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Evaluation of Analgesic and CNS Depressant Activities of *Grewia Paniculata* in *Swiss albino* Mice

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Abstract *Grewia paniculata* (Family: Malvaceae) used to treat inflammation, pain, fever, wounds and also in psychological disorders. The present study was designed to investigate the analgesic and CNS depressant activities of different crude extract of *Grewia paniculata*. The analgesic activity was evaluated by using acetic-acid induced writhing, hot plate and tail immersion tests and the CNS depressant activity was examined by observing the reduction of locomotor and exploratory activities in the thiopental induced sleeping time, hole cross and open field tests in mice at the doses of 250 and 500 mg/kg, b.w. For analgesic and CNS depressant activity Diclofenac sodium (25 mg/kg i.p), Aspirin (100 mg/kg p.o), Morphine (5 mg/kg i.p) and Diazepam (1 mg/kg i.p) were used as reference drug, respectively. All the extract displayed significant analgesic effect ($P<0.05$-0.001) in acetic acid and heat induced pain models in a dose dependent manner. The extract prolonged the latency period to the thermal stimuli in both hot plate and tail immersion test. The spontaneous motor activity was decreased by ($P<0.001$) by all natural products in both hole cross and open field test. Statistical studies revealed that all the extracts prolong the duration of the thiopental sodium induced sleeping time in mice. Altogether, these results suggest that all the extracts of *Grewia paniculata* possesses potent analgesic and CNS depressant properties, which support its use in traditional medicine and suggesting that the plant should be further investigated for its pharmacological active natural products.

Keywords: *Grewia paniculata, analgesic activity, CNS depressant activity*

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1. Introduction

Bangladesh is an attractive repository of various medicinal plants. Thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times in Bangladesh [1]. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs. *Grewia paniculata* (Family: Malvaceae) a medicinal plant of Bangladesh, is a large shrub or small tree, with purplish-red fruits. This plant is locally called "Fattashi" and grows in South and Southeast Asia including Bangladesh [2]. *Grewia* species are used in traditional medicine to alleviate inflammation and for blood, cardiac, digestive, and respiratory disorders in addition to fever [3]. Traditional beliefs claim it services the digestive system to work better and it is additionally employed for other health problems including colds, diarrhea, hepatitis, heat stroke and dyspepsia etc [4,5]. Besides many other uses, this plant is used in different painful conditions such as inflammation, pain, fever and wounds in Bangladesh [6,7]. The plant has an analgesic and sedative activity and also used in various psychological disorders [8]. Previous phytochemical studies on *Grewia* species are limited, with compounds of the anthocyanin, fatty acid, keto alcohol, and δ- lactone types having being isolated or detected [3]. Several compounds have been isolated from this plant, i.e., microgrewiapine [3], Microcosamines A and B [9], N-Methyl-*6β*-(deca-1',3',5'-trienyl)-*3β*-methoxy-*2β*-methylpiperidine [10], methyl*3β*-O-p-hydroxy-Ecinnamoyloxy-2α,23-dihydroxyolean-12-en-28-oate, sucrose epicatechin, 3-trans-feruloyl maslinic acid and maslinic acid [11]. Pharmacological properties such as analgesic and cytotoxic [12], antidiarrhoeal [13,14], antimicrobial, cytotoxic and larvicidal [15] activities have been reported. From the existing information it is evident that the plant may possess some important biological activities. To date, different fractions of *Grewia paniculata* have not been systemically studied. Therefore, as a part of our continuing studies [8,13] on natural products for their pharmacological properties we investigated different extracts of *Grewia paniculata* for their analgesic and neuropharmacological activity.

2. Materials and Methods

2.1. Collection of the Plant Material

The bark and leaf of *Grewia paniculata* were collected from the local area of Gazipur, Dhaka, Bangladesh in September, 2011. The collected materials were then identified by Bushra Khan, Principal Scientific Officer, Bangladesh National Herbarium, Mirpur, Dhaka and a voucher specimen has been deposited (DACB: 35,942) for future reference.

2.2. Extraction and Fractionation of the Plant Material

The plant parts were extracted by a cold extraction method. The dried bark (1000 gm) and leaf (400 gm) powder were extracted with 3000 ml (3L) and 1200 ml (1.2L) of ethanol for 72 h at room temperature. The extracts were filtered and evaporated on rotary evaporator under reduced pressure. Recovered solvent was again used for percolation for another 72 h. The process was repeated three times and weights of the crude extracts obtain from the bark and leaf of the *Grewia paniculata* were 132 g (yield 13.2%) and 16.94 g (yield 4.24%). The bark extract was further partitioned using first with petroleum ether, then chloroform and at the end ethanol. The ethanol extract of bark (EEB), as well as petroether fraction (PEF), chloroform fraction (CHF), ethanol fraction (EFB), and ethanol extract of leaf (EEL) were used for this investigation.

2.3. Chemicals and Reagents

The chemicals were used in this study and purchased from Merck, Germany. Diclofenac sodium, diazepam, aspirin, morphine and thiopental sodium also bought from local manufacturers.

2.4. Experimental Animals

Swiss albino mice of either sex, 3-4 weeks of age, weighing between 25-30 gm were used for in-vivo pharmacological screening. Mice were collected from the animal research branch of the International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were kept in standard environmental conditions (temperature: 25±2 ℃; relative humidity: 55±10 % and 12 hours light/ dark cycle). The animals were fed with standard food (ICDDR, B formulated) and water *ad libitum* and acclimatized to laboratory conditions for 14 days before the experiments. All the experimental animals were treated following the Ethical Principles and Guidelines for Scientific Experiments on Animals (1995) formulated by the Swiss Academy of Medical Sciences and the Swiss Academy of Sciences. The University Animal Research Ethical Committee approved the experimental protocol.

2.5. Acute Toxicity Study

Mice were divided into control and test groups (n=6). The test groups received the extract per orally at the doses of 500, 1000, 1500 and 2000 mg/kg. Then the animals were kept in separate cages and were allowed to food and *ad libitum*. The control group received the water. The animals were observed for possible behavioral changes, allergic reactions and mortality for the next 72 h [16].

2.6. Study of Analgesic Activity

2.6.1. Acetic acid-induced Writhing Test

The analgesic activity of the samples was studied using acetic acid-induced writhing model in mice. Test samples at a dose of 250 and 500 mg/kg p.o and vehicle (5ml/kg) were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid but Diclofenac sodium (25 mg/kg, i.p.) and Aspirin (100 mg/kg, p.o.) were administered 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 10 min [17].

2.6.2. Hot Plate Test

The hot plate method is a thermal pain model to test analgesic activity based on the procedure described by Eddy and Leimbach (1953) [18]. The mice were treated with vehicle (5ml/kg), extracts (250 and 500 mg/kg) or the reference drug Morphine (5 mg/kg, i.p.).Then the animals were placed on Eddy's hot plate kept at a temperature of 52±0.5 ℃ and the latency period was recorded. The response in the form of forepaw licking, withdrawal of the paws or jumping were recorded at 0, 30, 60 and 90 minutes following the treatments (n=6). A cut off time of 28 seconds was used to avoid damage to the paw.

2.6.3. Tail Immersion Test

The procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice [19]. The mice were treated with vehicle, extracts or the reference drug morphine and then 1-2 cm of the tail of the mice was immersed in warm water kept constant at 55±1 ℃. The reaction time was the time taken by the mice to deflect their tails and to get the latency period. The latency period was obtained thrice and the mean was used. The tail-immersion response was determined at 0, 30, 60 and 90 min after the treatments (n=6). A maximum immersion time of 28 seconds was maintained to prevent thermal injury to the animals.

2.7. Study of CNS Depressant Activity

2.7.1. Thiopental Sodium-induced Sleeping Time Test

The animals were randomly divided into control, standard and test groups consisting of six mice each. The test groups received of *Grewia paniculata* extracts at the doses of 250 and 500 mg/kg while standard group was treated with diazepam (1 mg/kg i.p.) and control with water (5 ml/kg). Thirty minutes later, thiopental sodium (40 mg/kg, i.p.) was administered to each mouse to induce sleep. The animals were observed for the latent period (time between pentobarbitone administration to loss of righting reflex) and duration of sleep (time between the loss and recovery of righting reflex) [20].

2.7.2. Hole Cross Test

The most consistent behavioral change is a hyperemotional response to novel environmental. The experiment was carried out as described by Takagi et al. (1971) [21]. A partition was fixed in the middle of a cage having a size of 30 × 20 × 14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. Mice were treated with control, standard or extract and were placed in one side of the cage. Then the number of passage of a mouse through the hole from one chamber to other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after the administration of the standard (i.p) and test drugs (p.o).

2.7.3. Open Field Test

Open field behavioral test is routinely used to evaluate both locomotor activity and emotionally in rodents. This experiment was carried out as described by Gupta et al. (1971) [22]. The animals were divided into control, standard and test groups containing 6 mice each. The test group received the extract at the doses of 250 and 500 mg/kg orally whereas the control group received water (5 ml/kg, p.o.) and standard group received diazepam (1 mg/kg, i.p.). In the board an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40 cm height. The number of squares passed anyway by the animals was counted for 3min started at 0, 30, 60, 90 and 120 min after oral administration of the test drugs.

2.8. Statistical Analysis

Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dennett's multiple comparisons. The results obtained were compared with control group; $p < 0.05$, 0.001 were considered to be statistically significant.

3. Results

3.1. Acute Toxicity

Oral administration of *Grewia paniculata* at the doses of 500–2000 mg/kg did not produce any mortality or noticeable behavioral changes in mice within 72 hr observation period. Therefore, it can be suggested that *Grewia paniculata* have low toxicity profile with LD_{50} greater than 2000 mg/kg.

3.2. Analgesic Activity

3.2.1 Acetic Acid-induced Writhing Test

Table 1. Effects of different extracts of *Grewia paniculata* on acetic acid-induced writhing test in mice.

Group	Dose (mg/kg)	No. of Writhings (Mean±SEM)	% of writhing	% of writhing inhibition
Control	5ml/kg	50.58±4.18	100.00	--
Diclofenac sodium	25	9.75±0.77**	19.27	80.72
Aspirin	100	19.25±4.12**	38.06	61.94
EEB	250	23.5±2.86**	46.46	53.54
	500	13.5±1.83**	26.69	73.30
PEF	250	23. 58±4. 02**	46.61	53.38
	500	22.25±4. 20**	43.99	56.01
CHF	250	27.5±2.41**	54.37	45.63
	500	20.58±2.85**	40.68	59.31
EFB	250	19±5.20**	37.56	62.44
	500	14.5±3.46**	28.67	71.33
EEL	250	17.33±2.65**	34.26	65.74
	500	17±4.32**	33.61	66.39

Control group received water (5 ml/kg, p.o.), standard groups received Diclofenac sodium (25 mg/kg, i.p.) and Aspirin (100 mg/kg, p.o.).Test groups were treated with 250 mg/kg and 500mg/kg of EEB, PEF, CHF, EFB and EEL extracts. Data expressed as Mean ± SEM, n = 6 in each group. Statistical analysis done by one way ANOVA followed by Dunnett's test. *p<0.05, **p<0.001, compared to control group. **EEB** =Ethanol extract of bark, **PEF**= Petroether fraction of bark, **CHF** =Chloroform fraction of bark, **EFB**=Ethanol fraction of bark and **EEL**=Ethanol extract of leaf.

The results of the acetic acid induced writhing test showed that the extracts of *Grewia paniculata* at all doses produced significant ($p < 0.001$) inhibition of writhing response (Table 1). In this test EEB at 500 mg/kg was found the highest 73.30% writhing response inhibitory effect. On the other hand PEF, CHF, EFB and EEL showed maximum 56.01%, 59.31%, 71.33% and 66.39% inhibition of writhing whereas the reference drug Diclofenac sodium and Aspirin displayed 80.72 and 61.94% inhibition of writhing, respectively.

3.2.2. Hot Plate Test

Hot plate test results shown in Table 2. All doses of the extracts were increased in latency time period when compared with the control. The results were found to be statistically significant ($p < 0.05$-0.001). At 90 min EEB produced the maximum 67.83% pain inhibition whereas the reference drug Morphine that produced 53.91% pain inhibition. Another PEF, CHF, EFB and EEL showed maximum 60.07%, 62.42%, 66.28% and 65.44% inhibition of thermal stimulus respectively.

3.2.3. Tail Immersion Test

The tail withdrawal reflex time following administration of the extracts of *Grewia paniculata* was found to increase with increasing dose of the sample. The result was statistically significant ($p < 0.05$-0.001). Tail withdrawal reflex data shows that at 90 min, the EEB and EEL produced maximum pain inhibition 80.31% and 80.55% while the reference drug Morphine produced 69.89% pain inhibition. The pain inhibition of thermal stimulus of PEF, CHF, EFB and showed maximum 78.17%, 82.03%,79.42 % respectively (Table 3).

3.3. CNS Depressant Activity

3.3.1. Thiopental Sodium Induced Sleeping Time Test

In thiopental sodium induced sleeping time test, all the doses of the extracts showed significant (p<0.05-0.001) dose dependent decrease in onset of sleep and increase in duration of sleep (Table 4) which was comparable with the control group. The EEL (500 mg/kg) was exhibited maximum 301.52% effect in duration of loss of righting reflex whereas the standard drug Diazepam (1 mg/kg) produced 199% effect. On the other hand EEB, PEF, CHF and EFB (500 mg/kg) were produced 231.42%, 192.22%, 249.51% and 294.74% effect in duration of loss of righting reflex respectively.

3.3.2. Hole Cross Test

The hole cross test showed that the depressing action of all the extracts was evident from the 2nd observation period in the test animals at the doses of 250 and 500 mg/kg (Table 5). Maximum depressant effect was observed from 3rd (60 min) to 5th (120 min) observation period. The EEB, PEF, CHF, EFB and EEL exhibited maximum 95.32%, 92.09%, 96.51, 96.34 and 96.34%% suppression of locomotor activity respectively whereas suppression of locomotor activity of the standard drug diazepam (1 mg/kg) produced 63.42% suppression. The results were statistically significant (p < 0.05-0.001) compared to control.

3.3.3 Open Field Test

In the open field test *Grewia paniculata* showed significant decrease of movement from 30 to 120 minutes (Table 6). The results were statistically significant (p < 0.001). Maximum depressant effect was observed from 3rd (60 min) to 5th (120 min) observation period. The EEB, PEF, CHF, EFB and EEL exhibited maximum 98.89%, 88.88%, 86.40%, 94.48% and 97.63% suppression of locomotor activity respectively whereas suppression of locomotor activity of the standard drug diazepam (1 mg/kg) produced 65.81% suppression.

Table 2. Effects of different extracts of *Grewia paniculata* on hot plate test in mice.

Group	Dose (mg/kg)	Mean Reaction Time (s)			
		0 min	30 min	60 min	90 min
Control	5 ml/kg	9.67 ±0.98	8.83 ±0.60	9.83 ±1.04	9.33 ±1.11
Morphine	5	10.83 ±1.76	17 ±2.09* (48.06%)	21.33 ±2.19** (53.91%)	16 ±1.80 (41.68%)
EEB	250	13.83 ±1.04	15.33 ±1.31 (42.40%)	24.67 ±2.49** (60.15%)	23.67 ±2.82** (60.58%)
	500	7.33 ±1.25	22 ±3.08** (59.86%)	28 ±2.96** (64.89%)	29 ±3.00** (67.83%)
PEF	250	9.17 ±1.25	12.83 ±1.42 (31.17%)	14 ±1.63 (29.78%)	19.83 ±1.94* (52.95%)
	500	10.67 ±0.80	15.17 ±2.29 (41.79%)	20.83 ±0.90** (52.81%)	27.5 ±3.23** (60.07%)
CHF	250	9.67 ±1.42	10 ±2.29 (11.7%)	11.83 ±1.53 (16.90%)	16.5 ±2.74 (43.45%)
	500	9.83 ±1.19	10.33 ±1.49 (14.52%)	19.33 ±2.72 (49.15%)	24.83 ±2.47** (62.42%)
EFB	250	7.17 ±1.73	10.33 ±1.85 (14.52%)	13.33 ±1.77 (26.26%)	13.17 ±1.34 (29.15%)
	500	8.17 ±1.01	14 ±1.55 (36.92%)	19.5 ±2.03* (49.59%)	27.67 ±3.38** (66.28%)
EEL	250	13.33 ±1.66	11.67 ±1.40 (24.34%)	20.17 ±2.94* (51.24%)	25.17 ±1.86** (62.93%)
	500	8.33 ±1.56	21 ±1.91** (57.95%)	23.67 ±2.66** (58.47%)	27 ±2.37** (65.44%)

Control group received water (5ml/kg, p.o.), standard group received Morphine (5 mg/kg, i.p.).Test groups were treated with 250 mg/kg and 500 mg/kg of EEB, PEF, CHF, EFB and EEL extracts. Data expressed as Mean ± SEM, n = 6 in each group. Statistical analysis done by one way ANOVA followed by Dunnett's test. *p<0.05, **p<0.001, compared to control group. **EEB** =Ethanol extract of bark, **PEF** = Petroether fraction of bark, CHF =Chloroform fraction of bark, **EFB** =Ethanol fraction of bark and **EEL**=Ethanol extract of leaf.

Table 3. Effects of different extracts of *Grewia paniculata* on tail immersion test.

Group	Dose (mg/kg)	Mean Reaction Time (s)			
		0 min	30 min	60 min	90 min
Control	5 ml/kg	1.77 ±0.11	1.66 ±0.15	1.83 ±0.24	1.77 ±0.11
Morphine	5	2.44 ±0.11	4.94 ±0.63** (66.39%)	5.11 ±0.40** (64.18%)	5.88 ±0.25** (69.89%)
EEB	250	3.38 ±0.16	3.55 ±0.52* (53.23%)	5.83 ±0.37** (68.61%)	7.49 ±1.18** (76.36%)
	500	2.05 ±0.29	5.55 ±0.68** (70.09%)	7.16 ±0.61** (74.44%)	8.99 ±0.67** (80.31%)
PEF	250	1.33 ±0.15	2.94 ±0.30 (43.54%)	5.99 ±0.36** (69.45%)	5.88 ±0.29** (69.89%)
	500	3.33 ±0.23	4.44 ±0.48** (62.61%)	7.38 ±0.75** (75.20%)	8.11 ±0.70** (78.17%)
CHF	250	2.61 ±0.38	3.27 ±0.30* (49.24%)	3.99 ±0.21** (54.14%)	4.55 ±0.49** (61.09%)
	500	1.88 ±0.11	3.33 ±0.24* (50.15%)	7.27 ±0.31** (74.83%)	9.85 ±0.81** (82.03%)
EFB	250	1.72 ±0.46	2.22 ±0.26 (25.23%)	2.99 ±0.33 (38.79%)	3.61 ±0.51* (50.97%)
	500	1.77 ±0.18	3.27 ±0.20* (49.24%)	6.94 ±0.58** (73.63%)	8.60 ±0.63** (79.42%)
EEL	250	4.22 ±0.26	3.05 ±0.29 (45.57%)	4.99 ±0.34** (63.33%)	6.44 ±0.34** (72.52%)
	500	1.66 ±0.08	6.27 ±0.47** (73.52%)	7.60 ±0.73** (75.92%)	9.10 ±0.65** (80.55%)

Control group received water (5ml/kg, p.o.), standard groups received Morphine (5mg/kg, i.p.). Test groups were treated with 250 mg/kg and 500 mg/kg of EEB, PEF, CHF, EFB and EEL extracts. Data expressed as Mean ± SEM, n = 6 in each group. Statistical analysis done by one way ANOVA followed by Dunnett's test. *p<0.05, **p<0.001, compared to control group. **EEB** =Ethanol extract of bark, **PEF**= Petroether fraction of bark, **CHF** =Chloroform fraction of bark, **EFB=**Ethanol fraction of bark and **EEL**=Ethanol extract of leaf.

Table 4. Effects of different extracts of *Grewia paniculata* on thiopental sodium induced sleeping time test in mice.

Group	Dose (mg/kg)	Onset of Sleep (minutes)	Duration of Sleep (minutes)	% effect
Control	40	2.06 ±0.64	66.33 ±8.04	100
Standard	1	1.72 ±0.20	132 ±8.31**	199
EEB	250	1.39 ±0.15	75.83 ±7.63	114.32
	500	1.36 ±0.06	153.5 ±11.53**	231.42
PEF	250	1.82 ±0.21	100.67 ±10.01	151.77
	500	1.46 ±0.13	127.5 ±14.93*	192.22
CHF	250	1.99 ±0.26	65.33 ±7.54	98.51

	500	1.35 ±0.09	165.5 ±6.43**	249.51
EFB	250	1.38 ±0.09	88.67 ±15.84	133.68
	500	1.63 ±0.19	195.5 ±14.82**	294.74
EEL	250	1.75 ±0.21	54.67 ±1.91	82.42
	500	1.33 ±0.04	200 ±12.81**	301.52

Control group received water (5 ml/kg, p.o), standard group received Diazepam (1mg/kg, i.p.). Test groups were treated with 250 mg/kg and 500 mg/kg of EEB, PEF, CHF, EFB and EEL extracts. Data expressed as Mean ± SEM, n = 6 in each group. Statistical analysis done by one way ANOVA followed by Dunnett's test. *p<0.05, **p<0.001, compared to control group. **EEB** =Ethanol extract of bark, **PEF**= Petroether fraction of bark, **CHF** = Chloroform fraction of bark, **EFB**=Ethanol fraction of bark and **EEL**=Ethanol extract of leaf.

Table 5. Effects of different extracts of *Grewia paniculata* on hole cross test in mice.

Group	Dose (mg/kg)	Number of movements (% of Number of movements inhibition)				
		0 min	30 min	60 min	90 min	120 min
Control	5 ml/kg	15.5 ±1.84	14.33 ±2.18	13.67 ±1.83	10.5 ±1.03	10 ±1.41
Diazepam	1	12.83 ±2.18	9.83 ±1.51* (31.40%)	5 ±1.80** (63.42%)	4 ±1.03** (61.90%)	3.67 ±1.14** (63.3%)
EEB	250	10.83 ±1.25	1.83 ±0.75 (87.23%)	1.83 ±0.54** (86.61%)	1.33 ±0.42** (87.33%)	1.83 ±0.54** (81.7%)
	500	11.83 ±1.90	0.67 ± 0.33** (95.32%)	0.67 ±0.21** (95.09%)	1.17 ±0.40** (88.86%)	0.83 ±0.16** (91.7%)
PEF	250	9.33 ±1.68	2.67 ± 1.20 (81.37%)	1.83 ±0.47** (86.61%)	1 ±0.44** (90.47%)	1.33 ± 0.76** (86.7%)
	500	9.17 ±1.49	2.5 ±1.76 (82.55%)	1.5 ±0.43** (89.02%)	0.83 ±0.47** (92.09%)	1 ±0.36** (90.00%)
CHF	250	9.5 ±1.45	1.33 ±0.49 (90.72%)	2 ±1.18** (85.37%)	1 ±0.36** (90.47%)	1.5 ±1.02** (85.00%)
	500	7.17 ±1.11	0.5 ±0.22 (96.51%)	1.67 ±0.42** (87.78%)	0.83 ±0.16** (92.09%)	0.83 ±0.16* (91.7%)
EFB	250	12.5 ±1.28	1.83 ±0.47** (87.23%)	1.83 ±1.04** (86.61%)	1.67 ±0.49** (84.09%)	1.67 ±0.88** (83.3%)
	500	7.67 ±1.23	1.5 ±0.34** (89.53%)	0.5 ±0.22** (96.34%)	0.83 ±0.31** (92.09%)	0.83 ±0.16** (91.7%)
EEL	250	11.17 ±2.16	1.17 ±0.60** (91.84%)	1.67 ±0.42** (87.78%)	1.67 ±0.33** (84.09%)	1.17 ±0.31** (88.3%)
	500	9.5 ±1.33	0.67 ±0.33** (95.32%)	0.5 ±0.34** (96.34%)	0.83 ±0.47** (92.09%)	0.67 ±0.49** (93.3%)

Control group received water (5ml/kg, p.o.), standard groups received Diazepam (1mg/kg, i.p.). Test groups were treated with 250 mg/kg and 500mg/kg of EEB, PEF, CHF, EFB and EEL extracts. Data expressed as Mean ± SEM, n = 6 in each group. Statistical analysis done by one way ANOVA followed by Dunnett's test. *p<0.05, **p<0.001, compared to control group. **EEB** =Ethanol extract of bark, **PEF**= Petroether fraction of bark, **CHF** =Chloroform fraction of bark, **EFB**=Ethanol fraction of bark and **EEL**=Ethanol extract of leaf.

Table 6. Effects of different extracts of *Grewia paniculata* on open field test in mice.

Group	Dose (mg/kg)	Number of movements (% of Number of movements inhibition)				
		0 min	30 min	60 min	90 min	120 min
Control	5ml/kg	112.83 ±6.24	112.5 ±5.67	103.33 ±11.39	99 ±3.95	90.67 ±1.52
Diazepam	1	131.33 ±7.71	72.67 ±17.16* (35.40%)	57 ±8.83** (44.84%)	53 ±14.94** (46.46%)	31 ±13.61** (65.81%)
EEB	250	65.83 ±5.42	14.67 ±7.16** (86.96%)	10.33 ±4.35** (90.00%)	5 ±2.87** (94.95%)	11.5 ±2.88** (86.32%)
	500	77.83 ±9.99	11.33 ± 4.56** (89.93%)	10.33 ±5.08** (90.00%)	2.5 ±1.96** (97.47%)	1 ±0.63** (98.89%)
PEF	250	73.17 ±17.07	15.67 ±4.66** (86.07%)	17.67 ±7.51** (82.89%)	18.33 ± 6.31** (81.48%)	20 ± 5.52** (77.94%)
	500	79.17 ±4.16	15.17 ±11.07** (86.52%)	15 ± 6.00** (85.48%)	11 ±5.01** (88.88%)	16.5 ± 5.28** (81.80%)
CHF	250	62.33 ±10.42	38.17 ±12.53** (66.07%)	19 ±5.15** (81.61%)	25.5 ±10.86** (74.24%)	21.67 ±7.86** (76.10%)
	500	78.67 ±9.70	35.17 ±9.62** (68.74%)	17.17 ±8.29** (83.38%)	20.5 ±9.51** (79.29%)	12.33 ±4.09** (86.40%)
EFB	250	89.67 ±6.96	24.83 ±5.84** (77.93%)	22.33 ±6.85** (78.39%)	21 ±6.09** (78.78%)	20.83 ±4.05** (77.03%)
	500	65.17 ±14.48	11.5 ±4.90** (89.77%)	8.33 ±3062** (91.94%)	6.67 ±3.19** (93.26%)	5 ±2.40** (94.48%)
EEL	250	69.33 ±9.12	4 ±1.84** (96.44%)	11.5 ±2.66** (88.87%)	17.17 ±7.76** (82.66%)	13.5 ±5.95** (85.11%)
	500	75.83 ±9.05	2.67 ±1.23** (97.63%)	4.83 ±1.04** (95.33%)	5.17 ±2.11** (94.77%)	8.17 ±4.56** (94.98%)

Control group received water (5ml/kg, p.o.), standard group received Diazepam (1mg/kg, i.p.). Test groups were treated with 250 mg/kg and 500mg/kg of EEB, PEF, CHF, EFB and EEL extracts. Data expressed as Mean ± SEM, n = 6 in each group. Statistical analysis done by one way ANOVA followed by Dunnett's test. *p<0.05, **p<0.001, compared to control group. **EEB** =Ethanol extract of bark, **PEF**= Petroether fraction of bark, **CHF** =Chloroform fraction of bark, **EFB**=Ethanol fraction of bark and **EEL**=Ethanol extract of leaf.

4. Discussion

The present study has shown to establish significant analgesic potential of *Grewia paniculata* using acetic acid induced writhing method for visceral pain by peripheral activity and hot plate and tail immersion test for pain mediated. The extracts also possess significant CNS depressant activity. No acute toxicity was observed after oral administration of *Grewia paniculata* even at the dose of 2000 mg/kg in mice.

The acetic acid-induced writhing test in mice is widely used to screen and study compounds for peripherally mediated analgesic activity. Peritoneal administration of acetic acid (0.7%) induces endogenous pain mediators, such as prostaglandins, histamine, serotonin (5-HT), bradykinin and substance P that sensitize pain nerve endings [23,24]. The released prostaglandins, mainly prostacyclin (PGI_2) and to lesser extent PGE_2 and $PGF_{2\alpha}$ have been held responsible for pain sensation [25]. In this study suggest that all the extracts of *Grewia paniculata* carry a significant peripherally mediated analgesic activity and also exert the activity probably by inhibiting the synthesis or action of prostaglandins. The hot plate test is widely used to assess analgesic activity of drugs. This test dose not directly measure the intensity of the noxious stimulus perceived by the animal, but also the animal's response to it and so may be affected non-analgesic drugs. Sedative and muscle relaxants may impair the ability to response and hence be wrongly considered to have analgesic activity [26]. This test examined response of mice in hot plate at 52 ±0.5 ℃ and cut off at 28 seconds. The validity of this method has been shown even in the presence of substantial impairment of motor performance. It is an established fact that any agent that causes a prolongation of the hot plate latency using this test must be acting centrally [27]. However, all the extracts of *Grewia paniculata* have prominent central analgesic action. The tail immersion test was considered to be selective to examine compounds acting through opioid receptor; all the extracts increased pain threshold which means basal latency, which indicates that it may act via

centrally mediated analgesic mechanism. Overall, the analgesic action of all the extracts of *Grewia paniculata* is assumed to be due to inhibition of prostaglandin synthesis and its role on both central and peripheral analgesic mechanism. The plant extracts may act as more significant analgesic activity.

The locomotor activity is a test to appraise the level of excitability of the CNS [28] and any decrease of this activity may be narrowly related to sedation resulting from depression of the central nervous system [29]. Gamma-amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. Different anxiolytic, muscle relaxant, sedative-hypnotic drugs are elucidation their action through GABAA, therefore it is possible that extracts of *Grewia paniculata* may acts by potentiating GABAergic inhibition in the CNS via membrane hyperpolarization which leads to a decrease in the firing rate of critical neurons in the brain or may be due to direct activation of GABA receptor by the extracts [30]. The locomotor activity is a measure of the level of excitability of the CNS and decrease of this activity may be closely related to sedation resulting from depression of the central nervous system [29]. Many research showed that plant containing flavonoids, saponins and tannins are useful in many CNS disorders [31]. Earlier investigation on phytoconstituents and plants suggests that many flavonoids and neuroactive steroids were found to be ligands for the $GABA_A$ receptors in the central nervous system which led to the assume that they can act as benzodiazepine like molecules [32]. Phytochemical investigations also showed the presence of Reducing Sugar, Steroids, Alkaloids, Tannins, Gums and Glycosides. In case of the thiopental sodium induced sedative test both the doses of the extracts produced a significant increase in the hypnotic effect, thus suggesting a profound sedative activity. This method is a very sensitive way to detect agents with CNS depressant activity [33]. The results from the CNS depressant tests indicated that it significantly decreased the locomotor activity as shown by the results of the hole cross and open field tests. While evaluating neuropharmacological activities of *Grewia paniculata*, it was found that all the extracts possesses central nervous system depressant activity as indicated by decreased exploratory behavior in mice.

5. Conclusion

Based on the results of the present study, it can be concluded that that all the extracts of *Grewia paniculata* possesses potent analgesic and CNS depressant properties, which support its use in traditional medicine. However, further studies are needed to understand the exact mechanisms of action and to isolate the compound (s) responsible for such activity.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. All listed authors read and approved the final manuscript.

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