ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Analgesic and antipyretic activity of methanolic extract of *Coccinia grandis* L. leaves in experimental animals.

Aggarwal Ashish S*[1], Suralkar Ujwala R[1], Chaudhari Sugandha G[3], Deshpande SV[2], Garud Aniket A[1], Talele Sandeep G[1]

[1]Department of Pharmacology, Padm. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune - 411018, Maharashtra, India
[2]Department of Pharmaceutical Chemistry, Padm. Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune-411044, Maharashtra, India.
[3]Department of Pharmacology, Dr. L. H. Hiranandani College of pharmacy, Ulhasnagar-421003, Mumbai, Maharashtra, India.

ABSTRACT

The present study investigates the analgesic and antipyretic activity of the methanol extract (50, 100 and 200 mg/kg) of *Coccinia grandis* L., leaves in rats and mice. Acetic acid induced writhing, Tail immersion and Hot plate models were used to evaluate analgesic activity and Yeast induced pyrexia model was used to evaluate antipyretic activity. Our findings show that oral administration of methanol extracts significantly inhibit acetic acid-induced writhing in mice in dose dependent manner but failed to show significant inhibition in Tail immersion and Hot plate models. Antipyretic study revealed that methanolic extract exhibits significant reduction in pyrexia that was comparable to standard drug.

Keywords: *Coccinia grandis* L., analgesic activity, antipyretic activity, Acetic acid.

Corresponding author
Email: ash.pharmacology@gmail.com

ISSN: 0975-8585

INTRODUCTION

Pain is an unpleasant sensation no doubt, but on the whole it is usually beneficial to man (or animal). It is mainly a protective mechanism for the body, occurs whenever any tissues are being damaged, and it causes the individual to react to remove the pain stimulus [1]. Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states [2].

Coccinia grandis L., of the family Cucurbitaceae is distributed in tropical Africa, commonly found in Pakistan, India and Srilanka. Coccina is a climber and trailer. Every part of this plant is valuable in medicine and various preparations have been mentioned in indigenous system of medicine for various skin diseases, bronchial catarrh, bronchitis and Unani systems of medicine for ring worm, psoriasis, small pox, scabies and other itchy skin eruptions and ulcers. Oil of this plant is used as an injection into chronic sinuses. Coccinia grandis leaves are reported for its antihyperlipidemic [3], antimicrobial [4], antitussive [5], antiulcer [6], antioxidant activity and fruits are reported for hepatoprotective [7], cerebral oxidative stress [8].

The aim of the present investigation was to evaluate the possible analgesic and antipyretic effects of the methanolic extract of the leaves of Coccinia grandis (ML) in experimental animals.

MATERIALS AND METHODS

Plant Material

The plant material consists of dried powdered leaves of *Coccinia grandis* L. Voigt. (Cucurbitaceae). The plant was collected in and around the farms of Chikhli, Tal-Haveli, Dist.-Pune, Maharashtra, India during the month of September-2008 and was authenticated by Joint Director, Botanical Survey of India, Western Circle,Pune-4110 01 (Ref No BSI/WC/Tech./ 2008/477 dated 3/10/2008).

Preparation of the extract

Air-dried powdered leaves (500gm) of Coccinia grandis L. were extracted with 2.0 L methanol by using Soxhlet apparatus. An exhausted marc was collected. After the removal of solvent by concentration under reduced pressure at 60^0C, 19.38 gm of methanolic residue were obtained, respectively.

Animals

Wistar rats (200-250 g) of either sex were used for present study. Animals were kept under a 12 h light/ 12 dark cycle, with free food and water ad libitum. Albino mice (Swiss strain) weighing 22-25 g were housed under standard laboratory condition in a group of six each.

Animals had free access to food and water. The Institutional Animal Ethical committee (IAEC) has approved the protocol of the study.

Vehicles

Plant extract, aspirin were suspended in 0.5% (w/v) carboxymethylcellulose sodium (CMC) and administered orally to animals. Acetic acid diluted separately in normal saline and injected intraperitoneally.

Phytochemical screening

Preliminary phytochemical screening of the crude methanolic extracts of *Coccinia grandis* L. leaves (ML) were carried out. Phytochemical screening of the extract was performed using the following reagents and chemicals [9] alkaloids with Dragendorff's reagent, flavonoids with the use of Mg and HCl; tannins with 1% gelatin and 10% NaCl solutions and saponins with ability to produce suds and hemolysis reaction.

Acute oral toxicity study

The acute toxicity was determined on albino mice by fixed dose method of OECD Guide line no 423 given by CPCSEA. Groups of 6 mice were administered test drug by oral route in the range of 300- 2000mg/kg and mortality was observed after 24 hr., in case of *Coccinia grandis* L. leaves, since there was death of one animal in the group treated with 300mg/kg dose and all the animals died in the group of animals treated with 2000mg/kg dose. Therefore 1000mg/kg was treated as LD_{50} and $1/20^{th}$, $1/10^{th}$ and $1/5^{th}$ (i.e. 50mg/kg, 100mg/kg and 200 mg/kg) of 1000mg/kg were selected for further study.

SCREENING METHODS FOR ANALGESIC ACTIVITY:

Acetic acid-induced writhing response in mice

Analgesic activity was evaluated on the acetic acid-induced writhing according to Koster et al. [10]. Male albino mice were divided in to five groups of six animals each. Group I served as control and received drugless (0.5%, w/v, CMC; 10 ml/kg) vehicle. Group II served as standard and were treated orally with standard drug, Aspirin (200 mg/kg). Group III, IV and V were treated orally with methanolic extract of 50, 100 and 200 mg/kg body weight respectively. The animals were pretreated with their respective treatment, 1 h prior to intraperitoneal injection of 1% (v/v) acetic acid (0.1 ml/10 g). Five minutes after the intraperitoneal injection of acetic acid, the number of writhing during the following 10 min was counted. [11].

$$\text{Percent inhibition} = 1 - (N_T/N_C) \times 100$$

Where N_T is average number of writhings in treated groups and N_C is average number of writhings in control groups.

Tail Immersion Method [12]

Healthy albino rats weighing about 150-200gm were taken. The animals were divided into five groups of 6 animals each. Group I served as control and received drugless (0.5%, w/v, CMC; 10 ml/kg) vehicle. Group II served as standard and were injected Diclofenac sodium (9 mg/kg) intraperitoneally. Group III, IV and V were treated orally with methanolic extract of 50, 100 and 200 mg/kg body weight respectively. They were divided into different groups, numbered and placed into individual restraining cages leaving the tail hanging out freely. The animals are then allowed to adapt in the cages for 30 minutes before testing. The lower 5cm portion of the tail was marked and immersed in a cup of freshly filled warm water of exactly 55 ^{o} C. Within a few seconds the rat reacts by withdrawing the tail. The reaction time was recorded by a stop watch. After each determination the tail was carefully dried. The reaction was determined before oral administration of respective treatments which was recorded as zero minutes reading. After the drug was administered the reaction time was recorded at an interval of 30, 60, 90, 120 and 150 mins. The cut off time of the immersion is 15 seconds. The mean reaction time was recorded for each group and compared with the value of standard drug.

Eddy's hot plate method

Male albino mice weighing 22-25g were taken. The animals were divided into five groups of 6 animals each. Group I served as control and received drugless (0.5%, w/v, CMC; 10 ml/kg) vehicle orally. Group II served as standard and were injected Diclofenac sodium (9 mg/kg) intraperitoneally. Group III, IV and V were treated orally with methanolic extract of 50, 100 and 200 mg/kg body weight respectively. The animals were individually placed on the hot plate maintained at 55°C, one hour after their respective treatments. The response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first. The cut off time for the reaction was 15 seconds 15. The test was carried before the treatment and at 30, 60 and 90 min after administration [13].

SCREENING METHOD FOR ANTIPYRETIC ACTIVITY:

Yeast induced pyrexia in rats

The animals were divided into five groups of 6 animals each. Group I served as control and received drugless (0.5%, w/v, CMC; 10 ml/kg) vehicle. Group II served as standard and were treated orally with Aspirin (100 mg/kg). Group III, IV and V were treated orally with methanolic extract of 50, 100 and 200 mg/kg body weight respectively. Initial rectal temperature of rats was recorded using tele thermometer. Hyperthermia was induced by subcutaneous injection of 10ml/kg of 20% aqueous suspension of brewer's yeast. After 18 hrs of yeast injection, the

animals showing a rise in temperature inferior to 0.7°C was discarded. The temperature was recorded at 1h, 2h, 3h and 4h after treatment [14].

Statistical analysis:

All the values were expressed as mean ± standard error mean (S.E.M.) and compared with corresponding control values. P values were analyzed using one-way ANOVA followed by Dunnett's t-test. $p<0.05$ was considered as statistically significant.

RESULTS

Phytochemical screening

Phytochemical screening of the crude methanolic extract of the leaves of *Coccinia grandis* revealed (Table 1) the presence of glycosides, flavonoids, alkaloids, phenols, tannins and terpenoids.

Table 1: Chemical constituents present in methanolic extract of *Coccinia grandis* L. leaves.

Test	Methanolic extract
Carbohydrates	-
Proteins and amino acids	-
Glycosides	+
Alkaloids	+
Phytosterols	+
Flavonoids	+
Tannins	+
Phenolic compounds	+
Gums and mucilage	-
Fixed oil & fats	-
Saponins	-
Volatile oils	-
Tri terpenoids	+

Analgesic activity:

Effect of ML in Acetic acid-induced writhing method

When compared to control animals, ML (100 and 200 mg/kg) significantly reduced the number of abdominal writhing in a dose dependent manner with 16.01 %($p<0.05$) and 23.5 % ($p<0.01$) inhibition, respectively, while the standard showed 41.50% ($p<0.001$) inhibition (Table 2). The analgesic activity was comparable to the effect of standard drug, Aspirin (200mg/kg).

ISSN: 0975-8585

Table 2: Analgesic effect of methanolic extract of on Acetic acid induced writhing method

Group	Dose(mg/kg)	No. of writhing	Inhibition (%)
I	Control	36.16± 0.47	-
II	Std 200 mg/kg	21.16 ± 1.19***	41.5
III	ML 50 mg/kg	33.03± 0.55*	8.7
IV	ML 100 mg/kg	30.33± 0.55*	16.12
V	ML 200 mg/kg	27.66± 0.49**	23.5

Results are presented as mean ± SEM, (n=6), *p< 0.05, **p < 0.01, ***p< 0.001 when compared to control.

Effect of ML in Eddy's hot plate method

In the present study, Diclofenac sodium, a centrally acting analgesic drug, produced an inhibitory effect on the nociceptive response in Hot plate method, while extract of *Coccinia grandis* L. failed to affect the response (Table 3).

Table 3: Analgesic effect of methanolic extract of on Eddy's hot plate method

Group	Treatment	Mean latency (s) in minutes (X±SEM)			
		0	30	60	90
I	Control	2.18±0.12	2.31±0.20	2.43±0.27	2.49±0.32
II	Std 9 mg/kg	2.25±0.35	6.91±0.20*	9.74±0.34*	11.32±0.22*
III	ML 50 mg/kg	2.79±0.20	2.94±0.34	3.10±0.45	3.19±0.40
IV	ML 100 mg/kg	3.11±0.36	3.28±0.28	3.42±0.18	3.55±0.20
V	ML 200 mg/kg	2.67±0.43	2.90±0.25	3.09±0.16	3.21±0.23

Results are presented as mean ± SEM, (n=6), *p< 0.001 when compared to control.

Effect of ML in Tail Immersion Method

In the present study, Diclofenac sodium, a centrally acting analgesic drug, produced an inhibitory effect on the nociceptive response in Tail Immersion Method while extract of *Coccinia grandis* L. failed to affect the response (Table 4).

Table 4: Analgesic effect of methanolic extract of on Tail immersion method

Treatment (mg/kg)	Tail flick latency in minutes (X±SEM)					
	0	30	60	90	120	150
I-Control	3.08± 0.4	3.1± 0.33	3.13± 0.6	3.01± 0.51	2.95± 0.34	2.95± 0.29
II-Std.	2.70± 0.40	4.08± 0.38*	4.35± 0.45*	4.70±0.19*	5.40±0.22*	5.47±0.31*
III-M(L) 50	3.31± 0.29	3.48± 0.30	3.51± 0.34	3.54± 0.42	3.55± 0.25	3.55± 0.20
IV-M(L)100	3.01± 0.35	3.19± 0.30	3.22± 0.40	3.27± 0.37	3.25± 0.32	3.28± 0.44
V-M(L)200	2.95± 0.22	3.10± 0.37	3.16± 0.31	3.23± 0.28	3.21± 0.34	3.22± 0.41

Results are presented as mean ± SEM, (n=6), *p< 0.001 when compared to control.

ISSN: 0975-8585

Antipyretic activity:

Effect of ML in Yeast induced pyrexia method

The methanolic extract of leaves of *Coccinia grandis* L. produced significant ($p<0.01$) reduction at the dose of 100mg/kg as compare to other two doses in yeast induced pyrexia. Methanolic extract of *Coccinia grandis* L. leaves exhibits significant reduction in pyrexia that was comparable to standard drug paracetamol (Table 5).

Table 5: Antipyretic effect of methanolic extract of on Yeast induced pyrexia method

Treatment (mg/kg	Before	0h	1h	2h	3h	4h
I-Control	38.49±0.07	39.41±0.09	39.58±0.12	39.61±0.23	39.53±0.25	39.71±0.15**
II-Std.	37.95±0.11	39.01±0.12	38.78±0.16	38.43±0.18**	38.12±0.29**	38.01±0.26**
III-M(L) 50	37.69±0.11	38.58±0.12	38.31±0.11*	38.17±0.19**	38.03±0.17**	37.86±0.22**
IV-M(L)100	38.10±0.09	39.07±0.15	38.86±0.12	38.63±0.11**	38.47±0.22**	38.21±0.15**
V-M(L)200	37.80±0.11	38.93±0.12	38.76±0.11	38.49±0.15*	38.28±0.24**	38.13±0.21**

Results are presented as mean ± SEM, (n=6), *p< 0.05, **p < 0.01 when compared to control.

DISCUSSION

The present study demonstrated that the methanol extract of *Coccinia grandis* given by oral route in rats and mice have shown analgesic and antipyretic properties, since they were able to inhibit acetic acid-induced writhing episodes and yeast induced pyrexia.

Acetic acid causes algesia by liberating endogenous substances and many others that excite pain nerve endings, and is a sensitive method of screening both peripheral and central analgesic efficacy of agents. Oral administration of the methanol extracts of *Coccinia grandis* significantly reduced the number of abdominal constriction following acetic acid, indicating analgesic activity in mice at the doses assayed. The results of this writhing test alone did not ascertain whether the antinociceptive effects are central or peripheral. Thus, to clear the mode of the inhibitory effect of these species on the nociceptive responses, the effect of the species under study on the Tail immersion and Hot plate method was examined. Thermal painful stimuli are known to be selective to centrally, but not peripherally, acting analgesic drugs. Methanolic extract of *Coccinia grandis* L. failed to show central analgesic action. These findings, therefore, suggest that the apparent analgesic action of the active compound(s) in the methanol extract of *Coccinia grandis* L. May be mediated through peripheral but not central mechanism(s).

Antipyretics are agents which reduce elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained [16, 17]. Most of the antipyretic drugs exhibit drug action by inhibiting COX-2 expression, thus inhibiting prostaglandin synthesis to reduce elevated temperature. It was found that the Methanolic

extract of *Coccinia grandis* at the doses of 100 and 200 mg/kg showed significant decrease in yeast-induced fever. This result seems to support the view that Coccinia grandis L. methanolic extract has some influence on prostaglandin biosynthesis because prostaglandin is believed to be a regulator of body temperature [12]. However further study is required to determine the mechanism of action.

CONCLUSION

An extensive search in Ethnopharmacology has taken place worldwide. The plant Coccinia grandis L was traditionally claimed for a large number of pharmacological actions and medicinal uses. In the present investigation, Phytochemical screening of the crude methanolic extract of the leaves of *Coccinia grandis* revealed the presence of glycosides, flavonoids, alkaloids, phenols, tannins and terpenoids. . The significant analgesic and antipyretic action may be attributed to the phytoconstituents present in it. The present study offered a scientific proof to the traditional use of *Coccinia grandis* L. However, further phytochemical studies are needed to isolate the active compounds responsible for these pharmacological activities.

REFERENCES

[1] Kanodia L, Das S. Bangladesh J Pharmacol 2008; 4:35-38.
[2] Chattopadhyay D, Arunachalam G, Ghosh L et al. J Pharm Pharmaceutical Sci 2005; 8:558-564.
[3] Geetu Singh, Prasoon Gupta, Preeti Rawat, Anju Puri, Gitika Bhatia, Rakesh Maurya. Phytomed 14 (2007) 792–798.
[4] Dewanjee S, Kundu M, Maiti A, Majumdar R, Majumdar A, Mandal SC. Trop J Pharm Res 2007; 6 (3): 773-778.
[5] Shakti Prasad Pattanayak, Priyashree Sunita. Bangladesh J Pharmacol 2009; 4: 84-87.
[6] Papiya Mazumder, Sasmul D, Nimbi R. Natural Product Radiance 2008; 7(1): 15-18.
[7] Vadivu R, Krithika A, Biplab C, Dedeepya P, Shoeb N, Lakshmi KS. Int J Health Res 2008; 1(3): 163-168.
[8] Umamaheswari M, Chatterjee TK. Pcog Res 2009; 1(1): 25-34.
[9] Trease GE, Evans WC. Pharmacognosy, Bailliere Tindall Press, London. 1983; 309–706.
[10] Koster R, Anderson M, De-Beer EJ. Federation Proceedings 1959; 18:418–420.
[11] Taber RI, Greenhouse DD, Rendell JK, Irwin S. J Pharmacol Exp Ther 1969; 169: 29–37.
[12] H G Vogel. Drug Discovery and Evaluation – Pharmacological Assays, 2 nd Edition, Springer, New York, 2002; 697.
[13] Eddy NB and Leimbach DJ. J Pharmacol Exp Ther 1953; (107):385-393.
[14] Abena A, Diatewa M, Gakosso G, Gbeassor M and Hondi Assah JM. Fitoterapia 2003; (74):231-236.
[15] Hendershot LC and Forsaith J. J Pharmacol Exp Ther 1959; 125: 237-240.
[16] Clark WG & Cumby HR. J Physiol 1975; 248: 625–638.
[17] Zeil R, Krupp P, Schorbaum E, Lomax P & Jacob J. Temperature regulation & drug action 1975; 233–241.