PharmacologyOnLine April 30, 2016

Archives • 2016 • vol.1 • 12-21

ANTINOCICEPTIVE, ANTI-INFLAMMATORY AND CENTRAL NERVOUS SYSTEM DEPRESSANT EFFECTS OF CRUDE ETHANOL EXTRACT AND ITS AQUEOUS, CHLOROFORM AND PETROLEUM ETHER FRACTIONS OF *DENDROPHTHOE FALCATA* (LINN.) STEM

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Abstract

Dendrophthoe falcata (Linn.) was a parasitic plant widely used to treat various ailments as traditional medicine. The objective of the present study was to investigate analgesic, anti-inflammatory and central nervous system (CNS) depressant effects of aqueous, ethanol, chloroform and petroleum ether extracts of *D. falcata* stem (ADFS, EDFS, CDFS and PDFS) on experimental mice. The analgesic activity of the extracts was determined by tail immersion and acetic acid induced writhing methods. Carrageenan-induced paw edema model was applied to find out anti-inflammatory potential. The CNS-depressant effect of the extracts were evaluated by using open field and hole cross tests. All the extracts had exhibited significant ($P^b<0.01$, $P^a<0.001$) analgesic, anti-inflammatory and CNS-depressant effects at dose dependent manner. Chloroform fraction showed maximum analgesic effect (77.58% 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) had shown by the chloroform fraction at 200mg/kg dose. On the other hand, ethanol extract had 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 120min of oral administration of the extract at 200mg/kg dose.

Key words: Dendrophthoe falcata;Analgesic; Anti-inflammatory; CNS-depressant effect.

Introduction

Dendrophthoe falcata (Linn.) belongs to the Loranthaceace family. It is commonly known as 'Vanda' in the Indian Ayurvedic System of Medicine [1]. It is a perennial climbing woody hemiparasitic [2], and evergreen shrubthat is frequently observed on different host plants. Bark of itis grey, leaves are thick, coriaceous, usually opposite 7.5 to 18 by 2-10 cm in shape, flower of the plant are stout, unilateral racemes often two from an axil pedicle. It comprises of 20 species where about 7 species are found in Indian subcontinent [3], that are indigenous to India, Sirilanka, China, Australia, Bangladesh, Malayasia, Myanmar, Thailand and Indo-china [1, 2]. Entire plant is extensively used 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. Preliminary phytochemical screening revealed the presence of carbohydrates, phytosterols, flavonoids, glycosides and phenolic compounds [2]. It is reported to contain biologically active substances such as quercetin, tannins, *β*-sitosterol, *β*-amyrin, oleanolic acid [3]. 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)* [1].

Nociception is a noxious stimulus that mediates through neural path. Mechanical, thermal, or chemical stimuli, for example, activate primary afferent nociceptors in the peripheral and central nervous system. After any stimulation, a nociceptor transmits signal to the brain via the spinal cord which causes perception of pain [4]. It not only reduces normal activities of the sufferer but also negatively impact quality of his/her life [5]. Inflammatory responses in the peripheral and central nervous systems play key roles in the development and persistence of many pathological pains [4]. It is a type of protective response of a variety of factors like physical and chemical factors, immunological factors, microbial infections, and tissue damage. The persistence of a process for remission of diseases may lead to various diseases that are associated with chronic inflammation like arthritis, atherosclerosis, cancer etc. Anxiety is associated with 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 [6]. So, there is a need to develop new analgesic and anti-inflammatory drugs as the currently available drugs are associated with severe side effect and many patients are resistance to these [7]. Medicinal plants have been used in traditional health care systems since prehistoric times and are still the most important health care source for the most of the world's population [8]. Medicinal plants are believed to be cost-effective and harmless source of novel biochemical constituents with strong therapeutic properties [9]. Therefore, there is a major research emphasis on discovering plants with analgesic, anti-inflammatory and CNS-depressant potential that may be safe and cost effective for the treatment of diseases associated with pain, inflammation and anxiety.

The present study was undertaken to investigate analgesic, anti-inflammatory and CNS depressant activities of the *D. falcate* stem extracts that may unveil the rationality of use of the plant as traditional medicine and potentiality of it in the herbal medicine.

Methods

Plant materials

For the investigation, *Dendrophthoe falcata* (Linn.)stem, 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. DACB-39432. The collected stem 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.

Extract preparation

Approximately, 800g of powdered material was placed in a clean, flat-bottomed glass container and soaked in ethanol. The container with their contents were sealed and kept for 7 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 extracts (20g). Ethanol extract was divided into two portions. One portion (4g) was poured into a glass vial to be tested

as crude ethanol extract, whereas the second portion (16g) was dissolved in 100 mL of ethanol, and partitioned successively with chloroform and petroleum ether and water. The fractions were then concentrated using a rotary evaporator to obtain chloroform (yield weight 4.5g), petroleum ether (yield weight 6.50g), and aqueous extracts (5.0). This process rendered a gummy concentrated reddish black color. The gummy extracts were transferred to a closed container for further use and storage.

Drugs and chemicals

Ethanol, chloroform, petroleum ether, acetic acid and Tween-80 were purchased 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.

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, Bio-safety and Bio-security 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 [10].

Analgesic activity

Acetic acid-induced writhing test

The analgesic activity of the extracts was studied by using acetic acid-induced writhing model in Swiss albino mice, described by Koster R. *et al.*[11]. The animals were divided into fourteen groups (n=6).Group I animals received vehicle (1% tween 80 in water, p.o.), group II received morphine sulfate at10 mg/kg body weight while animals of groups III-XIV received aqueous, ethanol, chloroform and petroleum ether extracts of the *D. falcata* stem (ADFS, EDFS, CDFS and PDFS).

Group III, IVand V received ADPS; group VI, VII and VIII received EDFS; group IX, X and XI received CDFS and group XII, XIII and XIV received PDFS at a dose of 50,100,200 mg/kg, b.w (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 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

Tail immersion test

Tail immersion test was done by the method described by Toma W. *et al.* [12]. This test is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail-withdrawal reflex. According to the method, vehicle (1% tween 80 in water, p.o.), morphine sulfate (10mg/kg, p.o.), ADFS, EDFS, CDFS and PDFS were administered to the mice of respective groups30 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 during drug treatment (0 min) and at 30, 60, 90, and 120 min of extract administration. 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 by using the following formula:

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

Anti-inflammatory activity

Carrageenan induced paw edema test

Anti-inflammatory activity of the extracts was evaluated by carrageenan-induced paw edema model [13]. 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 ADFS, EDFS, CDFS and PDFS. The reference group received

ibuprofen (10 mg/kg p.o.) 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 4h 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.

CNS depression activity

Open field test

The CNS depression effect of the extracts was evaluated by spontaneous locomotor activity of experimental mice which was described by Gupta *et al*. [14]. Eighty four albino mice were divided into fourteen groups (*n* = 6). Before 30min of the experiment, vehicle (1% tween 80 in water, p.o.),diazepam (2 mg/kg, p.o.), ADFS (50, 100 and 200 mg/kg, p.o.),EDFS (50, 100 and 200 mg/kg, p.o.), CDFS (50, 100 and 200 mg/kg, p.o.) and PDFS(50, 100 and 200 mg/kg, p.o.) were administered to respective group (I-XIV).The animals were placed on the floor of an open field (100 cm×100 cm×40 cm) that was 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.

Hole cross test

This test was performed by a method which was described by Takagi *et al*. [15]. According to the 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.), ADFS (50, 100 and 200 mg/kg, p.o.), EDFS (50, 100 and 200 mg/kg, p.o.), CDFS (50, 100 and 200 mg/kg, p.o.) and PDFS (50, 100 and 200 mg/kg, p.o.) were administered to the respective group. 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 at 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.

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.

Results

Analgesic activity

Acetic acid induced writhing method

All the extracts had reduced writhing response significantly ($P^a<0.001$) in dose dependant fashion. Standard drug morphine sulfate (10mg/kg) showed maximum (89.15%) inhibition followed by CDPS which showed 77.58% inhibition at 200mg/kg,b.w. The order of writhing inhibition of the extracts and the standard drug was MS>CDPS> PDPS>EDPS>ADPS. (**Table 1)**

Tail immersion method

In the radiant heat tail-immersion test, the tail-withdrawal reflex time of the mice was significantly ($p^b< 0.01$ and $p^a< 0.001$) elongated as dose dependent manner after administration of all the extracts. CDFS, among the extracts, had exhibited potent effect. MPE of it was 32.55%, 53.96%,

61.32%, and 79.42% at 30min, 60min, 90min and 120min, respectively at 200mg/ml dose. Order of tail withdrawal reflex time is CDFS> EDFS> PDFS> ADFS. **(Table 2)**

Anti-inflammatory activity

Paw edema volume (mL) of mice in vehicle control group was a steady level during the study period. But administration of ibuprofen or extracts significantly had reduced the edema volume according to dose size. Among the four extracts, CDFS exhibited maximum effect, and inhibition of edema volume was 43.75%, 64.55%, 83.33% and 91.02% at 1h, 2h, 3h and 4h, respectively. Besides, ADFS had shown lowest effect with 20.00%, 45.56%, 60.25% and 69.23% of edema volume at the 1h, 2h, 3h and 4h of the study period. Order of the edema volume inhibition was Ibuprofen>CDFS>PDFS>>EDFS> ADFS. **(Table 3)**

CNS depression activity

Open field test

In this test, movement of mice, compared with control group, was reduced significantly (P^a<0.001) after orally administration of the diazepam and extracts. The effect was significant from second observation (30 min) and continued up to the fifth observation (120 min) in dose dependent manner. At the fifth observation diazepam had shown 96.63% inhibition at 2mg/kg b.w. ADFS, EDFS, CDFS and PDFS showed71.09%, 87.85%, 84.74 % and 92.80% at a dose of200mg/kg, b.w, respectively. The order of movement inhibition of the extracts was Diazepam> PDFS>EDFS> CDFS> ADFS. (**Table 4)**

Hole-cross test

Here, number of passes of the diazepam and extracttreated mice through the hole, compared with the control mice, was reduced significantly at dose dependent fashion. Diazepam and all the extracts had shown significant reduction from second (30min) to fifth (120min) observation. The standard drug diazepam (2mg/kg,b.w.) gave 90.56% inhibition, and ADFS, EDFS, CDFS and PDFS (200mg/kg, b.w.) had shown 55.17%, 75.53%, 65.25% and 86.59% inhibition at the fifth (120min) observation. The order of hole-cross reduction was diazepam>PDFS>EDFS> CDFS> ADFS. **(Table 5)**

Discussion

D. falcata stem extracts have shown marked analgesic, anti-inflammatory and CNS depressant effects. Acetic acid-induced writhing and heat induced tail immersion tests are simple, reliable and well recommended protocols in evaluating analgesic property of a medicinal agent. 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 [16]. 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 [17]. Acetic acid causes inflammatory pain by inducing capillary permeability and liberating endogenous substances that excite pain nerve ending [16], 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 [18]. 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 [17]. 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 [19]. The analgesic activity of the extracts could be due to blockade of effect or release of endogenous inflammatory substances. Thus, the significant (p^a< 0.001) reduction of writhing indicates analgesic activity of the extracts by inhibition of prostaglandin synthesis or by action on prostaglandin [16] (**Table 1)**.The tail-withdrawal response, an acute pain model is predominantly selective for centrally acting analgesics, implicating supraspinal analgesic pathways which is similar to the action of opioid agonists.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 [20]. 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 [21]. The production of prostaglandins such as PGE2 and/or PGI2 is facilitated through COX-2 activity in inflammatory response . 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. All the extracts significantly ($p^a< 0.001$) reduced paw edema volume which suggested that the anti-inflammatory effect of the extracts on carrageenan-induced paw edema may act via the inhibitory activity of COX-2 [22](Table 3).

Locomotor activity is considered as an index of alertness and a reduction of it is an indicative of sedative or CNS depressant activity [23]. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system, 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 [24, 25]. 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 [26]. 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. It may also be due to enhanced affinity for GABA or an increase in the duration of the GABA-gated channel opening [27]. 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 of movements in dose dependant manner (Table 4, 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 [24].

It is well established that alkaloids, flavonoids, triterpene, steroidal saponins and tannins are potent analgesic and anti-inflammatory compounds [28] which exert their effect through inhibition of prostaglandin synthesis [29] leukocyte migration, arachidonic acid metabolizing enzymes (phospholipase A2, cycloxygenase and lipoxygenase) inhibition or by modulating proinflammatory gene expression . Research has shown that any plant having aforementioned phytochemicals act as CNS depressant agent, and are useful for the treatment of many CNS disorders. These agents are ligands of $GABA_A$ receptors in the CNS and act as benzodiazepine-like agents [23]. Literature review revealed that *D. falcata* stem extracts are rich of tannins, flavonoids, phenolic compounds, phytosterols, triterpenes, quercetin, β-sitosterol, triterpenes etc. bioactive compounds which are believed to be responsible for analgesic, anti-inflammatory and CNS-depressant effects of the extracts. 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.

Conflict of interest: Authors have declared that they have no conflict of interest.

Acknowledgments

The author is grateful to the head of animal division of ICDDR, B for providing required mice within time. The author is also grateful to the National Herbarium, Mirpur, Dhaka, Bangladesh assisting for the identification, and giving voucher specimen number of the plant.

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

Sample	Dose mg/kg,p.o.	Writhing number	Percent inhibition of writhing
Control(vehicle)	0.1ml/mice	47.20±5.41	00
Morphine sulfate	10mg/kg	5.12±1.10[a]	89.15
ADFS	50	34.60±5.23[a]	26.69
	100	25.60±4.40[a]	45.76
	200	17.55±3.25[a]	62.81
EDFS	50	27.14±2.05[a]	42.50
	100	20.32±3.50[a]	56.94
	200	14.22±2.10[a]	69.87
CDFS	50	22.20±3.11[a]	52.97
	100	15.65±2.21[a]	66.84
	200	10.58±2.43[a]	77.58
PDFS	50	25.37±4.27[a]	46.25
	100	17.10±3.33[a]	63.77
	200	12.50±2.11[a]	73.51

Data are 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.

Table 2. Analgesic effect of *D. falcata* stem 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. 11±0.10	2.15±0.12	2.34 ±0.15	2.8±0.25	3.10±0.21
Morphine sulfate	5	2.35±0.23	4.43±0.23 (28.40)[a]	5.84±0.42 (46.52)[a]	6.20±0.56 (51.15)[a]	8.95 ± 0.45 (86.50)[a]
ADFS	50	2.13±0.35	2.97±0.41 (3.04)	3.16±0.19 (13.08)[b]	3.87±0.23 (22.10)[a]	4.66±0.28 (32.14)[a]
	100	2.05±0.35	3.5±0.12 (11.57)[a]	4.16±0.31 (26.54)[a]	4.37 ±0.31 (29.18)[a]	5.39±0.37 (42.01)[a]
	200	2.21±0.61	4.10±0.20 (16.55)	4.67 ±0.38 (31.57)[a]	5.03± 0.42 (36.20)[a]	6.27±0.40 (52.11)[a]
EDFS	50	2.24±0.45	2.43±0.15 (1.01)	3.37±0.63 (15.54)[b]	4.43±0.17 (29.04)[a]	5.31±0.38 (40.25)[a]
	100	2.15±0.42	2.57±0.22 (2.44)	4.47±0.34 (28.73)[a]	5.07± 0.26 (36.46)[a]	6.03±0.90 (48.84)[a]
	200	2.35±0.23	2.37±0.42 (5.35)	4.92±0.26 (35.28)[a]	5.76±0.78 (45.98)[a]	6.92±0.84 (60.76)[a]
CDFS	50	2.19±0.2	3.86±0.23 (8.88)[a]	4.71 ±0.23 (30.84)a	5.12±0.39 (36.20)[a]	6.46±0.95 (53.72)[a]
	100	2.32±0.42	4.82±0.11 (21.38)[a]	5.51±0.47 (42.50)[a]	6.87±0.69 (59.92)[a]	7.38±0.85 (66.45)[a]
	200	2.36±0.1	2.73±0.11 (32.55)	6.46 ± 0.64 (53.96)[a]	7.03±0.78 (61.32)[a]	8.42±0.75 (79.42)[a]
PDFS	50	2.25±0.2	3.03±0.24 (4.84)[b]	3.53±0.27 (15.31)[a]	4.87±0.12 (32.85)[a]	5.88±0.37 (46.07)[a]
	100	2.39±0.36	4.19±0.30 (10.06)[a]	4.23±0.36 (25.54)[a]	5.88±0.24 (46.83)[a]	6.57±0.47 (55.74)[a]
	200	2.45±0.1	2.23±0.26 (23.65)	5.2±0.20 (36.92)[a]	6.39±0.41 (52.56)[a]	7.72±0.68 (70.03)[a]

Data are presented as mean ± SEM (n = 6); $p^c < 0.05$, $p^b < 0.01$, $p^a < 0.001$ compared with the vehicle control group. ANOVA followed by Dunnett's test is done in SPSS version 15.0

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 (Percent inhibition)				
		0h	1h	2h	3h	4h
Control	0.1ml	0.81±0.02	0.80±0.03	0.79±0.04	0.78±0.02	0.78±0.01
Ibuprofen	10	0.80±0.04	0.42±0.03[a] (47.50)	0.27±0.02[a] (65.82)	0.10±0.01[b] (93.82)	0.04±0.01[a] (94.87)
ADFS	50	0.79±0.02	0.73±0.02 (8.75)	0.64±0.03[b] (18.98)	0.52±0.01[a] (33.33)	0.44±0.02[a] (43.58)
	100	0.77±0.03	0.68±0.02[b] (15.00)	0.55±0.02[a] (30.37)	0.40±0.01[a] (48.71)	0.36±0.02[a] (53.84)
	200	0.80±0.03	0.64±0.03[b] (20.00)	0.43±0.01[a] (45.56)	0.31±0.02[a] (60.25)	0.24±0.01[a] (69.23)
EDFS	50	0.81±0.04	0.72±0.03[b] (10.00)	0.53±0.02[b] (32.91)	0.40±0.02[a] (48.71)	0.37±0.02[a] (52.56)
	100	0.79±0.03	0.67±0.02[b] (16.25)	0.45±0.01[a] (43.03)	0.36±0.02[a] (53.84)	0.25±0.02[a] (67.94)
	200	0.77±0.04	0.62±0.02[b] (22.50)	0.38±0.03[a] (51.89)	0.27±0.03[a] (65.38)	0.18±0.01[a] (76.92)
CDFS	50	0.82±0.03	0.57±0.04 (28.75)	0.43±0.03[b] (45.56)	0.30±0.02[a] (61.53)	0.18±0.02[a] (76.92)
	100	0.79±0.02	0.52±0.03[b] (35.00)	0.33±0.01[a] (58.22)	0.21±0.01[a] (73.07)	0.10±0.01[a] (87.17)
	200	0.81±0.04	0.45±0.04[b]	0.28±0.02[a] (64.55)	0.13±0.02[a] (83.33)	0.07±0.01[a] (91.02)
PDFS	50	0.80±0.03	0.63±0.04 (21.25)	0.52±0.03[b] (34.13)	0.42±0.02[a] (46.15)	0.20±0.02[a] (74.35)
	100	0.79±0.04	0.60±0.03[b] (25.00)	0.40±0.02[b] (49.36)	0.27±0.01[a] (65.38)	0.17±0.01[a] (78.20)
	200	0.78±0.04	0.54±0.03[b] (32.50)	0.30±0.02[a] (62.02)	0.19±0.01[a] (75.64)	0.13±0.02[a] (83.33)

Data are 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

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 (%) at 120 min
		0 min	30 min	60 min	90 min	120 min	
Control	0.1ml	143.45±8.65	136.25±7.15	126.34±8.11	117.45±6.47	112.65±6.48	--
Diazepam	2	146.30±9.12	63.10±8.12[a]	20.23±2.02[a]	7.25±2.17[a]	3.79±0.25[a]	96.63
ADFS	50	144.76±7.23	97.12±5.32[b]	73.21±6.47[a]	60.78±6.20[a]	49.77±5.32[a]	55.81
	100	146.55±8.63	86.37±7.25[b]	68.23±5.45[a]	51.36±7.40[a]	43.90±4.11[a]	61.02
	200	145.29±9.18	76.38±6.78[b]	52.72±6.12[a]	38.23±4.13[a]	32.56±2.34[a]	71.09
EDFS	50	143.70±9.52	75.36±8.10[a]	50.47±5.30[a]	44.10±5.32[a]	29.87±3.15[a]	73.48
	100	147.69±8.10	66.81±6.35[a]	38.23±4.75[a]	32.69±4.37[a]	22.45±2.02[a]	80.07
	200	153.26±9.12	49.84±5.23[a]	31.42±3.52[a]	23.56±2.35[a]	13.68±2.11[a]	87.85
CDFS	50	142.62±9.10	77.25±6.53[a]	64.39±7.52[a]	56.10±6.23[a]	38.63±3.15[a]	65.70
	100	149.10±8.69	71.30±5.02[a]	47.10±5.54[a]	42.60±3.27[a]	27.14±2.42[a]	75.90
	200	144.42±7.52	57.64±5.11[a]	38.37±4.26[a]	28.10±2.15[a]	17.19±2.18[a]	84.74
PDFS	50	149.63±6.89	62.89±5.90[a]	42.20±4.17[a]	33.44±4.23[a]	21.60±2.10[a]	80.82
	100	145.28±7.50	56.30±6.80[a]	31.52±3.50[a]	22.40±3.11[a]	12.27±2.36[a]	89.10
	200	148.23±8.46	41.27±4.25[a]	26.38±2.31[a]	17.36±2.01[a]	6.10±1.30[a]	92.80

Data are presented as the 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

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 (%) at 120min
		0 min	30 min	60 min	90 min	120 min	
Control	0.1ml	18.32±1.40	16.86±2.10	16.11±2.12	15.10±2.68	15.37±2.13	--
Diazepam	2	17.20±1.25	3.78±1.02^{a}	3.12±0.50^{a}	2.10±0.12^{a}	1.45±0.03^{a}	90.56
ADFS	50	18.35±2.40	13.34±2.35	10.70±1.72^{b}	9.10±1.15^{b}	8.24±1.13^{a}	46.08
	100	19.23±1.55	11.38±2.20	9.75±1.56^{b}	8.39±1.12^{a}	7.36±1.16^{a}	52.11
	200	18.52±2.35	10.42±2.12^{b}	8.53±1.50^{a}	7.16±0.40^{a}	6.89±1.54^{a}	55.17
EDFS	50	16.51±2.15	8.56±2.13^{b}	7.90±1.15^{a}	6.85±1.20^{a}	5.42±0.15^{a}	64.73
	100	17.54±2.17	7.59±1.15^{a}	7.45±0.87^{a}	6.75±1.12^{a}	4.56±0.52^{a}	70.33
	200	15.45±1.80	6.72±1.62^{a}	5.68±0.74^{a}	4.95±0.18^{a}	3.76±0.20^{a}	75.53
CDFS	50	18.23±2.46	10.26±1.30^{c}	8.73±1.12^{b}	7.30±1.65^{a}	6.86±1.07^{a}	55.36
	100	19.13±2.51	8.22±1.21^{a}	7.83±1.18a	6.89±1.12^{a}	6.13±0.62^{a}	60.11
	200	17.45±1.93	8.94±1.07^{a}	7.16±1.10a	5.94±1.34^{a}	5.34±0.55^{a}	65.25
PDFS	50	18.53±2.34	7.69±1.78^{c}	6.94±1.04^{c}	4.67±1.10^{a}	4.10±1.13^{a}	73.32
	100	16.76±2.27	6.02±1.50^{b}	5.68±0.22^{a}	4.04±0.45^{a}	3.02±0.56^{a}	80.35
	200	17.43±2.10	5.26±0.35^{a}	4.86±0.12^{a}	3.2±0.11^{a}	2.06±0.13^{a}	86.59

Data are presented as mean ± SEM (n = 6); $p^{c}< 0.05$, $p^{b}< 0.01$, $p^{a}< 0.001$ compared with the vehicle control group. ANOVA followed by Dunnett's test is done in SPSS version 15.0