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Research Article

ANALGESIC AND ANTI PYRETIC POTENTIAL OF METHANOLIC EXTRACT OF *KYLLINGA TRICEPS* ROTTB

Nishant Verma[1]*, Amit Chaudhary[1], Shamim Ahmad[2], Vipin Garg[3]

1.T. M. College of Pharmacy, Teerthanker Mahaveer University, Moradabad (U.P.) 244001
2.Translam Institute of Pharmaceutical Education & Research, Meerut, (U.P.), India-250001
3.Department of Pharmaceutical Technology, MIET, Meerut (U.P.) 250005

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Abstract

Kyllinga triceps is traditionally used to treat painful and inflammatory conditions. In the present study, analgesic and antipyretic activities of methanolic extract of Kyllinga triceps at different doses was studied using hot plate, acetic acid induced writhing and yeast induced hyperthermia method. Kyllinga triceps showed significant analgesic and antipyretic activities in all models studied. Results support the traditional use of the plant in the treatment of pain and fever.

Keywords: - : *Kyllinga triceps*, Analgesic, Antipyretic, Paracetamol, Pentazocine.

Introduction

Analgesics are primary need of patients to getrid of any kind of pain.[1] Pain is one of the basic symptoms of all human ailments which is a sensorial modality and primarily protective. Analgesics only relieve pain in a particular complaint without affecting its cause.[2] The most eminent analgesics include opiates and NSAIDs, but they are not helpful in all cases due to their adverse effects. Besides pain the fever is another most common symptom of sickness which is caused by increase in the body temperature of an individual at a particular time.[3] Herbal medicines are often used as remedies in these conditions since as a result of poverty medicines may be unaffordable.[4] It is a well known fact that herbal medicines may be sources of substances with better therapeutic potentials than some currently used orthodox medicines.[5]

Materials and methods

Collection and authentication of plant material

Fresh plant of *Kyllinga triceps* was collected in the month of April, 2011 from Venkateswara University, Tirupati, Andhra Pradesh, India. The plant was identified and authenticated by Dr. K. Madhava Chetty, Asst. Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. A voucher specimen has been deposited at the College of Pharmacy, TMU Moradabad. (TMU/Consult/2011-12/206) for future reference.

Preparation of the Extract

Fresh whole plant along with roots were shade dried reduced to moderately coarse poeder, loaded into soxhlet extractor and was subjected to successive extraction with Petroleum Ether, Benzene, Chloroform, Methanol and distilled water by hot extraction using Soxhlet apparatus at a temperature of 60˚C. The extracts were concentrated under reduced pressure using a rotary vaccuum evaporator to constant weight and preserved in desiccator for further studies.

Animals

Adult albino wistar rats of both sexes weighing between 200 to 250 gm were used for the study. Also albino mice of both sexes weighing between 20-25gm were used. They were housed in polypropylene cages and fed with standard diet and water *ad libitum*. The animals were exposed to an alternating 12 h light and dark cycle. All the experimental procedures and protocols involving animals were reviewed by the Institutional Animal Ethics Committee in accordance with the guidelines of CPCSEA.

Drugs and chemicals

Paracetamol (Batch no. RG332, Calpol) was

purchased from Glaxo Smith Kline Pharmaceutical Ltd. Mumbai, India. Pentazocine drug (Batch no. 3357, Fortwin) was obtained from Ranbaxy Limited. Aspirin (Batch no. 13007594, Ecosprin) USV Ltd Mumbai. Analytical grade chemicals including various organic solvents (Petroleum Ether, Benzene, Chloroform and Methanol) from Rankem, Pvt. Ltd., New Delhi were used for the successive extraction.

Preliminary phytochemical screening

The different extracts were subjected to qualitative phytochemical screening for the identification of the phytoconstituents. While Petroleum Ether, Benzene, Chloroform does not show any appreciable tests for the presence of different phytoconstituents, Petroleum Ether extract showed positive tests for the presence of alkaloids, glycosides, flavonoids. However Methanolic extract showed positive tests for alkaloids, glycosides, flavonoids, tannins and phenolic compounds. The plant is used to cure pain and fever, the analgesic and antipyretic activity of the methanolic extract of the plant at different doses levels (100 mg/kg, 200 mg/kg, 400 mg/kg) is being reported here.

Acute toxicity study

Acute toxicity study for the extract was carried out according to the method described in the literature.[6] The methanolic extract of root of *Kyllinga triceps* suspended in 5% gum acacia solution in doses of 100-2000 mg/kg was administered orally to animals. The animals were observed continuously for any change in autonomic or behavioral responses for first few hours and at 24 hour interval for a period of 72 hours. At the end of this period the mortality if any in each group was noted.

Antipyretic testing

Hyperthermia was induced in rats following the method of teotino et al., 1963.[7] Initial rectal temperatures of rats were recorded using a six channel tele-thermometer for 1 min. Rats were made hyperthermic by subcutaneous injection of 20% yeast suspension at a dose of 1 ml/100 gm body weight. When the temperature was at peak (18 hours after yeast injection) the rectal temperature were again recorded. Those animals that showed a rise in rectal temperature of more than 1.2^0 C were used.[8] Different doses of methanolic extract of Kyllinga triceps were given orally as a suspension prepared in 2% Tween 80 solution. Animals were divided into five groups of six animals each.First group received 1 ml of 2% Tween 80 solution orally and served as control. Second, third, fourth and fifth groups received standard antipyretic agent i.e. paracetamol suspension (100 mg/kg), methanolic extract (100 mg/kg), methanolic extract (200 mg/kg), metanolic extract (400 mg/kg) respectively. The rectal temperatures of animals were recorded at 30 minutes intervals for 4 hour following the administration of Tween 80, standard drug and plant extract.[9]

Analgesic activity

Hot Plate Method

The hot plate method described by Turner (1965) was followed for the assessment of analgesic activity. Albino mice were introduced to a hot plate maintained at 55 ± 0.5^0C. the reaction time to the thermal stimulus was recorded as the time interval from introduction of the animal to the plate until the first lick of the limbs or the first jump of the animals. The test groups received methanolic extract of kyllinga triceps at different dose levels prepared as suspension in 2% Tween 80 orally, the standard group received Pentazocine (10 mg/kg, i.p.)[10] and control group received only 1 ml of 2% Tween 80 solution. The reaction times were determined befor and after 30 minutes, 1 hour, 2 hours and 3 hours period with reference to the control group received only vehicle.

Acetic Acid Induced Writhing

Acetic acid induced writhing response in mice Acetic acid solution at a dose of 10 ml/kg (0.6%) was injected i.p. and the number of writhes during the following 15 minutes period was observed. The test groups received methanolic extract of Kyllinga triceps at different dose levels prepared as suspension in 2% Tween 80 orally, the standard group received Aspirin (10 mg/kg, i.p.) and control group received only 1 ml of 2% Tween 80 solution. Significant reductions in number of writhes by drug treatment as compared to vehicle treatment animals were considered as a positive analgesicresponse. The percent inhibition of writhing was calculated.[10]

$$\% \text{ Inhibition} = \frac{Wc - W_T}{Wc} \times 100$$

Statistical analysis

All the results obtained from various activities, as described above, were analyzed statistically by using Student's t test and $p<0.05$ were considered significant.[11]

RESULTS

Yield of plant extract

The 115 gm whole plant of *Kyllinga triceps* was taken for extraction by Soxhlet apparatus. Total 17.56 gm methanolic extract was obtained. The yield of methanolic extract was found to be 15.27 % w/w.

Phytochemical testing

Phytochemical testing showed that the methanolic extract of *Kyllinga triceps* contains alkaloids, glycosides, flavonoids, tannins and phenolic compounds.

Acute toxicity

No adverse effect and no mortality were observed in the animals during the period of study, 72 hours up to the dose 2000 mg/kg of methanolic extract of roots of *Kyllinga triceps.* Hence, there were no lethal effects in any of the groups. In the study, methanolic extract of *Kyllinga triceps* was administered at a dose of 100 mg/kg, which was determined as the most effective dose.

Anti-pyretic Activity

The anti-pyretic activity of the methanolic extract of Kyllinga triceps has been shown in table 1, which showed significant activity at 200mg/kg and 400 mg/kg dose levels. The results were comparable to that of Paracetamol a prototype anti-pyretic drug.

Analgesic Activity

Hot Plate Method

From the result it can be deduced that the extract has shown dose dependant activity. After administration of the methanolic extracts at all the three dose levels, threr is statistically significant increase in the hot plate reaction time. But the increase is comparable to the standard drug, Pentazocine only at 600 mg/kg dose level (Table 2).

Acetic Acid Induced Writhing

The methanolic extract at dose levels of 100, 200 and 400 mg/kg exhibited 30.55, 68.55 & 82.10 % inhibition of writhing as compared to that of 82.96% inhibition shown by Aspirin. It is quite evident from the result that the extract at 400 mg/kg showed comparable activity to that of Aspirin (Table 3).

Table.1. Effect of different doses of methanolic extract of Kyllinga triceps and paracetamol on yeast induced hyperthermia in rats.

	Rectal Temperature (^{0}C)						
	Initial before yeast injection	18 Hrs. after yeast injection	Time after drug administration (hrs)				
			0.5 hrs	1 hrs	2 hrs	3 hrs	4 hrs
Control	36.20± 0.059	38.21± 0.078	38.25± 0.071	38.30± 0.081	38.28± 0.046	38.23± 0.061^a	37.31± 0.069
Paracetamol (100 mg/kg)	36.18± 0.045	38.28± 0.061	37.39± 0.098^d	36.72± 0.078^d	37.35± 0.071^d	37.84± 0.098^c	37.11± 0.061^c
MEKT (100 mg/kg)	36.26± 0.087	38.25± 0.038	38.16± 0.045	38.13± 0.067	37.90± 0.068^a	38.20± 0.072	37.57± 0.078
MEKT (200 mg/kg)	36.18± 0.059	38.16± 0.088	37.84± 0.082^c	36.92± 0.071^d	37.60± 0.068^d	37.94± 0.059^b	37.10± 0.038
MEKT (400 mg/kg)	36.23± 0.057	38.28± 0.067	37.63± 0.062^d	36.86± 0.076^d	37.43± 0.051^d	37.73± 0.101^c	37.06± 0.092

Values are expressed as mean ± S.E.M. (n=6); significance at $p<0.05^a$, $p<0.02^b$, $p<0.01^c$, $p<0.001^d$ as compared to control.

Table 2 Effect of different doses of methanolic extract of Kyllinga triceps on Hot Plate reaction time in mice.

Groups	Dose (mg/kg)	Reaction Time (Seconds)				
		Initial	Time after drug administration (Hrs)			
			0.5 hrs	1 hr	2 hrs	3 hrs
Control		8.65± 0.0260	8.63± 0.0223	8.67± 0.0421	8.68± 0.0139	8.66± 0.0101
Pentazocine	10	8.70± 0.0139	24.67± 0.0795^{d}	31.74± 0.0213^{d}	36.61± 0.0261^{d}	30.65± 0.0493^{d}
MEKT	100	8.68± 0.0099	11.98± 0.0392^{d}	15.18± 0.0216^{d}	16.88± 0.0217^{d}	13.00± 0.0318^{d}
MEKT	200	8.70± 0.0207	18.98± 0.0491^{d}	22.57± 0.0212^{d}	27.63± 0.0212^{d}	20.53± 0.0281^{d}
MEKT	400	8.71± 0.0201^{c}	22.53± 0.0291^{d}	29.63± 0.0171^{d}	34.67± 0.0394^{d}	27.98± 0.0218^{d}

Values are expressed as mean ± S.E.M. (n=6); significance at $p<0.05^{a}$, $p<0.02^{b}$, $p<0.01^{c}$, $p<0.001^{d}$ as compared to control.

Table no 3 Effect of different doses of methanolic extract of Kyllinga triceps on Acetic acid induced writhing in mice.

S.N.	Groups	Dose (mg/kg)	No. of Writhings (Mean ± SEM)	% Inhibition
1	Control		37.66± 1.326	
2	Aspirin	10	6± 0.387^{d}	82.91
3	MEKT	100	26± 0.513^{d}	30.52
4	MEKT	200	11.5± 0.499^{d}	68.61
5	MEKT	400	6.33± 0.431^{d}	82.2

Values are expressed as mean ± S.E.M. (n=6); significance at $p< 0.05^{a}$, $p< 0.02^{b}$, $p<0.01^{c}$, $p<0.001^{d}$ as compared to control.

DISCUSSION

The present study establishes the anti-pyretic and analgesic activities of the methanolic extract of Kyllinga triceps in the used models. Since antipyretic and analgesic activities are commonly mentioned as characteristic of drugs or compounds which have an inhibitory effect on prostaglandin biosynthesis[12], the yeast induced hyperthermia in rat model was therefore employed to investigate the antipyretic activity of Kyllinga triceps. It was found that the methanolic extract at the dose of 400 mg/kg showed a significant decrease in rectal temperature similar to that shown by the standard drug paracetamol. This result seems to support the view that the extract has some influence on prostaglandin biosynthesis because prostaglandin is believed to be a regulator of body temperature.[13]

Likewise the analgesic activity of methanolic extract of the plant was evaluated using the hot plate method and writhing test in mice. The hot plate method is useful in detecting centrally acting analgesics[14] where as acetic acid induced writhing method is useful to detect peripheral analgesic effects. Acetic acid, which is used as an inducer for writhing syndrome, causes algesia by liberation of endogenous substances, which then excite the pain nerve endings. The fact that methanolic extract of Kyllinga triceps showed analgesic activity in both the studied models, indicate that this effect could be due to the presence of two components, one acting centrally and the other via peripheral route. From the above results it can be deduced that methanolic extract has shown dose dependent activity. As the phytochemical screening has shown the presence of alkaloids, glycosides,

flavonoids, tannins and phenolic compounds in the methanolic extract. Its potent activity may be attributed to the presence of these phytoconstituents. More detailed phytochemical studies are, however, necessary to identify the active principles and exact mechanism of action.

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REFERENCES

1. Semwal, D.K., Semwal, R.B., Semwal, R., Jacob, V., Singh, G., Analgesic and antipyretic activities of Gindarudine, a morphine alkaloid from Stephania glabra, Current Bioactive Compounds, 2011, (7), 214-217.
2. Mate, G.S., Naikwade, N.S., Chowki, C.S.A.A., Patil, S.B., Evaluation of anti-nociceptive activity of Cissus quadrangularis on albino mice, Int. J. Green Pharm., 2008, (2), 118-121.
3. Wolf, S.M., Fauci, A.S., Dale, D.C., Unusual etiologies of fever and evaluation. Annu. Rev. Med., 1975, (26), 277-281.
4. Soucat, A., Levy, B.D., De, B.X., Gbedonou, P., Lamarque, J.P., Bangoura, o., Camara, O., Gandaho, T., Ortiz, C., Kaddar, M., Knippenberg, R., Affordability, cost effectiveness and efficiency of primary health care: the bamako initiative experience in benin and guinea. Int. J. Health Plan Mgt, 1997, (12), S81-S108.
5. Wang, Y., Wang, X.,, Cheng, Y., A computational approach to botanical drug design by modeling quantitative composition activity relationship. Chem. Biol. Drug Design, 2006, 968), 166-172.
6. Litchfield, J.T., Wilcoxon, F., A simplified method of evaluating dose-effect experiments, J. Pharmacol. Exp. Ther., 1949, (96), 99-135.
7. Teotino, U.M., Friz, L.P., Gandini, A. Bella, D.D., Thiodirevative of 2,3-dihydro - 4H - 1,3-benzoxazin-4-one synthesis and pharmacological properties. J. Med. Chem. 1963, (6), 248-250.
8. Taesotikul T., Panthong A., Kanjanapothi D., Verpoorte R., Scheffer J.J.C. Antiinflammatory, antipyretic and antinociceptive activities of Tabernaemontana pandacaqui Poir. J. Ethanopharmacol 2003, (84) 31-35.
9. Narayanan N., Thirugnanasambantham P., Viswanathan S., Vijayasekaran V., Sukumar E. Antinociceptive, ant-inflammatory and antipyretic effects of ethanol extract of Clerodendron serratum roots in experimental animals. J. Ethanopharmacol 1999, (65), 237-241.
10. Puchchakayala G., Podili Laxmi, Bobbala Diwakar et al. Antinociceptive and anti-inflammatory effects of Cleome chelidonni linn. roots in experimental animals. Phcog. Mag. 2008, 4(13) 32-36.
11. Kulkarni S. K. Handbook of Experimental Pharmacology. Vallabh Prakashan, New Delhi, 2nd ed., 1993, 82-87.
12. Vane J. R. The evaluation of non-steroidal anti-inflammatory drug and their mechanism of action. Drug 1987, (33), 8-27.
13. Milton A. S. Prostaglandins and fever. Trends in Pharmacological Sciences 1982, (40), 490-492.
14. Radhakrishnan R., Zakaria M.N.M., Islam M. W. et al. Analgesic and Anti-Inflammatory Activities of Teucrium stocksianum. Pham. Bio. 2001, 39 (6): 455-459.

Correspondence Address:
Nishant Verma
Teerthanker Mahaveer College of Pharmacy,
Teerthanker Mahaveer University, Moradabad (U.P.) 244001
E-mail- nishantvermamiet@gmail.com
Phone no:- +919536359544