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Research Article

PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF *ANDROGRAPHIS PANICULATA* AND *VITEX NEGUNDO* FOR ANALGESIC ACTIVITY

DIVYA SEN*, MANOJ SAHU, LOKESH VERMA, JITENDRA BANWEER

Sager Institute of Research and Technology Pharmacy, Bhopal, Madhya Pradesh, India. Email: sendivya29@gmail.com

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ABSTRACT

Objective: Pain is defined physical or mental depending on it source of origin and pain treated with anti-anxiety, antidepressant, and analgesic medicine. *Andrographis paniculata* locally called kalmegh of Acanthaceae family and *Vitex negundo* locally called nirgundi of Lamiaceae, both medicinal plants which yield the therapeutic compound and herbal drug used cure diseases.

Methods: The analgesic activity was performed by hot plat and tail immersion method. The present study extract performs models plants 50 Swiss albino mice four groups of each five animals. This experimental animal administered with extract intraperitoneal at dose level 50, 100, and 300 mg/kg used as reference drugs diclofenac sodium-induced time test using albino mice as experimental animal.

Results: Medicinal plants ethanolic extract contains reducing carbohydrate, flavonoids, tannins, and triterpenoids. The sample comparable antibacterial cytotoxic activity. The different solvent soluble and mild activity herbal drug analgesic highest activity compared to standard drug, diclofenac sodium.

Conclusion: Analgesic models based different parameter for studies bioactive compounds further for isolation and purification compounds.

Keywords: Analgesia, Analgesic models, Nociception, Hot plate, Tail immersion

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INTRODUCTION

Ayurveda is the science of lifestyles, because India's historical tool for intensive care focuses on ones, ideas and illness [1]. The practice of Ayurveda medicine consisted of 80 stages divided into 180 chapters and numbered 314 flowers, which are used as medicine in India. India, round 15,000 medicinal flowers have been recorded six but traditional groups are using simplest 7000–7500 plant life for curing exclusive sicknesses [6].

Pain can be defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damages or described [4] as the major clinical, social, and economical problem in most communities around the world. There are three types of pain receptor stimuli. Mechanical a chemical stimulus would be, ex high pressure or stimulus [2].

Andrographis paniculata commonly known as "kalmegh" has wide array of properties that include antidiabetic, antidiarrheal, antimicrobial, and anti-inflammatory activities [10]. These plant traditionally used for different medicinal purposes in Asia and Europe remedy for a wide spectrum of AI as an herbal. The plant extract and andrographolide five major organs, including lungs, brain, liver, kidney, and intestine, achieved beside panel of cell line representatives [7]. *Vitex negundo* is commonly known as "Nirgundi" possesses antibacterial, anticancer, antifungal, antimicrobial, enzyme inhibiter anticonvulsant, and drug potentiating. From the perusal of literature, it appears that, of all the biological effects studied on [8] *A. paniculata* and *V. negundo*, the analgesic activities have been less investigated. Therefore, the present study was planned to evaluate the analgesic activity of *A. paniculata* and *V. negundo* leaves extract in experimental model [15].

METHODS

Plant materials and extraction

The medicinal plants *Andrographis paniculata* (leaves) and *V. negundo* (seeds) of collected from Botanical Garden of Sager Institute of Research and Technology-Pharmacy in the month of March 2020, and identified and certified by Dr. Saba Naaz (H.O.D., Department of Botany), Saifia Science College of Bhopal, M.P. Science, Madhya Pradesh, India. Accession number for specimen is (File no. 120/121/saif./sci./collage/ Bpl.) was authenticated and has been deposited in the herbarium of the institute.

Both medicinal plants (*A. paniculata* leaves and *V. negundo* seeds) were shade dried and powdered from stored in airtight container. For performing research work, animal models on the animals taken permission from the Institutional Animal Ethical Committee, Sager Institute of Research and Technology – Pharmacy, the assigned Reference No. is SIRT-P/19/01/IAEC34.

Preparation of ethanolic extract of *A. paniculat*a (leaves) and *V. negund*o (seeds). In the present study, plant materials were extraction using cold maceration method; the leaves and seeds were collected, plants raised properly. A bout 200 g of the powder (*A. paniculata* leaves) and 100 g (*V. negundo* seeds) was extracted with organic solvent ethanol and allowed standing for 3 days each.

The extract was filtered using Whatman No.1 filtered paper to remove all unextractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. Extract was transferred to beaker and evaporated in room temperature and excessive moisture removed was collected in airtight container.

Drugs and chemicals

Tablets of diclofenac sodium and tramadol of Novartis, Bhopal, were procured form local medical store. All other solvent and chemicals of analytical grade used were obtained from SD Fine Chemical, Bhopal.

Ethical clearance was taken from Institutional Animal Ethical committee of the institute before commencement of the study.

Animals

Experimental animals used in this study were albino mice weighing 20–25 g. All animals were procured from Sager Institute of Research Technology, Bhopal. The animals were housed in polypropylene cages, with dry paddy husk bedding and covered with stainless steel mesh lid. The environment of the room was maintained on a 12 h light/dark cycle at a constant room temperature of 26+2°C and relative humidity of 45–55% [11]. The mice had free access to standard mice chow diet, respectively. The mice were acclimatized to the surrounding for 1 week committee for the Purpose of Control and Supervision of Experiments on Animals, Bhopal [13].

Models

Hot plate test

The thermal nociceptive threshold in mice was assessed using a hot plate apparatus. The hot plate temperature thermostatically set at 52.5±0.5°C. The latency to licking or kicking of the fore or hind paws was recorded at various time periods after drug treatment. A cutoff time of 45 s was imposed to avoid tissue damage [12].

Experiment design

The perform process albino mice of either sex (No. = 6) weight 20–25 g were used. Mice exception on a hot plate temperature keep out 55±0.1°C and latency time >15 s during the hot plate having animals were excluded. The albino mice were divided by four groups, per group six mice. The I group treated with saline (10 mL/kg), II group with diclofenac sodium (15 mg/kg i.p.), and III group and IV group were treated with oral dose of 300 mg/kg of kalmegh and nirgundi extract of ethanol, respectively. Reference drug used diclofenac sodium for differentiation. After 15, 30, and 60 min administration dose, animals were dropped inside the cylinder onto latency time and the hot plate (hot plate without licking or flicking of hind limb or jumping mice remains which for time) was recorded in seconds. The cutoff time damage tissue damage of 45 s and set for all animals. The recorded by latency time per group at 0, 30, 60, 90 and 120 min follow by administered drug. Percent analgesia was calculated using the following formula.

$$\% \text{ Analgesia} = \frac{\text{Test latency-control latency}}{\text{Cut off time-control latency}} \times 100$$

A total of 30 animals divided into five groups (N=06) for analgesic activity

Group	Description	No. of animals required
1.	Control group	06
2.	Standard (diclofenac) 15 mg/kg	06
3.	Aqueous extract of *kalmegh*	18
4.	Aqueous extract of *Nirgundi*	18
	Total experimental animals required	50

Tail immersion test (TMT)

The immersion test as described by Sewell and Spencer [16] was used for thermal-induced pain. Each mouse was placed in a holder with its tail protruding. The tail was placed in a water bath at 55°C until the tail whipped or a flinch of the whole body occurred. A cutoff time of 7 s was imposed [18]. The latency time of the reaction was recorded to the nearest 0.1 s. All animals were tested for a control value and then restudied at the peak time for antinociceptive effect after drug treatment. These peak times were derived from preliminary experiments of antinociceptive response for each drug over a period of 100 min [17]. The analgesic response for each animal was calculated according to the following formula:

$$\% \text{ Antinociception} = \frac{T - C}{7 - C} \times 100$$

Where, C and T represent the tail whip reaction time in seconds before drug treatment (C) and at the peak time (T) after the treatment of the drug. The data are expressed as mean analgesic response + SEM [52].

The actual flick responses of mice, that is, time taken in second to withdrawn it's from hot water source were calculated and result was compared with control group [14].

Experimental design

The test was performed on albino mice of either sex (no-6) mice weight 20–25 g used. Albino mice a particular experience or from treatment pre-testing and divided into four groups, per group only six mice. I group was saline with treated (10 ml/kg), II group diclofenac sodium (15 mg/kg i.p.), III group treated with oral dose of 300 mg/kg of ethanol extract of kalmegh, and Group IV treated with oral dose of 300 mg/kg of the ethanol extract of nirgundi, respectively. Diclofenac sodium used as reference drug for comparison. Each mouse placed into cage in such way that their tail hangs freely. Warm water deep in albino mice tail 55°C until the tail licking or flinch the entire body especially in a way that has not been planned, a cutoff time surtax 7 s. The reaction was recorded latency time nearest 0.1 s. The latency time was recorded for each group after the oral administration of drug at 0, 15, 30, and 60 min.

Statistical analysis

The results were presented as mean±SEM. "Two-way ANOVA" with Dunnett's post-test was performed using GraphPad Prism version 9.3 00 for Windows (GraphPad software, San Diego California, USA).

RESULTS

Preliminary phytochemical studies revealed that the presence of alkaloids, carbohydrates, tannins, phenols, saponins, triterpenoids, and steroids in *A. paniculata* (leaves) and *V. negundo* (seeds) ethanol extract. They found to be non-toxic when administered orally to the Swiss albino mice with doses 50, 100, and 300 mg/kg, p.o., and the LD_{50} was found to be safe at the highest dose (50500 mg/kg, p.o.). The mice were treated with in *A. paniculata* (leaves) and *V. negundo* (seeds) *extracts* had significantly ($p<0.05$) reduced pain indices compared to the saline-treated group.

Central Analgesic Effect

1. Hot plate method (thermal stimulation)
2. In this study, *A. paniculata* (leaves) ethanol extract demonstrates an increase dose-dependent paw licking latency time and hamper sensation pain in an ornament parallel to standard drug, diclofenac sodium, 15 mg/kg, i.p. during that time, the effect of ethanol extract administration was shown at 100, 300, and 600 mg/kg p.o. The mice treated with *A. paniculata* (leaves) *ethanol extract* had significantly ($p<0.05$) reduced pain-related indices compared to the saline-treated group. Two-way ANOVA revealed that administration extract significantly affects the paw licking time as compare to saline treated group on hot plat (paw licking [F 2,10] = $p<0.05$). *Post hoc* indicates that ethanol extract (100, 300, and 600 mg/kg, p.o.) significantly increases paw, licking time ($p<0.05$) as compare to saline-treated group (Fig. 1).

Tail immersion test

The ethanol extracts of *A. paniculata* (leaves) *ethanol extract* demonstrate an increase dose-dependent paw licking latency time and hamper sensation pain in an ornament parallel to exhibited standard drug; *diclofenac* sodium (15 mg/kg, i.p.). The effect shown at 100, 300, and 600 mg/kg, p.o. The mice treated with *A. paniculata* had significantly ($p<0.05$) reduced pain-related indices compared to the saline-treated group.

Two-way ANOVA revealed that administration extract significantly affects the tail flicking time as compare to saline-treated group on tail immersion test (tail flicking [F 2,9] = $p < 0.5$). *Post hoc* indicates that ethanol extract (100, 300, and 600 mg/kg, p.o.) significantly increases tail, flicking time ($p<0.05$) as compare to saline-treated group (Fig. 2).

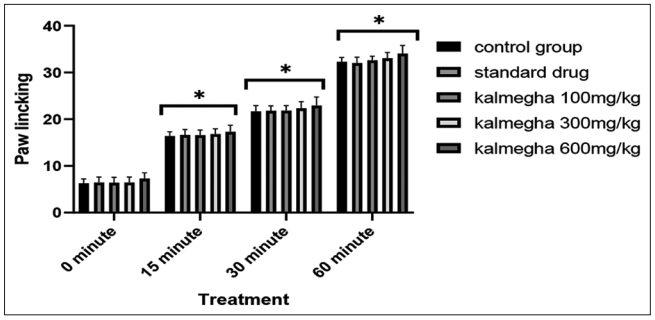

Fig. 1: **Effect of *Andrographis paniculata* (leaves) ethanolic extract on pain in hot plate test. Different group (n=6) of animal by administrated with saline (10 mL/kg, i.p.) or ethanolic extract on 0, 15, 30, and 60 min, these animals were subjected on hot plate test 45 s and pain-related indices were measured; each was represented mean±SEM of six mice in each group *p<0.05 versus control (two-way ANOVA followed by Bonferroni comparison test)**

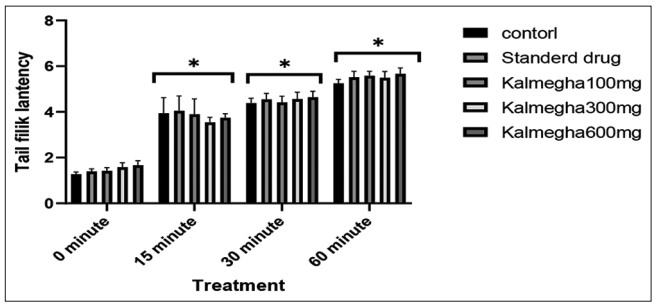

Fig. 2: **Effect of *Andrographis paniculata* ethanolic extract on pain in tail immersion test. Different group (n=6) of animal by administrated with saline (10 mL/kg, i.p.) or ethanolic extract on 0, 15, 30, and 60 min, these animals were subjected on tail immersion test 7 s and pain-related indices were measured each was represented mean±SEM of six mice in each group *p<0.05 versus control (two-way ANOVA followed by Bonferroni comparison test)**

Hot plate method (thermal stimulation)

In this study, *V. negundo (seed) ethanol extract* demonstrates an increase dose-dependent paw licking latency time and hamper sensation pain in an ornament parallel to standard drug; diclofenac sodium (15 mg/kg, i.p.) while the effect was shown at 100, 300, and 600 mg/kg p.o. The mice treated with *V. negundo (seed) ethanol extract*, had significantly ($p<0.05$) reduced pain-related saline-treated group.

Two-way ANOVA revealed that administration of ethanol extract significantly affects the paw licking time as compare to saline-treated group on hot plat (paw licking [F 5,10] = $p<0.05$). *Post hoc* indicates that ethanol extract (100, 300, and 600 mg/kg, p.o.) significantly decreases paw licking time ($p<0.05$) as compare to saline-treated group.

Tail immersion test

The ethanol extracts of *V. negundo (seeds) ethanol extract* demonstrate an increase dose-dependent paw licking latency time and hamper sensation pain in an ornament parallel to standard drug; diclofenac sodium (15 mg/kg, i.p.). The effect shown at 100, 300, and 600 mg/kg, p.o. The mice treated with *V. negundo* had significantly ($p < 0.05$) reduced pain-related indices compared to the saline-treated group.

Two-way ANOVA revealed that administration extract significantly affects the tail flicking time as compare to saline-treated group on tail immersion test (tail flicking [F 5,4]=$p<0.05$]. *Post hoc* indicates that ethanol extract (100, 300, and 600 mg/kg, p.o.) significantly decreases tail, flicking time ($p<0.05$) as compare to saline-treated group.

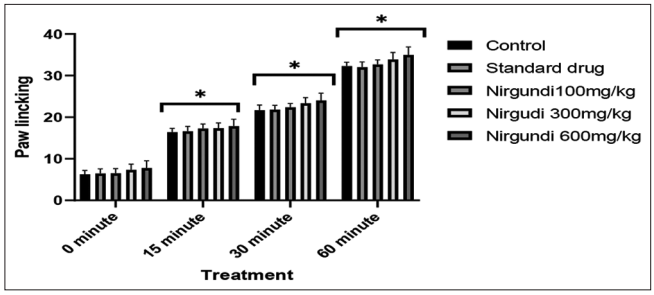

Fig. 3: Effect of *Vitex negundo* of (seeds) ethanolic extract on pain in hot plate test. Different group (n=6) of animal by administrated with saline (10 mL/kg, i.p.) or ethanolic extract on 0, 15, 30, and 60 min, these animals were subjected on hot plate test 45 s and pain-related indices were measured each was represented mean±SEM of six mice in each group *p<0.05 versus control (two-way ANOVA followed by Bonferroni comparison test)

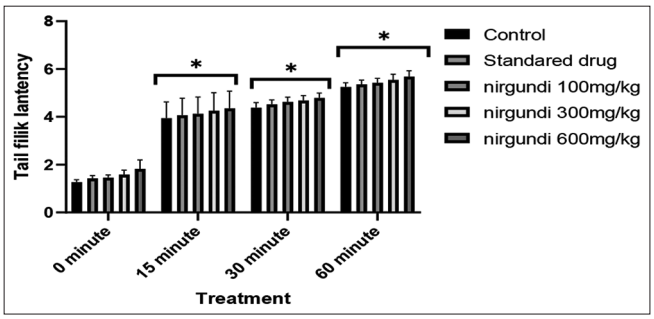

Fig. 4: Effect of Vitex negundo of (seeds) on pain in tail immersion test. Different group (n=6) of animal by administrated with saline (10 ml/kg, i.p.) or ethanolic extract on 0, 15, 30, and 60 min, these animals were subjected on tail immersion test 7 s and pain-related indices were measured each was represented mean ± SEM of six mice in each group *p<0.05 versus control (two-way ANOVA followed by Bonferroni comparison test)

CONCLUSION

The present study demonstrates the analgesic activity of two common seasoning plants *A. paniculata* leaves and *V. negundo* seed extract. The effect of analgesic activity may be due to its agonistic action on opioids receptors while the peripheral analgesic effect may be due to inhibition of PG generation.

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CONFLICTS OF INTEREST

No authors constrict.

AUTHORS' CONTRIBUTION

Guided by Manoj Sahu, Lokesh Verma helping analysis or data and result manuscript 44803 return by Divya and with some contribution of Dr. Jitendra Banweer

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No.

REFERENCES

1. Samy RP, Pushparaj PN, Gopalakrishnakone P. A compilation of bioactive compounds from ayurveda. Bioinformation 2008;3:100-10. doi: 10.6026/97320630003100, PMID 19238245

2. World Health Organization. Cancer Pain Relief and Palliative Care: Report of a WHO Expert Committee [Meeting held in Geneva from 3 to 10 July 1989]. Geneva: World Health Organization; 1990.
3. Das A, Panda S. Use of topical corticosteroids in dermatology: An evidence-based approach. Indian J Dermatol 2017;62:237-50. doi: 10.4103/ijd.IJD_169_17, PMID 28584365
4. Kulkarni SK, Akula KK, Deshpande J. Evaluation of antidepressant-like activity of novel water-soluble curcumin formulations and St. John's Wort paradigms of despair. Pharmacology 2012;89:83-90. doi: 10.1159/000335660, PMID 22343362
5. Sugiura YA, Terui NA, Hosoya YA. Difference in distribution of central terminals between visceral and somatic unmyelinated (C) primary afferent fibers. J Neurophysiol 1989;62:834-40. doi: 10.1152/jn.1989.62.4.834, PMID 2809705
6. Subhose V, Srinivas P, Narayana A. Basic principles of pharmaceutical science in ayurvĕda. Bull Indian Inst Hist Med Hyderabad 2005;35:83-92. PMID 17333665
7. Tiwari S, Singh A. Toxic and sub-lethal effects of oleandrin on biochemical parameters of fresh water air breathing murrel, Channa punctatus (Bloch.). Indian J Exp Biol 2004;42:413-8. PMID 15088692
8. Kumar S, Bajwa BS, Kuldeep S, Kalia AN. Anti-inflammatory activity of herbal plants: A review. Int J Adv Pharm Biol Chem 2013;2:272-81.
9. Patel PK, Sahu J, Chandel SS. A detailed review on nociceptive models for the screening of analgesic activity in experimental animals. Int J Neurol Phys Ther 2016;2:44-50.
10. Kumar SV, Kumar RS, Sudhakar P, Baskar N. Antioxidant, antinociceptive and anti-inflammatory activities of *Rhynchosia minima* (L) DC. Res J Pharm Technol 2020;13:1855-60.
11. Hijazi MA, El-Mallah A, Aboul-Ela M, Ellakany A. Evaluation of analgesic activity of *Papaver* libanoticum extract in mice: Involvement of opioids receptors. Evid Based Complement Alternat Med 2017;2017:8935085.
12. Yimer T, Birru EM, Adugna M, Geta M, Emiru YK. Evaluation of analgesic and anti-inflammatory activities of 80% methanol root extract of *Echinops* kebericho M. (*Asteraceae*). J Inflam Res 2020;13:647-58. doi: 10.2147/JIR.S267154, PMID 33061529
13. Lalrinzuali K, Vabeiryureilai M, Jagetia GC. Investigation of the anti-inflammatory and analgesic activities of ethanol extract of stem bark of Sonapatha *Oroxylum* indicum *in vivo*. Int J Inflam 2016;2016:8247014. doi: 10.1155/2016/8247014, PMID 26925290
14. Kumar JP, Shankar NB. Analgesic activity of *Mollugo pentaphylla* Linn. tail immersion method. Asian J Pharm Clin Res 2009;2:61-3.
15. Salwe K, Mirunalini R, Mano J, Manimekalai K. Evaluation of analgesic activity of *Murraya koenigii* and *Coriandrum sativum* leaves extract in animal model. Asian J Pharm Clin Res 2018;11:328-31.
16. Ahmad F, Khan RA, Rasheed S. Study of analgesic and anti-inflammatory activity from plant extracts of *Lactuca* Scariola and *Artemisia absinthium*. J Islamic World Acad Sci 1992;5:111-4.
17. Jain RA. To study the Attention Deficit Hyperactivity Disorder (ADHD) and to Evaluate the Cognitive, Hyperactive and Impulsive Behavior Against 6-OHDA Hbr Lesioned Sprague Dawley Neonates using Hyperaxe, Curcumin and Quercetin; 2012 ([doctoral dissertation]. Chennai: CL Baid Metha College of Pharmacy).
18. Basak A, Cooper S, Roberge AG, Banik UK, Chrétien M, Seidah NG. Inhibition of proprotein convertases-1,-7 and furin by diterpines of *Andrographis paniculata* and their succinoyl esters. Biochem J 1999;338:107-13. doi: 10.1042/bj3380107, PMID 9931305
19. Adedapo AA, Adeoye BO, Sofidiya MO, Oyagbemi AA. Antioxidant, antinociceptive and anti-inflammatory properties of the aqueous and ethanolic leaf extracts of *Andrographis paniculata* in some laboratory animals. J Basic Clin Physiol Pharmacol 2015;26:327-34. doi: 10.1515/jbcpp-2014-0051, PMID 25153591
20. Tiwari V. Phytochemical analysis and medicinal value of Kalmegh (*Andrographis paniculata* Nees). Indian J Appl Pure Biol 2017;32:283-8.
21. Statile L, Puig MM, Warner W, Bansinath M, Lovitz M, Turndorf H. Droperidol enhances fentanyl and sufentanil, but not morphine, analgesia. Gen Pharmacol 1988;19:451-4.
22. Divya S, Suresh J, Paramakrishnan N. Phytopharmacological review on *Vitex negundo* linn. Ann Rom Soc Cell Biol 2021;25:9496-508.
23. Smrity KK, Sultana S, Hassan MA, Hossain ML. Medicinal activity of *Vitex negundo* L.(Family: *Lamiaceae*) leaves extract: Assessment of phytochemical and pharmacological properties. J Pharmacogn Phytochem. 2019;8:3571-5.
24. Valdiani A, Kadir MA, Tan SG, Talei D, Abdullah MP, Nikzad S. Nain-e Havandi *Andrographis paniculata* present yesterday, absent today: A plenary review on underutilized herb of Iran's pharmaceutical plants. Mol Biol Rep 2012;39:5409-24. doi: 10.1007/s11033-011-1341-x, PMID 22198549
25. Mitra SK, Kannan R. A note on unintentional adulterations in ayurvedic herbs. Ethnobotanical Leafl 2007;11:11-5.