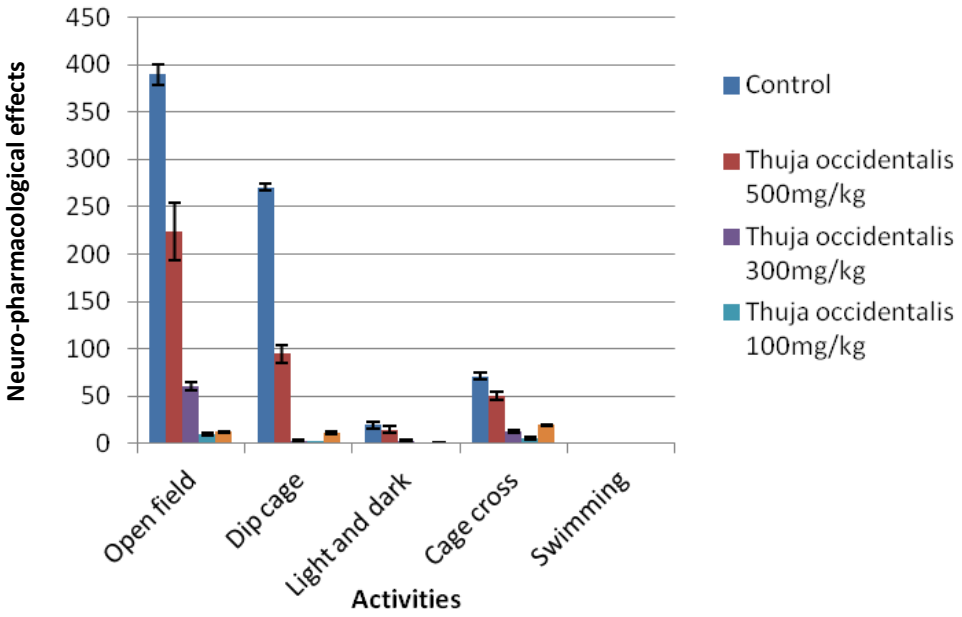

Figure 4. The neuro-pharmacological effects of 100, 300 and 500 mg/ml concentrations of *T. occidentalis* extract on mice in comparison with control and standard drug.

logical activities were observed to be significantly lowered in comparison to control and standard drug Diazepam (2 mg kg^{-1}) (Figure 6). The purpose of carrying out neuro-pharmacological studies is to explore the biological basis of the efficacy of crude extracts for the treatment of central nervous system disorders. These studies are mandatory for the formulation of safe and effective drugs. Most of the crude extracts with neuro-pharmacological activity possess quite non-specific action, affecting several different target receptors, ions, channels and transporters, likewise, the currently available most of the neuroactive drugs (Rang et al., 2007). The main neuro-pharmacological effects exhibited by crude extracts were as follows: reduction of anxiety and aggression,

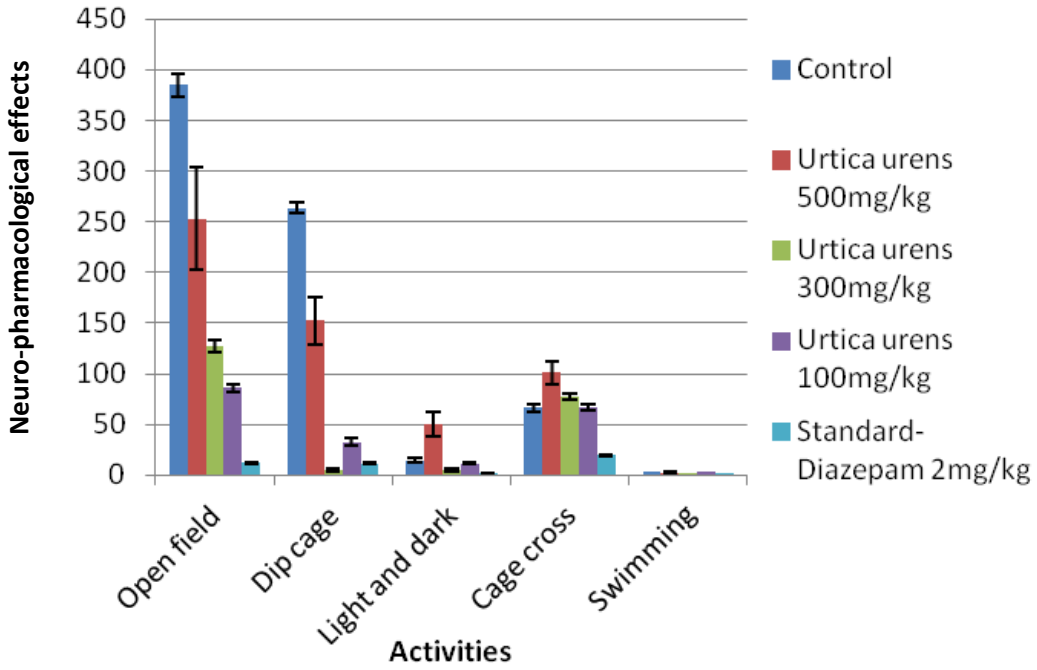

Figure 5. The neuro-pharmacological effects of 100, 300 and 500 mg/ml concentrations of *U. urens* extract on mice in comparison with control and standard drug.

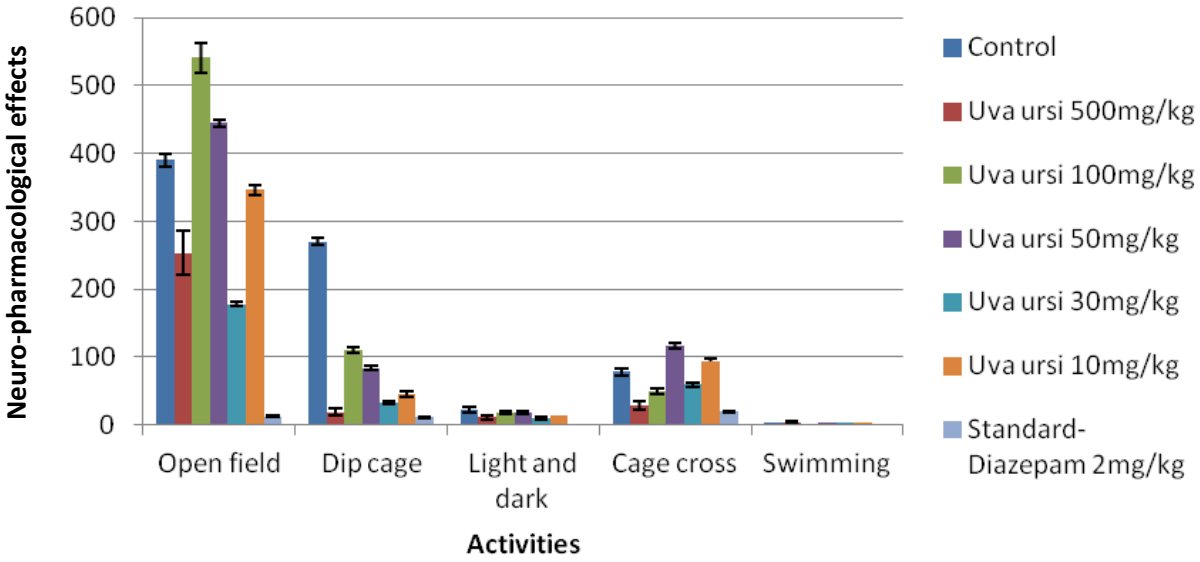

Figure 6. The neuro-pharmacological effects of 10, 30, 50, 100 and 500 mg/ml concentrations of *A. uva-ursi* extract on mice in comparison with control and standard drug.

sedation and induction of sleep as well as reduction of muscle tone and coordination (Argyropoulos et al., 2000) The neuro-pharmacological effects were observed at the dose of 100 and 300 mg/kg of *A. mellifica* extract. Extensive research was carried out on this insect drug by various researchers (Asafova et al., 1986; Ludyanskii, 1994). The pharmacologically active constituents reported are useful in the treatment of different diseases, for example Melittin is reported to influence the central nervous system; Phospholipase A, prevents neuronal cell death caused by prion peptides; Phospholipase B has detoxicating activity; Apamine stimulates the CNS; MCD stimulates CNS; Adolapin inhibits the specific brain enzymes (Shkenderov and Ivanov, 1983; Son et al., 2007; Urtubey, 2005).

Pharmacological effects of *D. purpurea* have been

reported by Ayuso et al. (1993) and Navarro et al. (1994). Our neuro-pharmacological results are found similar to the previously reported results by various researchers that may be due to the presence of digitoxin and minerals present in it (Benli et al., 2009; Negi et al., 2012).

Locomotor and exploratory activities of mice were significantly reduced by administration of *S. nigra* extract in comparison to control and standard drug that indicates that the extract of *S. nigra* acts through GABA receptor and this action may occur due to presence of following chemical constituents of *S. nigra*, that is, zicrin, prunasin, holocalin, potassium nitrate and choline. In general the change in pharmacological action occur in model experiments on introduction of *S. nigra* extract are reported due to rutin, quercetin, cyanicrin-3-glucoside, cyanidin-3-sumbubioside, sambunigrin, viburnic acid and vitamin A and C (Mahmoudi et al., 2014).

Anxiolytic effect of *T. occidentalis* was explored and the results were found to be similar to the research work carried out previously by Lokesh et al. (2011), Jahan (2010) and Alam (2009). Our research work revealed that *T. occidentalis* possessed pronounced depressive response in comparison to the other crude extracts, control and standard drug treated mice. Neuro-pharmacological action was observed in mice treated with *U. urens* extract may be due to the occurrence of butyric acid, acetylcholine and acetophenone (Tita et al., 1993).

Neuro-pharmacological activities on mice treated with *A. uva-ursi* extract showed significant depressive action in comparison to the control group. This response may be contributed by arbutin, methyl arbutin, hydroquinone and galloyl derivative of arbutin or related compounds (Beaux et al., 1999).

Conclusion

The conclusive remark on neuropharmacological activities of six crude drugs is that the chemical composition of each drug is different from each other but all are effective in reducing the hypothalamus (CNS) activity, therefore, a quick change/alteration in system behavior takes place which is neither toxic nor destructive in action. On the basis of these and other reported results it is recommended as a safe and effective drug.

Conflict of interest

Authors declared no conflict of interest.

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