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PHYTOCHEMICAL INVESTIGATIONS OF *DISMONDIUM GENGETICUM* PLANT EXTRACT AND ITS STUDY FOR ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT

In many different parts of the world, D. gangeticum has long been utilized as a medicine. Shalaparni root decoction is used as a snake bite treatment in India. The goal of this study was to determine the pharmacological properties of the Dismondium gengeticum leaf extract. Petroleum ether (PE) and 50% v/v ethyl alcohol were used to extract the dry and powdered leaf parts. The purpose of this study was to assess the phytochemical and pharmacological significance of the extract from Dismondium gengeticum leaves and investigate their anti-inflammatory properties. Formalin-induced rat paw edema was used to assess the anti-inflammatory properties of the standard and various test groups that included 50% ethanolic extract of Dismondium Gengeticum leaves. The evaluation's findings were observed using diclofenac sodium as the reference medication. A gradual edema was caused by the injection of formalin into the hind paw, which peaked five hours later. The paw thickness of the animals in Group I was 0.75 ±0.04 mm at t = 0. The animals in Group I had paws that were 1.78 ±0.04 mm thicker after 0.5 hours, 2.82 ±0.16 mm thicker after 1 hour, 3.86 ±0.28 mm thick after 2 hours, 4.95 ±0.38 mm thick after 3 hours, 5.02 ±0.22 mm thick after 4 hours, and 5.04 ±0.24 mm thick after 5 hours. Furthermore, test group IV, which contained 400 mg/kg body weight of a 50% ethanolic extract of Dismondium Gengeticum leaves, had comparatively higher activity than test group III. In the same way group III, test groups containing (200 mg/kg body weight) 50 % ethanolic extract of leaves of Dismondium Gengeticum compounds of this series showed insignificant anti-inflammatory activity except standard group and test group IV. Therefore, it can be recommended that future human research examine the harmonizing effect of combining diclofenac sodium with an ethanolic extract of Dismondium Gengeticum leaves on different bodily tissues.

Keywords: *Dismondium Gengeticum, leaves, ethanolic extract, anti-inflammatory activity, Shalaparni.*

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INTRODUCTION:

Inflammation is a defensive response which causes the different physiological adaptations which limits the tissue damage and removes pathogenic insult & Pain is an expected result of many diseases, medical care, surgical interventions and trauma. Pain is a complex experience which includes affective, cognitive and behavioral features, all of which are the result of mental process, as such; it represents psychological conditions [1-3].

D. gangeticum is traditionally used as therapeutics in many different geographical regions. In India, root decoction of *Shalaparni* is used as a snake bite remedy [4]. Root paste also traditionally taken orally as an antidote for snake and scorpion bites in the Dudhi block of District Sonebhadra, Uttar Pradesh, India [5]. Its root decoction is utilized in rheumatism with a dose of one spoon in Eastern Ghats of Andhra Pradesh, India [6]. Aqueous leaf extract is poisonous towards *E. coli.* in Odisha, India [7]. The plant's stem and leaf are used as a diuretic, antitoxic, vomiting and diarrhea as a decoction in the Chandra Prabha Wildlife Sanctuary, Chandauli District, Uttar Pradesh [8]. It is utilized in fever, vomiting, *Vata-Dosha* in Raipur District [9]. In tribal areas of Adilabad District, Telangana Region, plant roots, bark and leaves are used to treat fever and kidney disorders [8]. Inhabitant of eastern Ghats, Andhra Pradesh, India, its root decoction is traditionally utilized with half cup dose once daily for 2-3 months to alleviate respir-atory disease [10]. Paste of its stem bark is externally ap-plied in goiter daily once for 3-4 days by tribes of Chhattisgarh state [11]. Tonic "*Salampak*" prepared from *D gangeticum* is employed in gynecological diseases by tribals of Jhalod taluka of Dahod district, Gujarat, India [12]. Root is used in premature ejaculation in Bulamogi district, Uganda [13]. The root is also used for cough and cold in Deo-lapar forest range, Maharastra [14]. *D. gangeticum* is being utilized in Stomach ache and menstrual ache ethnomedici-nally by the Khasia community people in Moulibazar dis-trict of Bangladesh [8].

Desmodium gangeticum is reported as tonic, febrifuge, antipyretic, digestive, anti-catarrhal, diuretic, aphrodisiac and alterative. It is also potential in the treatment of typhoid, piles, asthma, bronchitis, dysentery, and biliousness. It is a bitter, sweet, thermogenic, nervine tonic, aphrodisiac, demulcent, anthelmintic, cardiac tonic, febrifuge, anti-inflammatory, diuretic, haemostatic, rejuvenating, and useful in neuromuscular and ophthalmic disorders, loss of appetite, flatulence, diarrhoea, dysentery, nausea, piles, helminthiasis. It is used in cardiac disorders, tuberculosis, cough, seminal weakness, urinary disorders, fever, debility, and gout. Whole plant shows many pharmacological activities like Medicinal Fabaceae of India; anti-inflammatory, anti-nociceptive, analgesic, anti-amnesic, anti-diabetic, anti-oxidant, anti-ulcer, antibacterial, wound healing, antipyretic etc. The root possesses antibacterial, antifungal, anti-inflammatory, analgesic, antileishmanial, immunomodulatory and CNS depressant activities [9-12].

Figure 1: Differents parts of *D. gangeticum*

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Flavonoids such as quercetin and rutin exhibit anti-inflammatory properties and antioxidants, enhancing cardiovascular health and immune system benefits [14, 15]. Flavonoids help in scavenging free radicals and protecting cells from damage [16]. Phenols like gallic acid, ellagic acid and vanillic acids play a crucial role in the plant's therapeutic effects, including its ability to support liver health, reduce inflammation, and potentially protect against chronic diseases [17]. Alkaloids are nitrogen-containing organic compounds known for their diverse pharmacological properties such as antimicrobial, anti-inflammatory, potentially anticancer properties, neuroprotective, and analgesic effects [18].

Having vast commercial medicinal use of *Desmodium gangeticum*, the study of the impact of drought stress on secondary metabolites will help the efficient use of the *Desmodium gangeticum* [L.] *Desmodium gangeticum [19]* is one of the essential herbs of ethnomedicine that have been used extensively either as a single drug or in combination with other drugs in the traditional system of medicines in India. Natural items with medicinal qualities have been used for as long as human civilization, and for a very long time, the primary sources of pharmaceuticals were mineral, plant, and animal goods. Alternative therapies and the therapeutic application of natural materials, particularly those produced from plants, have gained popularity in recent years [20].

The present study was intended to find out the pharmacological activities of the extract of leaves of Dismondium Gengeticum. The dry and powder portions of leaves were extracted with petroleum ether (PE) and 50% v/v ethyl alcohol. The aim of this research was to evaluate Phytochemical and Pharmacological importance of Dismondium Gengeticum leaves extract and examine their anti-inflammatory activity.

MATERIALS AND METHODS:

COLLECTION AND IDENTIFICATION OF PLANT MATERIALS

Leaves of plant of ***Dismondium Gengeticum*** was collected from Bithoor, Kanpur Uttar Pradesh India. The plant material was identified and authenticated taxonomically at Department of Pharmaceutical Technology, University of North Bengal Pin 734013 A voucher specimen of the collected sample was deposited in the departmental herbarium for future reference and it was authenticated by Dr. Bapi Ray Sarkar, Associate Professor.

EVALUATION OF PHARMACOGNOSTIC PARAMETERS

Routine pharmacognostic studies including organoleptic tests, macroscopic and microscopic observations were carried out to confirm the identity of the materials as well documented by some researchers [12-16,21-23].

EXTRACTION OF PLANT MATERIALS

Extraction of *Dismondium Gengeticum* leaves

The freshly collected leaves (1 kg) of *Dismondium Gengeticum* were washed with potable water and finally rinsed with distilled water and shade-dried. Then collected and cleaned parts of plant were dried in tray drier under controlled conditions at 35°C ±2 °C and powdered it. The powdered plant materials (500g) was macerated with petroleum ether to remove fatty substances, the marc was further exhaustively extracted with of 50% ethanol for 3 days. The extract was separated by filtration and concentrated on rotavapour (Buchi, USA) and then dried in lyophilizer (Labconco, USA) under reduced pressure. All chemicals and reagents used including the solvents were of analytical grade [21-23].

PLANT EXTRACT – EXTRACTION PROCEDURE:

1. The plant materials were moved promptly into the research facility, cleaned with deionized water and chose plant parts were independently conceal dried for multi week.

2. Each shade dried plant parts were powdered with the assistance of processor. Fine powder of each example was put away in clean compartment to be utilized for Soxhlet extraction following the technique for Subramanian and Nagarjan, (1969) in various polar solvents chose.

3. The dried plant material was pounded into fine powder utilizing a processor (blender). About 10 gm of powdered material was separated in Soxhlet extraction mechanical assembly progressively with various solvents (250 ml) as per their expanding extremity for 18 hours at a temperature not surpassing the breaking point of the individual dissolvable.

4. The acquired concentrates were separated by utilizing Whatman No. 1 channel paper and afterward accumulated at 40° C by utilizing an evaporator. The remaining concentrates were put away in fridge at 4° C in little and sterile glass bottles.

5. Percent extractive qualities were determined by the accompanying equation.

Percent Extracts = (Weight of dried extract / Weight of dried plant material)x100

Percentage Yield:

The percentage yield of ethanolic extract of *Dismondium Gengeticum* leaves was calculated. The extract obtained was further subjected to phytochemical screening and pharmacological investigations.

% Yield of extract = (Weight of extract X 100)/Weight of powder taken

PRELIMINARY PHYTO-CHEMICAL TESTS

Preliminary qualitative phytochemical screening of 50% ethanolic extract of *Dismondium Gengeticum* leaves were performed for alkaloids, carbohydrates, flavonoids, glycosides, triterpenoids, resin, saponins, steroids and tannins [12-16 and 21]. All the tests were done in

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triplicate except the foreign matter and moisture content.

PHARMACOLOGICAL SCREENING ON ANIMAL MODELS

ANIMALS AND ENVIRONMENT CONDITION

All the animals used for the study were healthy and active in their cage. Studies were carried out using male Wistar rats weighing 170-200 g. They were obtained from isted uppliers of from CPCSEA listed of Shri Ramnath Singh college of Pharmacy, Gormi, Bhind, Gwalior The rats were group housed in polyacrylic cages (38×23×10cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2 ^{0}C) and relative humidity 50% (±10%), with a dark and light cycle of 12 ± 1 h. They were allowed free access to standard dry pellet diet (Amrut, India) and water *ad libitum* and kept in quarantine for a week to acclimatize with animal house facility.

All procedures described were reviewed and approved by the institutional committee for ethical use of animals.

PHRMACOLOGICAL EVALUATION

Pharmacological evaluation of the extract of leaves of *Dismondium Gengeticum* **-**

A) Acute toxicity study following OECD guidelines [24]
B) Anti inflammatory activity in formalin induced rat paw edema method.

Prior to study proposal detailing the experimental protocol was submitted to Institutional Animal Ethics committee and was cleared Approval No. RNS/05/2025.

Animals used:-

a) **For acute toxicity studies:** Adult, healthy, overnight fasted, male albino mice, weighing between 35-45 grams.

b) **For anti-inflammatory activity:** Adult, healthy, overnight fasted, male albino rats, weighing between 200-250 grams.

Animals used for the experiment were maintained in the animal house facility of the institution under standard animal house conditions (followed to CPCSEA guideline), and were fed with commercial feed pellets and water *ad libitum*. Animals were acclimatized to the laboratory condition for a week prior to start the experiments.

All stock solution of either extract of leaves of *Dismondium Gengeticum* and standard drugs were prepared fresh on the day of experiment with DMSO (Dimethyl sulfoxide) [25].

Acute toxicities study:-

Acute toxicities were carried out by using Wistar albino mice, following OECD text guideline 423(2001) usage in defined dosage and results allowed the substances to be ranked and classified according to globally harmonized system (GHS) for the classification of chemicals which can cause acute toxicities [24].

Extract of leaves of Dismondium Gengeticum was dissolved in DMSO and it was administered to the animal (n=6) in the dose of 1000and 2000 mg/kg body weight orally (through an oral tube no-09), placed individually in plastic cages and observed at least one during first 30 minutes and periodically during 24 hours. Special attention given during first two hours treated animals observed by an observer blind to the treatment protocol. Since no mortality was observed in this dose range, hence this was considered as safer dose and no further toxicity study done at higher doses.

Anti-inflammatory activity:-

Chemicals used-

1) Diclofenac sodium

2) 1% formalin was prepared from commercial formaldehyde solution.

The activity was determined by formalin induced rat hind paw oedema method. Diclofenac sodium was used as the standard drug & the % inhibition of oedema was determined. Formalin (0.1 ml of 1%) was used as phlogistic agents and produced inflammation at the injected site of the animals. A compound may be anti-inflammatory agents, if it is effectively suppresses phlogistic agent induced paw edema.

Procedure:-

Adult, healthy animal of either sex over night fasted were randomly assigned to Group I to Group IV Groups (n=6).

Group I animal received only the vehicle orally.

Group II animal received Diclofenac sodium i.p (dose 5 mg/kg) (standard drugs in reference).

Group III & Group IV animals received the sample of *extract of leaves of Dismondium Gengeticum* (dose 200 & 400mg/kg) under test dissolved in DMSO administered orally.

Pedal edema was induced by injecting 0.1ml of freshly prepared 1% formalin solution into the planter apnoneurosis of right hand paw. Change in paw volume was measured immediately after zero hour, half an hour, one hour, two hours, three hours, four hours and five hours following injection using digital calipers. The treatment was continued for 6 consecutive days and the edema was measured on 1 and 6 days. The percentage inhibition of paw edema was calculated for all the models using the following formula.

% inhibition = [(Vc-Vt)/Vc] × 100. Where, Vc represent paw volume of treated control group, V_t represent paw volume of test or Diclofenac sodium.

Inflammation was indicated by significant ($P<0.05$) increase in paw thickness. The paw thickness of the groups treated with the test compound and that of the group treated with diclofenac sodium (standard drug) was compared with the negative control group. Significant reduction of the paw thickness (expressed as mean ± S.E.M) among the treated groups compared to the control group was used to indicate oedema inhibition (anti-inflammatory activity) [22,23,25].

Procedure:

Adult, healthy animal of either sex over night fasted were randomly assigned to Group I to Group X Groups (n=6).

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Group I	**Control**	Animals received only the vehicle orally.
Group II	**Standard**	Animals received Diclofenac sodium i.p (dose 10 mg/kg) (standard drugs in reference).
Group III	**Test (200mg/Kg b.w. administered orally)**	Treated with mixtures of 50% ethyl alcohol *extract of leaves of Dismondium Gengeticum* dissolved in DMSO.
Group IV	**Test (400mg/Kg b.w. administered orally)**	Treated with mixtures of 50% ethyl alcohol extracts of *extract of leaves of Dismondium Gengeticum* dissolved in DMSO.

Statistical analysis:-

All the results were expressed as mean ± SEM, and were subjected one way ANOVA followed by Dunnet test (post test). Values were considered significant when $p<0.05$. The potency of test compound was also determined by measuring its percentage inhibition/ reduction in compare with standard as well treated groups.

RESULTS AND DISCUSSION

Fresh leaves of the selected plant were collected, washed and after drying up properly it was subjected for the analysis of various physicochemical parameters. % of foreign organic matter of *Dismondium Gengeticum* was found in the range of 0.32-0.98 and loss on drying was found in a range of 6.44-8.59**.** The moisture content plays vital role in storage of the drugs and as it varies a lot the extractive values will vary which will affect the actual dose of the active constitute, as well as due to higher level of moisture content that may be affected on the rate detoriation of crude drugs. Determination of loss on drying in crude drugs also represents the presence of moisture level in the materials. If the moisture content is very less then that may be preserved the materials up to long duration and also the dose of the crude drugs is requires very lower level compare to the crude drugs having large amount of moisture.

The total ash of the leaves of *Dismondium Gengeticum* was found 13.13-14.97 while acid insoluble ash and water-soluble ash values were in the range of 2.24-2.29 and 6.34 to 6.48 respectively. Ash values are helpful in determining the quality and purity of crude drug, especially in the powdered form. The ash value of the leaves of *Dismondium Gengeticum* was found 3.42. Total ash reflects the care taken in its preparation as all traces of organic matter are removed during ash formation and usually consists of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. A higher limit of acid insoluble ash imitates the cases where silica may be present or when the calcium oxalate content of the drug is very high.

Extractive values of crude drugs are supportive for their estimation, particularly when the constituents of a drug cannot be willingly predictable by any other method. Further, these values point out the nature of the constituents present in a crude drug. The percentages of water soluble extractive values and alcohol soluble extractive values (w/w) of leaves of *Dismondium Gengeticum* were 9.8-10.68 and 11.38-12.54 respectively.

PERCENTAGE YIELD:

The percentage yield of ethanolic extract of leaves of *Dismondium Gengeticum* was found to be 9.5% (w/w). Further the qualitative phytochemical analysis of the ethanolic extract of leaves of the plant were conducted for confirming the presence or absence of alkaloids, tannic acids, glycosides, saponins, phytosterols, flavonoids, terpenoids, triterpenoids, resins, phenolic compounds & tannins, carbohydrates and proteins & amino acids.

PRELIMINARY PHYTOCHEMICAL ANALYSIS:

50% ethanolic extract of leaves samples of the selected plant were subjected to various qualitative tests for the identification and the presence of various phytochemical constituents. The present studies were carried out for the ethanolic extract of leaves of *Dismondium Gengeticum* and which revealed that the presence of medicinally active metabolites in the samples. The phytochemical character of leaves of the *Dismondium Gengeticum* investigated is summarized in table form. It can be clearly stated and concluded from table mentioned for the phytochemical evaluation data of the leaves extract of the plant. It has been sheen that presence of alkaloids and flavonoids in the extract of pet ether but it has been sheen that presence of alkaloids, protein and amino acid, steroids and terpenoids and flavonoids in the extract of 50% ethyl alcohol of leaves of Dismondium *Gengeticum* but tannic acids, glycosides, anthraquinone glycosides, cardiac glycosides, saponins, resins and tannin were absent in the extract of 50% ethyl alcohol of leaves of Dismondium *Gengeticum*.

Microscopy

The macroscopic and organoleptic evaluation is an easy method and is carried out at the time of plant collection. The various observed features of the leaves were recorded. The result of powder microscopy reveals the presence of various parts like epidermis, xylem, pith and palisade tissues.

PHARMACOLOGICAL SCREENING ON ANIMAL MODELS

Pharmacological evaluation of 50% ethanolic extract of leaves of *Dismondium Gengeticum* includes [21-25] –

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ACUTE TOXICITY STUDY

Table 1: Data showing the determination of acute toxicity of 50% ethanolic extract of leaves of *Dismondium Gengeticum* plant

Treatment / Dose	Total mice taken	Mortality (after 72 hr.)
		Inference of ethanolic extract of leaves of *Dismondium Gengeticum*
1000mg/kg	6	0
2000mg/kg	6	0

Acute toxicity study following OECD guidelines 423(2001)

Acute toxicity studies carried out prior to pharmacological evaluation studies of 50% ethanolic extract of leaves of *Dismondium Gengeticum* using OECD Test Guideline 423(2001) [24]. 50% ethanolic extract of leaves of *Dismondium Gengeticum* was found to be safe up to 2000 mg/kg body weight.

The 50% ethanolic extract of **leaves of *Dismondium Gengeticum*** has shown 0 % mortality (**Table 1**) at a dose corresponds to 1000 & 2000 mg/kg body weight after observing continuously for 8 hours and finally overnight mortality recorded. Behavior of the animals including body weight and any other toxic symptoms also observed for 72 h and the animals were kept under observation up to 14 days.

Acute toxicity study exposed the non-toxic nature of the ethanolic extract of leaves of the plant. There was no mortality or any toxic effects observed at the maximum tested dose level of 2000 mg/kg after 14 days.

From the acute toxicity study, it can be concluded that the ethanolic extract of leaves of the selected plant (*Dismondium Gengeticum*) has no fatal effect up to 2000mg/kg body weight after oral administration in rats. Hence, as per the literature guidelines 1/10th of the dose was set to assess the anti diabetic activity of the selected plant (*Dismondium Gengeticum*).

Finally it can be concluded from the acute toxicity study that the ethanolic extract of leaves of the selected plant (*Dismondium Gengeticum*) were safe and sound still at doses as high as 2 g/kg b.w. of rats.

Since no mortality was observed in this dose range of 1000to 2000mg/Kg b.w., hence this was considered as safer dose and no further toxicity study done at higher doses. Acute toxicity studies carried out prior to pharmacological evaluation studies of all the extracts using OECD Test Guideline to find out the safe and toxic dose. All the extracts of both the plants were found to be safe up to 2000 mg/kg body weight. Finally, 200 mg /kg b.w. and 400 mg /kg b.w. were selected for this study and acute toxicity study revealed the nontoxic nature for each selected plant extracts, which was tabulated in **table 1.**

Anti inflammatory activity in formalin induced rat paw edema method of different groups:

Table 2: Anti inflammatory activity in induced rat paw edema method of different groups: Change in paw edema volume in mm (Mean ± SEM) at various time intervals.

Group No.	Compound	Dose	Change in paw edema volume in mm (Mean ± SEM) at various time intervals						
			0 min	30 min	60 min	120 min	180 min	240 min	300 min
Group I	Control (Vehicle	DMSO	0.75 ±0.04	1.78 ±0.04	2.82 ±0.16	3.86 ±0.28	4.95 ±0.38	5.02 ±0.22	5.04 ± 0.24
Group II	Standard drug: Diclofenac sodium	5 mg/kg body weight	0.74 ±0.02	00.76 ±0.04	0.78 ± 0.04	0.80 ± 0.06	0.81 ± 0.08	0.82 ± 0.08	0.82 ± 0.08
Group III	Test sample: 50% ethanolic extract of leaves of Dismondium Gengeticum	200 mg/kg body weight	0.76 ±0.06	1.06 ± 0.06	1.78 ±0.08	1.92 ±0.1	2.84 ±0.06	3.83 ±0.04	4.39 ±0.26
Group IV	Test sample: 50% ethanolic extract of leaves of Dismondium Gengeticum	400 mg/kg body weight	0.74 ±0.04	0.98 ± 0.04	1.03 ± 0.13	1.26 ±0.16	1.32 ± 0.19	1.3 ± 0.22	1.04 ±0.04

*All values are expressed as means ± SD (=6), The observations are expressed as mean ± S.E.M.., * P < 0.05, ** P < 0.01, †P < 0.001, (ANOVA followed by Dunnett's test) as compared to control was considered as accepted values and significant.*

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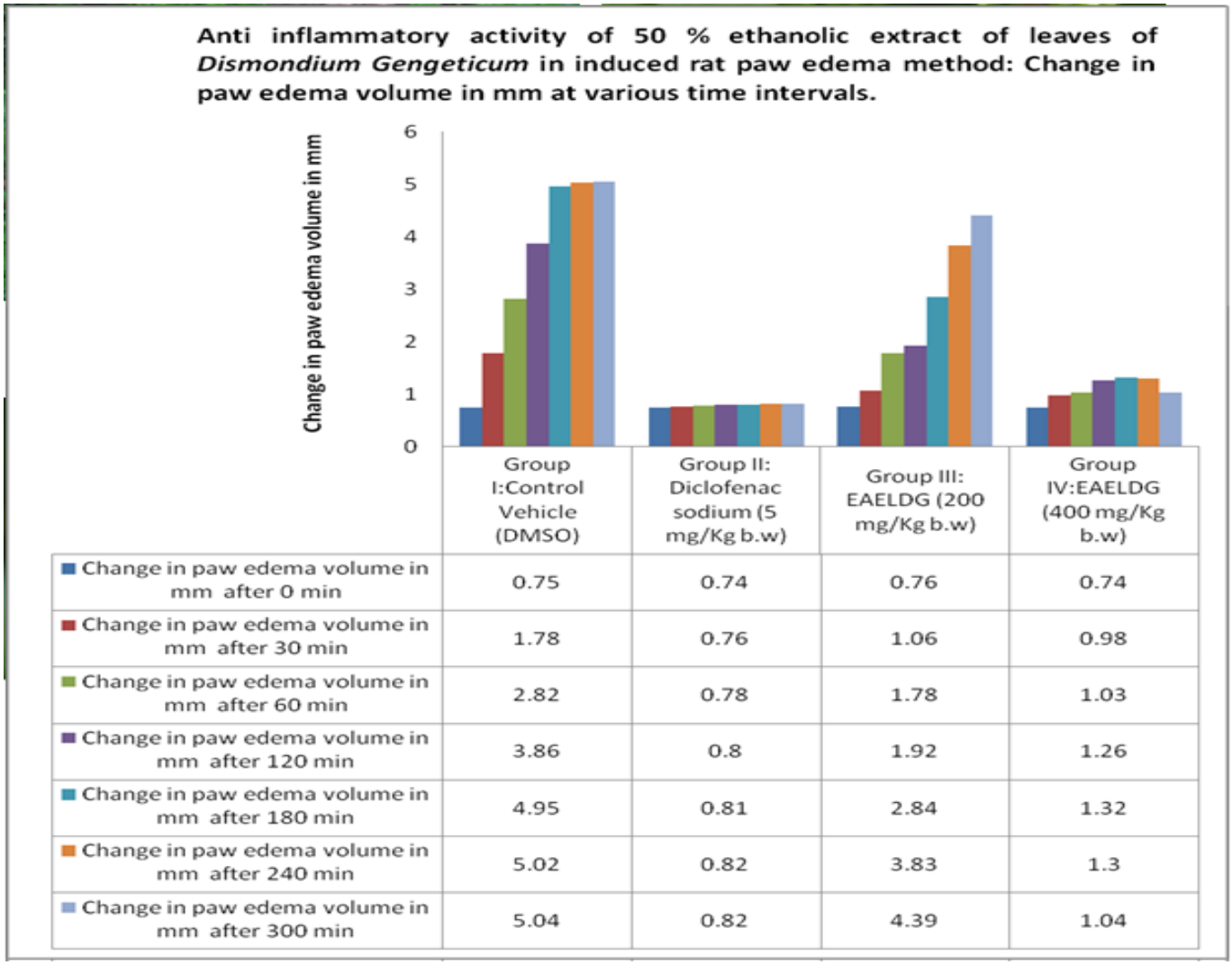

Figure 2: Histogram showing Anti inflammatory activity of standard & different test groups containing 50 % ethanolic extract of leaves of *Dismondium Gengeticum*: % inhibition in paw thickness at various time intervals.

Table 3: Anti inflammatory activity in induced rat paw edema method of different groups: % inhibition in paw thickness at various time intervals

Group No.	Compound	Dose	% inhibition in paw thickness at various time intervals					
			30 min	60 min	120 min	180 min	240 min	300 min
Group II	Diclofenac sodium	5 mg/kg body weight	57.30	72.34	79.27	83.63	83.66	83.73
Group III	Test sample: 50% ethanolic extract of leaves of *Dismondium Gengeticum*	200 mg/kg body weight	40.44	36.87	50.25	47.62	23.7	12.89
Group IV	Test sample: 50% ethanolic extract of leaves of *Dismondium Gengeticum*	400 mg/kg body weight	44.94*	63.47	67.35*	73.33*	74.1*	79.36**

All values are expressed as means ± SEM (n=6), P<0.05, P**<0.01 compared to control,Student t-test (Unpaired).*

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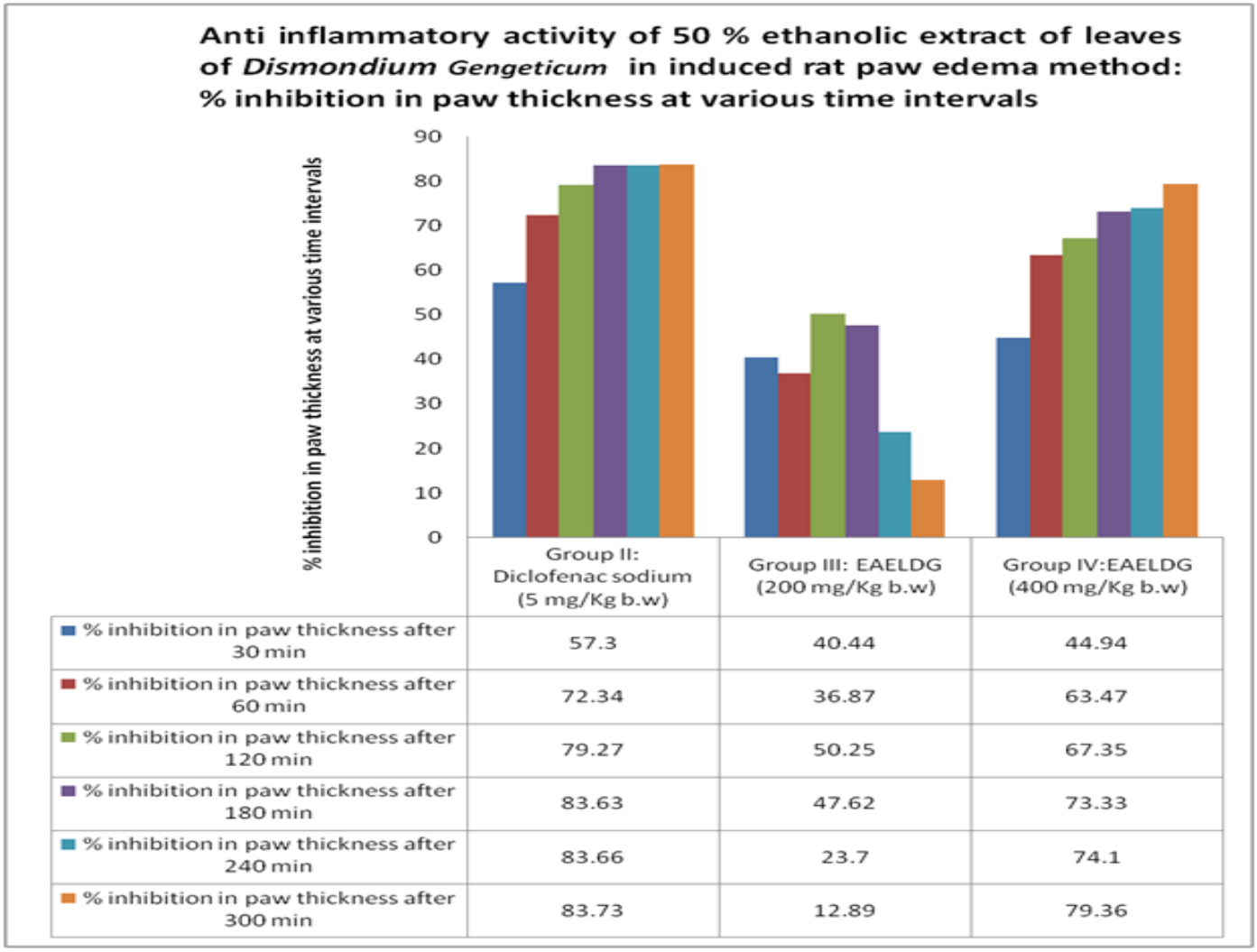

	Group II: Diclofenac sodium (5 mg/Kg b.w)	Group III: EAELDG (200 mg/Kg b.w)	Group IV:EAELDG (400 mg/Kg b.w)
% inhibition in paw thickness after 30 min	57.3	40.44	44.94
% inhibition in paw thickness after 60 min	72.34	36.87	63.47
% inhibition in paw thickness after 120 min	79.27	50.25	67.35
% inhibition in paw thickness after 180 min	83.63	47.62	73.33
% inhibition in paw thickness after 240 min	83.66	23.7	74.1
% inhibition in paw thickness after 300 min	83.73	12.89	79.36

Figure 3: Histogram showing Anti inflammatory activity of standard & different test groups containing 50 % ethanolic extract of leaves of *Dismondium Gengeticum*: Change in paw edema volume in mm (Mean ± SEM) at various time intervals.

Anti inflammatory activity:

Anti inflammatory activity in induced rat paw edema method: Change in paw edema (Mean ± SEM) and % inhibition in paw thickness at various time intervals are shown in **tables 2 and 2.** Anti inflammatory activity in induced rat paw edema method. Histogram is showing Anti inflammatory activity of standard & different test groups containing 50 % ethanolic extract of leaves of *Dismondium Gengeticum*: Change in paw edema volume in mm (Mean ± SEM) and % inhibition in paw thickness at various time intervals are shown in **figures 3 and 4.**

The anti-inflammatory activity of the standard & different test groups containing 50 % ethanolic extract of leaves of *Dismondium Gengeticum* has been evaluated by using formalin-induced rat paw edema method. The results of the evaluation have been observed by taking diclofenac sodium as the standard drug.

Some earlier reported researchers also studied on carrageenan-induced inflammation are a useful experimental model of acute inflammation for detecting orally active anti-inflammatory agents [21-23, 25]. Injection of formalin into the hind paw induced a progressive edema reaching its maximum at 5 hours. In case of Group I animals paw thickness found at t = 0 was 0.75 ±0.04 mm. Group I animals showed an increase in paw thickness of 1.78 ±0.04 mm (t = 0.5 hours), 2.82 ±0.16 mm (t = 1 hour), 3.86±0.28 mm (t = 2 hours), 4.95 ±0.38 mm (t = 3 hours), 5.02 ±0.22 mm (t = 4 hours) and 5.04 ±0.24 mm (t = 5 hours). Moreover, group IV, test groups containing (400 mg/kg body weight) 50 % ethanolic extract of leaves of *Dismondium Gengeticum* is exhibited fairly better activity compared to another test group III. In the same way group III, test groups containing (200 mg/kg body weight) 50 % ethanolic extract of leaves of *Dismondium Gengeticum* compounds of this series showed insignificant anti-inflammatory activity except standard group and test group IV.

Some earlier reported researchers also studied on carrageenan-induced inflammation are a useful experimental model of acute inflammation for detecting orally active anti-inflammatory agents [24]. Whereas in Group II, animals showed an increase in paw thickness of 0.74 ±0.02 mm (t = 0.0 hours), 0.76 ±0.04 mm (t = 0.5 hour), 0.78±0.04 mm (t = 1 hour), 0.80 ±0.06 mm (t = 2 hours), 0.81& 0.82 ±0.08 mm (t = 3 & 4hours) and 0.82 ±0.08 mm (t = 5 hours). Moreover, group IV (Treated with mixtures of (test groups containing dose of 400 mg/kg body weight) 50 % ethanolic extract of leaves of *Dismondium Gengeticum* dissolved in DMSO.) is exhibited fairly better activity compared to standard and another extracts with dose of

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200 mg/kg body weight. In the same way group IV extracts with higher doses showed significant anti-inflammatory activity. The group of II, IV was treated by Diclofenac sodium (10 mg/kg b.w.) and 50 % ethanolic extract of leaves of *Dismondium Gengeticum* (400 mg/kg body weight) showed maximum inhibition of edema after 5hrs, which was 83.72 & 79.36% respectively, compared with other treated groups (III) with dose of 200 mg/kg body weight.

Conclusion:

Further studies will be carried out in future to identify the active anti-inflammatory principles of this plant that are responsible. It can be thereby suggested further for the investigations on humans to study the harmonizing effect of the amalgamation of ethanolic extract of leaves of *Dismondium Gengeticum* and diclofenac sodium on other body tissues.

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