ABSTRACT
The herbal drug Aftimooni has been used for relief of anxiety and insomnia, however, no pharmacological studies have been reported. Therefore, the drug Aftimooni was given orally to rats and mice to study its action on central nervous system. It is observed that Aftimooni significantly potentiated pentobarbitone induced sleeping time at the dose of 1.070ml/kg (5 times greater dose than human dose). Moreover, it significantly increased the rearing and number of square crossed in open field test. In elevated plus-maze, Aftimooni showed an anxiolytic effect by increasing the time spent in open arm. Additionally, a significant and dose dependent inhibition on writhing response induced by acetic acid was observed at the doses of 0.214ml/kg and 0.428ml/kg. However, Aftimooni did not affect immobility time in forced swimming test. These observations suggest that the drug Aftimooni possesses potential anxiolytic, hypnotic, exploratory and analgesic effects.

Keywords: Aftimooni; Writhing Response: Anxiolytic; Hypnotic; Exploratory; Analgesic.

INTRODUCTION
In practice of Unani medicine, the compound preparations are found to be more effective than a single drug treatment (Bopaiah et al., 2000). Some of the herbal preparations are used to treat psychiatric disorders.

Aftimooni is one of the herbal formulations produced by Hamdard Laboratories (Waqf) Pakistan. It is thick and brownish syrup of aromatic odor. Human dose is 15 ml/ 70 kg/ day i.e., 0.214 ml/kg. It is used for the treatment of anxiety and other nervous disorders. It contains the following active ingredients, the percent composition is in paranthesis. Cassia angustifolia (14%), Cuscuta reflexa (7%), Lavandula stoechas (7%), Nelumbium nuciferum (7%), Nepeta hindostana (7%), Ocimum basilicum, leaves (9.5%), Ocimum basilicum, seeds (7%), Onosma bracteatum (14%), Polypodium vulgare (7%), Rosa damascena (4.7%), Terminalia chebula (7%) and Viola odorata (7%).

Most of the constituent components are well known to be used in the traditional system of medicine for the treatment of nervous disorders but these do not prove the claim of the compound drug Aftimooni as such. The petroleum ether extract of Cuscuta reflexa Roxb. stem (PECR) showed significant reduction in spontaneous activity, exploratory behavior, muscle coordination and in 30 degrees inclined screen tests along with significant analgesic and hypnotic properties (Pal et al., 2003). Lavandula stoechas is used to treat anxiety (Holmes, 1998; Kenner, 1998). Two constituents of Lavadula stoechas, coumarin and camphor have anti-inflammatory and anxiolytic effects respectively (Bhattacharjee, 2001). The nelumbium nuciferum embryo is primarily used for nervous disorders, insomnia with restlessness and hypertension (Nguyen, 2001). Its alcoholic extract is reported to have marked anti-inflammatory activity (Khan et al., 1997). In Unani system of medicine Nepeta hindostana is used as sedative (Zeng et al., 1980) and tonic (Hao and Yang, 1986, Vogel...
et al., 1969, Kitagawa et al., 1978, Kaneko et al., 1985). Some constituents of Ocimum basilicum, like terpenes have sedative, hypnotic and anticonvulsant effects (Ismail, 2006), 1, 8- cineol exerts anticonvulsant, hypnotic and muscle relaxant activities (Santos and Rao, 2000). Eugenol is reported to exert an anticonvulsant, anesthetic, sedative and muscle relaxant effects (Boissier et al., 1967, Dallmeier and Wajda, 1981), Estragole and terpenoid ether have sedative, anticonvulsant, antiinflammatory and immunostimulant properties (Franchome and Penoel, 1995, Tisserand and Balacs, 1995 and Oliveira et al., 2001). The plant Onosma bracteatum has been reported to have tranquilizing activity (Tandala et al., 1986). The decoction of plant Onosma bracteatum is refrigerant tonic and is used in the treatment of rheumatism, restlessness and febrile excitement (Chopra et al., 1986). Aqueous extract of the root of Polypodium vulgare decreased the spontaneous motor activity, prolonged the pentobarbitone induced hypnosis (Mann, 1989). Rosa damascena is also used to produce hypnosis (Rakhshanda and Hosseini, 2006). Terminalia chebula is used in paralysis, headache, epilepsy, loss of memory, melancholia and in rheumatism and neuralgia (Nadkarni, 1976). An orally administered compound containing viola odorata has an ability to reduce anxiety and increase mental alertness (Ott and Dobberstein, 2003).

So, in order to investigate the neuropharmacological activity of product Aftimooni, the present study was designed using rats and mice.

**MATERIALS AND METHODS**

**Animals**

Experiments have been carried out on Sprague Dawley rats (220-250 g) and NMRI mice (25-30g) of either sex. The animals were housed under a standard light/dark cycle at the animal house of Dr. HMI Institute of Pharmacology and Herbal Sciences, Hamdard University at 28 °C with standard food pellets and water provided ad libitum. Animals were kept in plastic cages under standard conditions of temperature, relative humidity and light/dark cycles (12/12 h). Animals were acclimatized to laboratory conditions before test. Each animal was used once in the experiment with exception of open field and plus maze. The experiments were performed between 0900 and 1700 h.

**Drugs**

Aftimooni was obtained from Hamdard Laboratories (Waqf). For pharmacological studies three doses of Aftimooni used were 0.214ml/kg (human dose), 0.428ml/kg (double human dose) and 1.070 ml/kg (5 times human dose). Imipramine (25 mg/kg, p.o.) manufactured by Novartis Pharma (Pakistan) Ltd, pentobarbital sodium (40mg/kg, i.p.) from Abbott and 0.6% glacial acetic acid (10ml/kg, i.p.) (Scharlau Chemie S.A. Spain).

**Pharmacological Studies of Aftimooni**

**Potentiation of pentobarbital-induced sleep in rats**

The rats were divided into three groups (n = 6), the first group served as control received normal saline while the other two groups received drug Aftimooni (Aft) at the doses of 0.428ml/kg (double human dose) and 1.070 ml/kg (5 times human dose). Thirty minutes later pentobarbital sodium (40mg/kg, i.p.) was administered to each rat to induce sleep, each rat was observed for the sleep onset that is loss of righting reflex over 1 minute was considered to be asleep (Wambebe, 1985; Rolland et al., 1991). The sleep latency was recorded by the injection of pentobarbital sodium to the sleep onset and the sleeping time was recorded from the loss of righting reflex to recovery (Ramirez et al., 1998).

**Open field test**

This method is used to evaluate possible sedative or stimulating activities of animals (Carlini et al., 1986). The open field apparatus consisted of a square area (80cm × 80cm) with walls 37.5cm high. The floor was marked with lines that divided it into 25 squares (16cm × 16cm).
Elevated plus-maze
This test has been widely validated for measuring anxiolytic and anxiogenic like activities in rodents (Lister, 1987). The apparatus was made of Plexiglas and consisted of two opposite open arms (40cm x 10cm x 0.25cm) and two closed arms (40cm x 10cm x 17cm), extending from a central platform (5cm x 5cm), to give the apparatus a plus sign appearance. The maze was elevated to a height of 60 cm above the floor.

Experimental protocol of Open field and elevated plus-maze tests
Two groups of rats (n= 6, in each group) were treated with double dose of Aftimooni (0.428ml/kg p.o.) and with 0.9 % normal saline.

After 60 minutes of administration, each animal was placed in the centre of the arena in an open field. The number of squares crossed (with all four paws), rearing and grooming were observed for 5 minutes. After this, same animal was tested in elevated plus-maze. The rat was placed at the center of the maze, facing an open arm and allowed to explore the maze for 5 minutes. The following parameters were scored in each arm: the time spent, number of arm entries, protected and unprotected head dips, and rearing (Wijeweera et al., 2006).

The plus maze was carefully wiped with a wet towel after each animal performance.

Antinociceptive (analgesic) testing
Acetic acid – induced writhing syndrome
The antinociceptive property of Aftimooni was tested using the model of writhing response in mice as described by Bentley et al (1983). Two doses of Aftimooni (Aft) were given to two groups of mice (n= 6 in each group), compared with the control group of mice (n= 6), treated with normal saline. The writhing syndrome was elicited by an intraperitoneal injection of 0.6% acetic acid at the dose of 10ml/kg. Test substance and control vehicle were given orally to the mice 60 min before the administration of acetic acid. The number of writhes was noted for 30 minutes.

Forced swimming test
NMRI mice were divided into four groups having ten animals in each group. This test was performed according to the method described by (Porsolt et al., 1977) with slight modifications. One hour after p.o. administration of single dose of Aftimooni (Aft) (0.214ml/kg), double dose (0.428ml/kg), positive control (Imipramine) and saline, mice were individually forced to swim in a transparent glass vessel (17cm high, 20 cm in diameter) filled with 13 cm of water at 25-28 °C. The total duration of immobility (seconds) was measured during the last 4 min of a single 6 min test session. Mice were considered immobile when they made no further attempts to escape except the movements necessary to keep their heads above the water.

Statistical Analysis:
The results were expressed to compare the values of control and treated by using standard statistical tools i.e. Mean, Standard Error and Standard Deviation. The Percent control and changes were compared using analysis of variance followed by Student’s t-test. In all the tests, the criterion for level of significance was considered as (p< 0.05).

RESULTS
Pharmacological Studies of Aftimooni
Potentiation of pentobarbital- induced sleep in rats
Aftimooni significantly increased pentobarbital sleeping time (Fig. 1) at the dose of 1.070 ml/kg (5 times greater than human dose) whereas at the dose 0.428ml/kg (double dose) increased the pentobarbione sleeping time non- significantly when compared to their control group.

Open field test
Comparison with the control group showed that Aftimooni at a dose of 0.428ml/kg (double human dose) significantly increased the rearing and number of squares crossed while the grooming was non significantly increased (Fig. 2).
Elevated plus-maze

The double human dose of Aftimooni (0.428ml/kg) significantly increased the time spent in open arm but increase in the time spent in closed arm was non significant. There was increase in open arm entries, unprotected head dips, protected head dips and rearing but this increase was non significant. The same dose also produced non significant decrease in closed arm entries, when compared to the saline treated group (Fig. 3a & b).

Antinociceptive (analgesic) testing of Aftimooni

Acetic acid – induced writhing syndrome

The effect of Aftimooni on the writhing response in mice is shown in (Fig. 4). It was found that both doses (single dose and double dose) of Aftimooni caused an inhibition on the writhing response induced by acetic acid. An increase in the dose of the Aftimooni resulted in a greater inhibition as compared to the control group treated with normal saline.
Forced swimming test

All animals treated with 0.214ml/kg of Aftimooni (human dose) and 0.428ml/kg of Aftimooni (double dose) did not prolong struggling time in forced swimming test and showed no antidepressant behavior ($p > 0.05$), while animals administered with the standard antidepressant drug, IMI, showed a significant ($p < 0.01$) decrease in time of immobility when compared to their control animals (Fig. 5).

**DISCUSSION**

Aftimooni is a herbal product and is used by general practitioners as an anxiolytic and hypnotic. However, there is absence of scientific report about the evaluation of its neuropharmacological effects. In the test of pentobarbital-induced sleep, the 1.070 ml/kg (5 times human dose) of Aftimooni significantly increased the potentiation of sleeping time of pentobarbital (40mg/kg i.p) for a period of $474.66 \pm 45.01$ min (Mean $\pm$ S.E.M., $n=6$) when compared with control group ($332.14 \pm 56.39$ min). In the open field test, Aftimooni significantly increased the number of rearing at the dose of 0.428ml/kg as shown in Fig. 2. It is conceded that rearing is a function of excitability of the central nervous system (Masur et al., 1971) and the significant

Fig. 3. Effect of Aftimooni (0.428 ml/kg) on (a) T. sp. O. A. – Time spent in open arm and T. sp. C. A – Time spent in closed arm (b) Open arm entries, closed arm entries, Unphd — unprotected head dips and rearing. * $p < 0.05$ compared with saline treated group, $n=6$. 

![Plus maze activity](image)
increase in the number of squares crossed showed its anxiolytic behavior which is usually accompanied by some behavioral disinhibition (Trevor et al., 2005).

An anxiolytic activity of Aftimooni at the dose 0.428ml/kg has been demonstrated (Fig. 3a & b). This drug was able to significantly increase the time spent in open arm (97.8±28.7). This result indicates anxiolytic activity when compared with control group (27.6 ± 13.3). These findings raised the possibility that the anxiolytic and other sedative effects may be exerted by different phytoconstituents possibly acting through different receptor subtypes or different affinity for the relevant receptors. Linalool is one of the phytoconstituents identified in *Rosa damascena* and *Ocimum basilicum* (ingredients of Aftimooni) has marked effects at the central nervous system, including hypnotic and anticonvulsant properties (Elisbetsky et al., 1995) in dose dependent manner. In addition, linalool has also shown anxiolytic and sedative effects in human subjects (Sugawara et al., 1998).

Our results regarding antinociceptive activity (analgesic activity) showed dose-dependent inhibition of acetic acid induced writhing. There is 67% inhibition at 0.214ml/kg and 79% inhibition at the dose of
0.428ml/kg (Fig. 4). However more work is required necessary in order to verify its mechanism of action to elucidate the active principles involved with its analgesic activity.

The drug Aftimooni did not show any antidepressant effects in the forced swimming test (Fig. 5).

CONCLUSION

It is concluded that drug Aftimooni showed significant hypnotic, anxiolytic, exploratory and analgesic effects at the doses of 1.070ml/kg, 0.428ml/kg, 0.428ml/kg, and (0.214ml/kg & 0.428ml/kg) respectively on rats and mice.

REFERENCES


Council of Scientific and Industrial Research, New Delhi.


