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Research Article

ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF ETHYL ACETATE EXTRACT OF THE AERIAL PARTS OF *ARGIMONE MEXICANA*

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ABSTRACT

This study demonstrates the Anti-oxidant and Anti-inflammatory activity of ethyl acetate extract of the aerial parts of *Argimone Mexicana*. Inflammation was induced in mice by Croton oil induced ear edema, Croton oil induced capillary permeability and Cotton pellet granuloma pouch. Ethyl acetate extract of *Argimone Mexicana* was found to be certain chemicals like alkaloids and fixed oils. The lethal dose (LD_{50}) selected was 2000 mg/kg body weight. Hence experimental doses were selected as 1/10 and 1/5 dose of *Argimone Mexicana* extract. Anti-oxidant and Anti-inflammatory activity of ethyl acetate extract of the aerial parts of *Argimone Mexicana* was compared with standard anti-inflammatory agent Indomethacin. The present investigation revealed that, *Argimone Mexicana* exhibit statistically significant anti-oxidant activity and extract at low dose (200 mg/kg) and high dose (400 mg/kg). The results showed statically significant reduction in ear edema, capillary permeability and in granuloma. This activity may be due to the presence of alkaloids and fixed oils.

Keywords: *Argimone Mexicana,* Indomethacin, Anti-oxidant, Anti-inflammatory activity.

INTRODUCTION

Inflammation was defined as the local response of living mammalian tissues to injury due to any agent. It is a body defense reaction in order to eliminate or limit the spread of injurious agent as well as to remove the consequent necrosed cells and tissues. The agents causing inflammation may be as under: Physical agents like heat, cold, radiation and mechanical trauma, Chemical agents like organic and inorganic poisons, Infective agents like bacteria, viruses and their toxins and Immunological agents like cell-mediated and antigen antibody reactions.[1]

There are a number of synthetic anticonvulsant drugs are available for use in the management, control and treatment of individuals with anti oxidant and anti-inflammatory property. However, most of the synthetic drugs process many toxic adverse effects. Therefore, there is great need for the development of cheap, effective and safe anti oxidant and anti-inflammatory drugs from the plants and other sources. In traditional systems of medicine, *Argimone Mexicana* is a well

known plant drug used for antioxidant and anti-inflammatory property. *Argimone Mexicana* is a Annual erect, glaucous herb up to 1m tall with pricky stem which exudes yellow latex when cut. Leaves: Sessile, alternate, sinuate pinnatified with prickly teeth, often variegated, grey to bluish green. Flowers: 4 cm to 5 cm in length, bright yellow, white or creamy white. Fruit: Capsule dehiscing by pores (3x1.5 cm), ellipsoid. Science, Horticulture, Medicine.. The plant contains alkaloids which are used as a mild pain killer. The fresh yellow, milky acid sap is known to contain protein dissolving substances which can be used in the treatment of warts, cold sores, cutaneons affections, skin diseases, itches etc.[2]

The root is known to be alterative and can be used in the treatment of chronic skin diseases. The seeds are known to be demulcent, emetic expectorant and laxative. An infusion, in small quantities can be used as a sedative for children, but caution is advised since the oil in the seeds is strongly purgative, the seed can also be used as an antidote to snake poisoning[3]

MATERIALS AND METHODS

Plant material: Aerial parts of *Argimone Mexicana* were collected. The collected plant material was shade dried to retain its vital phytoconstients and then subjected to size reduction for further extraction process.

Extraction: The aerial parts of *Argemone mexicana* were shade dried and granulated.500gm of the granulated parts were extracted with pet. ether, chloroform and ethyl acetate in Soxhlet apparatus by simultaneous extraction for 48 hours separately. The barch extract *was* concentrated in vacuum. The extract was studied for preliminary phytochemical analysis.

In-vitro antioxidant activity using DPPH

1 DPPH Radical Scavenging Assay: For DPPH radical scavenging activity different concentration of EAEAM (10, 20, 40, 60, 80 & 1OOµg/ml) and different concentration of Ascorbic acid was prepared. After that these dilution were mixed with 0.5 ml DPPH solution (4mg in 100 ml methanol) and incubated for 30 min at room temperature in dark condition. After incubation measured the absorbance at 517 nm using methanol as a blank.[4]

$$\% \text{ inhibition of DPPH radical} = \frac{\text{Control - (sample with DPPH- sample without DPPH)}}{\text{Control}} \times 100$$

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is a body defense reaction in order to any agent. It is a body defense reaction in order to eliminate or limit the spread of spread of injurious agent as well as to remove the consequent cells and tissues.

Group I- served as control group and administered vehicle 10% DMSOat dose 1mlP.O.

Group II- served as standard and received indomethacin at the dose10 mg/kg P.O.

Group III- served as the test received EAEAM at the dose of 200mg/kg P.O..

Group VI- served as the test received EAEAM at the dose of 400mg/kg P.O.

1 Croton oil induced ear edema[5]

Principle: This method was been describe primarily as for the concomitant assessment of the antiphlogostic and thymolytic activities of topically applied steroids.

Reagent composition:

A. Ethanol, Pyridine, Ethyl ether, croton oil.
B. Indomethacin 10 mg/ kg, oral. prepared as a stock solution

Procedure: An irritant solution was prepared by dissolving 4 parts croton oil in a solvent mixture of 10 parts ethanol, 20 parts of pyridine, 66 parts ethyl ether. *Argimone mexicana* and Indomethacin were dissolved in the same vehicle (irritant).

The left ear was kept untreated to serve as control. One hr. later group I and II received croton oil solution. After 4 hour animals were

decapitated. AN 8 mm cork borer was used to punch out discs from both the treated as well as control ears. The punches were weighted immediately after decapitation and difference in weight was use to assess the inflammatory response.

2) Croton oil-induced capillary permeability in mice[6]

Principle: This method was been describe primarily as for the concomitant assessment of the antiphlogostic and thymolytic activities of topically applied steroids.

Reagent composition:

- **A)** 1 N KOH. 0.6 N H3PO4-acetone (5:13), 1% evans blue
- **B)** Indomethacin 10 mg/ kg, oral. prepared as a stock solution

Procedure: Mice were treated with croton oil as described in the Croton oil-induced ear edema in mice section. 3 h after blazing, 0.2 ml of 1% evans blue was injected via the tail vein. The animals were sacrificed by cervical dislocation 1 h later and a plug (8mmin diameter) was removed from ear. The plugs were soaked in 1 ml of 1 N KOH at 37 °C for 24 h, added 9 ml 0.6 N H3PO4-acetone (5:13), and centrifuged at 3000 rpm for 15min.The supernatants were detected at 620 nm.

3 Cotton pallet induced granuloma pouch[7]

Principle: The foreign body granulomas were provoked in rats by subcutaneous implantation of pellets of compressed cotton, after several days, histological giant cells and undifferentiated connective tissue san is observed besides the fluids infiltration. The dried pellets after removed. (Gerhard vogel, 2002)

Reagent composition

C. Sterilized cotton pellet (each weighing 20 mg implanted s.c.)
D. Indomethacin 10 mg/ kg, p.o. prepared as a stock solution.

Procedure: This study carried out by cotton pellet implantation method in rats with under light ether anesthesia, sterile cotton (Bengal Surgical, limited, Kolkata) pellet (20mg) were implanted subcutaneously in the axilla and groin regions of the rats. The animals were treated orally *Argimona mexicana* extract (200,400mg/kg) daily for seven days. Animals in the control group received normal DMSO. Indomethacin (10 mg/kg i.p) was given to animal in the control group. They were sacrificed on day 8, the cotton pellet removed, free from extraneous tissue and dried overnight at 60^0 C and weighed. The percentage inhibition calculated.

RESULTS AND DISCUSSION

The phytochemical estimation of ethyl acetate extract of *Argimone mexicana* was containing alkaloids and fixed oil.Then acute toxicity study was carried out and no. animal died upto 2000mg/kg .So the dose of the extract was selected as $1/5^{th}$ and $1/10^{th}$ dose for study.

Then antioxidant scavenging activity of ethyl acetate extract of *Argimone mexicana* was studied and the results were exhibiting statistically significant antioxidant activity. The ethyl acetate extract of *Argimone mexicana* (200mg/kg) group showed statistically significant reduction in ear edema when compaired to disease control group.The maximum % inhibition of ear edema in 200mg/kg treated group was 56.46% ($P<0.05$) The ear weights significantly decreased when compared to the control group.The % inhibition of ear edema in 400mg/kg treated group was 66.67% at $p<0.05$.

Ethyl acetate extract of the aerial parts of *Argimone mex*icana for the standard drug indomethacin showing statistically significant reduction in ear edema.The ear weights significantly decreased for the control group.The % inhibition of ear edema in indomethacin treated group was 76.92% at $p<0.05$.

Ethyl acetate extract of the aerial parts of *Argimone mex*icana were tested at a different dose 200mg/kg and 400mg/kg showing statistically significant reduction in capillary permeability.

The ethyl acetate extract of *Argimone mexicana* (200mg/kg) group showed statistically significant reduction in permeability when compaired to disease control group.The maximum % inhibition of permeability in 200mg/kg treated group was 40.13% ($P<0.05$)The permeability significantly decreased when compared to the control group.The % inhibition of capillary permeability in 400mg/kg treated group was 72.36% at $p<0.05$.

Ethyl acetate extract of the aerial parts of *Argimone mex*icana for the standard drug indomethacin showing statistically significant reduction in ear permeability.The permeability significantly decreased for the control group. The % inhibition of ear edema in indomethacin treated group was 87.20% at $p<0.05$.

***In-vitro* assay (Antioxidant activity)**

Table 1: Results of the effects of EAEAM using DPPH assay

S. No.	Concentration	% inhibition
1	4	47.43
2	6	51.34
3	8	53.9
4	10	56.89
5	20	59.67
6	30	63.43
7	40	67.21

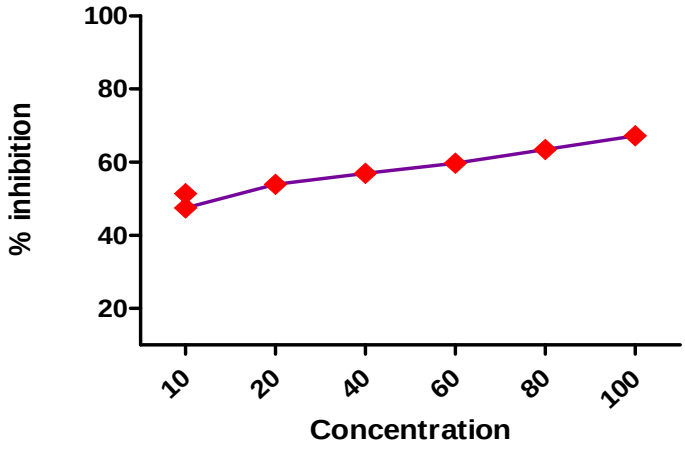

Fig. 1: Diagrammatic representation of the effects of EAEAM by using DPPH Assay

Croton oil induced edema

Table 2: Results of the effects of EAEAM and indomethacin against croton oil induced edema response in mice

S. No.	Treatment	Ear edema (mm)	% inhibition
1	Control	6.50±0.223	------
2	EAEAM (200 mg/kg)	2.83±0.307***	56.46
3	EAEAM (400 mg/kg)	2.16±0.477***	66.67
4	Indomethacin(10mg/kg)	1.50±0.562***	76.92

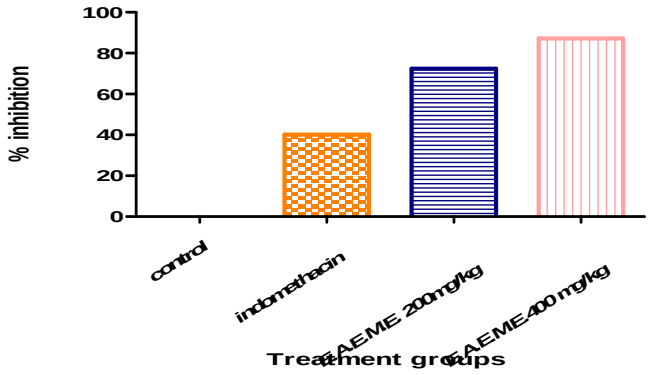

Fig. 2: Diagrammatic representations of the results of the effects of EAEAM and indomethacin against percentage inhibition croton oil induced edema response mice

Croton oil induced capillary permeability

Table 3: Results of the effects of EAEAM and indomethacin against Croton oil induced capillary permeability response in mice

S. No.	Treatment	Concentration (µg/ml)	% inhibition
1	control	0.2681±0.0225	----------
2	EAEAM (200 mg/kg)	0.1605±0.0255***	40.13
3	EAEAM (400 mg/kg)	0.0741±0.0178**	72.36
4	Indomethacin (10mg/kg)	0.0343±0.0212***	87.20

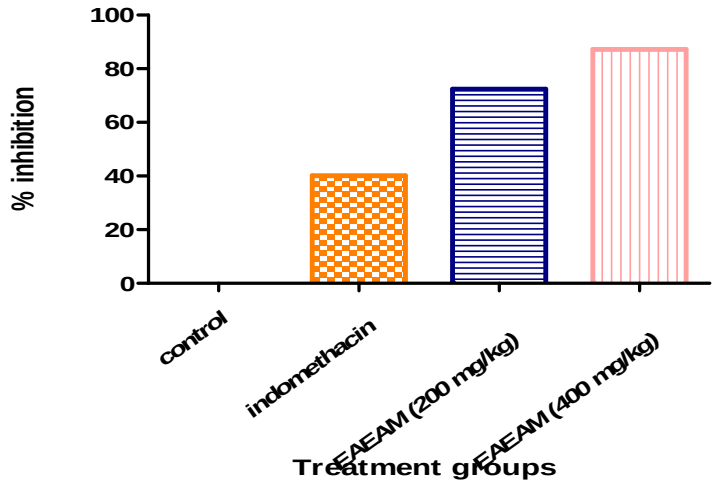

Fig. 3: Diagrammatic representation of the results of the effects of EAEAM and indomethacin against percentage inhibition of Croton oil induced capillary permeability response in mice

Cotton pellet Induced granuloma

Table 4: Results of the effects of EAEAM and indomethacin against Cotton pellet induced granuloma response in rats

S. No.	Treatment	Granuloma weight	% inhibition
1	Control	60.50±1.352	--------
2	EAEAM (200 mg/kg)	50.67±1.022***	16.25
3	EAEAM (400 mg/kg)	44.17±1.249***	26.997
4	Indomethacin(10mg/kg)	39.16±1.609***	35.26

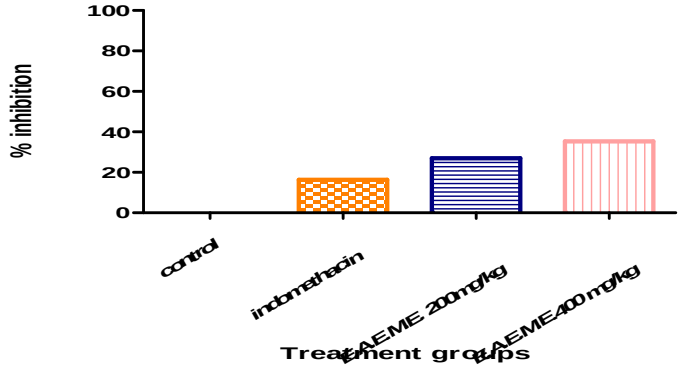

Fig. 4: Diagrammatic representation of the results of the effects of EAEAM and indomethacin against percentage inhibition of Cotton pellet induced granuloma response in rats

CONCLUSION

From all the study we conclude that the ethyl acetate extract of the aerial parts of *Argimone mexicana* showed anti-inflammatory activity and antioxidant activity.

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