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Evaluation of antinociceptive and anti-inflammatory properties of *Desmodium gangeticum* (L.) in experimental animal models

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ABSTRACT

The present study was carried out to investigate the anti-inflammatory and antinociceptive activity of the ethanolic extract of Desmodium gangeticum leaves. We evaluate the ethanolic extract against thermal (Eddy's hot-plate & Tail flick test) and chemical (Acetic acid & Formalin) induced nociceptive response as well as anti-inflammatory activity against Carageenan induced paw oedema. The oral administration of DG extract (50, 100, 200mg/kg), positive control morphine (5mg/kg i.p.) and aspirin (300mg/kg o.p.) inhibited acetic acid-induced writhing by 25.92%, 55.12%, 68.13%, 85.61% and 72.19% (P<0.05), respectively. The highest dose of the DG extract increases the latency period by 37.65% in Eddy's hot-plate and 28.26% (P < 0.05) in tail fick test. In formalin induced nociceptive pain, 200mg/kg dose of extract inhibits 29.67% (P < 0.05) of neurogenic pain and in late inflammatory phase as compared to diclofenac (45.31%) & indomethacin (44.39%), DG extract shows 30.87%(100mg/kg), 42.78%(200mg/kg) inhibition. In carageenan induced paw oedema model, DG extract shows 15.68% (50mg/kg), 24.5% (100mg/kg) and 45.09% (200mg/kg) inhibition after 6h, where indomethacin shows 51.96%. After 24h, 200mg/kg extract dose and indomethacin were equipotent by inhibiting 43.86 %, 46.57%, respectively. These results indicate the presence of anti-inflammatory and antinociceptive principles in the ethenolic extract of Desmodium gangeticum, and reinforce the plant's potential therapeutic use against pain and inflammatory diseases.

Key words: Antinociceptive activity; Anti-inflammatory activity; *Desmodium gangeticum*; oedema; indomethacin.
Abbreviations: DGE: Ethanolic extract of *Desmodium gangeticum.*

INTRODUCTION

Pain and inflammation is a pathophysiological response of mammalian tissues to a variety of stimulants including infectious organisms, toxic chemical substances, physical injury or tumor growth [1]. The non-steroidal anti-inflammatory drugs (NSAIDs) such as acetyl salicylic acid, paracetamol, ibuprofen etc., and their new congeners, like celecoxib selective COX-2 inhibitors hamper early steps of prostaglandin biosynthesis pathway that untune the inflammation and pain response. Narcotics like morphine and its congeners act by intervening to CNS related mechanism of pain [2, 3]. But the side and toxic effects of the currently available anti-inflammatory and analgesic drugs has restricted their use. Gastric ulceration, renal damage form NSAIDs [4,5]; CNS depression, addiction and constipation from narcotic [6,7]; cardiac abnormalities from newer specific COX-2 inhibitors such as rofecoxib and celecoxib are more common dose related side effects [8]. Therefore, a need arises for the development of newer anti-inflammatory agents probably from the natural origin with more powerful activity and with lesser side effects to substitute the current chemical therapy.

Desmodium gangeticum (L.) is a small perennial shrub belongs to "Fabaceae" family. *D.gangeticum* (L.) (DG) also known as Salpan, Salpani (Hindi) and Shalparni (Sanskrit) that growing throughout India. Shalparni is a sub-erect, under-shrub 2–3 ft high with irregular angled, branched woody stem. 1–2 cm long leaves are unifoliate or trifoliate, flowers small pink to purple in colour [9, 10].

Phytochemical investigation revealed that plant contains tryptamines, phenethylamines like alkaloids and their N-oxides [11]; gangetin, gangetinin, desmodin, and desmocarpin like pterocarpanoids [12]; phospholipids, sterols, and flavone glycosides have also been reported [13]. Recently new aminoglucosyl glycerolipid group have been reported by PK Mishra et al. (2005) [14]. Pharmacological studies shows that *D.gangeticum* (L.) posses anti-catarrhal, antiemetic, bitter tonic, febrifuge, digestive properties [10]. *D.gangeticum* has great therapeutic value in typhoid, piles, inflammation, asthma, bronchitis, and dysentery treatment. *D.gangeticum's* alkaloids showed smooth muscle stimulant, anticholinesterase, CNS stimulant, depressant responses and antileishmanial activities [15, 16, 17]. Aqueous extract of the plant root has also been shown to have hypocholesterolemic and antioxidant effects in isoproterenol induced myocardial infraction [18]. *D.gangeticum* extract has potent antioxidant activity observed against DPPH, nitric oxide, ferryl-bipyridyl and hypochlorous acid [19].

MATARIALS AND METHODS

2.1. Plant Material

Fresh aerial parts of *D.gangeticum* were collected from their natural habitats in and around Dehradun. The plant was authenticated from Botanical Research survey of India, Dehradun (Voucher Speci. BSD112718). Aerial parts of *D.gangeticum* were air dried at room temperature and powdered coarsely. 200gm of the pulverized plant was extracted with 90% ethanol using a soxhlet apparatus. The extract was filtered, pooled and first concentrated on rotavapour. The yield was 12.7% (w/w). The extract of *D.gangeticum* (DGE) was administered as a suspension in 2% Gum acacia to the animals.

2.2. Preliminary Phytochemical Tests
Preliminary phytochemical screening method was carried out on the standard screening method of Trease and Evans (1983) [20].

2.3. Animals
Male Wistar rats (150–250 g) and Swiss albino mice (20–25 g) male/female were used. Animals were procured from the animal house, Laboratory Animal Resource, Division of Animal Genetics, IVRI, Izatnagar, Bareilly, India and acclimatized to laboratory condition at Animal House, G.R.D (P.G) I.M.T, Dehradun, India at room temperature 24±2°C with a 12h/12h/light/dark cycle and 70% RH. The animals were kept in polypropylene cages and maintained on balanced ration provided by standard dry pellet diet (Hindustan Lever, Mumbai, India) and water ad libitum**.** All animals were treated in accordance with the guideline for the Care and Use of Laboratory Animals (NIH Publication No.86-23, revised 1985) with the permission of institute ethical committe. All the animals were acclimatized to the laboratory environment for 5 days before the experiment.

2.4. Drugs
We selected the same commercial brands prescribed to humans as Analgesics. Carageenan was purchased from S.D. Fine Chemicals Limited, Bombay. All the solutions were prepared fresh in pyrogen free water used as a drug solvent in hospital. All other chemicals were of analytical grade and purchased from Merck.

2.5. Antinociceptive Activity
2.5.1. Hotplate Test
The hotplate test was performed to measure response latencies according to the method previously described [21]. Each mice was dropped gently on the hot plate maintained at 55.0 ± 0.5°C and the time taken for the mouse to lick the paw was recorded. Mice with baseline latencies of <5s or >30s were eliminated from the study. Each group of animal acted as its own control. The reaction time following the administration of the DGE (50, 100, 200mg/kg, p.o.), Aspirin (300mg/kg), Morphine (5mg/kg, s.c.), Naloxone + DGE (1mg/kg, i.p. + 200mg/kg), Naloxone + Morphine (1mg/kg, i.p. + 5mg/kg, s.c.) and 1% CMC (10ml/kg, o.p.) was measured at 0, 0.5, 1, 2, 3, 4, and 6 h after a latency period of 30min. A latency period of 20 sec was defined as complete analgesia and the measurement was terminated if it exceeded the latency period in order to avoid injury.

The percentage analgesic activity was calculated using the formula:

Percentage analgesic activity: [1-(Ta/Tb)] X100

Ta and Tb are latency periods of control and test group animals.

2.5.2. Acetic Acid-Induced Writhings
The antinociceptive activity of DGE was assessed using writhing test (abdominal constriction test) [22]. Mice (n=6) were randomly selected and treated with 10 ml/kg of 1% acetic acid (i.p). DGE extract (50, 100, 200mg/kg, p.o.), Aspirin (300mg/kg, o.p.), Morphine (5mg/kg, s.c.), Naloxone +DGE (1mg/kg, i.p. + 200mg/kg, p.o.), Naloxone + Morphine (1mg/kg, i.p. + 5mg/kg,

s.c.) and 1%CMC (10ml/kg, o.p.) were administered 30min prior to treatment with acetic acid. The writhing was counted for 30min after a latency period of 5min.

The percentage analgesic activity was calculated as follows: [1-(N/N`)] X100

where N represents the average number of writhing/stretching of control group and N` the average number of writhing/stretching of test group.

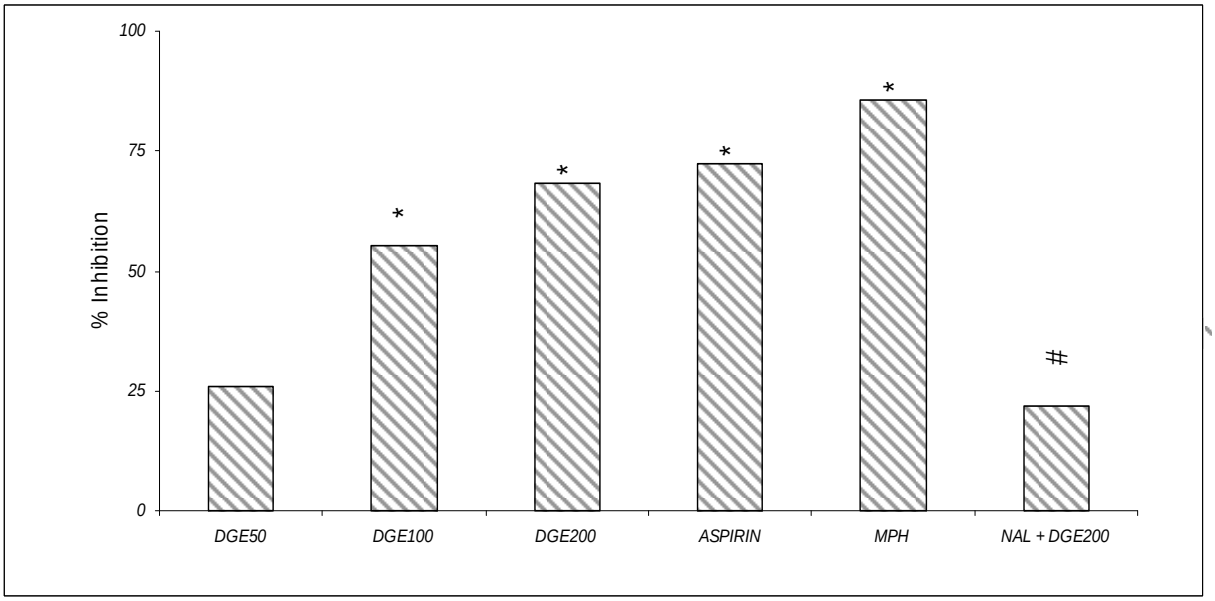

Fig 1. Percentage inhibition of acetic acid induced writhing by different doses of *Desmodium gangeticum* ethanolic extract in mice

DGE50- DG extract 50mg/kg p.o.; DGE100- DG extract 100mg/kg p.o.; DGE200- DG extract 200mg/kg p.o.; Aspirin-300mg/kg p.o.; MPH-morphine 5mg/kg s.c.; NAL+DGE- Naloxane 1mg/kg s.c. + DG extract 200mg/kg p.o..

2.5.3. Formalin test

The test was performed as described by Hunskaar and Hole (1987) with little modification [23]. Nine groups each consisting of 6 mice, were randomly selected. Mice in group 1 (control) was administered with 1% CMC (10ml/kg, o.p.), while mice in groups 2–4 were treated with 50, 100, 200mg/kg, p.o., of the DGE extract. Mice in groups 5–7 were treated with Diclofenac (5.64mg/kg, i.p.), Indomethacin (80mg/kg, i.p.), Morphine (10mg/kg, i.p.), and groups 8-9 treated with Naloxone (1mg/kg, s.c.) 15 minutes prior to administration of DGE (200mg/kg, p.o.) and Morphine (10mg/kg, i.p.), respectively 30 minutes prior to administration of 0.02 ml of 2.5% formalin into the sub-planter space of the right hind paw and the duration of paw licking was determined 0–5 minutes (1st Phase or neurogenic phase) and 15–30 minutes (2nd phase or inflammatory phase) after formalin administration. The 1st phase is regarded as the neurogenic mechanism and the 2nd phase is the inflammatory phase.

2.5.4. Tail flick method

Tail flick was conducted as described by Dykstra and Woods (1986) [24]. This involved immersing extreme 3 cm of the rat's tail in a water bath containing water at a temperature of 55±0.5 ◦C. Within a few minutes, the rats reacted by withdrawing the tail. The reaction time was recorded with a stopwatch. Each animal served as its own control and two readings were obtained for the control at 0 and 10min interval. The average of the two values was the initial reaction time (Tb). The test groups were given DGE (50, 100, 200mg/kg, p.o.), Aspirin (300mg/kg, p.o.), Morphine (5mg/kg, s.c.), Naloxone +DGE (1mg/kg, i.p. + 20mg/kg, p.o.) and 1% CMC (10ml/kg, o.p.). The reaction time (Ta) for the test groups was taken at intervals 0.5, 1, 2, 3, 4 and 6 h after a latency period of 30min following the administration of the DGE and drugs.

2.6. Anti-inflammatory Activity

DG extract was evaluated for anti-inflammatory activity against carageenan-induced rat paw oedema method [25]. Male wistar rats (150-200 g) were randomly distributed (n=6), and treated with DGE (50, 100, 200mg/kg, p.o.), standard drug Indomethacin (10mg/kg, p.o.) and 1% CMC (10ml/kg, o.p.). After 1 h, 0.1 ml of 1% w/v suspension of carageenan was injected into the sub-plantar region of the right hind paw to all the three groups. The paw volume, up to the tibiotarsal articulation, was measured using a plethysmometer at 1, 2, 3, 4, 6 and after 24h of carageenan injection, and mean increase in paw volumes were noted.

2.6. Statistical Analysis

The results obtained were presented as means ± SEM and analyzed using analysis of variance (ANOVA) followed by Dunnet test. The level of significance was set at 95%, $P<0.05$ for all treatment carried out compared to control group.

RESULTS

3.1. Preliminary Phytochemical Tests

Our preliminary phytochemical tests showed that flavonoid glycosides, pterocarpanoids, lipids, glycolipids, aminoglucosyl glycerolipid and alkaloids were present in the extract.

3.2. Hot-plate Test

The results of the hot plate test revealed that the reaction time was dose dependently increasing from 11.94% (50mg/kg), 23.71% (100mg/kg) and 37.65% (200mg/kg) with DG extract. Maximum effect was observed after 3 hr of dosing, where morphine shows 42.73% and DG extract (200mg/kg, p.o.) 37.65% increment in latency period ($P < 0.05$). Pretreatment with naloaxone (1mg/kg, s.c.) drastically reduced the analgesic potentials of morphine where as for DGE 200mk/kg, ~22% (3h) analgesic activity was abolished. Aspirin at 300mg/kg did not offer any protection against the heat induced pain (Table 1).

Table 1. Effect of *Desmodium gangeticum* leaves extract (DGE) on pain induced by Hot Plate in rat

TIME	CONTROL	DGE50	DGE100	DGE200	ASPIRIN	NAL + DGE200	MPH	NAL + MPH
0 hr	4.72 ± 0.04	4.75 ± 0.03	4.81 ± 0.03	4.78 ± 0.03	4.75 ± 0.06	4.71 ± 0.03	4.69 ± 0.03	4.71 ± 0.04
0.5 hr	4.69 ± 0.02	5.02 ± 0.05*	5.32 ± 0.04*	5.38 ± 0.04*	4.83 ± 0.06	4.89 ± 0.04	6.57 ± 0.07*	4.62 ± 0.05 □
1 hr	4.71 ± 0.04	5.11 ± 0.02	5.69± 0.03*	6.31 ± 0.04*	4.78 ± 0.05	5.03 ± 0.05#	7.30 ± 0.05*	4.79± 0.05 □
2 hr	4.60 ± 0.04	5.21 ± 0.03	6.03 ± 0.03*	6.93 ± 0.03*	4.93 ± 0.05	5.17 ± 0.04#	7.87 ± 0.07*	4.66 ± 0.04 □
3 hr	4.57 ± 0.02	5.19 ± 0.04	5.83 ± 0.04*	7.33 ± 0.05*	4.82 ± 0.04	5.36 ± 0.05#	7.98 ± 0.08*	4.57 ± 0.05 □
4 hr	4.59 ± 0.03	5.13 ± 0.02	5.51± 0.04*	6.99 ± 0.03*	4.91 ± 0.04	5.15 ± 0.09	7.82 ± 0.05*	4.75 ± 0.04 □
6 hr	4.59 ± 0.04	4.99 ± 0.04	5.02 ± 0.03	6.68 ± 0.05	4.90 ± 0.05	4.90 ± 0.06	6.69 ± 0.05*	4.79 ± 0.04

DGE50: Ethanolic extract of Desmodium gangeticum (L.) at a dose 50mg/kg;
DGE100: Ethanolic extract of Desmodium gangeticum (L.) at a dose 100mg/kg;
DGE200: Ethanolic extract of Desmodium gangeticum (L.) at a dose 200mg/kg;
Aspirin at a dose300mg/kg p.o.;
NAL+ DGE200: Naloxane 1mg/kg s.c. + DG extract 200mg/kg p.o.;
MPH: Morphine at a dose 5mg/kg s.c.;
NAL+ MPH: Naloxane 1mg/kg s.c. + Morphine at a dose 5mg/kg s.c..
** P>0.05 when compared to control,*
 # P>0.05 when DGE20 compared to NAL+ DGE20,
□ P>0.05 when MORPHINE compared to NAL+ MORPHINE.

3.3. Acetic Acid-Induced Writhings

DGE significantly reduced writhing and stretching induced by 1%, 10mg/kg acetic acid (Table 2). A significant and dose dependant writhing inhibition was observed as 25.92%, 55.12% and 68.13% ($P < 0.05$) at 50, 100, 200mg/kg of DGE respectively while aspirin (300mg/kg) had 72.19% ($P < 0.05$) and morphine (a centrally acting analgesic) had 85.62% ($P < 0.05$). Pretreatment of naloxone blocked the protective effect of morphine but higher dose of DGE still shows inhibitory effect, percentage inhibition was only 21.87% ($P < 0.05$).

Table 2. Effect of *Desmodium gangeticum* leaves extract (DGE) on Acetic Acid-Induced Writhings in Mice

Treatment	Control	DGE50	DGE100	DGE200	Aspirin	MPH	NAL + DGE200	NAL + MAP
No. of Writhing	84.1 ± 1.31	62.3 ± 1.43	37.74 ± 1.56*	26.8 ± 1.69*	23.38 ± 1.72*	12.09 ± 1.05*	65.7 ± 1.57#	78.59 ± 1.72 □
% Inhibition	…..	25.92	55.12	68.13	72.19	85.62	21.87	6.55

DGE50: Ethanolic extract of Desmodium gangeticum (L.) at a dose 50mg/kg;
DGE100: Ethanolic extract of Desmodium gangeticum (L.) at a dose 100mg/kg;
DGE200: Ethanolic extract of Desmodium gangeticum (L.) at a dose 200mg/kg;
Aspirin at a dose300mg/kg p.o.;
NAL+ DGE200: Naloxane 1mg/kg s.c. + DG extract 200mg/kg p.o.;
MPH: Morphine at a dose 5mg/kg s.c.;
NAL+ MPH: Naloxane 1mg/kg s.c. + Morphine at a dose 5mg/kg s.c..
** P>0.05 when compared to control,*
 # P>0.05 when DGE20 compared to NAL+ DGE20,
□ P>0.05 when MORPHINE compared to NAL+ MORPHINE.

3.4. Formalin test

Result of antinociceptive effect against formalin given in Table 3. DG extract had analgesic effects on both first neurogenic phase (0–5min) and second inflammatory phases (15–30min) of formalin induced pain. Its neurogenic phase of pain was effectively (29.67%) blocked only at 200mg/kg ($P < 0.05$), whereas all the doses of DGE significantly block the inflammatory pains.

DG extract was found to be more effective in 2nd phase of formalin test and inhibits the inflammatory pain 30.87% (100mg/kg), 42.78% (200mg/kg). In second phase, 200mg/kg dose of DG extract shows 42.78% inhibition and was equally effective to diclofenac (45.31%) and indomethacin (44.39%).

Table 3. Effect of *Desmodium gangeticum* leaves extract (DGE) on Formalin induced pain

Treatment	Control	DGE50	DGE100	DGE200	Diclofenac	Indomethacin	MPH	NAL+DGE200	NAL+MPH
1st Phase	67.37±1.73	62.35±0.83	57.73±1.31	47.38±1.71*	66.5±1.73	65.13±1.34	39.09±1.36*	61.47±1.33#	66.73±1.72†
% Inhibition	…….	7.45	14.30	29.67	1.29	3.32	52.36	8.75	0.94
2nd Phase	142.13±1.38	117.73±1.76	105.25±1.63	96.32±1.64*	89.73±1.89*	88.03±1.35*	107.53±1.52	124.56±1.30	119.43±1.89†
% Inhibition	…….	17.16	30.87	42.78	45.31	44.39	24.34	12.36	15.97

DGE50: Ethanolic extract of Desmodium gangeticum (L.) at a dose 50mg/kg;
DGE100: Ethanolic extract of Desmodium gangeticum (L.) at a dose 100mg/kg;
DGE200: Ethanolic extract of Desmodium gangeticum (L.) at a dose 200mg/kg;
Diclofenac at a dose 5.46mg/kg i.p.;
Indomethacin at a dose 80mg/kg i.p.;
NAL+ DGE200: Naloxane 1mg/kg s.c. + DG extract 200mg/kg p.o.;
MPH: Morphine at a dose 5mg/kg s.c.;
NAL+ MPH: Naloxane 1mg/kg s.c. + Morphine at a dose 5mg/kg s.c..
** P>0.05 when compared to control,*
 # P>0.05 when DGE20 compared to NAL+ DGE20,
† P>0.05 when MORPHINE compared to NAL+ MORPHINE.

3.5. Tail flick

Latency period for tail withdrawal was increasing in dose dependent manner and highest effect was observed after 3-4hr of dosing. Latency period was increased 16.19(50mg/kg), 19.7(100mg/kg) & 21.57(200mg/kg) after 3h of oral administration of DG extract. The inhibitory effect of the DG extract was maximum (28.26%) between 3 and 4h post-dosing with the dose of 200mg/kg. The antinociceptive property of the DG extract at 200mg/kg (18.65–28.26%) was not as effective as that of morphine (55.14%-65.2%). The analgesic activity of the DG extract was blocked by naloxone while aspirin had no effect on this test (Table 4).

Table 4. Effect of *Desmodium gangeticum* leaves extract (DGE) in Tail Flick Test on rat

Time	Control	DGE50	DGE100	DGE200	ASPIRIN	MPH	NAL +DGE200
0 hr	2.37 ± 0.03	2.41 ± 0.04	2.38 ± 0.03	2.37 ± 0.03	2.35 ± 0.05	2.39 ± 0.05	2.36 ± 0.06
0.5 hr	2.49 ± 0.04	2.61 ± 0.02	2.65 ± 0.03*	2.73 ± 0.03*	2.87 ± 0.03*	7.05 ± 0.06*	2.41 ± 0.04
1 hr	2.61 ± 0.04	2.74 ± 0.04*	3.08 ± 0.04*	3.13 ± 0.04*	2.73 ± 0.04*	7.43 ± 0.06*	2.63 ± 0.03#
2 hr	2.71 ± 0.02	3.01 ± 0.04*	3.29 ± 0.04*	3.37 ± 0.04*	2.93 ± 0.03*	7.67 ± 0.03*	2.75 ± 0.04#
3 hr	2.69 ± 0.02	3.21 ± 0.02*	3.35 ± 0.03*	3.43 ± 0.03*	2.95 ± 0.05*	7.73 ± 0.09*	2.81 ± 0.05#
4 hr	2.64 ± 0.04	3.28 ± 0.05	3.18 ± 0.04*	3.38 ± 0.04*	2.89 ± 0.05*	6.85 ± 0.04*	2.83 ± 0.04
6 hr	2.66 ± 0.04	3.04 ± 0.03	3.11 ± 0.02*	3.27 ± 0.02*	2.97 ± 0.04*	5.93 ± 0.07*	2.77 ± 0.05

DGE50: Ethanolic extract of Desmodium gangeticum (L.) at a dose 50mg/kg;
DGE100: Ethanolic extract of Desmodium gangeticum (L.) at a dose 100mg/kg;
DGE200: Ethanolic extract of Desmodium gangeticum (L.) at a dose 200mg/kg;
Aspirin at a dose 300mg/kg p.o.;
NAL+ DGE200: Naloxane 1mg/kg s.c. + DG extract 200mg/kg p.o.;
MPH: Morphine at a dose 5mg/kg s.c.;
** P>0.05 when compared to control,; # P>0.05 when DGE20 compared to NAL+ DGE20,*
† P>0.05 when MORPHINE compared to NAL+ MORPHINE.

3.6. Anti-inflammatory Activity-Carageenan induced paw oedema

The average right paws volumes are presented in Table 5. For the control group, the injection of the phlogistic agent caused localised oedema, after 30min. The swelling increased progressively after 6h to a maximum 43.13% and remained obvious to 20.54% in control group 24h after injection. Pretreatment with DG extract shows significant dose dependent reduction in carrageenan-induced paw oedema to 13.75%(50mg/kg), 23.75%(100mg/kg) and 27.5%(200mg/kg) after 3h post-dosing interval. After 6h of treatment paw oedema was abolished significantly upto 15.68%, 24.5% and 45.09% and continued for 24h upto 27.39%, 35.61% & 43.83% at 50, 100, 200mg/kg respective doses while indomethacin showed 51.95% protection at 6h and 46.57% at 24h respectively (Table 5). Anti-inflammatory effect of DG extract was continuing even after 24h of dosing.

Table 5. Effect of *Desmodium gangeticum* leaves extract (DGE) on Carrageenan-Induced Paw Oedema in rat

Time	Control	DGE50	DGE100	DGE200	Indomethacin
1 hr	0.58 ± 0.03	0.56 ± 0.04	0.53 ± 0.03	0.51 ± 0.04	0.47 ± 0.03*
2 hr	0.67 ± 0.03	0.63 ± 0.04	0.56 ± 0.03	0.55 ± 0.05*	0.49 ± 0.04*
3 hr	0.80 ± 0.03	0.69 ± 0.04	0.61 ± 0.04*	0.58 ± 0.02*	0.51 ± 0.05*
4 hr	0.83 ± 0.04	0.72 ± 0.04*	0.67 ± 0.04*	0.63 ± 0.03*	0.57 ± 0.05*
6 hr	1.02 ± 0.03	0.86 ± 0.05*	0.77 ± 0.04*	0.56 ± 0.06*	0.49 ± 0.04*
24 hr	0.73 ± 0.05	0.53 ± 0.04*	0.47 ± 0.04*	0.41 ± 0.04*	0.39 ± 0.04*

DGE50: Ethanolic extract of Desmodium gangeticum (L.) at a dose 50mg/kg;
DGE100: Ethanolic extract of Desmodium gangeticum (L.) at a dose 100mg/kg;
DGE200: Ethanolic extract of Desmodium gangeticum (L.) at a dose 200mg/kg;
Indomethacin at a dose 80mg/kg i.p.;
** P>0.05 when compared to control,*

DISCUSSION

The present study demonstrated that the ethanolic extract of leaves of *D.gangeticum*, given by oral route in mice/rats have shown analgesic properties when assayed in two chemical (acetic acid-induced writhing and formalin test) and two thermal (hotplate and tail flick test) models of nociception and anti-inflammatory properties in carageenan-induced paw oedema animal model.

Acetic acid is a widely used chemical for the evaluation of peripheral antinociceptive activity [26]. Intraperitoneal injection of acetic acid indirectly induces the contraction of the abdominal muscles accompanied by extension of the forelimbs and elongation of the body. In this model, pain is generated indirectly via endogenous mediators, such as bradykinin, serotonin, histamine, substance P, and PGs, which all stimulates the peripheral nociceptive neurons. The mechanism of acetic acid writhing response through nociceptive neurons stimulation is related to the prostaglandins system. Thus, these nociceptive neurons are also sensitive to non-steroidal anti-inflammatory drugs, along with centrally acting drugs [22, 27]. Our results showed that orally administered DG extract shows 25.92%, 55.12% and 68.13% ($P < 0.05$) inhibition of acetic acid induced writhing at 50, 100, 200mg/kg, respectively. These effects may be attributed to PG synthesis inhibition. Acetic acid test is a non-selective antinociceptive model since acetic acid acts indirectly by inducing the release of endogenous mediators. Thus, the results of this writhing test alone did not ascertain whether the antinociceptive effects are central or peripheral.

To clear the mechanism of antinociceptive effect of DG extract, formalin test was performed on wistar rats. It is well known that formalin produces a distinct biphasic nociception pain, a first phase (0-5min) corresponding to acute neurogenic pain, and a second phase (lasting from 15-30min after formalin injection) corresponding to inflammatory pain responses [23]. It is generally accepted that drugs which act mainly centrally, such as narcotics, inhibit both phases of formalin-induced pain while peripherally acting drugs, such as non-steroidal anti-inflammatory drugs and corticoids, inhibit mainly the second phase of formalin-induced nociception [28, 29]. DG extract at the dose of 200mg/kg, inhibited both phases of the formalin test, but in 1st phase DG extract was not as effective as morphine (5mg/kg, i.p.). DG extract more prominently inhibits the inflammatory pain, at 50, 100, 200mg/kg doses and highest dose (200mg/kg) was equipotent to diclofenac (5.64 mg/kg, i.p.), indomethacin (80mg/kg, i.p.).

Thermal antinociceptive test, Eddy's hot-plate test and tail flick test were also performed to evaluate the possible central antinociceptive effects of DG extract. The hot-plate test used to evaluate central pain at the supra-spinal and spinal levels in which C, Aδ type I, and Aδ type II-sensitive fibers, mediates the pain [21] and tail flick response is a spinal reflex that is selective for centrally acting analgesic compounds, like pethidine [30, 31]. DG extract doses of 50, 100, and 200 mg/kg significantly increased latencies in the hot-plate model as well as in tail flick test, where aspirin like NSAIDs fail to produce response. DG extract was about to equipotent to intraperitonial administered morphine in eddy's hot-plate experiment. These results revealed that DG extract might exert pain relief action through the central nervous system. Pretreatment of naloxone in morphine treated animals diminishes morphine effect, but the not in DG extract treated animals. Therefore, antinociceptive experiment against thermal and chemical stimulus revealed that antinociceptive effect of DG extract might be due to central and peripheral analgesic activity. This peripheral analgesic activity might be the reason that naloxone could not block the DG extract induced latency period to thermal (hot-plate and tail flick model) as well as in chemical like acetic acid & formalin induced pain. Peripheral analgesic properties, activity might be probably linked to their anti-inflammatory effects.

The carrageenan-induced rat paw edema is a suitable test for evaluating anti-inflammatory /anti-edematous effect of natural products. Carrageenan induced oedema development is a biphasic event; an initial early phase (90–180min) involves the release of histamine and 5HT, and the later phase (270–360min) of edema is due to activation of kinin-like substances and release of prostaglandins, protease and lysosomes [32, 33, 34]. Prostaglandins (PGs) play a major role in the development of the second phase oedema, around 3h [35]. NSAIDs, indomethacin effectively inhibits the carageenan induced paw oedema and shows 51.96% inhibition of paw oedema. DG extract shows slow onset of action, therefore after 6 hour of treatment 200mg/kg dose was inhibits 45.09% paw oedema and after 24 hour of treatment equally effective to indomethacin.

The antinociceptive and anti-inflammatory activities exerted by this extract may be attributed to the presence of secondary metabolites like flavonoids, pterocarpinoids, alkaloids and their N-oxides. Flavonoids also have anti-inflammatory effects through its inhibition of the cyclooxygenase pathway [36]. Pterocarpinoids has also been reported to have antioxidant activity [19]. That the extract inhibited neurogenic and non-neurogenic pains as well as narcotic

pains may in part explain the mechanisms of its action and these effects are due to the present of pterocarpinoids, flavonoids and other component in the extract.

CONCLUSION

In conclusion, the results of the present anti-inflammatory and analgesic study justify the ethanopharmacological use of *Desmodium gangeticum*. Further experimental studies should be carried out to correlate the pharmacological activities with the chemical constituents.

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