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ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF STEM BARK OF *ANNONA RETICULATA* LINN.

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ABSTRACT

The present study was to evaluate the analgesic and anti-inflammatory activity of stem bark extract of *Annona reticulata.* The analgesic activity was assessed in three models Writhing test, Tail flick test, Tail immersion test by using Pentazocine as standard and anti-inflammatory activity was performed against carrageenan and histamine induced paw oedema using Ibuprofen as standard. The results of the study indicated that the aqueous extract of the stem bark of *Annona reticulata* at the dose of 300mg/kg possesses significant analgesic and anti-inflammatory activity at all tested models.

KEY WORDS: *Annona reticulata*, Analgesic, anti-inflammatory activity, histamine induced.

1.INTRODUCTION

Annona reticulata Linn, belong to the family Annonaceae, is a small deciduous or semi-ever green tree reaching 8-10 m tall with an open, irregular crown. It is a native of the West Indies occurs wild and also cultivated throughout India. Its root is a drastic purgative, root decoction is applied externally on head for head ache and taken internally for nervous shock(Pullaiah and Chennaiah, 1997). Seed, leaf, stem and roots are insecticidal, antihelmenthic, suppurant and are used against inflammatory tumors. Leaf decoction is used for nervous shock, indigestion and abdominal pain (Weniger, 1986) and leaf paste is applied externally for boils (Girach, 1994). Leaf is used as an antiphlogistic (Watt and Breyer-brandwijk, 1962), blood disorders and rheumatism (Villar, 1997). Bark is a powerful astringent and used as a tonic, vermifuge, antidysentric, amenorrhea (Comerford, 1996) and fevers (Hodge and Taylor, 1956). Infusion of the bark is used to treat diarrhea (Heinrich, 1992). Fruit is antihelmenthic (Nayar, 1955) and antidysentric (Logan, 1973). Seed is astringent and vermifugal. Essential oil and fruit decoction is mixed with cobwebs and used as a post partum medicine (Velazco, 1980).

The phytochemical studies revealed that the presence of alkaloids (Chang, 1995; Yang and Cheng, 1987) phytosterols (Oguntimein, 1987; Jirovetz, 1998) fixed oils, phenolic compounds, tannins, proteins and flavonoids (YU, 1997; VU, 1994). The stem bark reported to contain Isoquinoline alkaloids (Forgacs, 1981) terpenoids (Etse, 1987) and flavonoids (Hisham, 1994). Keeping this in view after extensive literature survey available from all scientific sources revealed no information about the pharmacological validation of the analgesic and anti-inflammatory activity. Thus the present study deals with the anti-inflammatory and analgesic activity by using different experimental models.

2.MATERIALS AND METHODS

Plant collection and authentication: The stem bark (3 kg) was collected from matured trees from Eturunagaram, Warangal district and authenticated by Prof. V.S. Raju, Department of Botany, Kakatiya University, Warangal. A voucher specimen has been deposited in the Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam. The collected plant material was shade-dried and powdered.

Preparation of extract: The shade dried and powdered plant material (500 g) was refluxed with distilled water for 2 h. The extracts were concentrated under vacuum which afforded a solid residue (yield: 12.86% w/w).

Experimental animals: Adult Wistar albino rats of either sex weighing between 160-180 g (for anti-inflammatory study) and adult Swiss albino mice of either sex weighing between 25-30g (for gross behavioural and analgesic screening) were used. The animals were maintained with standard diet and water *ad libitum* under experimental conditions.

Gross Behavioural and toxicity Studies: Groups of six mice of mixed sex were kept under laboratory conditions and allowed free access to water. The extract, at concentration of 1000, 2000 and 3000 mg/kg, suspended in 0.5%w/v sodium carboxy methyl cellulose and were administered orally. After administration of the test samples, the animals were observed continuously for first four hours for behavioural changes (Seth, 1972) and at the end of 72 h for mortality if any. However, no mortality was observed in the animals.

Analgesic activity: The analgesic activity of the aqueous extract of A. reticulata stem bark was assessed in three models Writhing test, Tail flick test and Tail immersion test at dose levels of 100 and 300 mg/kg respectively. Pentazocine (30 mg/kg) was used as reference standard. In all analgesic experiments, adult Swiss albino mice (food and water ad

libitum) of either sex were used. Forty-five minutes before testing, the animals were placed in acrylic cages. The test substances (suspended in 0.5% w/v sodium CMC in normal saline) were given intraperitoneally 30 min before the algogenic procedures.

Writhing Test: This test was carried out as per the method suggested by Turner (1965). The selected mice were made to writhe by single intraperitoneal injection of 0.6% v/v aqueous acetic acid (0.1 ml/kg). Test substances were administered 30 min before injection of acetic acid. Animals were kept under observation immediately after acetic acid injection, for two consecutive 5 min periods. The number of writhes (full extension of hind paws) were recorded and tabulated in Table-1.

Tail flick Test: This test was carried out using an analgesiometer. Analgesia was assessed with a tail flick apparatus. Baseline latency (reaction time) was obtained with three measurements, after each measure animals were returned to the observation chambers for 5 min. The mean of these three measurements is the pre drug latency time. At this point, animals presenting two measures 6 min were discarded from the study. The test substances were administered intraperitoneally immediately after the third pre drug measure. Fifteen minutes after words, another set of four measures was taken at 15, 30, 45 and 60 min and the reaction considered as the post-drug reaction time. A cut-off time of 10 sec was used to prevent tissue damage. The results were tabulated in Table-2

Tail immersion Test: This test was carried out as suggested by Srinivas Reddy (2009). Prior to the analgesic experiments, the animals were screened for sensitivity test by immersing tip of the tail gently in hot water maintained at 55 – 55.5^{0}C. The animals which lifted the tail from hot water within 5 sec were selected for the study. The test samples were administered intraperitoneally and the reaction time was measured at 15, 30, 45 and 60 min respectively. The results were recorded in Table-3.

Anti-inflammatory activity:

Carrageenan induced paw oedema: The anti-inflammatory activity of the aqueous extract of *A. reticulata* stem bark was assessed as per the method suggested by Winter (1962). The selected wistar albino rats were housed in groups of six in each in acrylic cages under laboratory conditions and fasted over night. The test samples were suspended in 0.5% w/v sodium carboxy methyl cellulose and administered orally 30 min before injection of carrageenan (0.1 ml of 1%w/v solution) in normal saline into the sub planter region of left hind paw of each rat. The contra lateral paw was injected with an equal volume of saline. All the groups of animals received one of the following through oral route. 0.5%w/v sodium CMC (0.2 ml/100g), aqueous extract (100 or 300 mg/kg) and Ibuprofen (10 mg/kg). The paw swelling was calculated by a plethysmograph as the volume of mercury displaced by the inflamed paw (ml). The paw volume was measured at 0 h, 1h, 2 h and 4 h respectively. The percentage inhibition of paw oedema was calculated in the various groups by using the formula:

Percentage inhibition= Vc- Vt/ Vc X 100, Where Vc and Vt denote mean increase in paw volume of control and drug-treated animals respectively. Observations were presented in Table-4.

Histamine induced paw oedema: The procedure carried out was same as above with a little difference in the use of the edematogenic agent. Histamine (0.1% w/v in normal saline) was injected into the paw of each rat at a dose of 0.1ml to induce oedema. The paw volume was measured at 0 h, 1 h, 2 h and 4 h respectively. The anti-inflammatory effect was expressed as percent inhibition of oedema. The results were reported in Table 5.

Statistical analysis: The mean value ± SEM was calculated for each parameter. The statistical significance was determined by using the Student's *t*- test.

3.RESULTS AND DISCUSSION

The gross behavioural and toxicity studies of the tested extracts revealed marked analgesia at all tested dose levels but no mortality was observed in any of the above doses at the end of 72h.

Pain and inflammation are prelude to repair process. The aqueous extract exhibited potent analgesic effects against chemical and thermal noxious stimuli. This is evidenced by significant reduction in the number of acetic acid induced writhing episodes and increase in the reaction time by thermal stimuli at the tested dose of 300 mg/kg. However, at a dose of 100 mg/kg, the extract did not show appreciable reduction in the number of writhing episodes and increase of reaction time in thermal stimuli.

The chemical mediator's viz. histamine, 5-HT, bradykinin and PEG_1 are involved in the genesis of acute inflammation. Anti-inflammatory drugs produce anti-inflammatory effect by selective or non-selective inhibition of inflammatory activity of these chemical mediators. In the following experiment, the oedema suppressant activity exhibited by the aqueous extract may be due to the inhibitory effects on the release of histamine, 5-HT and kinin like substances which are reported to be released from the mast cell degradation during the first hour of carrageenan induced artificial paw oedema. The anti-inflammatory activity was further confirmed from the results obtained by histamine induced paw oedema.

Pretreatment with aqueous extract (300 mg / kg, p.o.) showed significant oedema suppressant activity ($p < 0.001$) against carrageenan and histamine induced paw oedema like that of Ibuprofen. The aqueous extract reduced inflammation by 49.32% and 46.4% in both the models respectively. However, the extract at 100 mg/kg, p.o. did not produce significant anti-inflammatory activity. The results indicate that the aqueous extract of the *A. reticulata* stem bark is endowed with effective analgesic and anti-inflammatory activities.

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Table 1- Effect of aqueous extract of *A. reticulata* stem bark on Writhing induced by acetic acid in rats

Group	Treatment	Dose	Number of Writhngs			% inhibition
			5-10 min	10-15min	Total	
I	0.5%w/v Sodium CMC	0.1ml/10g	8.167±0.85	6.0±0.781	14.167±0.660	-
II	Pentazocine	30mg/Kg	3.5±0.390	2.5±0.206	6.0±0.265*	57.648
III	Aq.extract of *A.reticulata*	100mg/Kg	7.0±0.625	5.67±0.607	12.67±0.476	10.567
IV	Aq.extract of *A.reticulata*	300mg/Kg	4.167±0.548	3.167±0.436	7.334±0.379*	48.232

Results expressed as Mean ± SEM from six observations, * $P < 0.001$

Table 2-Effect of aqueous extract of *A. reticulata* stem bark in rats in the Tail flick test

Group	Treatment	Dose	Basal reaction time (sec)	Reaction time (sec)			
				15min	30min	45min	60min
I	0.5%w/v Sodium CMC	0.1ml/10g	2.0±0.32	2.33±0.191	2.5±0.206	2.66±0.194	2.65±0.198
II	Pentazocine	30mg/Kg	2.15±0.27	4.1±0.407*	5.5±0.38**	6.82±0.724**	8.15±0.596**
III	Aq.extract of *A.reticulata*	100mg/Kg	2.17±0.28	2.6±0.203	2.83±0.280	3.16±0.367	4.32±0.192
IV	Aq.extract of *A.reticulata*	300mg/Kg	2.64±0.191	3.6±0.204*	4.66±0.508*	5.82±0.548**	6.74±0.72**

Results expressed as Mean ± SEM from six observations, *$P < 0.01$, ** $P < 0.001$

Table 3- Effect of aqueous extract of *A. reticulata* stem bark in rats in the Tail immersion test

Group	Treatment	Dose	Basal reaction time (sec)	Reaction time (sec)			
				15min	30min	45min	60min
I	0.5%w/v Sodium CMC	0.1ml/10g	2.6±0.204	2.16±0.27	2.36±0.192	2.5±0.204	2.66±0.193
II	Pentazocine	30mg/Kg	2.32±0.191	4.16±0.362*	5.6±0.313**	7.1±0.235**	8.6±0.205**
III	Aq.extract of *A.reticulata*	100mg/Kg	2.68±0.192	3.18±0.152	3.33±0.193	4.23±0.314	4.83±0.24
IV	Aq.extract of *A.reticulata*	300mg/Kg	2.7±0.203	3.6±0.204*	4.76±0.384**	6.68±0.452**	8.53±0.306**

Results expressed as Mean ± SEM from six observations, *$P < 0.01$, ** $P < 0.001$

Table 4- Effect of aqueous extract of *A.reticulata* stem bark on carrageenan induced paw oedema in rats

Group	Treatment	Dose	Volume of mercury displaced in ml				%inhibition of paw oedema at 4h
			0h	1h	2h	4h	
I	0.5%w/v Sodium CMC	0.2ml/100g	0.973±0.034	1.267±0.034	1.52±0.034	1.663±0.36	-
II	Ibuprofen	10mg/Kg	0.973±0.022	1.28±0.024	1.42±0.026	1.313±0.02*	52.79
III	Aq.extract of *A.reticulata*	100mg/Kg	0.986±0.03	1.23±0.023	1.46±0.016	1.56±0.017	17.89
IV	Aq.extract of *A.reticulata*	300mg/Kg	1.004±0.027	1.22±0.03	1.4±0.035	1.34±0.036*	49.32

Results expressed as Mean ± SEM from six observations, * $P < 0.001$

Table 5- Effect of aqueous extract of *A.reticulata* stem bark on histamine induced paw oedema in rats

Group	Treatment	Dose	Volume of mercury displaced in ml				%inhibition of paw oedema at 4h
			0h	1h	2h	4h	
I	0.5%w/v Sodium CMC	0.2ml/100g	1.01±0.012	1.34±0.018	1.56±0.019	1.723±0.027	-
II	Ibuprofen	10mg/Kg	1.027±0.018	1.26±0.018	1.41±0.027*	1.36±0.03**	54.07
III	Aq.extract of *A.reticulata*	100mg/Kg	1.01±0.022	1.25±0.018	1.48±0.02	1.64±0.019	11.63
IV	Aq.extract of *A.reticulata*	300mg/Kg	1.023±0.02	1.29±0.027	1.44±0.029*	1.41±0.019**	46.4

Results expressed as Mean ± SEM from six observations, *$P < 0.01$, **$P < 0.001$

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