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Evaluation of Analgesic, Anti-inflammatory and CNS Depressant Potential of *Dendrophthoe falcata* (Linn.) Leaves Extracts in Experimental Mice Model

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Abstract

Plants are extraordinary reservoir of bioactive natural products, many of which exhibit chemical and structural features on outstanding biological activities in animals. *Dendrophthoe falcata* (Linn.) is an important parasitic plant extensively used in folk medicine. In this regard, the aim of this study was to investigate the possible analgesic, anti-inflammatory and central nervous system (CNS) depressant activities of ethanol, aqueous, chloroform and petroleum ether extracts of *D. falcata* (Linn.) leaves (AEDFL, EEDFL, CEDFL and PEDFL) on Swiss albino mice. The analgesic activity was determined by tail immersion and acetic acid induced writhing methods at a dose of 50, 100 and 200 mg/kg, p.o. using morphine sulfate as a standard drug at a dose of 5mg/kg of body weight. Carrageenan-induced paw edema model was applied to find out anti-inflammatory potential at the same doses of analgesic activity. Here ibuprofen was treated as a standard drug at 10mg/kg body weight. The CNS depressant effect of the extracts were evaluated by using open field and hole cross tests. All the extracts exhibited significant ($P^b < 0.01$, $P^a < 0.001$) analgesic, anti-inflammatory and CNS depressant effects at dose dependant manner. Petroleum ether extracts (PEDFL) showed maximum analgesic effect (77.45% inhibition of abdominal writhing and 82.12% elongation of tail withdrawal time) at 200mg/kg dose. Moreover, the highest anti-inflammatory potential (90.24% inhibition of paw edema volume) was showed by petroleum ether extract at 200mg/kg dose. On the other hand, ethanol extract (EEDFL) proved to have significant (97.33% and 85.91% inhibition of locomotion in open field and

in hole cross test respectively) CNS-depressant activities after 120 min of oral administration of the extract at 200mg/kg dose.

Keywords: *Dendrophthoe falcata,* Analgesic, Anti-inflammatory, CNS depressant.

1. Introduction

Dendrophthoe falcata (Linn.) belongs to the family of Loranthaceace and is commonly known as 'Vanda' in the Indian Ayurvedic System of Medicine [1, 2]. It is a perennial climbing woody hemiparasitic [3], and evergreen shrub that is frequently observed on different host plants such as *Mangifera indica, Melia azadirecta, Psidium guayava etc.* Bark of *D. falcata* is grey. Its leaves are thick, coriaceous, much variable in shape usually opposite 7.5 to 18 by 2-10 cm. Flower of the plant are stout, unilateral racemes often two from an axil pedicle. These are ovate sub-acute, concave and scarlet or orange or less commonly pink or white in color. Anthers are linear, equal in length to the free portion of the filament. Berries of *D. falcata* are 8-13 mm long ovoid oblong, pink, smooth crowned by a cup- shaped calyx. *D. falcata* comprises of 20 species where about 7 species are found in Indian subcontinent [4], that are indigenous to tropical and sub-tropical regions especially in India, Sirilanka, China, Australia, Bangladesh, Malayasia, Myanmar, Thailand and Indo-china [2,3].

Entire plant is used extensively in traditional system of medicine as an aphrodisiac, astringent, narcotic and diuretic. It is applied for the treatment of pulmonary tuberculosis, asthma, menstrual disorders, swellings, wounds, ulcers, strangury and psychic disorders [3]. Leaf paste is used in skin diseases. Its paste is applied on boils setting, dislocated bones and extracting pus [2]. It is also used in renal and vesical calculi and vitiated conditions of *kapha* and *pitta*. *D. falcata* is reported to have antilithiatic, cytotoxic, immunomodulatory, antimicrobial, antioxidant and hepatoprotective activity. In the traditional system of medicine *D. falcata* is recommended for the treatment of epilepsy and paralysis [1]. Also, it has antibiofilm and anti-quorum effects against a wide variety of microorganisms [5]. The plant is also proved to have anti-fertility efficacy in Wistar female rats [6,7], as well as anticancer effect against human breast cancer [4,8].

Preliminary phytochemical screening revealed the presence of carbohydrates, phytosterols, flavonoids, glycosides and phenolic compounds [3]. It is reported to contain biologically active substances such as quercetin, tannins, β-sitosterol, β-amyrin, oleanolic acid [4]. Several active chemical constituents such as stigmasterol, kaempferol, quercetin–3-O-rhamnoside, rutin, and myricetin and their glycosides, (+)-catechin, leucocyanidin, gallic acid, chebulinic acid and some pentacyclic triterpenes: kaempferol-3-O-α-L-rhamnopyranoside and quercetin-3-O-α-L-rhamnopyranoside, etc are isolated and identified in it (*D. falcata)* [2]. Moreover, three new pentacyclc triterpenes such as -(3ß-acetoxy-1ß-(2-hydroxy-2-propoxy)-11α-hydroxy-olean-12-ene, 3ß-acetoxy-11α-ethoxy-1ß-hydroxy-olean-12-ene , and 3ß-acetoxy-1ß-hydroxy-11α-methoxy-0lean-12-ene have been reported in the plant [9, 10].

Inflammation is a crucial and necessary function of the innate immune system, protecting the host against pathogens and initiating a specific immune response. Hence, inflammation is a complex process that is frequently associated with pain [11]. Anxiety is being explained as a psychological and physiological state marked by cognitive, somatic, emotional and behavioral elements. Together, these components provoke a disagreeable emotion associated with fear, worry as well as restlessness. Therefore, it can be an obstacle in everyday life [12]. It is proved that prolong use of synthetic drugs of all classes are not only harmful but also very expensive to develop. So, the associated complexities of synthetic drugs have lead to a shift towards locating natural resources showing therapeutic potentials [13]. These conventional medicines are associated with unpleasant side effects or toxic effect such as gastrointestinal disturbances [14], and have shown only limited success against

chronic diseases [15]. Drugs are increasingly being developed from natural origins, offering a very promising approach to identify novel therapeutic agents [16]. Natural products have long been used in traditional medicine to treat inflammation and other inflammation-related diseases, and the raw materials of these products are often used to develop new drugs [17,18].

The present study was undertaken to investigate analgesic, anti-inflammatory and CNS depressant activities of aqueous, ethanol, chloroform and petroleum ether extracts of *D. falcata* leaves in Swiss albino mice that may unveil the rationality of use of the plant as traditional medicines and potentiality of it in the herbal medicine.

2. Materials and methods

2.1 Plant materials

For the investigation, *Dendrophthoe falcata* (Linn.) leaves, mistletoe of *Swietenia fabrilis* tree were collected from Joypurhat, Bangladesh in September, 2012 and identified by experts of the Bangladesh National Herbarium, Dhaka, where a voucher specimen has also been retained with accession no. 39432. The collected leaves were cleaned and dried for one week in electric oven, and pulverized into a coarse powder using a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark, and dry place until further analysis.

2.2 Extract preparation

Approximately, 800g of powdered material was placed in a clean, flat-bottomed glass container and soaked in ethanol and similarly 400g of the powder was soaked in distilled water. Both of the containers with their contents were sealed and kept for 5 days. Then the extraction was carried out by using an Ultrasonic Sound Bath accompanied by sonication (40 minutes). The entire mixture, then, underwent a coarse filtration by a piece of clean and white cotton material. Then the extracts were filtered through Whitman filter paper (Bibby RE200, Sterilin Ltd., UK) and were concentrated to obtain the ethanol (12g) and aqueous (4g) extracts. Ethanol extract was divided into two portions. One portion (2g) was poured into a glass vial to be tested as crude ethanol extract, whereas the second portion (10g) was dissolved in 100 mL of ethanol, and partitioned successively with chloroform and petroleum ether. The fractions were then concentrated using a rotary evaporator to obtain chloroform fraction (yield weight 1.5g), and petroleum ether fraction (yield weight 2.60g). This process rendered a gummy concentrated reddish black color. The gummy extracts were transferred to a closed container for further use and storage.

2.3 Drugs and chemicals

Ethanol, chloroform and petroleum ether were used as solvent for the extraction of the leaves and acetic acid was used in writhing test. These chemicals were purchased from Merck, Germany. Tween-80 was also collected from Merck, Germany. Ibuprofen, diazepam and morphine sulfate were collected from Square Pharmaceuticals Ltd. Bangladesh. All the chemicals used in these investigations were of analytical reagent grade.

2.4 Animals

Swiss albino mice of either sex weighing approximately 25-30g were used for these experiments. These mice were purchased from the animal research branch of International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). After their purchase, the mice were kept in standard environmental conditions (25.0 ±2.0°C & 55-65% relative humidity and 12 h light/dark cycle) for four weeks to acclimate and fed ICDDR, B formulated rodent food and water *ad libitum*. The experimental procedures involving animals were conducted in accordance with the guidelines of Southeast University, Dhaka, Bangladesh. The study protocol was approved by Institutional Animal, Medical Ethics, Biosafety and Biosecurity Committee of the University. The set of rules followed for animal experiment were approved by the institutional animal ethical committee and handled in accordance with international guidelines for care and use of laboratory animals [19].

2.5 Analgesic activity

2.5.1 Acetic acid-induced writhing test

The analgesic activity of the extracts (AEDFL, EEDFL, CEDFL and PEDFL) was studied by using acetic acid-induced writhing model in Swiss albino mice, described by Koster R. *et al.* [20]. The animals were divided into fourteen groups with six mice in each group. Group I animals received vehicle (1% tween 80 in water, p.o.), animals of group II received morphine sulfate at 5 mg/kg body weight while animals of groups III-XIV received extracts of the leaves. Group III, IV and V received AEDFL at a dose of 50,100, 200 mg/kg body weight (p.o.). Group VI, VII and VIII received EEDFL at a dose of 50, 100, 200 mg/kg body weight (p.o.). Group IX, X and XI received CEDFL at a dose of 50,100, 200 mg/kg body weight (p.o.). Group XII, XIII and XIV received PEDFL at a dose of 50, 100, 200 mg/kg body weight (p.o.). After 30 minutes of vehicle, standard drug and extracts administration, 0.6% v/v acetic acid was administered into the peritoneum of each animal. The writhing response, which consists of a contraction of the abdominal muscle together with a stretching of the hind limbs, was determined for 20 minutes after a latency period of 5 minutes. The Percentage reduction of abdominal constriction indicates the percentage protection against it which was taken as an index of analgesia. It was calculated as:

$$\% \text{ Inhibition} = [(N_c - N_t) / N_c] \times 100$$

Where, N_c = number of writhing of the control group, N_t = number of writhing of the treated group

2.5.2 Tail immersion test

Tail immersion test was done by the method described by Toma W. *et al.* [21]. This test is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail-withdrawal reflex in mice. According to the method, vehicle (1% tween 80 in water, p.o.), morphine sulfate (5mg/kg, p.o.), AEDFL (50, 100 and 200 mg/kg, p.o.), EEDFL (50, 100 and 200 mg/kg, p.o.), CEDFL (50, 100 and 200 mg/kg, p.o.) and PEDFL (50, 100 and 200 mg/kg, p.o.) were administered to the mice of respective groups 30 min before the experiment. About 3 cm tail of each mouse was immersed in warm water kept constant at 55 ± 1°C. The reaction time was the time taken by the mouse to deflect the tail. The latency period of the tail-withdrawal response was taken as the index of analgesia and was determined before drug treatment and at 30, 60, 90, and 120 min after oral administration of the extracts with respective dose. To determine the baseline, each animal was tested before administration of drug/extracts. A cut off period of 10 s was maintained to avoid tail tissue damage. The results of the tail immersion test are expressed as a percentage of the maximal possible effect (%MPE), which was calculated using the following formula:

$$\%\text{MPE} = [(\text{Post drug latency} - \text{pre drug latency}) / (\text{Cut off period} - \text{pre drug latency})] \times 100$$

2.6 Anti-inflammatory activity

2.6.1 Carrageenan induced paw edema test

Anti-inflammatory activity of the extracts was evaluated by carrageenan-induced paw edema model [22]. Swiss albino mice (25-30g) of both sexes were divided into fourteen groups of six animals in each. The test groups received 50, 100 and 200 mg/kg body weight p.o. of the extract AEDFL, EEDFL, CEDFL and PEDFL. The reference group received ibuprofen (10 mg/kg p.o.) body weight while the control group received vehicle (1% tween 80 in water, p.o.). After 30 min, 100µl 1% carrageenan suspension in normal saline was injected into the sub-plantar tissue of the left hind paw of each animal. The paw volume was measured before and after carrageenan injection at 1, 2, 3 and 4 hrs using a Plethysmometer 7150 (Ugo Basile, Italy). The extant of reduction of paw volume revealed the ability of inflammation reduction. The percentage inhibition of inflammation was calculated by the following formula:

$$\% \text{ Inhibition} = [(V_0 - V_S)/V_0] \times 100$$

Where, V_0 is the average paw edema volume of the control group, V_S is average paw edema volume of the treatment and standard group.

2.7 CNS depression activity

2.7.1 Open field test

The effect of the aqueous, ethanol, chloroform and petroleum ether extracts on the spontaneous locomotor activity of the experimental animals was evaluated by the method, described by Gupta *et al*. [23]. Eighty four albino mice were divided into fourteen groups (*n* = 6). Vehicle (1% tween 80 in water, p.o.), diazepam (2 mg/kg, p.o.), AEDFL (50, 100 and 200 mg/kg, p.o.), EEDFL (50, 100 and 200 mg/kg, p.o.), CEDFL (50, 100 and 200 mg/kg, p.o.) and PEDFL (50, 100 and 200 mg/kg, p.o.) were administered to different groups (I- XIV) before 30minutes of experiment. The animals were placed on the floor of an open field (100 cm×100 cm×40 cm) divided into a series of squares of black and white color. The number of squares moved by each animal was counted for 5 min at 0, 30, 60, 90 and 120 min during the study period. Number of movement reduced by extracts revealed the extant of depressant, and the percentage inhibition of square movement was calculated at 120 min by the following formula:

$$\% \text{ Inhibition} = [(N_0-N_S)/N_0] \times 100$$

Where, N_0 is the average number of square traveled by control group, N_S is the average number of square traveled by treatment or standard group.

2.7.2 Hole cross test

The method described by Takagi *et al*. [24, 25], was applied for this study. According to this method, eighty four albino mice were divided into fourteen groups (*n* = 6). As like open field test, vehicle (1% tween 80 in water, p.o.), diazepam (2 mg/kg, p.o.), AEDFL (50, 100 and 200 mg/kg, p.o.), EEDFL (50, 100 and 200 mg/kg, p.o.), CEDFL (50, 100 and 200 mg/kg, p.o.) and PEDFL (50, 100 and 200 mg/kg, p.o.) were administered to the respective groups. Then gradually all animals were placed in a chamber of a box having a size of 30 × 20 × 14 cm where a partition was made at the middle of the cage. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. After the treatment, the total number of passages of each mouse through the hole from one chamber to other was counted for a period of 5 min on 0, 30, 60, 90 and 120 min during the study period. CNS depressant activity was assessed by the reduced number of passages of mice through the hole and percentage protection of movement was calculated at 120 min by the following formula:

$$\% \text{ Inhibition} = [(N_0-N_S)/N_0] \times 100$$

Where, N_0 is the average number of passage by control group, N_S is the average number of passage by treatment or standard group.

2.8 Statistical analysis

All the values were expressed as the mean ± SEM (Standard Error Mean) of three replicate experiments (n = 6 mice per group). The analyses were performed by using SPSS statistical package for WINDOWS (version 15.0; SPSS Inc, Chicago) and $P^b<0.01$, $P^a<0.001$ were considered to be statistically significant compared to vehicle control group. Statistical significance (p) calculated by ANOVA followed by Dunnett´s test.

3. Results

3.1 Analgesic activity

3.1.1 Acetic acid induced writhing method

The result in **Table 1** showed the effect of oral administration of AEDFL, EEDFL, CEDFL and PEDFL (50, 100, 200 mg/kg) in acetic acid-induced abdominal writhing test. All the extracts have reduced writhing-response significantly ($P^a < 0.001$) in dose dependant fashion. Morphine sulfate (5mg/kg) was used as standard drug that reduced writhing number to 5.39±1.52 and exhibited maximum inhibition (88.47%). PEDFL showed maximum analgesic effect among the extracts. It exhibited 44.98% (25.72±3.20), 64.86% (16.42±2.20) and 77.45% (10.54±1.43) inhibition of abdominal writhing at

50,100 and 200mg/kg, p.o. doses. AEDFL, EEDFL and CEDFL showed 57.75% (19.75±4.21), 66.73% (15.55±2.10) and 61.06% (18.20±2.10) at 200mg/kg dose respectively. The order of analgesic potential among the four extracts was PEDFL> EEDFL> CEDFL> AEDFL.

Table 1: Analgesic effect of *D. falcata* leaves extracts and morphine sulfate in acetic acid-induced writhing test.

Sample	Dose mg/kg,p.o.	Writhing number	Percentage inhibition of writhing
Control (vehicle)	0.1ml/mice	46.75±4.50	00
Morphine sulfate	10mg/kg	5.39±1.52[a]	88.47
AEDFL	50	37.25±4.18[a]	20.32
	100	27.60±3.40[a]	40.96
	200	19.75±4.21[a]	57.75
EEDFL	50	30.32±3.45[a]	35.14
	100	21.30±3.23[a]	54.43
	200	15.55±2.10[a]	66.73
CEDFL	50	34.54±3.45[a]	26.11
	100	24.12±4.50[a]	48.40
	200	18.20±2.10[a]	61.06
PEDFL	50	25.72±3.20[a]	44.98
	100	16.42±2.20[a]	64.86
	200	10.54±1.43[a]	77.45

Each value is presented as mean ± SEM (n = 6); $p^a < 0.001$ compared with the control group. ANOVA followed by Dunnett's test is done in SPSS version 15.0

3.1.2 Tail immersion method

In the radiant heat tail-immersion test, the leaves extracts prolonged the heat stress tolerance capacity of treated mice. The tail-withdrawal reflex time of mice by the hot water-induced pain was significant after administration of AEDFL, EEDFL, CEDFL and PEDFL. All the extracts at 50, 100 and 200 mg/kg doses showed statistically significant ($p^b < 0.01$, $p^a < 0.001$) elongation of reaction time with the increase order of dose compared with control group (vehicle). Morphine sulfate (5mg/kg,p.o.) was used as standard drug which showed 26.69% (4.37±0.28), 45.05 % (5.78±0.10), 51.17% (6.25±0.30) and 94.53% (9.58±0.45) MPE at 30 min, 60 min, 90 min and 120 min respectively. PEDFL showed highest and AEDFL showed lowest effects among the extracts. At 200mg/kg dose, PEDFL exhibited 24.20% (4.02±0.97), 49.04% (5.98±0.66), 60.45% (6.88±0.61) and 82.12% (8.59±0.24) MPE; EEDFL exhibited 17.77% (4.08±0.47), 38.81% (5.59±0.91), 52.36% (6.57±0.65) and 75.00% MPE (8.20±0.6); CEDFL exhibited 25.40% (4.07±0.19), 30.50% (4.47±0.73), 49.87% (6.52±0.52), 57.23% (6.6±0.08)MPE; AEDFL showed 13.92% (3.26±0.62), 27.45% (4.32±0.4), 45.40% (5.725±0.4) and 51.53% MPE (6.205±0.41) at 30

min, 60 min, 90 min and 120 respectively. The order of tail withdrawal reflex time was PEDFL> EEDFL> CEDFL> AEDFL (**Table 2**).

Table 2: Analgesic effect of *D. falcata* leaves extracts and morphine sulfate in tail immersion test.

Sample	Dose mg/kg.p.o.	Response time (s) (%MPE)				
		0 min	30 min	60 min	90 min	120 min
Vehicle	0.1ml/mice	2. 13±0.19	2.10±0.05	2.24 ±0.29	2.5±0.40	2.80±0.09
Morphine sulfate	5	2.32±0.35	4.37±0.28[a] (26.69)	5.78±0.10[a] (45.05)	6.25±0.30[a] (51.17)	9.58±0.45[a] (94.53)
	50	2.25±0.23	2.37±0.50[b] (1.54)	3.20±0.3[a] (12.25)	4.19±0.24[a] (25.03)	4.49±0.33[a] (28.90)
AEDFL	100	2.29±0.2	3.07±0.52[a] (10.11)	3.42±0.58[a] (14.65)	4.8±0.2[a] (32.55)	5.47±0.4[a] (41.24)
	200	2.17±0.42	3.26±0.62[a] (13.92)	4.32±0.4[a] (27.45	5.725±0.4[a] (45.40)	6.205±0.41[a] (51.53)
	50	2.27±0.1	2.85±0.05[b] (7.50)	3.8±0.3[a] (19.79)	4.41±0.50[a] (27.68)	5.22±0.25[a] 38.18)
EEDFL	100	2.37±0.2	3.42±0.42[a] (13.76)	4.24±0.2[a] (24.50)	5.19±0.22[a] (36.95)	6.17±0.11[a] (49.80)
	200	2.8±0.36	4.08±0.47[a] (17.77)	5.59±0.91[a] (38.81)	6.57±0.65[a] (52.36)	8.20±0.6[a] (75.00)
	50	2.25±0.1	2.85±0.05[b] (7.74)	3.05±0.42[a] (10.32)	4.17±0.2[a] (24.77)	4.62±0.3[a] (30.58)
CEDFL	100	2.34±0.45	3.14±0.34[a] (10.44)	3.99±0.3[a] (21.54)	5.00±0.42[a] (34.72)	5.79±0.54[a] (45.03)
	200	2.05±0.42	4.07±0.19[a] (25.40)	4.475±0.73[a] (30.50)	6.525±0.52[a] (49.87)	6.6±0.08[a] (57.23)
	50	2.03±0.35	2.74±0.27[b] (8.90)	3.49±0.02[a] (18.31)	4.19±0.50[a] (27.10)	5.30±0.56[a] (41.02)
PEDFL	100	2.07±0.35	3.8±0.25[a] 21.81)	4.47±0.03[a] (30.26)	5.68±0.1[a] (45.52)	6.59±0.52[a] (56.99)
	200	2.11±0.61	4.02±0.97[a] (24.20)	5.98±0.66[a] (49.04)	6.88±0.61[a] (60.45)	8.59±0.24[a] (82.12)

Each value is presented as mean ± SEM (n = 6); $p^b < 0.01$, $p^a < 0.001$ compared with the vehicle control group. ANOVA followed by Dunnett's test is done in SPSS version 15.0

3.2 Anti-inflammatory activity

Table 3 represents the anti- inflammatory activity of AEDFL, EEDFL, CEDFL and PEDFL. All the extracts showed statistically significant ($p^b < 0.01$, $p^a < 0.001$) anti-inflammatory activity as dose dependant manner. Data are presented as volume (mL) of paw edema and inhibition (%) of it (paw edema volume). Ibuprofen (5mg/kg) was used as standard drug which showed maximum inhibition (96.34%) of paw volume at 4 hour of observation. PEDFL exhibited 18.98% (0.64±0.01), 25.31% (0.59±0.01) and 36.70% (0.5±0.03) inhibition at 1 h and it (inhibition) continued up to the fifth observation (4 h) where this fraction produced 70.73% (0.24±0.03), 81.70% (0.15±0.01) and 90.24% (0.08±0.02) inhibition of paw edema volume at 50, 100, 200mg/kg dose respectively.

This fraction exhibited highest anti-inflammatory potential among the four extracts. After oral administration of 200mg/kg dose EEDFL showed 27.84%, 56.25%, 76.54% and 87.80% inhibition; AEDFL exhibited 26.58%, 48.75%, 71.60% and 80.48% inhibition, as well as CEDFL showed 25.31%, 46.25%, 69.13% and 76.82% inhibition of paw volume at 1 h, 2 h, 3 h and 4 h respectively. The order of paw volume inhibition was PEDFL> EEDFL> AEDFL> CEDFL.

Table 3: Effect of p.o. administration of *D. falcata* leaves extracts on carrageenan-induced paw edema.

Sample	Dose mg/kg	Mean edema volume in mL (Percentage inhibition)				
		0 h	1 h	2 h	3 h	4 h
Control	0.1ml	0.77±0.03	0.79±0.02	0.80±0.02	0.81±0.01	0.82±0.03
Ibuprofen	10	0.79±0.01	0.46±0.01^a(41.72)	0.26±0.02^a(67.50)	0.05±0.03^a(93.82)	0.03±0.01^a (96.34)
	50	0.79±0.02	0.69±0.01^b(12.65)	0.61±0.01^b(23.75)	0.49±0.01^a(39.50)	0.3±0.01^a (63.41)
AEDFL	100	0.78±0.01	0.64±0.01^b(18.98)	0.49±0.02^a(38.75)	0.32±0.03^a(60.49)	0.29±0.01^a (64.63)
	200	0.8±0.01	0.58±0.02^a(26.58)	0.41±0.03^a(48.75)	0.23±0.01^a(71.60)	0.16±0.01^a (80.48)
	50	0.81±0.03	0.65±0.02^b(15.18)	0.58±0.01^a(27.50)	0.39±0.01^a(51.85)	0.27±0.03^a(67.07)
EEDFL	100	0.79±0.03	0.6±0.01^b (24.05)	0.47±0.02^a(41.25)	0.29±0.01^a(64.19)	0.18±0.01^a (78.04)
	200	0.75±0.01	0.57±0.02^a(27.84)	0.35±0.01^a(56.25)	0.19±0.02^a(76.54)	0.10±0.02^a (87.80)
	50	0.82±0.02	0.73±0.03(7.59)	0.63±0.01^b(21.25)	0.51±0.01^a(37.03)	0.35±0.01^a (57.31)
CEDFL	100	0.8±0.01	0.67±0.02^b(15.18)	0.53±0.01^a(33.75)	0.37±0.03^a(54.32)	0.32±0.02^a(60.97)
	200	0.78±0.02	0.59±0.02^a(25.31)	0.43±0.03^a(46.25)	0.25±0.01^a(69.13)	0.19±0.02^a(76.82)
	50	0.8±0.01	0.64±0.01 (18.98)	0.5±0.01^a (37.5)	0.36±0.01^a(55.55)	0.24±0.03^a(70.73)
PEDFL	100	0.76±0.03	0.59±0.01^a(25.31)	0.43±0.02^a(46.25)	0.27±0.03^a(66.66)	0.15±0.01^a (81.70)
	200	0.82±0.02	0.5±0.03^a(36.70)	0.3±0.03^a(62.50)	0.15±0.01^a(81.48)	0.08±0.02^a(90.24)

Each value is presented as mean ± SEM (n = 6); $p^b < 0.01$, $p^a < 0.001$ compared with the vehicle control group. ANOVA followed by Dunnett's test is done in SPSS version 15.0

3.3 CNS depression activity

3.3.1 Open field test

Aqueous, ethanol, chloroform and petroleum ether extracts (AEDFL, EEDFL, CEDFL and PEDFL) of the *D. falcata* leaves exhibited statistically significant ($p^a < 0.001$) reduction of movements (square of open field) of the test mice for respective group compared with vehicle control group. The results were significant from second observation (30 min) and continued up to fifth observation (120 min) in dose dependant manner (200mg>100>50mg). Among the four extracts, EEDFL (200mg) showed maximum effect which is similar with the effect of standard drug diazepam (2mg). EEDFL (200mg) showed 174.67±8.70 movements at 0 min of treatment. 30 min after oral administration, sharp reduction ($p^a < 0.001$) of locomotion (43.14±6.26) was observed by the extract, and in fifth observation (120min) movement number was only 4.09±3.61 where inhibition of movement was 97.33%. On

the other hand, AEDFL (200mg) showed least effect. It showed 175.35±9.33, 110.23±6.3 and 16.42±4.33[a] movements in 0 min, 30 min and 120 min respectively, and showed 89.30% inhibition after 120 min. Standard drug diazepam (2mg) exhibited 178.21±7.32 movement at 0 min. It showed 67.29±8.42 and 4.39±0.55 movements in 30 min and 120 min respectively where inhibition of movement was 97.14% (120min). The order of CNS depressant effect among the four extracts was EEDFL> PEDFL> CEDFL> AEDFL (**Table 4**).

Table 4: CNS depressant activity of different fractions of *D. falcata* leaves by open field test in mice.

Sample	Dose mg/kg .p.o.	Number of movements					% Inhibition of movements after 120 min
		0 min	30 min	60 min	90 min	120 min	
Control (vehicle)	0.1ml	180.45±7.63	176.67±6.11	173.82±6.83	167.72±5.58	153.58±5.37	--
Diazepam	2	178.21±7.32	67.29±8.42[a]	23.45±3.02[a]	8.23±2.12[a]	4.39±0.55[a]	97.14
	50	173.63±9.12	137.68±6.30[a]	57.22±8.07[a]	49.56±6.23[a]	37.22±4.34[a]	75.75
AEDFL	100	169.45±8.40	125.20±8.2[a]	43.32±8.42[a]	32.37±7.47[a]	21.30±5.14[a]	86.13
	200	175.35±9.33	110.23±6.3[a]	38.12±7.02[a]	18.04±5.43[a]	16.42±4.33[a]	89.30
	50	177.20±9.50	77.35±8.30[a]	40.27±6.35[a]	27.56±5.30[a]	17.65±3.10[a]	88.50
EEDFL	100	170.27±8.77	58.87±6.33[a]	32.28±5.55[a]	16.75±6.32[a]	9.25±2.12[a]	93.97
	200	174.67±8.70	43.14±6.26[a]	21.49±4.50[a]	12.29±3.34[a]	4.09±3.61[a]	97.33
	50	179.31±9.80	88.41±9.93[a]	59.50±7.57[a]	37.45±6.43[a]	22.10±3.13[a]	85.61
CEDFL	100	173.29±7.50	71.30±7.83[a]	47.54±6.50[a]	26.43±5.01[a]	13.39±2.56[a]	91.28
	200	182.34±7.23	49.20±6.21[a]	35.5±3.87[a]	22.43±4.01[a]	10.23±2.33[a]	93.33
	50	183.88±9.60	79.27±8.23[a]	50.20±7.56[a]	41.08±6.78[a]	23.07±3.10[a]	84.97
PEDFL	100	183.45±5.54	73.38±5.22[a]	39.38±3.54[a]	25.84±3.20[a]	11.36±2.02[a]	92.60
	200	170.59±5.29	45.30±5.69[a]	27.40±4.06[a]	17.11±3.13[a]	6.79±2.38[a]	95.57

Each value is presented as the mean ± SEM (n = 6); $p^a < 0.001$ compared with the vehicle control group. ANOVA followed by Dunnett's test is done in SPSS version 15.0

3.3.2 Hole-cross test

In the hole-cross test, the number of passes of tested mice through the hole was reduced significantly ($p^b < 0.01$, $p^a < 0.001$) by AEDFL, EEDFL, CEDFL and PEDFL compared with control group. Except 50mg of AEDFL, all of

the fractions and crude ethanol extracts exhibited CNS depressant potential from second observation (30 min) to fifth observation (120 min) in dose dependant fashion (200mg>100>50mg). Percentage inhibition of movements was calculated after 120 min of drug and extracts treatment in respect to control (vehicle) group. In case of diazepam treated mice, number of movements was 15.20±1.55 in 0 min which sharply reduced to 4.28±1.12 in 30 min. This reduction of movements was continued up to 120 min where inhibition of movements was 91.94%. Among the four extracts, crude ethanol portion showed maximum inhibition (85.91%) whereas aqueous extract exhibited minimum inhibition (71.40%) at 200mg/kg dose. After 30 min of oral administration of AEDFL, EEDFL, CEDFL and PEDFL, number of movements were 10.13±2.15, 5.28±1.92, 7.10±0.30 and 6.20±1.0 which reached to 4.08±1.55, 2.01±0.22, 3.39±0.53 and 2.59±1.54 respectively after 120 min at 200mg dose. The order of CNS depressant activity was EEDFL> PEDFL> CEDFL> AEDFL (**Table 5**).

Table 5: CNS depressant activity of different fractions of *D. falcata* leaves by hole cross test in mice.

Sample	Dose mg/kg. p.o.	Number of movements					% Inhibition of movements after 120 min
		0 min	30 min	60 min	90 min	120 min	
Control	0.1ml	16.37±1.44	15.56±1.50	14.71±2.32	14.10±2.62	14.27±1.10	--
Diazepam	2	15.20±1.55	4.28±1.12[a]	2.34±0.58[a]	1.95±0.22[a]	1.15±0.01[a]	91.94
AEDFL	50	17.35±3.42	15.30±3.15	11.15±2.50[a]	9.10±1.15[a]	8.20±2.12[a]	42.53
	100	19.45±1.50	13.33±2.24[b]	9.45±1.50[a]	6.5±1.02[a]	5.89±1.76[a]	58.72
	200	18.5±1.55	11.45±2.10[a]	6.5±0.50[a]	5.1±0.43[a]	4.08±1.55[a]	71.40
EEDFL	50	15.5±1.11	10.13±2.15[a]	6.17±1.10[a]	5.75±1.25[a]	4.57±0.55[a]	67.97
	100	16.5±1.11	7.39±1.45[a]	5.47±0.80[a]	5.15±1.02[a]	3.39±0.32[a]	76.24
	200	14.25±1.50	5.28±1.92[a]	3.39±0.50[a]	3.10±0.10[a]	2.01±0.22[a]	85.91
CEDFL	50	15.5±2.32	12.12±2.51[a]	10.2±1.64[a]	9.54±2.10[a]	7.25±1.23[a]	49.19
	100	14.5±1.32	9.0±1.55[a]	7.0±0.52[a]	6.81±1.01[a]	5.39±1.52[a]	62.22
	200	15.68±1.40	7.10±0.30[a]	5.5±0.02[a]	3.76±0.01[a]	3.39±0.53[a]	76.24
PEDFL	50	19.0±3.36	11.21±2.33[a]	9.18±2.12[a]	7.30±1.60[a]	6.01±1.27[a]	57.88
	100	18.0±1.57	8.25±1.31[a]	6.78±1.42a	5.39±1.52[a]	4.09±1.02[a]	71.33
	200	20.5±1.88	6.20±1.0[a]	4.5±1.50a	3.0±0.34[a]	2.59±1.54[a]	81.85

Each value is presented as mean ± SEM (n = 6); $p^b < 0.01$, $p^a < 0.001$ compared with the vehicle control group. ANOVA followed by Dunnett's test is done in SPSS version 15.0

4. Discussion

Results of the present study showed that the *D. falcata* leaves extracts have marked analgesic, anti-inflammatory and CNS depressant activities that have disclosed the effectiveness of the plant in folk medicine. Pain and inflammation are associated with the pathophysiology of various clinical conditions such as arthritis, cancer, vascular diseases, asthma, multiple sclerosis, colitis, inflammatory bowel disease and atherosclerosis. Many natural products are used in traditional medical systems to relieve the symptoms from pain and inflammation [26].

Acetic acid-induced writhing and heat-induced tail immersion tests are simple, reliable and well recommended protocols in evaluating medicinal agents for their analgesic property. Analgesics can act both on peripheral or central nervous system. Peripherally acting analgesics act by blocking the generation of impulses at chemoreceptors site of pain, while centrally acting analgesics not only raise the threshold for pain, but also alter the physiological response to pain and suppress the patient′s anxiety and apprehension [27]. Abdominal writhing is associated with local peritoneal receptor. This behavior results from the activation of acid-sensitive ion channels (ASICs) and transient receptor potential vanilloid-1 (TRPV1) localized in afferent primary fibers [28]. Acetic acid causes inflammatory pain by inducing capillary permeability and liberating endogenous substances that excite pain nerve ending [27], where the pain sensation is generated by producing localized inflammatory response due to release of free arachidonic acid from tissue phospholipids via cyclo-oxygenase (COX), and producing prostaglandin specifically PGE2 and PGF2α. The level of lipoxygenase products may also increase in peritoneal fluids [29]. More specifically, acetic acid injection induces a release of TNF-α, interleukin-1β (IL-1β) and interleukin-8(IL-8) by resident peritoneal macrophages, mast cells, prostanoids and bradykinin [28].These prostaglandin and lipoxygenase products are responsible for inflammation and pain. Substance(s) inhibiting the writhing response will have analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition [30]. Non steroidal anti-inflammatory drugs (NSAIDs) can inhibit COX in peripheral tissues and therefore interfere with the mechanism of transduction of primary afferent nociceptors. The mechanism of analgesic activity of *D. falcata* leaves extracts could be probably due to the blockade of the effect or the release of endogenous substances that excite pain nerve endings similar to that of Morphine sulfate and NSAIDs. Thus, the statistically significant ($p^a < 0.001$) reduction in the number of writhing indicates that AEDFL, EEDFL, CEDFL and PEDFL might exert analgesic activity by inhibition of prostaglandin synthesis or by action on prostaglandin [27] (**Table 1)**.

Tail immersion model is considered as an acute pain model. The tail-withdrawal response of mice is predominantly considered to be selective for centrally acting analgesics, implicating supraspinal analgesic pathways which is similar to the action of opioid agonists [31].The significant increase ($p^b < 0.01$, $p^a < 0.001$) of tail-withdrawal time by the extracts suggests the involvement of central mechanisms of their analgesic effects (**Table 2**). Tail immersion monitors a spinal reflex involving μ_2- and δ-opioid receptors. Therefore, the results of the study indicated that the central analgesic effect of *D. falcata* may be prominent on μ-opioid receptors [31,32].

Carrageenan-induced paw edema is a convenient and well established animal model for the evaluation of anti-inflammatory effect of any natural product or synthetic chemical compound. The edema formed by carrageenan in paw has two phases. The initial phase (1-2 h) is predominately a non-phagocytic edema followed by a second phase (2–5 h) with increased edema formation that remained up to 5 h. The inflammatory stimuli of carageenan induce the release of inflammatory mediators such as histamine, serotonin and bradykinin on vascular permeability in the initial phase. The late phase or second phase edema is attributed to the release of prostaglandin, bradykinin, protease, lysosome-like substances, growth factors and neurogenic factors [33-35]. The production of prostaglandins

such as PGE2 and/or PGI2 is facilitated through COX-2 activity in inflammatory response [36]. As described by Loram *et al*. [37], inflammation induced by carrageenan involves cell migration, plasma exudation and production of mediators, such as interleukin (IL)-1β, IL-6 and tumor necrosis factor-alpha (TNF-α), a cytokine-induced neutrophil. These mediators are able to recruit leukocytes in several experimental models [38]. In addition, there are evidences showing that inflammation and/or tissue damage can induce the release of glutamate from primary afferents, enhancing the activation of excitatory amino acid receptors in the peripheral tissue. The activation of glutamate, *N*-Methyl-D-aspartate (NMDA), receptors causes the secretion of inflammatory mediators such as TNF-a and IL-1b that in turn can sensitize peripheral nerve endings and activate the immune response [11]. Recently, it has also been reported that NO was produced by isoform of nitric oxide synthase (iNOS) which is involved in the inflammatory response on paw edema. The anti-inflammatory drugs such as aspirin and ibuprofan have been found to inhibit prostaglandin production that is the main reason of anti-inflammatory action in carrageenan-induced paw edema [36,39]. All the extracts (AEDFL, EEDFL, CEDFL and PEDFL) significantly ($p^b < 0.01$, $p^a < 0.001$) reduced paw edema volume in dose dependant manner **(Table 3).** From the results, it is suggested that the anti-inflammatory effect of the extracts on carrageenan-induced paw edema may act via the inhibition of NO production, along with the inhibitory activity of COX-2 [36].

CNS depressant effect of *D. falcata* leaves was studied using two neuropharmacological models (Open field and Hole cross). The results of the study provided evidence that the extracts reduced locomotor activity confirming its CNS depressant potential. Locomotor activity is considered as an index of alertness and a reduction of it is an indicative of sedative or CNS depressant activity [40]. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system [41], which is also involved in other physiological functions related to behavior and in various psychological and neurological disorders such as epilepsy, anxiety, depression, parkinson syndrome, and alzheimer's disease [42]. Diverse drugs such as anxiolytic, muscle relaxant and sedative-hypnotic exhibit their action via GABA. These drugs might modify the GABA system, induce anxiolysis or hypnosis in animals, at the level of the synthesis of it by potentiating the GABA-mediated postsynaptic inhibition through an allosteric modification of GABA receptors, and by direct increase in chloride conductance or indirectly by potentiating GABA-induced chloride conductance with simultaneous depression of voltage activated Ca^{++} currents like barbiturates [43].Therefore, it is predictable that extracts of *D. falcata* leaves may act by potentiating GABAergic inhibition in the CNS via membrane hyper-polarization leading to a reduction in the firing rate of critical neurons in the brain or it may be due to direct activation of GABA receptors by the extracts [41]. It may also be due to enhanced affinity for GABA or an increase in the duration of the GABA-gated channel opening [25]. In addition, the study on locomotor activity, as measured by hole cross and open field tests, showed that all doses of the extracts significantly ($p^b < 0.01$, $p^a < 0.001$) decreased the frequency and the amplitude of movements in dose dependant manner from 2nd observation (30 min) and continued up to the 5th observation (120 min) period (**Table 4 and 5**). Since the locomotor activity is a measure of the level of excitability of the CNS, this decrease in spontaneous motor activity could be attributed to the depressant effect of the plant extracts with the presence of compound(s) having CNS depressant potential [44].

It is well established that alkaloids, flavonoids and tannins are potent analgesic compounds [45]. Flavanoids exert their effect through inhibition of prostaglandin synthatase [46]. Daniela T. *et al.* showed the analgesic potential of a triterpene by inhibiting acetic acid-induced viscerosomatic nociception and cellular migration. In addition, it exhibited anti-inflammatory effect by reducing leukocyte migration and carrageenan-induced cytokine release, namely tumor necrosis factor a (TNF-a) and interleukin-1b (IL-1b) [11]. Various flavonoid derivatives including quercetin have

been reported to inhibit the activity of arachidonic acid metabolizing enzymes (phospholipase A2, cycloxygenase and lipoxygenase), and some flavonoids have proved to exert anti-inflammatory actions by modulation of proinflammatory gene expression, such as COX-2, i-NOS and several pivotal cytokines [36]. Steroidal saponins are significant inhibitor of inflammatory cytokines by activated macrophages [47]. Oxyresveratrol a potent antioxidant has been reported to exert anti-inflammatory activity through inhibition of iNOS/NO production, PGE2 synthesis and NFκB activation [48]. Epicathechin and (+) catechins are reported to have many biological effects including anti-inflammatory response [33]. Flavone and isoflavone suppresses inflammatory response by inhibiting NF-κB [49]. Control of different cellular processes such as inflammation or apoptosis are modulated by polyphenols [50]. Tannins are known to be useful for the prevention of cancer as well as treatment of inflamed or ulcerated tissues [45].

Research has shown that plants containing flavonoids, saponins and tannins are useful for the treatment of many CNS disorders [40]. Earlier investigation of the phytoconstituents of medicinal plants suggests that many flavonoids and neuroactive steroids are ligands for $GABA_A$ receptors in the central nervous system which suggests that they can act as benzodiazepine-like agents [40]. It has also been reported that some flavanoids exhibit high affinity binding to the benzodiazepine site of $GABA_A$ receptors. Therefore, the CNS-depressant activity may be due to the phytoconstituents present in the different extracts of the *D. falcata* leaves. Raihan M.O. *et al.* reported that β-sitosterol act as $GABA_A$ agonists [51]. Several plants have been reported to have CNS depressant activity due to the presence of triterpenoids, saponins and flavonoids. Triterpenoid saponins are reported to have agonistic/facilitatory activities at $GABA_A$ receptor complex [43,44,52]. This is supported by their behavioral effects in animal models of CNS depression and anxiety.

Literature review revealed the presence of tannins, flavonoids, phenolic compounds, phytosterols, triterpenes, quercetin, β-sitosterol, triterpenes etc. bioactive compounds in the extracts of *D. falcata* leaves which are believed to be responsible for analgesic, anti-inflammatory and CNS-depressant effects of the plant. There is no strict evidence about which substances are exactly responsible for these effects. However, further studies are necessary to find out the key compound(s) responsible for these effects, and to identify the mechanism of action and the pharmacodynamics of these effects. More research and clinical trials could help to identify agents with more potent CNS depressant effects.

Conclusion

In conclusion, the results presented in this study revealed that crude ethanol extract and aqueous, chloroform as well as petroleum ether fractions of *D. falcate* (Linn.) leaves possess significant analgesic, anti-inflammatory and CNS depressant potentials. Petroleum ether (PEDFL) and ethanol extracts (EEDFL) have shown highly significant activities in mice than chloroform (CEDFL) and aqueous (AEDFL) extracts. These results further support the traditional use of this plant in medicine. The potential of the extracts as analgesic, anti-inflammatory and CNS depressant activities may be due to the presence of phytoconstituents like flavonoids, tannins, phenolics etc. However, more detail phytochemical analysis is necessary to isolate and characterize the active compounds which are responsible for these activities, and that will give a way to draw the proper mechanisms of action of these activities.

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