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ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF METHANOLIC EXTRACT OF *ACALYPHA FRUTICOSA* FORSSK. IN EXPERIMENTAL ANIMALS

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ABSTRACT

The present study was carried out to screen methanolic extract of aerial parts *Acalypha fruticosa* Forssk for analgesic and anti-inflammatory activities in experimental animals. Acute toxicity study was carried out in female Sprague Dawley rats and Swiss mice as per OECD guideline 423. Upon ascertaining its safety, the extract of the test drug was screened for analgesic and anti-inflammatory activities in Sprague Dawley rats and Swiss mice through various models. The test extract showed significant analgesic and anti-inflammatory activities at 250 and 500 mg per kg body weight.

KEYWORDS: Acalypha fruticosa, methanolic extract, rats, mice, analgesic, anti-inflammatory.

INTRODUCTION

Many species of acalypha are used traditionally to treat various ailments and the *Acalypha fruiticosa* Forssk. a member of Euphorbaceae is used in treatment of skin ailments, stomach cramps, indigestion and poisonous bites.[1] Macerated leaves are used to treat ocular infections and leaf sap is used to combat respiratory problems.[2] The stem and leaves are used to treat wound and skin ailments.[3]

The methanolic extract of *Acalypha fruticosa* aerial parts revealed the presence of flavonoid, phenol, Tannins, alkaloid and steroid.[4] Methanolic extract of Acalypha fruiticosa has remarkably reduced the tumor carcinoma and viable cell count mice with experimental Ehrlich's Ascitis carcinoma.[5] In another study, the chloroform extract of the test drug significantly protected the mice from electric shock as demonstrated by electric shock method and pentylenetetrazole induced convulsion method.[6] Assessment of anti-snake venom effects of Acalypha fruticosa leaves against Indian saw scaled Viper has been demonstrated using envenomed Wistar albino rats.[7]

MATERIALS AND METHODS

Test drug

Phytochemical analysis of the test extract has been carried out at Quality Control Laboratory of the Institute and which revealed presence of proteins, tannins, glycosides, alkaloids and flavonoids.

Animals

Sprague Dawley rats and swiss albino mice of either sex were used in the trial and the trial was conducted at Experimental animal facility of National Ayurveda Research Institute for Panchakarma, Cheruthuruthy, Thrissur vide approval No. IAEC/NRIP/2015-16/02 by Institute animal Ethics Committee.

Experimental design

Toxicity studies

In acute toxicity study (Acute toxic class method), 3 female animals were exposed to the test drugs at dose of 2000 mg/kg body weight orally once and the animals were observed for mortality and clinical signs of toxicity for 14 days. Next set of 3 female animals were also exposed at same dose level once and were observed for a period of 14 days.[8]

Efficacy studies

Studies to evaluate analgesic activity and anti-inflammatory activities of were carried out in 5 groups of animals each containing 3 male and 3 female animals.

1. CTL- Control group (distilled water)
2. LD - Low dose group (100 mg / kg Body weight)
3. AD- Average dose group (250 mg / kg Body weight)
4. HD - High dose group (500 mg / kg Body weight)
5. STD - Standard drug group.

Analgesic activity was evaluated with 4 experimental models i.e., radiant heat method (SD rats). hot plate method (Swiss mice), acetic acid induced writhing

method (Swiss mice) and formaldehyde induced paw licking method (Swiss mice).

(a). Tail flick Method
The basal reaction time in seconds for tail flicking response prior to drug administration was compared with that of post drug administration at different time intervals in rats between control, test groups and standard groups (Ibuprofen) as per the method of Gujral and Khanna.[9]

(b). Hot plate method
Animals were individually placed on a hot plate maintained at constant temperature (55^0 C) and the time taken by the animals for showing reactions such as paw licking and jump response is noted. The basal reaction time prior to drug administration was compared with that of post drug administration at different time intervals in mice between control, test groups and standard groups (Ibuprofen) as per the method of Eddy and Leimbach.[10]

(c) Formaldehyde induced paw licking method
Animals were administered with vehicle, test drug in different doses and standard drug Indomethacin (10 mg per kg body weight) orally. Thirty minutes post administration, 20 µl of 1% formaldehyde was injected subcutaneously under surface of the hind paw and the time spent for licking the paw injected with formaldehyde was counted for 5 min post formaldehyde injection.[11]

(d). Acetic acid writhing
3% acetic acid was used to induce writhing in mice and writhing response in animals is produced by intra peritoneal injection of acetic acid was taken as criteria to assess analgesic activity of test drugs (Witkin et al, 1961). Indomethacin (10 mg per kg body weight) administered group served as reference standard.[12]

Anti-inflammatory activity was evaluated with 2 experimental models i.e. Carrageenan induced hind paw oedema method and Formaldehyde induced hind paw oedema in SD rats.

(a) Carrageenan hind paw oedema method
0.1 ml of 1% Carrageenan in normal saline was used as phlogistic agent and was injected beneath plantar aponeurosis of right hind paw of rats to induce oedema. Control, test drug in different doses and standard drug (Ibuprofen) were administered 45 minutes prior to Carrageenan injection were injected. The increase in paw volume was noted 3 hours post carrageenan administration by fluid displacement method using plethysmometer and compared between the groups.[13]

(b) Formaldehyde induced hind paw oedema
Vehicle, test drug in different doses and standard drug (Ibuprofen) were administered to SD rats. 45 minutes later, hind paw oedema was be induced by injecting 0.1 ml of 1% formaldehyde beneath plantar aponeurosis of right hind paw of rats. The increase in paw volume was noted at 3 hours post formaldehyde injection by fluid displacement method using plethysmometer and compared between the groups.[14]

RESULTS AND DISCUSSION

Toxicity study
The methanolic extract of the *Acalypha fruticosa* did not produce mortality or clinical signs of toxicity in female SD rats and female swiss mice upon single oral administration at dose of 2000 mg /kg body weight.

Tail flick Method
Significant analgesia (P≤ 0.05) was observed at 120- and 240-minutes post test drug administration in animals which received average dose test drug group as compared to control group. Similarly, significant analgesia (P≤ 0.01) was observed at 240-minutes post test drug administration in animals which received high dose test drug group as compared to control group (Table 1)

Table 1: Effect of *Acalypha fruticosa* methanolic extract on latency of tail flick response in rats.

Groups	Reaction time in Sec (Mean ± SEM) at various time intervals post drug administration			
	Initial	60 Min	120 Min	240 Min
CTL	8.0±0.36	8.333±0.56	7.83±0.60	7.83±0.48
LD	7.17±0.31	7.83±0.40	7.50±0.50	8.67±0.56
AD	6.50±0.22	7.0±0.26	7.50±0.22*	7.67±0.33*
HD	6.67±0.21	7.67±0.21	8.0±0.26*	8.33±0.42**
STD	7.17±0.17	7.33±0.21	8.17±0.17**	8.83±0.17****

* P<0.05 ** P<0.01 **** P.0001

Hot plate method
Significant analgesia (P≤ 0.05) was observed at 120- and 240-minutes post test drug administration in animals which received average dose test drug group as compared to control group. Similarly, significant analgesia (P≤ 0.01) was observed at 120- and 240-minutes post test drug administration in animals which received high dose test drug group as compared to control group (Table 2)

Table 2: Effect of *Acalypha fruticosa* methanolic extract on hot plate analgesia in mice.

Group	Reaction time in Sec (Mean ± SEM) at various time intervals post drug administration			
	Initial	60 Min	120 Min	240 Min
CTL	10.00±0.52	10.50±0.43	9.83±0.48	10.17±0.40
LD	9.50±0.56	10.17±0.48	10.0±0.26	9.83±0.17
AD	8.0±0.45	8.50±0.34	9.67±0.50*	9.83±0.54*
HD	8.0±0.45	8.50±0.43	10.17±0.54**	10.50±0.43**
STD	9.83±0.31	11.33±0.42*	12.33±0.33***	13.17±0.48****

* P<0.05 ** P<0.01 *** P<0.001 **** P.0001

Formaldehyde induced paw licking method

Significant reduction in duration of paw lickings were observed in mice which received average dose (P<0.05) and high dose (P<0.01) of test drug as compared to control (Table 3).

Table 3: Effect of *Acalypha fruticosa* methanolic extract on paw licking test in mice.

Groups	Paw licking in seconds (Mean ±SEM)
CTL	48.33±3.19
LD	43.67±3.73
AD	36.67±2.04*
HD	33.67±1.91**
STD	29.33±1.99***

* P<0.05 ** P<0.01 *** P<0.001

(d). Acetic acid writhing

The test drug significantly (P<0.01) reduced the number of writhings in animals at high dose level as compared to control. (Table 4).

Table 4: Effect of *Acalypha fruticosa* methanolic extract acetic acid induced writhing in mice.

Groups	No. of writhing (Mean ±SEM)
CTL	26.17±1.64
LD	25.50±1.61
AD	23.67±1.71
HD	19.17±0.94**
STD	17.67±0.80***

** P<0.01 *** P<0.001

Carrageenan induced hind paw oedema method

Significant reduction in paw volume was observed in animals which received test drug at average ($P \leq 0.05$) and high dose ($P \leq 0.01$) as compared to those in control group. (Table 5)

Table 5: Effect of *Acalypha fruticosa* methanolic extract on carrageenan induced hind paw oedema in rats.

Groups	Increase in paw oedema in ml (Mean ± SEM)	Percentage reduction
CTL	0.44±0.03	--
LD	0.40 ±0.03	10.00
AD	0.33±0.02*	25.94
HD	0.30±0.02**	32.71
STD	0.21±0.03****	53.38

* P<0.05 ** P<0.01 **** P.0001

Formaldehyde induced hind paw oedema method

Significant ($P \leq 0.01$) reduction in paw volume was observed in animals which received test drug at average and high dose as compared to those in control group. (Table 6)

Table 6: Effect of *Acalypha fruticosa* methanolic extract on formaldehyde induced hind paw oedema in rats.

Groups	Increase in paw oedema in ml (Mean ± SEM)	Percentage reduction
CTL	0.41±0.03	--
LD	0.40 ±0.02	2.82
AD	0.31±0.01**	23.8
HD	0.30±0.01**	27.4
STD	0.22±0.02****	46.8

** P<0.01 **** P.0001

The methanolic extract of the *Acalypha fruticosa* showed significant analgesic activity in both tail flick method and hot plate method. The increase in latency of tail flick and paw licking/ jumping response might be due to increase in the threshold for pain and alteration of physiological response to pain like centrally acting analgesics.[15]

The extract significantly reduced the duration of paw licking consequent to injection of formaldehyde into the paw of mice. It also significantly decreased the stretching episodes in mice against acetic acid induced writhing in mice. These properties suggest the peripheral analgesic action of the test drug by inhibition of inflammatory cytokines and interleukins and presence of alkaloids in the extract may be attributed to these nociceptive activities.[16]

The methanolic extract of the test drug showed significant anti-inflammatory activity in both carrageenan and formaldehyde induced hind paw oedema methods and this might be due to presence of flavonoids in the extract.[17] The anti-inflammatory activity may be attributed to antioxidant potential of *Acalypha fruticosa* methanolic extract by counteracting reactive oxygen species (ROS) and preventing damage to cellular macromolecules.[18]

CONCLUSION

The methanolic extract of the *aerial parts of acalypha fruticosa* Forssk showed significant analgesic activity as screened through tail flick method, hot plate method, formaldehyde paw licking method and acetic acid induced writhing method. The results of the study revealed the extract is effective at both central and peripheral models of pain. In addition, the extract also exhibited significant anti-inflammatory activity as demonstrated through carrageenan and formaldehyde hind paw oedema method.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding publication of this paper.

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REFERENCES

1. Kirtikar K.R and Basu B.D. Indian medicinal plants volume III. Bishen Singh Mahendra pal Singh, Dehradun, 1980; 2261-2262.
2. Schmelzer G.H, Acalypha fruticosa Forssk. In: Medicinal plants. PROTA, Schmelzer G.H and Gurib-Fakim, A. [Eds.] Prota 11[1]: Wageningen, Netherlands. 2007.
3. Fleurentin J and Pelt J-M. Repertory of drugs and medicinal plants of Yemen. J. Ethnopharmacol, 1982; 6: 85-108.
4. Amina El shaibany, Molham al Habori, Shaza Al Massarani, Adenan Al Adhi and Andreas Michalsen. Hypoglecemic activity of Acalypha fruticosa Forsssk extracts in normal rats. Trop. J. Pharm. Res., 2015; 14(8): 1445-1450.
5. Sivakumar T, Venkatachala Murti M.N and Kumutha P. Evalluation of antitumor and antioxidant acivity of Acalypha fruiticosa in Ehrlich's Ascitis carcinoma bearing Swiss albino mice. Res. J. Pharm., Biol. Chem., 2010; 1(4): 191- 199.
6. Sumalatha Govindu and Sreedevi Adikay. Evaluation of antiepileptic activity of chloroform extract of acalypha fruticosa in mice. Phcog Res., 2014; 6(2): 108-112.
7. Malathi Ramaswamy, Sivakumar Doraikannu and Chandrasekhar Solaimuthu. Assessment of anti-snake venom effects of Acalypha fruticosa leaves against Indian saw scaled viper using envenomed Wistar albino rats. Int. j. sci., 2019; 6(3): 31-40.
8. OECD guideline 423 OECD guidelines for testing of chemicals section 4 Health effects OECD france, 2001.
9. Gujral M.C. and Khanna B.K. Comparative evaluation of some narcotic analgesics. Journal of scientific and Industrial research, 1956; 168: 11-13.
10. Eddy NB, Leimbach D. Systematic analgesics II. Diethyl butenyl and diethienylbutyl amines. Journal of Pharmacology and experimental therapeutics, 1953; 107: 387–93.
11. Hunskaar S and Hole K. The formalin test in mice-dissociation between inflammatory and non-inflammatory pain. Pain., 1997; 30: 103–114.
12. Witkin L B, Herbner C F, Gaddi F, O'Keefe E, Spitaletta P and Plumer A. J. Pharmacology of 2-aminoindane hydrochloride (SU- 8629). A potent non- narcotic analgesic. J Pharmacology and Experimental Therapeutics, 1961; 133: 400–8.

13. Winter, C.A, Risley, E.A. and Nuss, G.W. Carrageenan induced oedema in hind paw of the rat as an assay of anti-inflammatory drugs. Proceedings of Society of Experimental Biology and Medicine, 1962; III.: 544-547.
14. Turner R.A. Screening methods in Pharmacology Vol.-1, Academic Press, New York, 1965; 26-34.
15. Shreedhara C, Vaidya V, Vagdevi H, Latha K, Muralikrishna K, and Krupanidhi A, "Screening of Bauhinia purpurea Linn. for analgesic and anti-inflammatory activities," Indian J Pharmacol, 2009; 41(2): 75–79.
16. Mohammad Shoaib, Syed Wadood Ali Shah, Niaz Ali, Ismail Shah, Shafi Ullah, Mehreen Ghias, Muhammad Nawaz Tahir3, Farah Gul, Sohail Akhtar, Abd Ullah1, Wajid Akbar1and Asad Ullah. Scientific investigation of crude alkaloids from medicinal plants for the management of pain. BMC Complementary and alternative Medicine, 2016; 16: 178.
17. Anand Kumar M, S. Paulsamy S, Sathish Kumar P and Senthil Kumar P. Preliminary phytochemical studies for the quantification of secondary metabolites of medicinal importance in the plant, acalypha fruiticosa Forssk. J. Appl. & Nat. Sci, 2009; 1(10): 41-43.
18. Rajkumar V, Gunjan Guha, R. Ashok Kumar R. Therapeutic potential of Acalypha fruticosa. Food and Chemical Toxicology, 2010; 48: 1709-1713.