PHARMACOGNOSTICAL, *IN VIVO* ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *GREWIA NERVOSA*

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Abstract

Background: *Pain is usually related to inflammation, the most common manifestation of many diseases. Grewia nervosa leaves for the purpose of validating its ethnomedicinal uses.*

Aim & Objectives: *The present study was carried out for pharmacognostical, in vivo analgesic and anti-inflammatory activity of hydroalcholic extract of leaves of Grewia nervosa belonging to the family of Tiliaceae.*

Methods: *In this study various standardisation of microscopic, physicochemical, fluorescence analysis and phytochemical screening was performed for hydroalcholic extract using standard procedure. The acute oral toxicity study was done by OECD guidline. The analgesic activity of extract was evaluated by hot plate method using albino mice and anti-inflammatory property of hydroalcoholic extract of leaf was tested by carrageenan induced acute paw odema in rats. The extract was evaluated at 100 and 200 mg/kg doses. Morphine 5 mg/kg for hot plate method, Indomethacin 10 mg/kg for paw odema test as a standard drug.*

Results & Discussion: *The pharmacognostical studies on the leaf of Grewia nervosa gave valuable information about quality and purity of the drug. Phytochemical screening of extracts revealed the presence of carbohydrates, alkaloids, saponins, phenols, tannins and flavonoids. The acute oral toxicity study shown no toxic sign and death at the dose 2000 mg/kg. The analgesic and anti-inflammatory effect of hydroalcholic extract (200 mg/ kg) shown significant ($p < 0.01$) effect.*

Conclusion: *Results clearly demonstrated that hydroalcoholic extract of Grewia nervosa displayed potent analgesic and anti-inflammatory effect. Future studies are need to isolate and characterize the active principle of Grewia nervosa.*

Keywords: *Grewia nervosa, Pharmacognostical, Analgesic, Anti-inflammatory.*

1. Introduction

Medicinal plants are used as traditional medicines to treat different diseases [1] and capability of producing biologically interesting and valuable chemical constituents.[2][3] Medicinal plants and a number of plants derived extracts are used against diseases in various system of medicine. Plant derived natural products such as flavonoids, terpenes and alkaloids [4][5] have considerable attention in recent years due to their pharmacological properties including analgesic, inflammatory and anti-pyretic activities. Anti-inflammatory activity of many plants attributed to their sterol, triterpene [6] and flavonoids contents.[7] Prolonged administration of anti-inflammatory drugs produces adverse effects. Herbal drugs have lesser side effects and safe, are replaced by synthetic drugs.

During the last few years, a great deal of interest has been given to medicinal plants as potential therapeutic agents in the treatment of pain and inflammation. Pain is usually related to inflammation, the most common manifestation of many diseases.[8] *Grewia nervosa* is to considered to have therapeutic potential, however, the plant has been proven to insecticidal, larvicidal and free radical scavenging activities.[9][10][11] The present study is to investigate the analgesic and anti -inflammatory properties of hydroalcoholic extract of *Grewia nervosa* leaves for the purpose of validating its ethnomedicinal uses.

2. Materials and Methods

Collection and authentication of plant material

The leaves of *Grewia nervosa* L were collected from Malappuram, Kerala during the month of December- January. The collected plant material was identified and authenticated by a Taxonomist DR. G. V. S Murthy of Botanical survey of India (BSI), Agricultural university, Tamilnadu, Coimbatore. The Authenticated vocher specimen (Ref No: BSI/SRC/5/23/2018/Tech/833) was deposited in the herbarium of Division of Pharmacognosy, JKK Munirajah Medical Research Foundation, College of Pharmacy, Tamilnadu, India.

Preparation of hydroalcoholic extract

The freshly collected leaves of *Grewia nervosa* (200 g) materials were cleaned to remove dust and the dried under shade. After that, leaves were ground into coarse powder. The dried le of plant were defatted with petroleum ether (50-60^{o}C) and extracted with ethanol (70% v/v) in a soxhlet extractor. Each time

before extracting with the next solvent the material was dried in hot air oven. After completion of extraction the solvent was evaporated using a rotary vacuum-evaporator. The resulting dry hydroalcoholic extract percentage yield 11.16 % (w/w). The residue was lyophilized and store at -70^{0}C. The dried extract was used for further studies.

3. Pharmacognostical evaluation

Macroscopic evaluation

The leaves of *Grewia nervosa* subjected to macroscopic studies which comprised of organoleptic characteristics like color, odour, taste, shapes, texture, fracture of the drug. These parameters are useful in quality control of the crude drug and were evaluated as per standard guideline.[12]

Microscopic evaluation

Fresh leaves of *Grewia nervosa* were selected for the microscopical studies. A thin section was taken from the bark and stained with Chloral hydrate solution and concentrated hydrochloric acid in 1:1 ratio and observed under trinocular microscope.[13]

Powder microscopy

Preliminary examination and behavior of the powder with different chemical reagents were carried out with the procedure described in Khandelwal.[14]

Physiochemical evaluation

Physicochemical constants of the powder bark have been done to evaluate the quality and purity of the drug. Various physicochemical parameters Ash values and extractive values were calculated.[15][16]

Fluorescence analysis

A fluorescence characteristic of the powdered was observed in visible light (365 nm) and UV light (254 nm) respectively under the ultraviolet chamber.[17][18] The observed colour was noted.

Preliminary phytochemical studies

The plant extract was subjected to preliminary phytochemical screening for the detection of various plant constituents present.[19][20]

4. Pharmacological Studies

Animals

Swiss albino mice (20-25 g) and Male Wistar rats (180-200 g) were used for assessment of analgesic activity and anti- inflammatory activity respectively. Animals were breed and maintained at Central Animal Facility of the Institute under standard housing conditions of temperature 25 ± 2°C, relative

humidity 55- 65% and light and dark cycles of 12 h, respectively. Animals were provided with standard pellet diet and water. Principles of laboratory animal care guidelines were followed and prior permission was sought from the Institute Animal Ethics Committee for conducting the experiments.

Acute toxicity study

Acute oral toxicity studies were performed according to Organization for Economic Co-operation and Department (OECD).[21] Swiss albino male mice (n=6/each dose) were selected by random sampling technique. Animals were fasted for 12 hrs with free access to water only. Hydroalcoholic extract of Grewia nervosa (dissolved distilled water) were administered orally at a dose of 5 mg/kg and mortality was observed for 3 days. However, the mortality was observed in only one mice out of six animals, then the same dose was repeated with higher doses such as 50, 300, 500, 1000 and 2000 mg/kg. General behaviors such as motor activity, tremors, convulsion, straub reaction, aggressiveness, piloerection, loss of lighting reflex, sedation, muscle relaxation, hypnosis, analgesia, ptosis, lacrimation, diarrhea and skin colour were observed for the first one hour and after 24 hours of drug administration.

Analgesic activity

The analgesic effect of *Grewia nervosa* was studied in mice using hot plate method by Eddy et.al.[22] Animals were divided into four groups(n=6) of Swiss albino mice (20-25 g) were placed on a plate and maintained 15-20 min at room temperature for environmental adaptation. Food was withdrawn for the prior night of the experiment and water was allowed *ad libitum.* Control group received the vehicle (distilled water, 10 ml/kg, *po*.) Positive control group received Morphine (5 mg/kg), whereas groups III and IV were administered with hydroalcoholic extract of *Grewia nervosa* (100 and 200 mg/kg, *po.* respectively). Each animal was individually placed gently on Eddy's hot plate at 55^0C. The responses of reaction time were recorded at 30,60 and 90 min after drug administraion.

The anti-inflammatory activity

The anti-inflammatory activity of hydroalcoholic leaf extract against carrageenan induced acute paw oedema in rats by Winter et al and Kulkarni et.al.[23][24] Male albino rats (180-200 g) were divided into four groups (n=6). They were fasted prior night of the experiment and water was allowed *ad libitum.* A mark was made on both the hind paws just beyond tibio-dorsal junction, so that every time the paw is dipped in the mercury column upto the fixed mark to ensure constant paw volume. The fist control group received distilled water (10 ml/kg), while the second group was treated with Indomethacin (10 mg/kg), the third group were administered with hydroalcoholic extract of *Grewia nervosa* (100 and 200 mg/kg

respectively). Acute inflammation was produced by sub plantar administration of 0.1 ml of 1% carrageenan in left hind paw of rats. The right paw served as reference non-inflammed paw for comparison. The paw volume of both legs of control and plant extract were noted at 0,30,60,120 and 180 min after carrageenan injection. Reduction in the paw volume was noted.

Statistical Analysis:

All the values were statistically analysed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. Comparison between control and drug treated groups were considered to be significant. All values are expressed as mean ± SEM of six animals.

5. Results

Pharmacognostical studies

Macroscopic evaluation

Grewia nervosa, a shrub, the youngest leaf was brown in colour and matured leaf was green in colour, elliptic, oblong or ovate, lanceolate in shape. The base is rounded or coordinate and the margins are sub entire to serrulate. Apex acute to acuminate and petiole upto 1 cm long [Figure1].

Microscopic evaluation

The cross section of leaf shows a typical dorsiventral pattern. In cross section the leaf appears normal. In lamina portion the upper epidermis showed single layered epidermal cells, which are barrel shaped. The upper epidermis is followed by palisade cells. The palisade cells are elongated and smaller in size. The palisade layer is 3 to 4 layered and it is followed by spongy parenchyma cells. The spongy parenchyma layer is delimited by lower epidermis, which is also single layered. In mid rib area a large central vascular bundle is present. The protoxylem points are facing towards the upper epidermis, which in turn surrounded by phloem. The vascular bundle is surrounded by parenchymatous cells [Figure 2]. The parenchymatic zone consists of some stored materials.

Powder microscopy

The microscopical structured obtained in the leaf of *Grewia nervosa* was shown the following features, such as stellate trichome, unicellular trichome with epidermal cells, vessel elements, broken piece of epidermis, bundle of tracheids, oil globules, piece of epidermis were observed [Figure 3a–g]. The above studies enabled to identify the plant material for further investigation which may form an important aspect.

Figure 1: Leaves of *Grewia nervosa*

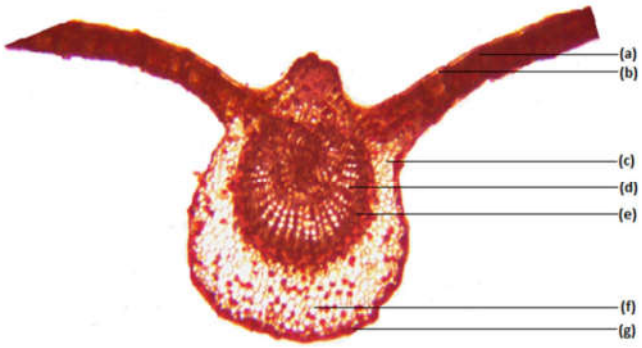

Figure 2: Transverse section of leaves of *Grewia nervosa*

a) Palisade cell, b) Upper Epidermis c) Spongy Parenchyma d) Xylem e) Phloem f) Collenchyma g) Lower Epidermis

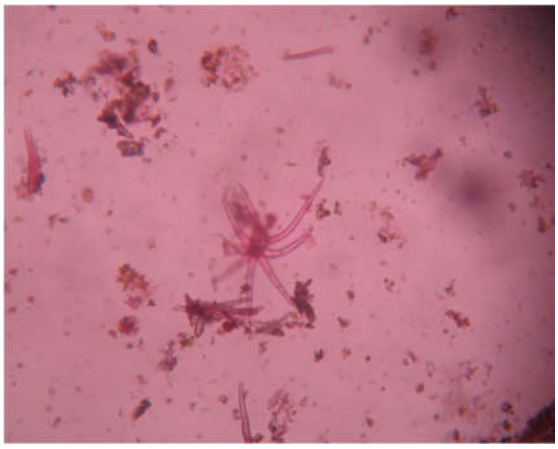

Figure 3a: Stellate trichome

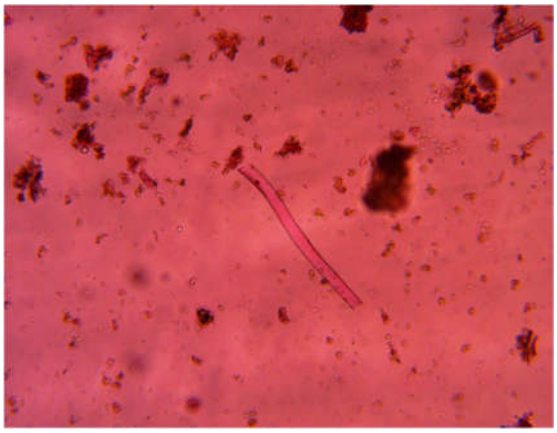

Figure 3b: Unicellular trichome with epidermal cell

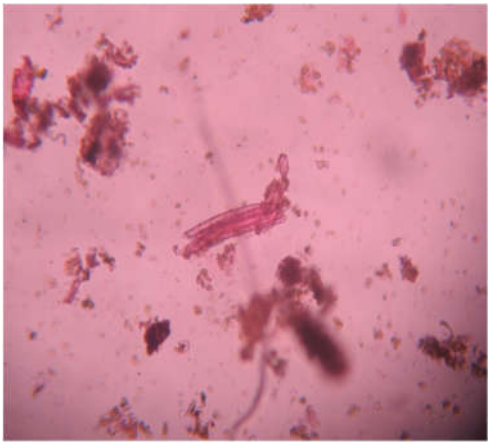

Figure 3c: Vessel element

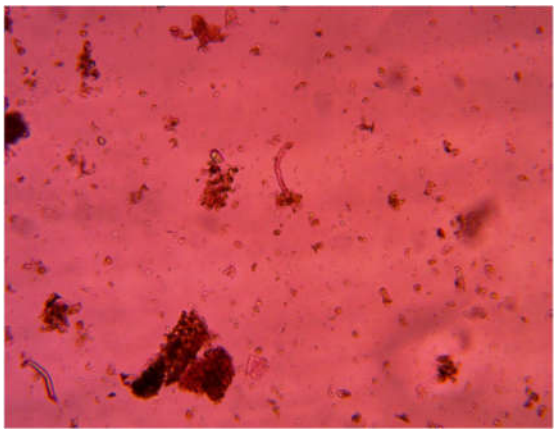

Figure 3d: Broken piece of epidermis

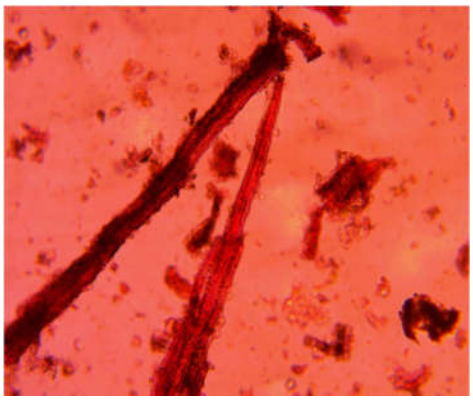

Figure 3e: Bundles of tracheids

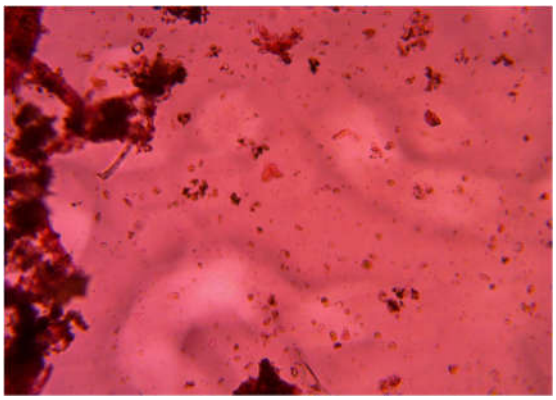

Figure 3f: Oil globules

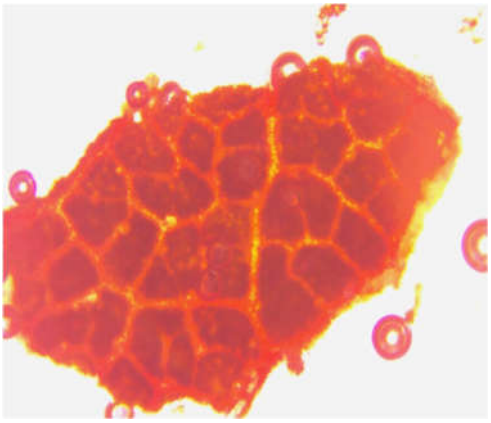

Figure 3g: Piece of epidermis

Physiochemical evaluation

Analytical parameters of ash value revealed the percentage of total ash value, acid insoluble ash value, water soluble ash value and extractive values were shown in Table 1 and 2 respectively.

Fluorescence analysis

The fluorescence behaviour of the powdered samples as such as well as after treatment with different reagent solutions towards ordinary light and ultra violet light (both long 365 nm and short 254 nm wavelengths) was observed and exhibited characteristic colours were shown in Table 3.

Preliminary phytochemical studies

Preliminary phytochemical studies of hydroalcoholic extract of *Grewia nervosa* revealed the presence of carbohydrates, alkaloids, saponins, phenols, tannins and flavonoids.

Table 1: Ash value of powdered leaves of *Grewia nervosa*

Ash Parameter	Percentage (%w/w)
Total Ash	3.3 %
Acid insoluble Ash	0.33 %
Water soluble Ash	1 %

Table 2: Extractive value of leaves of *Grewia nervosa*

Extracts	Percentage (%w/w)
Alcohol soluble extract	6.3 %
Water soluble extract	11.33 %

Table 3: Fluorescence analysis of powdered leaf of *Grewia nervosa*

Organic solvents	Visible/Day light	Shorter wave length 254 nm	Longer wave length 365 nm)
Powder+ Methanol	Light Green	Dark Green	Pink
Powder+ 1%Glacial acetic acid	Green	Green	Dark Brown
Powder+ 10%Sodim hydroxide	Dark Brown	Black	Black
Powder+ Dilute ammonia	Brown	Green	Black
Powder+1M Sulphuric acid	Light Brown	Green	Black
Powder+1M Hydrochloric acid	Pale Yellow	Pale Green	Light Brown
Powder+ 10% ferricchloride	Light Brown	Pale Green	Black
Powder+ Iodine	Reddish Brown	Black	Black
Acetone+ Methanol	Light Green	Green	Reddish Brown

Acute toxicity study:

The acute toxicity showed that rats receiving hydroalcoholic extract of *Grewia nervosa* at 2000 mg/kg orally exhibited normal behavioural, neurological and autonomic profiles. No mortality or toxicity of any nature was observed during 24 h and thereafter up to 14 days observation. This indicates that approximate LD_{50} value of extract was more than 2000 mg/kg, orally and it is relatively safe and non-toxic to rats.

Analgesic activity

The analgesic effect of hydroalcoholic extract of *Grewia nervosa* using hot plat method in mice to

determine the central analgesic activity of extract. In this method all the test doses of extract and the standard drug morphine produced significant central analgesic activity ($p < 0.01$) when compared with control shown in Figure 4.

Anti-inflammatory activity

Carrageenan induced paw edema model was used for the evaluation of anti-inflammatory activity of hydroalcoholic extract of *Grewia nervosa*. Carrageenan induced rat paw edema was markedly inhibited by extract (200 mg/kg) and indomethacin (10 mg/kg). The extract produced a significant ($p < 0.01$) acute-inflammatory effect in rats [Figure 5]**.**

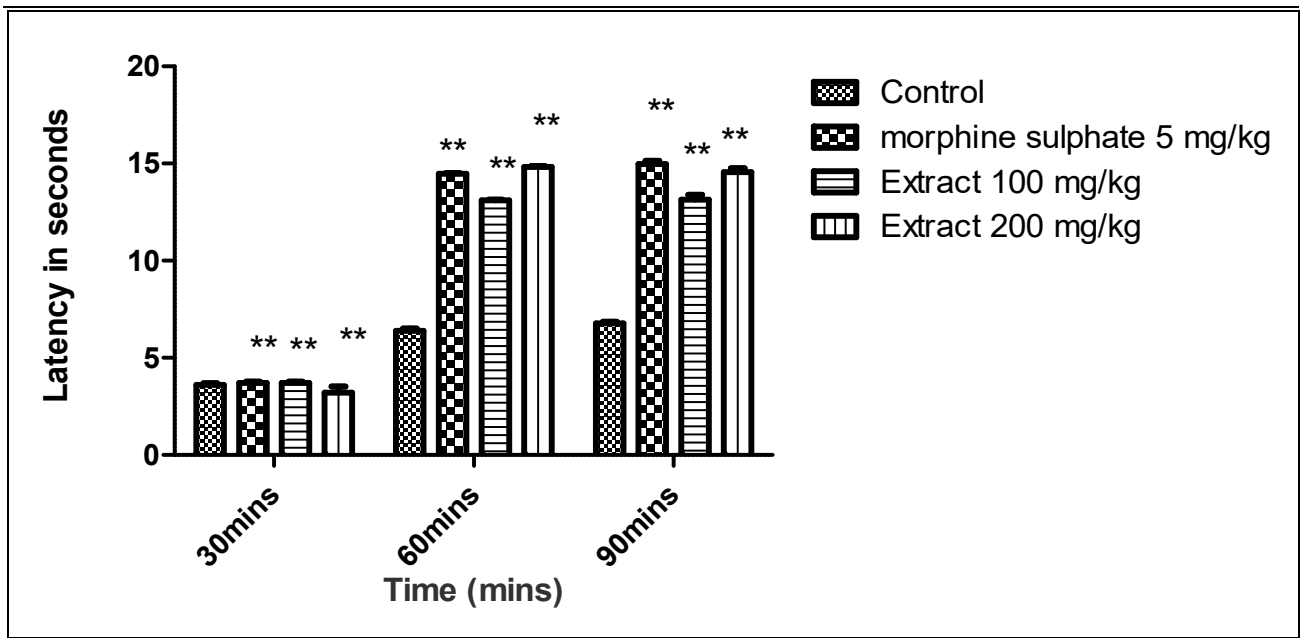

Figure 4. Analgesic activity of hydroalcoholic extract of *Grewia nervosa* in hot plate method

Each value in the results are expressed as mean ± S.E.M of 6 rats. **p <0.01 significantly different was compared with control group (ANOVA followed by Tukey-kramers test).

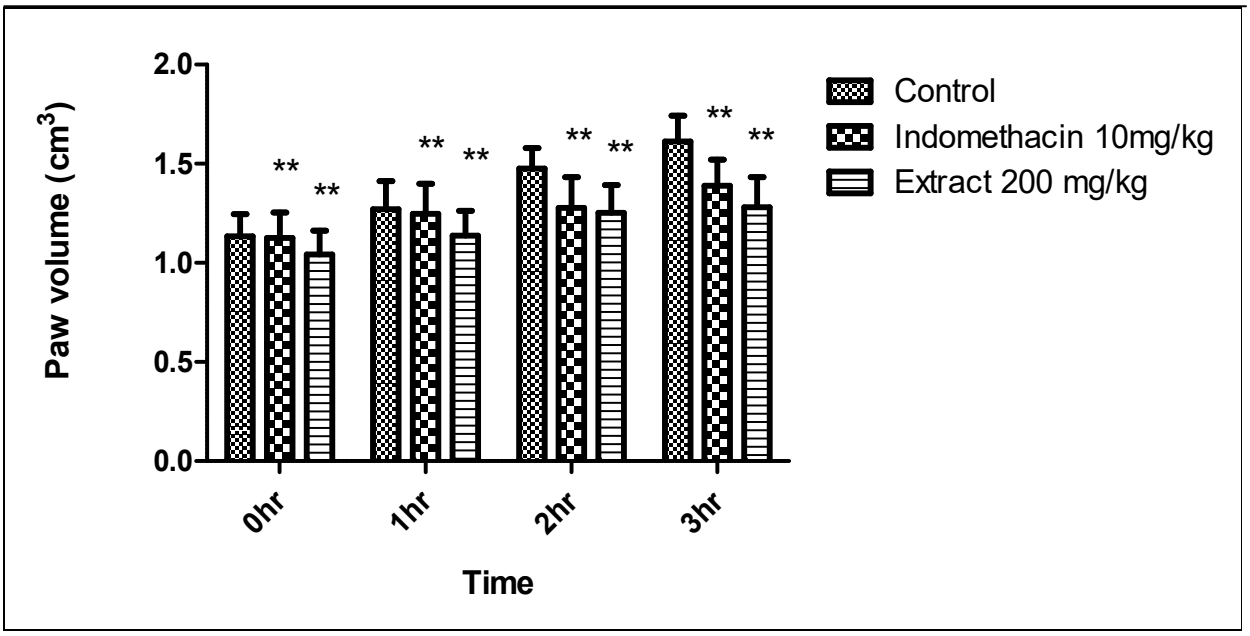

Figure 5. Anti-inflammatory activity of hydroalcoholic extract of *Grewia nervosa* in carragenan induced paw edema in rats.

Each value in the results are expressed as mean ± S.E.M of 6 rats. **p <0.01 significantly different was compared with control group (ANOVA followed by Tukey-kramers test).

6. Discussion

The plant *Grewia nervosa* has been studied to give a report on pharmacognostical, preliminary phytochemical and analgesic and anti-inflammatory activity. The pharmacognostical studies made on the leaf of *Grewia nervosa* like microscopical, macroscopical characters, powder analysis, ash value, extractive value and fluorescence analysis gave valuable information about quality and purity of the drug.

The preliminary phytochemical investigation showed the presence of carbohydrates, alkaloids, phenols, flavonoids and saponin in hydroalcoholic extract. No mortality was observed upto 2000 mg/kg in acute toxicity study. Medicinal plants which have been widely used to treat different pain conditions and inflammation. Anti-inflammatory activity of many plants attributed to their sterol and triterpens [25] or flavonoids contents.[26] Carragenan induced paw edema is a frequently used method for screening of acute inflammatory potentials of various natural and synthetic products to determine the possible mechanism involved in inflammation.[27] The present study indicates the hydroalcoholic extract of *Grewia nervosa* possess potent analgesic and anti-inflammatory activity, which is in accordance with its traditional use both the hot plate and carrageenan induced paw

edema methods have been found to be suitable for evaluation of centrally acting analgesic and anti-inflammatory. Analgesic effect of the extract was demonstrated experimental models using Eddy's hot method using thermal stimuli, an increase in reaction time is generally considered as an important parameter of analgesic activity. The extract 200 mg/kg shown more significant analgesic activity when compared with control was dose dependent. Anti-inflammatory effect the extract was demonstrated by carrageenan induced paw edema method shown the inhibition of contraction of at doses of 100 mg/kg and 200 mg/kg, a maximum inhibitory effect at 3rd h of treatment. Anti-inflammatory activities of plants are due to the secondary metabolites, so the estimation of anti-inflammatory potential also confirms the existence of phytoconstituents in the plant extract.[28]

7. Conclusion

Our results clearly demonstrated that hydroalcoholic extract of *Grewia nervosa* displayed a potent analgesic and anti-inflammatory effect may be due to the presence of alkaloids, saponins, phenols and flavonoids. Future studies are need to isolate and characterize the active principle of *Grewia nervosa*. the results provided a validation of the plant used as a remedy in painful and inflammation conditions.

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Nil

Conflicts of interest

There are no conflicts of interest.

References

1. Ridtitid W, Sae-Wong C, Reanmongkol W, Wongnawa M. Antinociceptive activity of the ethanolic extract of kaempferia galanga linn. In experimental animals. J Ethnopharmacol 2008; 118(2):225–230.

2. Uddin G, Rauf A, Siddiqui BS, Shah SQ. Preliminary comparative phytochemical screening of Diospyros lotus Stewart. Middle-East Journal of Scientifc Research 2011;10(1):78–81.

3. Rauf A, Uddin G, Siddiqui BS, Muhammad N, Khan H. Antipyretic and antinociceptive activity of Diospyros lotus L. in animals. Asian Pacifc Journal of Tropical Biomedicine 2014;4(1): S382–S386.

4. Osawa T, Kawakishi S, Namiki M. Antimutagenesis and anticarcinogenesis mechanism II. New

york:Plenum press; 1990:139-153.

5. Keith MW. Sally AL. Michael WS. Thomas JG. Garry MM. Needles contain amounts of taxol comparable to the stem bark of Taxus brevifolia: analysis and isolation. J Nat Prod 1990; 53: 1249-1255

6. Ahmad MM, Qureshi S, Shah A, Qazi NS, Rao RM, Albakiri M. Anti-inflammatory activity of Caralluma tuberculata alcoholic extract. Fitoterapia 1983; 46:357-360.

7. Parmar NS and Ghosh MMN. Current trends in flavonoid research. Indian J Pharm 1978;12: 213-228.

8. Tripathi KD. Essential of medical pharmacology. 6th ed. New Delhi:Jaypee Brothers medical publishers; 2010:184-201.

9. Bandara KA, Kumar V, Jacobsson U and Molleyres LP. Insecticidal piperidine alkaloid from Microcos paniculata stem bark. Phytochemistry 2000; 54:29– 32.

10. Fan H, Yang GZ, Zheng T, Mei ZN, Liu XM, Chen Y et al. Chemical constituents with free-radical- scavenging activities from the stem of Microcos paniculata. Molecules 2010; 15:5547–5560.

11. Feng SX, Lin LD, Xu HH. and Wei XY. Two new piperidine alkaloids from the leaves of Microcos paniculata. J Asian Nat Prod Res 2008; 10:1155–1158.

12. World Health Organization. Quality control methods for herbal materials. Geneva: World Health Organization; 2011.

13. Trease GE, Evans WC. Text Book of Pharmacognosy. 15th ed. St. Louis, MO: Elsevier; 2009.

14. Khandelwal KR. Practical pharmacognosy: techniques and experiments. Maharashtra: Niral Prakashan; 2008.

15. Alam F, Najum us Saqib Q. Pharmacognostic standardization and preliminary phytochemical

studies of *Gaultheria trichophylla*. Pharm Biol 2015;53(12):1711–1718.

16. Singh P, Khosa RL, Mishra G, et al. Pharmacognostical evaluation of aerial parts of *Graptophyllum pictum* (L.) Griff. (Syn: *Justicia picta Linn*.): A well-known folklore medicinal plant. Anc Sci Life 2015;34(4):223–229.

17. Kataria S, Rao SK, Bhandari A, et al. Pharmacognostical standardization of *Corchorus depressus* (L.) Stocks (*Tiliaceae*)-A promising ethnomedicinal plant. Indian Journal of Traditional Knowledge 2013;12(3):489–497.

18. Chase CR, Pratt R. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J Am Pharmacol Assoc 1949; 8:324-31.

19. Harborne JB. (1984). Phytochemical investigation of certain medicinal plants. 2nd ed. London: Chapman & Hall; 1984:37-40.

20. Goswami P, Islam R, Khongmalai E, Sarkar B R, Sen S, *et al*. Pharmacognostical and preliminary phytochemical screening of leaves of *Polygonum muricatum*. Int J Res Pharm Pharm Sci 2017; 2(3): 6-10.

21. Ecobicon DJ. The basis of toxicology testing. New York:CRC Press; 1997:43-86.

22. Eddy N B, Leimbach D. Synthetic analgesics. II. Dithienylbutenyl- and dithienylbutylamines. The Journal of Pharmacology and Experimental Therapeutics 1953;107(3):385–393.

23. Winter CA, Risely EA, Nus GN. Carrageenan induced oedema in hind paw of the rat as an assay for anti- inflammatory drugs. Pro Soc Exp Biol Med 1962; 111:544-547.

24. Kulkarni SK, Dandiya PC. Influence of intraventricular administration of norepinephrine, Dopamine and 5-hydroxytryptamine on motor activity of rats. Indian J Med Res 1975;63(3):462- 468.

25. Ahmad MM, Qureshi S, Shah A, Qazi NS, Rao RM, Albakiri M. Anti-inflammatory activity of

Caralluma tuberculata alcoholic extract. Fitoterapia 1983; 46:357-360.

26. Parmar NS, Ghosh MMN. Current trents in flavonoids research. Indian J Pharm 1978; 12:213- 228.

27. Ameya G, Gure A, Dessalengn E. Antimicrobial activity of Echinops Kebericho against human pathogenic bacteria and fungi. Afr. J Trad Compl Altern Med 2016;13(6):199-203.

28. Sengar N, Joshi A,Prasad SK, Hemalatha S. Anti-inflammatory, analgesic and anti-pyretic activities of standardized root extract of *Jaminum sambac*. J Ethnopharmacol 2015; 160:140-148.

29. Osawa T, Kawakishi S, Namiki M. Antimutagenesis and anticarcinogenesis mechanism II. New york:Plenum press; 1990:139-153.

30. Osawa T, Kawakishi S, Namiki M. Antimutagenesis and anticarcinogenesis mechanism II. New york:plenum press; 1990:139-153.