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Neuropharmacological Activities of *Abies pindrow* Aerial Parts in Mice

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Abstract: The methanol extract (200 or 400 mg/kg, *p.o.*) and ethyl acetate fraction (25 or 50 mg/kg, *p.o.*) of *A. pindrow* aerial parts were screened for anticonvulsant, antidepressant, locomotor, hypnotic and antistress activities. The methanol extract (ME) and ethyl acetate fraction (EAF) could not reduce duration of MES-induced tonic extensor phase with respect to the standard drug, phenytoin (20 mg/kg, *i.p.*). Both ME and EAF showed significant reduction of time spent in immobile state in forced swim test and did not stimulate locomotion in an open field model, thereby confirming their specific antidepressant activity. In cold swim test, ME and EAF showed antistress activity comparable to diazepam (1 mg/kg, *i.p.*). None of the test doses of ME and EAF could significantly increase duration of sleep in mice as compared to the control group. Phytochemical screening of ME and EAF showed presence of flavonoids as major class of phytoconstituents.

Keywords: Anticonvulsant, Antidepressant, Antistress, Himalayan fir, Pinaceae, Hypnotic.

1. INTRODUCTION

Mental disorders in the current situation have become serious problem in health management (Mukherjee & Roy, 1990). During the last two decades, synthetic psychotropic drugs have been used effectively in the treatment of mental disorders. But the chronic use of these synthetic psychotropic drugs leads to memory impairment, addiction, dependence, autonomic, endocrine, allergic, hematopoietic and neurological side effects (Handa, 1995).

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Natural products especially higher plant derived products represent over 50% of all drugs in clinical use (Balandrin *et al*., 1993). About 3.5 to 4 billion people in the world relied on plants as source of drugs for ailments (Farnsworth, 1988), as they are safe, efficacious and good compatibility with the body (Kamboj, 2000). A number of plants are known to be used since long in treatment of mental illness but have not been validated scientifically (Bhattacharya & Haldar, 2013). *Abies pindrow* Royle is one of such plants.

A. pindrow (Pinaceae) popularly known as Silver Fir, is found in Western Himalayas (Kirtikar & Basu, 1975). Traditionally, the plant has been used in the treatment of nervous disorders (Quattrocchi, 2012). The plant has been reported to exhibit anti-inflammatory, antiulcer, antibacterial, and bronchoprotective activities (Singh & Pandey, 1997; Singh *et al*., 2000). Though few studies have been shown antistress and analgesic activities of plant but these studies are too preliminary to justify its traditional claims. *A. pindrow* has been reported to contain chalcone glycosides, flavonoids, fatty acids, hydrocarbons and terpenoids (Burdi *et al*., 2007; Samejo *et al*., 2010; Tiwari & Minocha, 1980; Tripathi *et al*., 1996). The authors have previously reported antianxiety activity in ME and EAF of *A. pindrow* aerial parts at the doses of 400 and 50 mg/kg, *p.o.*, respectively, using elevated plus maze model of anxiety (Kumar & Kumar, 2015).

The available literature reveals that *A. Pindrow* has not been investigated systematically for neuropharmacological activities. Thus, the present investigation was undertaken to investigate detailed neuropharmacological activities of methanol extract of *A. pindrow* aerial parts and its EAF.

2. MATERIALS AND METHODS

2.1 Collection and autentication of plant material

Abies pindrow aerial parts were taken from Gulaba Kothi, Manali at a height of 2000-2100 m, India in September, 2012. Identification was confirmed by Dr. Sunita Garg, Chief Scientist and Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi (Reference no. – NISCAIR/RHMD/Consult/2013/2242/23, dated 21/05/2013).

2.2 Animals

For carrying out pharmacological and toxicological studies, laca mice (either sex) were purchased from the Central Research Institute, Kasauli, India, having body weight 20-25 g. The laboratory pellet diet and water *ad libitum* were

provided to animals. The animal experiments were carried out after taking approval from Institutional Animal Ethics Committee, Punjabi University, Patiala (107/ 99/CPCSEA/2013-52, dated 18/10/2013). Before the start of experiment, the animals were acclimatized to laboratory conditions daily for 1 h for seven days continuously. All the experiments were carried out from 9 AM to 12 PM as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals. Six animals were used in the group The animals were kept on fasting overnight before use. The doses was administerd with Oral cannula .

2.3 Preparation of Extracts and Fraction

The crude extracts and fraction of *A. pindrow* aerial parts were made using LR grade methanol (S.D. Fine Chemicals, Mumbai, India), ethyl acetate (E Merck, Delhi, India) and petroleum ether (60-80 ºC) (RFCL Ltd., New Delhi, India).

Aerial parts of *A. pindrow* were dried, powdered and extracted in a Soxhlet apparatus using solvents in increasing order of polarity *viz.,* petroleum ether and methanol.

The methanolic extract ME (20 g) was uniformly suspended in water in RBF. To the solution, ethyl acetate was added and stirred continuously eor 30 min at 50 ºC. The ethyl acetate layer was then separated. This procedure was repeated for 10 times. All ethyl acetae layers were pooled and concentrated under reduced pressure which was then dried and stored in a vacuum desiccator. The solvents from crude extracts were recovered using Rota evaporator (BUCHI, Switzerland). Both ME and EAF were subjected to preliminary phytochemical screening using standard procedures (Farnsworth, 1966).

2.4 Vehicle and Standard Drugs

Various test doses were prepared using distilled water and Tween 80 (2%) as a vehicle. 0.2 to 0.25 ml volume is administerd to the mice. Phenytoin sodium injection (20 mg/kg, *i.p.*), imipramine (15 mg/kg, *i.p.*), thiopentone sodium (80 mg/kg, *i.p.*), diazepam (2 mg/kg, *i.p.*) and diazepam (1 mg/kg, *i.p.*), were used as standard anticonvulsant, antidepressant, hypnotic, CNS depressant and antistress drugs respectively.

2.5 Acute Toxicity Studies

Acute oral toxicity study was performed as per OECD guidelines-423 (Organization for Economic Cooperation and Development) (Anonymous, 2001; Biswas *et al*., 2014). Methanol extract of *A. pindrow* aerial parts was administerd orally,

afterwards animals were observed individually for behavioural profile (alertness, restlessness, irritability and fearfulness), neurological profile (spontaneous activity, reactivity, touch response and pain response) and autonomic profile (defecation and urination) for at least once during the first 30 min and periodically during the first 24 h, with special attention given during the first 4 h and daily thereafter, for a total of 14 days. A total of six mice were used and each received a single oral dose of 2000 mg/kg/*p.o.* (limit test). Animals were kept overnight fasting prior to drug administration and food was withheld for further 3-4 h.

2.6 Experimental Design

Animals were divided into six (I-VI) groups.

Group I - Control (vehicle 0.25 ml, *p.o.*).

Group II - Standard drug treated (Standard).

Group III - Test group received ME (200 mg/kg, *p.o.*).

Group IV - Test group received ME (400 mg/kg, *p.o.*).

Group V - Test group received EAF (25 mg/kg, *p.o.*).

Group VI - Test group received EAF (50 mg/kg, *p.o.*).

2.7 Anticonvulsant Activity

Anticonvulsant activity was evaluated using maximal electroconvulsive shock (MES) test. Tonic hind limb extension was induced in mice by applying maximal electroshock stimulus (50 mA for 0.2 sec) through ear-clip electrodes of electroconvulsometer (Swinyard *et al*., 1952). The test substances were initially administered to mice. After 45 minutes the electric shock was given to mice, and duration of tonic extensor phase of convulsions and percentage protection of animals were recorded.

2.8 Antidepressant Activity

This activity was evaluated using Plexiglas cylinder (height 40 cm; diameter 18 cm) containing water upto the height of 15 cm and temperature at 25±2 °C. After 1 h of administration of test substances mice were forced to swim. (Kaur *et al*., 2014). Mice were allowed to swim for 6 min. The total duration of immobility (floating in the water in a slightly hunched but upright position, its nose above the surface) was noted during the test period.

2.9 CNS Depressant / Locomotor Activity

The apparatus composed a grey PVC wall surrounding a square wooden arena with dimensions 40×40×40 cm and the floor was divided into 25 squares

marked by black lines. The mice were placed individually into the central part of the open field apparatus and the number of crossings and rearings were recorded for 5 min, after 30 min of administration of the test substances. (Campos *et al*., 2005)

2.10 Hypnotic Activity

After 30 min of administration of vehicle and test extracts, thiopentone sodium (80 mg/kg, *i.p.*) was administered to induce sleep in mice (Kaur *et al*., 2014). The time taken for onset of duration of sleep was noted for all the animals. After induction of sleep, mice were placed in the inverted position. When sedation was over, the mice came to normal posture, and their sleep time was noted. This interval between loss and recovery of righting reflex was recorded as the duration of sleep. The time interval between injection of thiopentone sodium and start of sleep/loss of righting reflex was recorded as latency time.

2.11 Antistress Activity

After 1 h of administration of test drugs mice were forced to swim in a Plexiglas cylinder (height 40 cm; diameter 18 cm) containing water upto the height of 15 cm, and maintained at 10±2 °C (Kaur *et al*., 2014). Mice were allowed to swim for 6 min. The total duration of immobility was noted during this test period.

2.12 Statistical Analysis

Results are expressed as mean ± standard deviation (SD) and the extracts were compared with standard drug and control by one way analysis of variance (ANOVA) followed by Student-Newman-Keul's test (Scheffer, 1980).

3. RESULTS

The methanol extract (ME) of *A. pindrow* aerial parts was prepared by extracting properly identified plant in a Soxhlet apparatus with methanol, after defatting with petroleum ether (60-80 ºC). Yield of ME was found to be 14.80%. Phytochemical investigation was done for the ME of plant which showed the presence of flavonoids as one of the major class of phytoconstituents. Thus, flavonoid-rich fraction was prepared by partitioning methanol extract with ethyl acetate using standard procedure. Yield of EAF was found to be 20.45% in relation to the methanol extract.

In acute toxicity study, the mice treated with ME did not show any lethality and toxic reactions until the end of the study period, therefore, the tested dose is said to be "unclassified" under the toxicity scale. Therefore, at a doses of 200 and 400 mg/kg methanol extract which are $1/10^{th}$ and $1/5^{th}$ of dose tested

for acute toxicity study, respectively, were selected for neuropharmacological studies. Further, the doses of EAF were selected based on its percentage yield obtained from ME.

The ME and EAF of *A. pindrow* aerial parts were subjected to anticonvulsant activity in mice using MES test. The decreased duration of MES-induced tonic extension phase in the mice and percentage protection of animals were noted after oral administration of ME (200 or 400 mg/kg), EAF (25 or 50 mg/kg), phenytoin (20 mg/kg, *i.p.*) and the control (vehicle) as shown in Table 1. The ME and EAF exhibited significant anticonvulsant activity at all doses as equivalent to control, but the activity was not comparable to the standard drug, which completely abolished duration of tonic extension in mice and protected all animals from MES-induced convulsions. The ME (400 mg/kg) and EAF (50 mg/kg) reduced duration of tonic extension to 17 and 16.50 sec, respectively, with respect to control (23.79 sec).

The antidepressant activity of ME and EAF of *A. pindrow* aerial parts was evaluated using despair swim test. Table 2 shows the time spent by mice in immobile state after acute treatment with ME (200 or 400 mg/kg, *p.o.*), EAF (25 or 50 mg/kg, *p.o.*), imipramine (15 mg/kg, *i.p.*) and the control (vehicle, *p.o.*). The ME and EAF reduced the duration of immobility at 400 and 50 mg/kg, respectively as depicted by despair swim test, but the locomotor activity was not increased as found in open field test (Table 3). These findings confirmed the antidepressant activity of ME and EAF. Both ME and EAF

Table 1: Anticonvulsant activity of *A. pindrow* using MES test.

Treatment	**Dose (mg/kg)**	**Meann time spent in extensor phase (sec)±SD**	**% Protection of animals**
Control	Vehicle	23.79±1.75a	66.66
Standard drug (Phenytoin)	20	0±0*	100
Methanol Extract	200	16.00±1.41*a	33.33
	400	17.00±1.78*a	50
EAF	25	17.00±1.41*a	50
	50	16.50±1.87*a	66.66

n = 6; The data is expressed as Mean±SD; **P<0.05* vs Control; [a]*P<0.05* vs Standard; one way ANOVA followed by Student-Newman-Keul's test.

significantly reduced rearing and crossings with respect to control in open field test suggesting its CNS depressant activity.

The ME and EAF of *A. pindrow* aerial parts were subjected to hypnotic activity in mice using thiopentone sodium-induced sleeping assay. The mean latency time and duration of sleep after acute oral administration of ME (200 or 400 mg/kg), EAF (25 or 50 mg/kg) and the control (vehicle) have been shown in table 4. None of the test doses of ME and EAF could significantly increase duration of sleep in mice as compared to the control group.

The antstress activity in was determined using cold swim test. The mean immobility time of the mice after acute administration of ME (200 or 400 mg/

Table 2: Antidepressant activity of *A. pindrow* using despair swim test.

Treatment	Dose (mg/kg)	Meann immobility time (sec)±SD
Control	Vehicle	223.16±4.75a
Standard drug (Imipramine)	15	44.00±2.82*
Methanol extract	200	74.50±3.72*a
	400	47.33±2.16*
EAF	25	68.33±2.16*a
	50	43.67±3.14*

n = 6; The data is expressed as Mean±SD; **P<0.05* vs Control; [a]*P<0.05* vs Standard; one way ANOVA followed by Student-Newman-Keul's test.

Table 3: Locomotor activity of *A. pindrow* using open field test.

Treatment	Dose (mg/kg)	Meann number of squares crossed±SD	Meann number of rearings±SD
Control	Vehicle	55.85±6.20a	15.11±3.50a
Standard drug (Diazepam)	2	41.31±7.45*	8.24±2.58*
Methanol extract	200	48.47±5.69*a	10.85±2.23*a
	400	42.15±4.85*	8.51±1.58*
EAF	50	49.30±6.64*a	9.99±1.57*a
	100	41.85±4.49*	8.34±2.99*a

n = 6; The data is expressed as Mean±SD; **P<0.05* vs Control; [a]*P<0.05* vs Standard; one way ANOVA followed by Student-Newman-Keul's test.

kg), EAF (25 or 50 mg/kg), diazepam (1 mg/kg, *i.p.*) and the control (vehicle) is shown in Table 5. The ME although significantly reduced time spent by mice in immobile state as compared to control but activity was comparable to standard drug. On the other hand, EAF (50 mg/kg) significantly reduced time spent by mice in immobile state and was found equivalent to standard drug.

Table 4: Hypnotic activity of *A. pindrow* using thiopentone sodium-induced sleeping assay.

Treatment*	Dose (mg/kg)	Meann latency time (min)±SD	Meann duration of sleep (min)±SD
Control	Vehicle	1.45±0.36	59.00±1.41
Methanol extract	200	1.50±0.34	59.33±2.42
	400	1.46±0.20	61.66±2.80
EAF	25	1.60±0.18	61.16±2.78
	50	1.51±0.29	61.50±4.27

n = 6; The data is expressed as Mean±SD; **P<0.05* vs Control; one way ANOVA followed by Student-Newman-Keul's test.
*Thiopentone sodium (80 mg/kg, *i.p.*) was injected intraperitoneally to all groups of mice treated with control, methanol extract and EF.

Table 5: Antistress activity of *A. pindrow* using cold swim test.

Treatment	Dose (mg/kg)	Meann immobility time (sec)±SD
Control	Vehicle	172.00±9.75a
Standard drug (Diazepam)	1	37.11±5.14*
Methanol extract	200	73.94±4.02*a
	400	53.66±3.32*a
EAF	25	52.80±4.44*a
	50	41.23±2.42*

n = 6; The data is expressed as Mean±SD; **P<0.05* vs Control; [a]*P<0.05* vs Standard; one way ANOVA followed by Student-Newman-Keul's test.

4. DISCUSSION

The ME and EAF of *A. pindrow* aerial parts significantly inhibited tonic extension in mice in comparison to control but not as that of standard drug, suggesting its mild anticonvulsant activity against generalized tonic-clonic and

cortical focal seizures with respect to control (Swinyard *et al*., 1952). When the mice were forced to swim in a restricted space, from which they cannot escape, after administration of test drugs significantly reduced duration of immobility. The available literature reveals that minor or major tranquilisers do not affect immobility in forced swim test (FST) but reduce motor activity (Porsolt *et al*., 1977). Psycho-stimulants can reduce immobility in FST like antidepressant drugs, but in contrast, psycho-stimulants show marked motor stimulation in locomotor activity test. Thus, test drugs should also be assessed for motor activity to infer their specific antidepressant activity. The present work showed that the reduction of immobility in despair swim test was not associated with stimulation of locomotor activity in open field test, thus confirmed specific antidepressant activity of test drugs. The ME and EAF significantly decreased number of rearings and crossings in open field test with respect to control suggesting their CNS depressant activity. These observations further support previously reported work of authors on anxiolytic activity of ME and EAF of *A. pindrow* aerial parts (Kumar & Kumar, 2015). The test drugs did not exhibit hypnotic activity. When mice are individually kept in a cold environment leads to a sharp increase in the level of adrenocorticoids. This increased level of neurotransmitter induces stress in animals (Komiya *et al*., 2006). The ME and EAF significantly reduced time spent by the animals in immobile state in cold environment as compared to control group, thus, inferring its antistress activity.

Both ME and EAF did not exhibit hypnotic activity. The literature reveals that anxiolytics selectively modulate α_2 - and / or α_3 - containing $GABA_A$ receptors, whereas α_1 – subtype is involved in sedation (Atack, 2005; Rudolph & Mohler, 2006). On the basis of observations that the ME and EAF exhibit significant anxiolytic activity without sedation, it is suggested that these drugs are selective agonists for α_2 - and / or α_3 – containing $GABA_A$ receptors.

Preliminary phytochemical screening showed presence of flavonoids as major group of phytoconstituents in ME and EAF of *A. pindrow* aerial parts. A large number of flavonoids have been reported to possess varied neuropharmacological activities. Few of these are quercetin (Saaby *et al*., 2009), apigenin (Kumar & Sharma, 2006), hesperidin (Marder *et al*., 2003) and chrysin (Wolfman *et al*., 1994). Our results are in agreement to these reports that flavonoids of *A. pindrow* aerial parts are responsible for neuropharmacological activities of the plant.

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