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EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY EFFECTS OF ETHANOLIC LEAVES EXTRACT OF AMARANTHUS ROXBURGHIANUS

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Abstract:
Amaranthus roxburghianus is one of the traditionally well-known plants with outstanding therapeutic properties, and is used mostly in treating different diseases in India. Thus, based on these medicinal properties, various investigations have been undertaken in order to appraise the pharmacological activities and the chemical composition of these species. Here, we elucidate the analgesic and anti-inflammatory activity of Amaranthus roxburghianus ethanolic leaves extract. phytochemical screening of Amaranthus roxburghianus extract showed the presence of alkaloids, Carbohydrates, Glycosides, Flavonoids, Tannins, Proteins, Amino Acids. the ethanolic leaves extract of Amaranthus roxburghianus, possess peripheral and central analgesic activity in animal model. The Amaranthus roxburghianus leaves extract shows anti-inflammatory activity in different animal model. Flavonoids and tannins are the major constituents of Amaranthus roxburghianus leaves, which may be responsible for its Analgesic, Anti-inflammatory activity.

KEYWORDS: *Amaranthus roxburghianus, Phytochemical screening, Analgesic activity, Anti-inflammatory activity*

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INTRODUCTION:

Cerebral and coronary artery diseases are the leading causes of death around the world. More and more researchers have demonstrated that abnormal inflammatory cells form a plaque and play an essential role in the pathogenesis and progression of atherosclerosis [1]. Anti-inflammatory agents have been shown to have good effects on the prevention and treatment of atherosclerosis and coronary artery diseases [2]. Therefore, developing novel anti-inflammatory drugs is very important today.

Analgesic drugs operate in different ways on the peripheral and central nervous systems. Narcotic drugs, such as morphine, have analgesic activity, which inhibits the delivery of pain impulses [3, 4]. Peripheral drugs include paracetamol and non-steroidal anti-inflammatory drugs (NSAIDs). Only NSAIDs possess analgesic and anti-inflammation activity due to the mechanism of inhibiting cyclooxygenases (COXs) for the decrease in prostaglandin (PG) production, which consequently reduces pain and inflammation. However, the NSAIDs used clinically are often of limited application because of their common side effects, such as gastrointestinal (GI) hemorrhage. As to COX, COX-1 is thought to provide cytoprotection, whereas COX-2 inhibitor may have selective anti-inflammatory properties and lack GI side effects.

Amaranthus roxburghianus is one of the traditionally well-known plants with outstanding therapeutic properties, and is used mostly in treating different diseases in India. Amaranthus roxburghianus is commonly, called as Prince's feather (English) chirikoora (Telugu). It is also distributed in Occasional weed of waste lands and cultivated lands [5]. The high antioxidant capacity of Amaranthus roxburghianus has been attributed to high levels of polyphenolic compounds specially alkaloids [6].

Non–steroidal anti-inflammatory drugs (NSAIDs) are used worldwide for their wide range of activity. However, its side effects are high. Natural products from medicinal plants have more pharmacological significance with improved efficacy and lesser side effects. Hence the present work was aimed to explore the use of extract of Amaranthus roxburghianus species with proper validation.

MATERIALS AND METHODS:

Collection, identification and Authentication of plants:

The whole plant of Amaranthus roxburghianus collected in the month of February, 2021 from chittur dist. The plant is identified and authenticated by Prof. Madhav Shetty, Dept. of botany, Taxonomist, SV University, Tirupati. A voucher was kept in the Department of Pharmacognosy for reference.

Extraction procedure:

The coarse powder was packed tightly in the soxhlet apparatus and extracted with ethanol for 72 hours with occasional shacking maintained at 60ºc throught out the extraction process. The extract was concentrated. The resulting ethanolic extract of the Amaranthus roxburghianus was subjected to phytochemical study**.** The percentage hield of extraction is shown in Table 1

Preliminary Phytochemical Screening:

The condensed extracts were used for preliminary screening of phytochemicals such as cholesterol, alkaloid, flavanoids, saponin, cardiac glycosides and terpenoids [7-8]. The phytochemical screening shown in Table 2.

Screening Procedure:

Test for flavanoids

Add a few drops of concentrated HCL and Mg turning to 1 ml of ethanol extract. Appearance of pink or magenta-red colour indicates the presence of flavanoids.

Test for cholesterol :

To 2 ml of the extract 2 ml of the chloroform was added in a dry test tube. Then 10 drop of acetic anhydride and 2 to 3 drops of con. H2SO4 was added. A red colour changed to blue green colour.

Test for Alkaloids:

To the extract added 1% HCl and 6 drops of Mayer's reagent and Dragendorff reagent. Any organic precipitate indicated the presence of alkaloids in the sample.

Test for terpenoids:

5ml of each extract was added to 2ml of chloroform and 3ml of con.H2SO4 to form a monolayer of reddish-brown coloration of the interface was showed to form positive result for the terpenoids.

Test for cardiac glycoside:

5mlof each extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of con.H2SO4. A brown ring of the interface indicated a deoxysugar characteristic of cardenolides. A violet ring might appear below the brown ring whereas acid layer, a greenish ring might

form just gradually throughout thin Layer.

Test for steroids:
2 ml of acetic anhydride was added to 0.5 g of ethanolic extract of each sample with 2ml of H2SO4. The colour change from violet to blue or green indicated the presence of steroids

Test for Saponins :
The extract with 20 ml of distilled water was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam indicated the presence of saponins.

Pharmacological studies :
Swiss albino mice weighing 20-25 gm wistar rats weighing 150- 200 gm were used for this study. The experiments were carried out in Jeeva life Sciences (Jeeva life Sciences have in house breeding). On arrival, the animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2ºc and relative humidity of 30-70%. A12: 12 light: day cycle was followed. All animals were allowed to free access to water and bed with standard commercial pelleted chow. All the experimental procedures are protocols used in this study were reviewed by Institutional Animal Ethics Committe, proposal number and were accordance with the guidelines of the IACE. The study was conducted after obtaining from committee for the purpose of control and supervision on animals (CPCSEA) and institutional animal ethics committee (IAEC), proposal number CPCSEA/IAEC/JLS/15/05/21/06.

Acute Toxicity Studies:
The acute toxicity was performed according to OECD guidelines. The selected albino rats were used for toxicity studies. The animals were divided into five groups of three in each. The animals were fasted overnight prior to the acute experimental procedure. Extract was given orally to rats at the graded doses like 5, 50, 100, 1000 & 2000 mg/ kg body weight. Immediately, after dosing, the animals were observed continuously for first four hours for behavioural changes were closely observed for hyperactivity, ataxia, convulsion, salivation, tremors, diarrhoea, lethargy, sleep. They were then kept under observation up to 14 days after drug administration to determine the mortality, if any. One-tenth and one-fifth of the maximum tolerated dose (200 and 400 mg/ kg, body weight) of ethanol leaf extract of Amaranthus roxburghianus. selected to evaluate analgesic, anti-inflammatory studies in rats. [9]

EXPERIMENTAL PROCEDURE:

Pharmacological studies:
Analgesic activity:
Hot plate method in mice
The hot plate assay method was employed for the purpose of preferential assessment of possible centrally medicated analgesic effects of ethanolic extract of Amaranthus roxburghianus. The central analgesic drug pentazocine was used for positive control group. In this experiment, four groups (n=6) of Swiss albino mice (20-25 g) were placed on a hot plate maintained at room temperature for 15 min. Food was withdrawn on the preceding night of the experiment. Group I- Normal Control received CMC (0.5%), and Group II- standard treated with pentazocine (3 mg/kg i.p), whereas group III and IV-animals were treated orally with ethanolic extract of Amaranthus roxburghianus (200 and 400 mg/kg respectively) [10]
Group I: Normal control (CMC)
Group II: Standard (Pentazocine 3 mg/kg)
Group III: Test Drug I (Ethanolic leaf extract of Amaranthus roxburghianus)
Group IV: Test Drug II (Ethanolic leaf extract of Amaranthus roxburghianus
Each animal was then individually placed gently on Eddy's hot plate at 55ºC. Latency to exhibit nociceptive responses such as licking paws or jumping off the hot plate were determined at 30, 60, 90 and 120 min after administration of the drugs or vehicle.

Tail immersion test:
This method assessment was used to evaluate the centrally medicated analgesic effects of ethanolic extract of Amaranthus roxburghianus. The wistar rats were divided into four groups each consists of six animals. They were placed into individual restraining cages leaving the tail hanging out freely. The lower 5cm portion of the tail is marked and this part of the tail was immersed in a water bath containing water at a temperature of 55± 0.5 ºC. Withdrawing the tail from the hot water showed the analgesic effect. The reaction time was noted on a stop-watch. Each animal served as control. The average of the two values was the initial reaction time. Group –II served as standard and received pentazocine (3 mg/kg, i.p) The Group III and IV were treated orally with ethanolic extract of Amaranthus roxburghianus (200 mg/kg and 400mg/kg) respectively, [11]
Group I : Normal Control
Group II: Pentazocine (3 mg/kg)
Group III: Test Drug I (Ethanolic extract leaf Amaranthus roxburghianus)

Group IV: Test Drug II (Ethanolic extract leaf Amaranthus roxburghianus)
The reaction time of the groups were taken at 0, 30, 60, 90 and 120min. The cut off time of the immersion was 15seconds. The reaction time was measured.

Acetic acid induced writhing response in mice:
This method was used to preferentially evaluate possible peripheral analgesic effects of ethanolic extract of Amaranthus roxburghianus Four groups of Swiss albino male mice (n=6) were fasted overnight prior to start the experiment with free access to water. The peripheral analgesic drug Diclofenac sodium (10 mg/kg) was used as a positive control. Group-I Normal Control received CMC (0.5%) Group-II was treated with Diclofenac Sodium (10mg/kg), whereas Group III and IV were treated orally with the ethanolic extract of Amaranthus roxburghianus at a dose of 200 mg/kg and 400mg/kg respectively.
Group I: Normal Control
Group II: Diclofenac Sodium (10mg/kg)
Group III: Test Drug I (Ethanolic extract leaf Amaranthus roxburghianus)
Group IV: Test Drug II (Ethanolic extract leaf Amaranthus roxburghianus)
After 30 min of treatment, the mice were injected intra peritoneally with 0.1 ml of 1% acetic acid solution to induce the characteristic writhings. The mice were then placed in an observation box and the numbers of writhing were counted in a 5min period. The response of the extract and Diclofenac sodium treated groups was compared with those of animals in the control group.

Anti-inflammatory activity:
Carrageenan-induced paw edema in rats :
For this experiment, the rats (120-150g) were divided into four groups (n=6). The group I received 0.5% CMC (10ml/kg), while the GroupII received Indomethacin (10mg/kg). The Group III and IV were treated orally with the ethanolic extract of Amaranthus roxburghianus at a dose of200 mg/kg and 400 mg/kg orally.[12]

Group I: Normal Control (CMC)
Group II: Indomethacin (10mg/kg)
Group III: Test Drug I (Ethanolic extract leaf Amaranthus roxburghianus)
Group IV: Test Drug II (Ethanolic extract leaf Amaranthus roxburghianus)
Acute inflammation was produced by injecting 0.1 ml of 1% (w/v) carrageenan suspension into the sub planter region of the right hind paw ofthe rats. The animals were pretreated with the drug 1hour before the administration of carrageenan.The paw thickness was measured at 1, 2, 3 and 4 h after carrageenan injection by using digital vernier callipers.

Cotton pellet induced granuloma method in rats[12]:
Cotton pellets, weighing 5mg each were sterilized. Under ether anaesthesia, the pellets were introduced subcutaneously through a skin incision on the back of the animals. Starting from 30 min after the implantation of cotton pellet for all the rats, Group-I normal control received CMC (0.5%) orally. Group-II was treated with Dexamethasone (1 mg/kg), whereas Groups III and IV were treated orally with 200 mg/kg and 400 mg/kg of ethanol extract Amaranthus roxburghianus. .
Group I : Normal Control (CMC)
Group II : Dexamethasone (1mg/kg)
Group III: Test Drug I (Ethanolic extract leaf Amaranthus roxburghianus)
Group IV: Test Drug II (Ethanolic extract leaf Amaranthus roxburghianus)

The test drugs were administered daily for 7days. On the 8th day, the animals were sacrificed with diethyl ether. The granulomas were removed and the weighed.

Statistical examination of data:
Results were communicated as mean ± S.E.M. The measurable contrast between the gatherings in the term of the mean rate of wound mending was determined as far as ANOVA mean ± S.E.M. The thing that matters was viewed as noteworthy if $P< 0.05$.
Data wereanalyzed by using One-way ANOVA followed by Dennett's test

RESULTS AND DISCUSSION:

Percentage yield of extraction

Table no1: Percentage yield

S.No	Type of extraction	Percentage yield
1.	70% Eathanol	17.54%

Quantitative phytochemical analysis of extracts

Table no2: Quantitative phytochemical analysis

Parameters	value
1. Alkaloid	-
2. Carbohydrates	+
3. Glycosides	+
4. Flavonoids	+
5. Tannins & Phenolic compounds	-
6. Proteins	+
7. Saponins	-
8. Sterols or Triterpenes	+

the qualitative phytochemical analysis of ethanolic extract of Amaranthus roxburghianus. It contains alkaloids, Carbohydrates, Glycosides, Flavonoids, Tanins Proteins, Amino Acids

pharmacological activity:

Analgesic activity:

Hot plate Method in Mice

The analgesic activity of ethanolic leaves extract of Amaranthus roxburghianus was assessed using hot plate method in Swiss albino mice. The ethanolic leaves extract of Amaranthus roxburghianus showed significant analgesic activity at 200 and 400 mg/kg. Analgesic activity was comparable with standard drug pentazocine. Among the two doses, 400 mg/kg showed maximum analgesic activity at reaction time 120 min (7.2±0.44) is slightly lower than the standard drug pentazocine (9.9±0.34) in this analgesic testing model, pentazocine significantly prolonged the

reaction time of animals with relatively extended duration of stimulation, confirming centrally active drugs. In the present study, all extracts showed significant ($p<0.05$ and $p< 0.01$) analgesic activity but among the two doses, 400 mg/kg showed highest analgesic activity at reaction time120 min

Table no3: Analgesic effect of ethanolic extract of Amaranthus roxburghianus on hot plate test in Swiss albino mice

GROUP	Paw licking or jumping in seconds			
	30min	60min	90min	120min
Group-I Control(no drug)	2.2±0.22	2.6±0.12	2.9±0.21	2.8±0.10
Group-II Pentazocine (3mg/kg)	2.8±0.18	6.9±0.62**	9.8±0.64**	9.9±0.34**
Group-III (200mg/kg)	2.7±0.20	3.7±0.15*	4.6±0.21**	4.1±0.41**
Group-IV (400mg/kg)	2.8±0.14	5.8±0.37**	7.4±0.39**	7.2±0.44**

Values were mean ± SEM, (n=6), *P<0.05 **P<0.01 Vs control

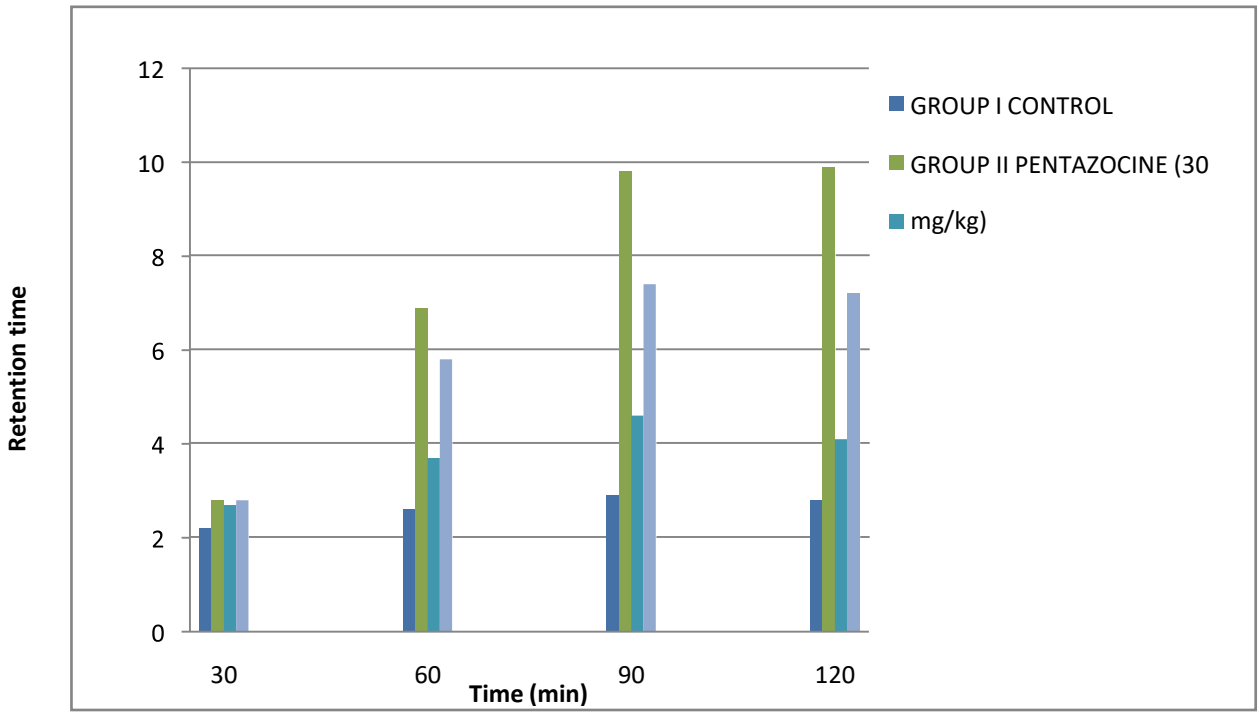

Fig.1 Analgesic effect of ethanolic leaves extract of Amaranthus roxburghianus on hot plate method in mice.

Tail Immersion Method:

There was a significant reduction of pain full sensation due to tail immersion in warm water. The maximum inhibitory effect of Showed significant ($p< 0.01$) at 90 min post dose in 400 mg/kg. The maximum anti- nociceptive properties of the plant extract (3.5±0.04) were not as effective as that of pentazocine, 3 mg/kg (5.8±0.06)

Table no4: Analgesic effect of ethanolic leaves extract of Amaranthus roxburghianus on tail immersion method in rats

GROUP	Mean latency to tail immersion in seconds				
	0 min	30min	60min	90min	120min
Group-I Control	1.5±0.04	1.4±0.02	1.6±0.01	1.6±0.03	1.7±0.04
Group II Pentazocine(3mg/kg)	1.8±0.06	2.6±0.04**	4.2±0.02**	5.8±0.06**	5.4±0.02**
Group III (200mg/kg)	1.2±0.02	1.9±0.01*	2.1±0.04*	2.4±0.02	2.8±0.04*
Group IV (400mg/kg)	1.4±0.01	2.0±0.04*	2.6±0.01**	3.5±0.04**	3.2±0.01**

Values were mean ± SEM,(n=6), *P<0.05 **P<0.01 Vs control.

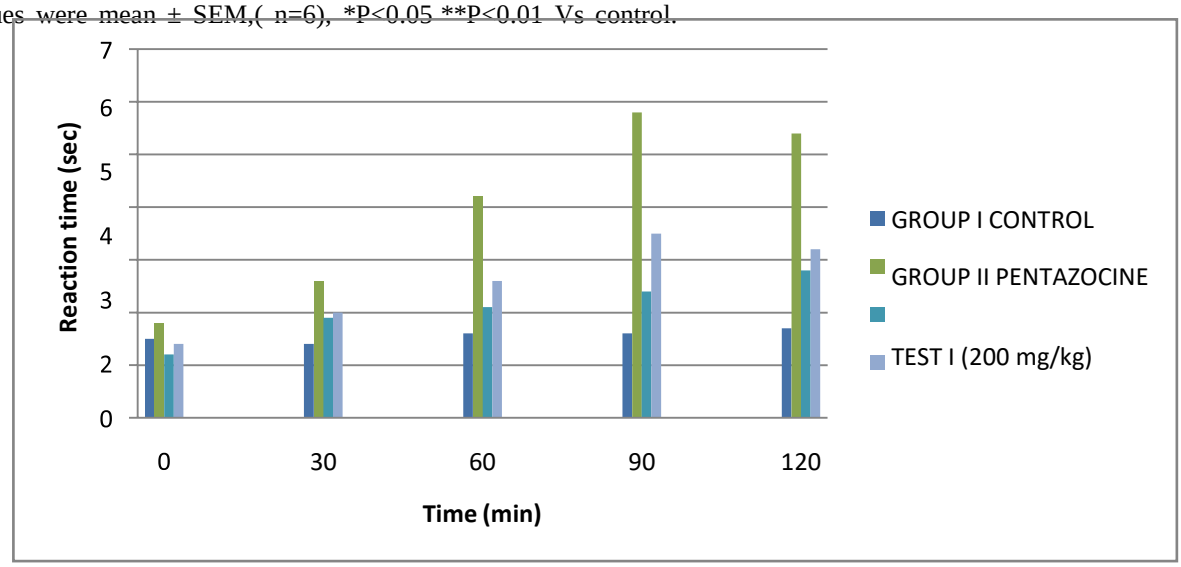

Fig. 2 Analgesic Effect of Ethanolic Leaves Extract Of Amaranthus roxburghianus On Tail Immersion Method In Rats.

Acetic Acid- Induced Writhing Response in Mice:

The oral administration of ethanolic leaves extract of Amaranthus roxburghianus Showed a dose dependent analgesic activity. Injection of acetic acid into control mice produced 51.4±6.4 writhes. Pre- treatment with ethanolic extract of Amaranthus roxburghianus at doses of 200 and 400 mg/kg reduced the number of writhes 39.4±2.4 (23.34 % protection) and 31.2±2.1 (39.29 % protection) respectively. Among the two doses 200, 400 mg/kg showed the slightly lower analgesic activity than standard drug Diclofenac Sodium 22.8±1.9 (55.64 % protection) it was observed that the onset of writhing was delayed and duration of writhing was shortened.

Table no5: Analgesic effects of ethanolic leaves extract of Amaranthus roxburghianus, (on acetic acid writhing test in Swiss albino mice

GROUP	Number of writhes	% Inhibition
Group-I Control	51.4±6.4	___
Group-II Diclofenac Sodium (10mg/kg)	22.8±1.9**	55.64
Group-III (200mg/kg)	39.4±2.4**	23.34
Group-IV (400mg/kg)	31.2±2.1**	39.29

Values were mean ± SEM, (n=6), **P<0.01 Vs control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

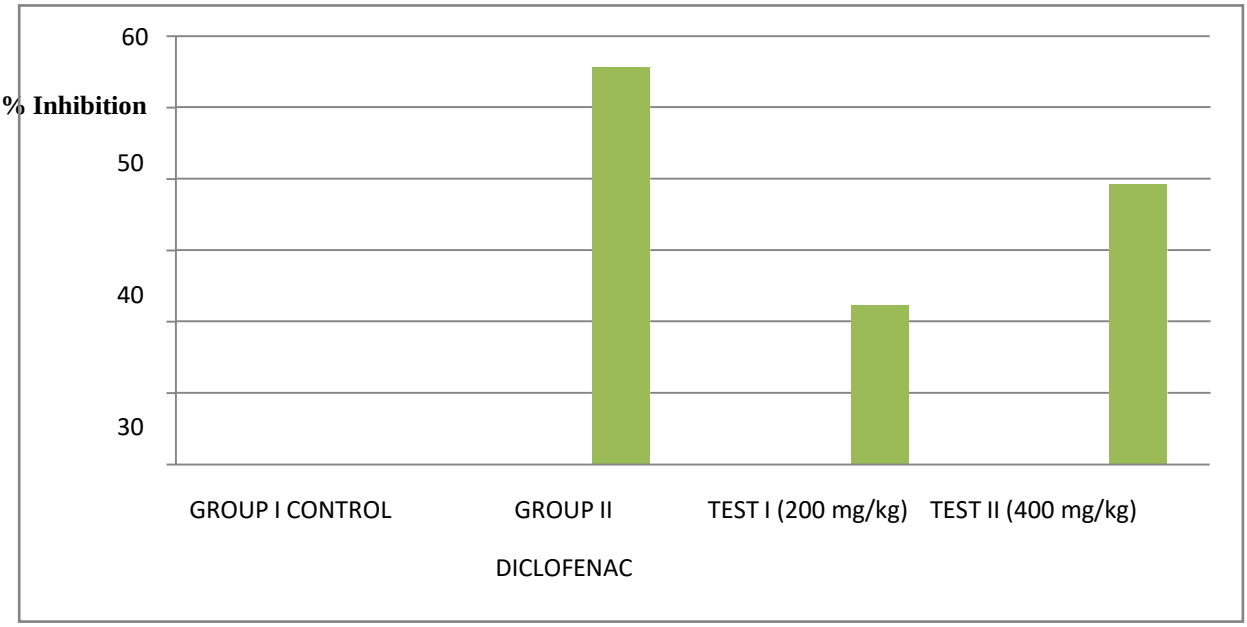

Fig. 3 Analgesic effect of ethanolic leaves extract of Amaranthus roxburghianus, on acetic acid induced writhing response in mice. Results are expressed as a percentage of inhibition.

Anti-Inflammatory Activity

Carrageenan-Induced Paw Edema in Rats:

The anti-inflammatory effect of the ethanolic leaves extract of Amaranthus roxburghianus on carrageenan – induced hind paw edema as shown in Table 4. The ethanolic leaves extract of Amaranthus roxburghianus at doses 200 and 400 mg/kg produced a significant effect aganist carrageenan induced inflammatory effect. The dose of 400 mg/kg exhibited a significant inhibition of 48 % after 3 h, the effect increased after 3h (52%). Anti-inflammatory activity of ethanolic extract of Amaranthus roxburghianus showed significant and similar to that of indomethacine (10 mg/kg).

Table no6: Anti-inflammatory activity of ethanolic extract of Amaranthus roxburghianus on Carrageenan induced paw edema method in Wistar rats

GROUP	Paw thickness in mm					% Inhibition at 3hr
	0 hr	1hr	2hr	3hr	4hr	
Group-I Carrageenan (control)	1.4±0.03	3.4±0.06	4.9±0.06	6.4±0.05	4.8±0.02	------
Group-II Indomethaci n (10mg/kg)	1.4±0.04	2.2±0.03**	2.9±0.04**	3.1±0.02**	2.2±0.04**	52
Group-III (200mg/kg)	1.2±0.02	3.0±0.04	4.2±0.03	4.7±0.01*	3.5±0.04**	27
Group-IV (400mg/kg)	1.1±0.01	2.7±0.04**	3.5±0.02*	3.3±0.06**	2.8±0.04**	48

Values were mean ± SEM, (n=6), *P<0.05, **P<0.01 Vs control.

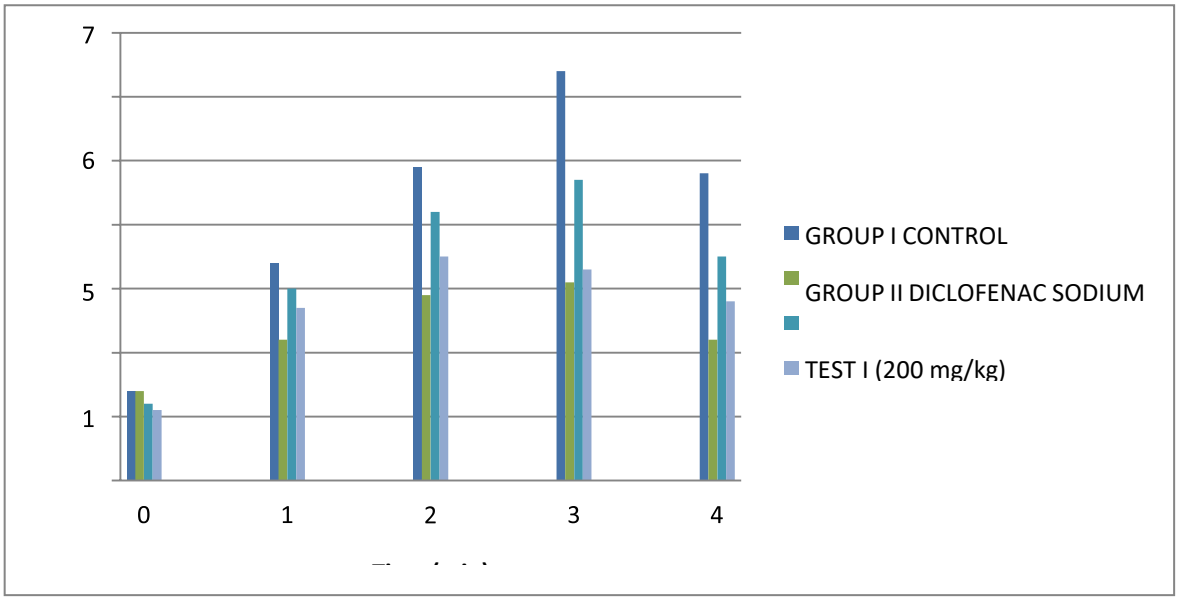

Fig. 4 Anti-inflammatory activity of ethanolic leaves extract of Amaranthus roxburghianus, on carrageenan induced paw edema method in Wistar rats

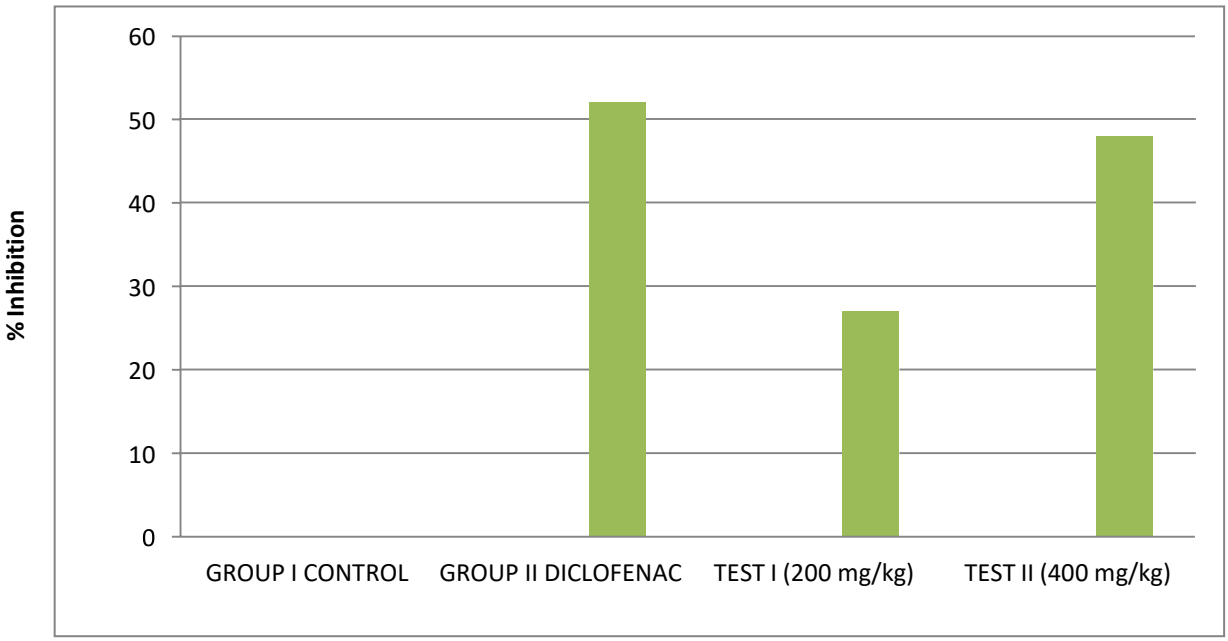

Fig. 5 Anti-inflammatory activity of ethanolic leaves extract of Amaranthus roxburghianus , on carrageenan induced paw edema method in Wistar rats. Results are expressed as a percentage inhibition.

Cotton Pellet-Induced Granuloma Method in Rats

The anti-inflammatory effect of the ethanolic leaves extract ofAmaranthus roxburghianus assessed by using cotton pellet induced granuloma method in Wistar rats. The ethanolic leaves extract of Amaranthus roxburghianus Showed significant anti-inflammatory activity at 200 and 400 mg/kg dose. After 7 days, the mean weight of granulomatous tissue surrounding the threads was significantly lower for the group treated with Amaranthus roxburghianus extract as compared tothe control group. Among the two doses 400 mg/kg showed maximum decreased formation of granuloma tissue. The results indicate that Amaranthus roxburghianus at dose level of 200mg/kg and 400 mg/kg produced a significant decrease in the weight of granuloma 38.16±0.04 (7.4% inhibition) and 34.58±0.04 (16.1% inhibition) respectively. Among the two dose 400 mg/kg showed the slightly lower reduced weight of granumola than standard drug dexamethazone 28.92±0.04 (29.8% inhibition)

Table no7: Anti-inflammatory activity of ethanolic extract of Amaranthus roxburghianus oncotton pellet induced granuloma pouch model Wistar rats

GROUP	Granuloma weight (mg)	% Inhibition
Group-I Control	41.24±0.04	___
Group-II Dexamethazone (1mg/kg)	28.92±0.04**	29.8
Group-III 200mg/kg	38.16±0.04**	7.4
Group-IV 400mg/kg	34.58±0.04**	16.1

Values were mean ± SEM, (n=6), **P<0.01 Vs control

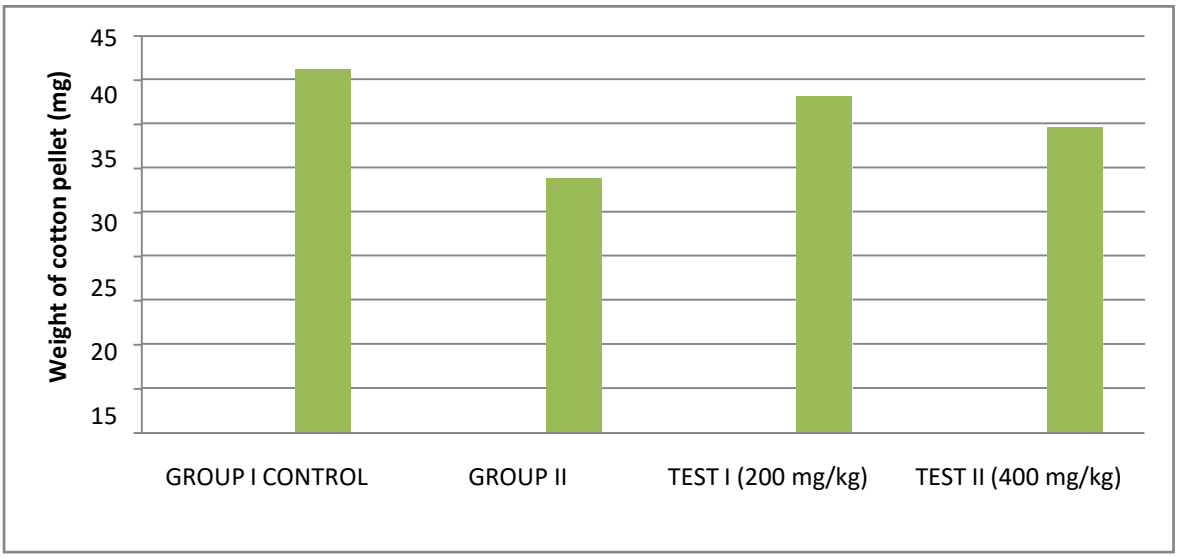

Fig. 6 Anti-inflammatory activity of ethanolic leaves extract of Amaranthus roxburghianus, on cotton pellet-induced granuloma in rats

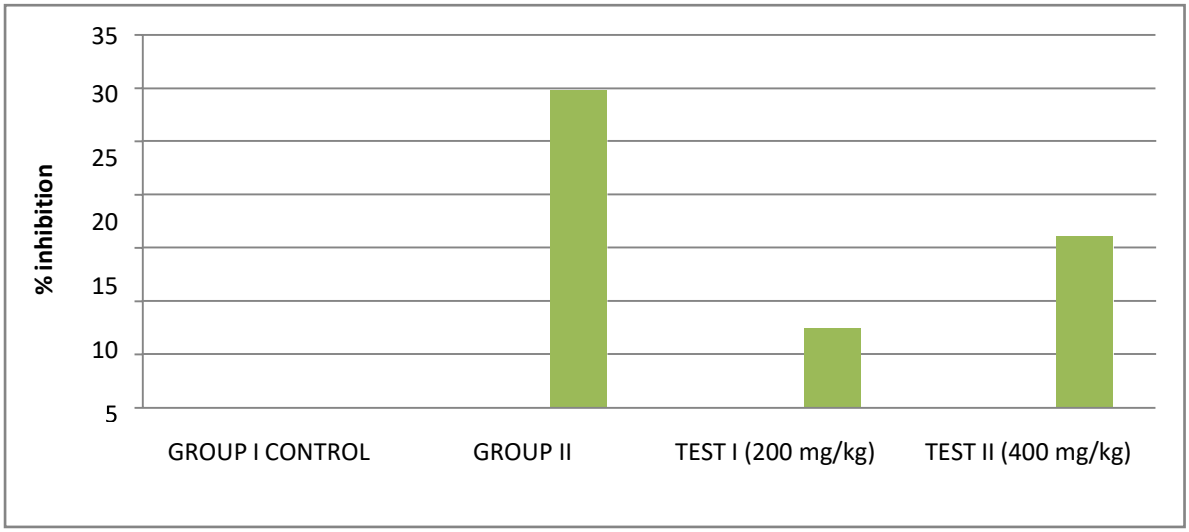

Fig. 7 Anti-inflammatory activity of ethanolic leaves extract of Amaranthus roxburghianus , on cotton pellet-induced granuloma in rats. Results are expressed as apercentage of inhibition

SUMMARY AND CONCLUSION:

The present study entitled "Evaluation of analgesic, anti-inflammatory effect ethanolic leaves extract of Amaranthus roxburghianus" deals with the exploration of pharmacological and phytochemical screening of the selected Indian medicinal plant Amaranthus roxburghianus belonging to the family Fabaceae. The results obtained from the preliminary phytochemical screening of Amaranthus roxburghianus extract showed the presence of Carbohydrates, Glycosides, Flavonoids, Proteins, Sterols, Triterpenes as shown in Table 1. It was reported that the flavonoids frequently found in plants possess analgesic, anti-inflammatory activity. The plant was collected and got authentified. Approval was obtained from committee for the purpose of control and supervision of experimental animals (CPCSEA) and institutional animal ethics committee (IAEC), proposal number CPCSEA/IAEC/JLS/15/05/21/07. The plant was shade dried and crushed. It was pulverized and extracted with ethanol using soxlet apparatus. The resulting extract was concentrated. The study of the plant Amaranthus roxburghianus was done by using mice with the oral doses of 5, 50, 100, 1000 & 2000 mg/ kg body weight of extract and no mortality was observed for 24 hours. Thus, dose was identified as per OECD 423 Guidlines.As for the analgesic effect, the leaf extract appears to act via the central and peripheral mechanisms of analgesia by using hot plate, tail immersion and Acetic acid induced writhing animal model. Anti-inflammatory effect of plant extract was done by using carrageenan- induced paw edema in rats and cotton pellet granuloma techniques.The Present study showed that the ethanolic leaves extract of Amaranthus roxburghianus, possess peripheral and central analgesic activity in animal model. The Amaranthus roxburghianus leaves extract shows anti-inflammatory activity in different animal model. Flavonoids and tannins are the major constituents of Amaranthus roxburghianus leaves, which may be responsible for its Analgesic, Anti-inflammatory activity.

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