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EVALUATION OF ANTI-INFLAMMATORY PROPERTIES OF VARIOUS FORMS OF *ABRUS PRECATORIUS* L.

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The maximum anti-inflammatory activity both *in vitro* and *in vivo* was at highest concentrations levels. Further the anti-inflammatory activity *in vivo* system tends to increase with time. This clearly indicates that the significant effect observed during later stages, can be due to the inhibition of prostaglandins. The cotton pellet granuloma method in rats at 200 mg/kg of methanol insoluble fraction of white form was better than the crude red fractions. The chemical constituents like triterpenoids, saponins, and flavanoids present in the seeds may be responsible for this anti-inflammatory activity.

Keywords : *Abrus precatorius;* Anti-inflammatory; Medicinal plants.

Introduction

Inflammation is associated with the cardinal signs of redness, heat, pain and swelling[1]. This is abomeostatic phenomenon[2]. When a tissue is injured the body adopts this mechanism to contain the inflammation that subsides within a few days, it is known as acute inflammation. The mediators of inflammation are histamine and kinin systems; and prostaglandins[3]. The earlier phase of Carrageenin inflammation is maintanied by histamine and kinin systems and sustained inflammation is maintained by prostaglandins[4]. When a tissue is injured with a noxious agent, leucoyctes migrate to the inflammed site and wage a war to neutralise the ill effects of the foreign agents[5,6]. In the process, the lysosomes present in these cells get damaged resulting in the release of the hydrolytic enzymes that cause extensive damage to the surrounding tissue[7,8].

The *Abrus precatorius* L. (Fabaceae) were extensively studies for various biological activities. It is a climbing shrub, widely distributed all over tropical India in hedges and among bushes on open lands [9-11]. Among variously coloured seed types [12] two common seed types namely red and black coloured seeds (red form) and the white coloured seeds (white form) were selected for this study.

The roots, leaves and seeds are used for medicinal purposes in *Ayurveda, Siddha* and *Unani*. Seeds used in eye disease; jaundice; pain, poisoning, fainting; arthritis and leucoderma[13-19]. Aerial parts extract were also used for treating certain infections like leucorrhoea, gonorrhoea, diarrhoea and dysentery[20,21].

Materials and Methods

Collection of Plant Materials - Fresh seeds of red and white forms of *A. precatorius* were collected from Maruthamalai Hills of Coimbatore district and Mettur, Salem district, Tamilnadu. India and identified at the Botanical Survey of India, Coimbatore. The voucher specimens are kept for reference (A.S. 1001 to 1008) in our department herbarium.

Animals - Adult albino rats initially weighing between 180-220 grams were obtained from the animal house, J.S.S. College of Pharmacy, Ootacamund. Throughout the experimental period, the animals were housed in large spacious acrylic cages. The animals were provided with food and water *adlibitum*. They were maintained at a temperature range of 20- 25°C.

Diet - The commercial pelleted animal feed marketed by Brookbond Lipton India Limited, Bangalore was supplied to the animals throughout the experimental period.

Preparation of extract- Shade-dried, seed powder of each form was extracted with 50% aqueous ethanol in cold maceration method [22, 23] at room temperature separately. After filtration, the marc was extracted twice in the same conditions. Ethanol was removed under vacuum and the aqueous residue was lyophilized to dry. Extracts (Crude-50% ethanolic extract) were fractioned in petroleum ether, chloroform and methanol. The crude (50% ethanolic extract of red form and white form) and methanol soluble and insoluble fractions of crude (red form and white form) were stored in desiccators for pharmacological experiments.

In vitro anti-inflammatory - Inhibition of bovine serum albumin denaturation and anti-inflammatory activity: The test compounds were dissolved in minimum amount of dimethyl formamide and diluted with phosphate buffer (0.2μ pH 7.4). Final concentration of dimethyl formamide in all solutions was 2%. Test solution (1ml) containing different concentration of drug were mixed with 1ml of 1 mmol per litre bovine serum albumin in phosphate buffer and incubated at 27°C for 15 minutes. Denaturation was induced by keeping the reaction mixture, in a water bath at 60°C for 10 minutes. After cooling the turbidity was measured at 660 nm (Schimadzu- U.V. Visible Recording Spectrophotometer 160-A). Percentage inhibition of denaturation was calculated from control. Each experiment was performed in triplicate and the average value was taken. The percentage inhibition was calculated by using the following formula[24].

Percentage inhibition =100 (V/V 100)

In vivo Anti-inflammatory - Acute models - Carrageenin induced rat paw edema : Adult *Wistar* albino rats of either sex weighing 180-220 gms were divided into 16 groups of 6 animals each. The group 1 served as solvent control received 0.3 %w/v of carboxyl methyl cellulose (Sodium salt) as a fine suspension (1ml/kg) orally. Group 2 served as positive control received Ibuprofen (100 mg/kg) in a similar manner. Group 3 to Group 5 received 125,250,500 mg/kg of crude extract of red form; Group 6 to Group 9 received 100 mg, 200 mg/kg methanol soluble and insoluble fraction of red form. Group 10 to group 12 received 125, 250, 500 mg/kg of crude extract of white form, group 13 to group 16 received 100 mg, 200 mg/kg of methanol soluble and insoluble fractions (crude white form) respectively in a similar manner.

All the extracts and fractions were suspended in 0.3% w/v of carboxyl methyl cellulose and administered as a fine suspension orally. One hour after the administration of test compounds, 0.1ml of 1% w/v. carrageenin in normal saline, was injected in the plantar region of the left hind paw of control as well as Ibuprofen treated group. The right paw served as reference, non-inflammed for comparison. The paw volume of both control and Ibuprofen treated rats at 30, 60, 120, 240 min, were measured after carrageenin challenge. The per cent difference in the right and left paw volumes of each animal in control and Ibuprofen treated groups were calculated and compared the mean per cent change in paw volume in control drug, and test compound treated animals and expressed as per cent oedema inhibition by the drug and the test compounds [25].

The anti-inflammatory effect was calculated using the following formula:

$$\%A = \frac{(\%\,Ic - \%I)}{\%I} \times 100$$

Where, Ic is the mean inflammation in controls.

Per cent inflammation (% I):

$$\%I = \frac{(\%\,V_f - V_i)}{V_i} \times 100$$

Where, V_f is the final volume of the hind paw and V_i is the initial volume of the hind paw.

$$I = \frac{\sum \%I}{n} \pm S_m$$

Chronic Anti- Inflammatory model:-Cotton-pellet granuloma technique: The anti-inflammatory activity of the crude extracts of both forms at various dose levels were studied by cotton pellet granuloma technique in rats.

Adult *Wistar* albino rats of either sex weighing 180-220 gms were divided into 16 groups of 6 animals each. The group 1 served as solvent control received 0.3%w/v of carboxyl methyl cellulose as a fine suspension (1ml/kg) orally. Group 2 served as positive control receiving Ibuprofen (100 mg/kg) in a similar manner. Group 3 to group 5 received 125, 250, 500 mg/kg of crude extract red form, group 6 to group 9 received 100 mg, 200 mg/kg methanol soluble and insoluble fraction of red form. Group 10 to group 12 received 125, 250, 500 mg/kg of crude extract white form, group 13 to group 16 received 100 mg, 200 mg/kg of methanol soluble and insoluble fraction (crude - white form) respectively in a similar manner.

The test compounds were administered orally once in the morning around 10.00 AM. The granuloma was induced by implanting sterilized cotton pellets weighing 10mg and placing it in the groin region subcutaneously under pentothol sodium anaesthesia (40 mg/kg; i.p.) The granuloma was allowed to develop over a period of 7 days, and the pellets were recovered on the 8th day along with the granulomatous tissue and weighed (wet weight).

The granuloma was then dried at 50°C and weighed. The process is repeated until a constant dry weight was obtained. Then the percentage inhibition of the formation of granuloma was calculated comparing the solvent control group using the formula [26].

$$\%= \frac{(\%Ic - \%I)}{\%I} \times 100$$

Where, Ic is the mean inflammation in controls.

Results and Discussion

In the *in vitro* studies the inhibition of bovine serum albumin denaturation indicated that crude and all other fractions showed more than 60% inhibition except the methanol soluble fraction of white form at 1000 µg/ml. In particular, the methanol insoluble portion of the red form showed the activity of 75% at 1000 µg/ml concentration. Also the same was evident with the crude extract of red form that it showed 83.33 % inhibition at 1000 µg/ml concentration and was equipotent with the positive control Ibuprofen at the same concentration.

Table 1. Anti-inflammatory activity of crude and its fractions of red and white forms of *A. precatorius* seeds by inhibition of bovine serum albumin denaturation.

Compound (Drug)		Absorbance ± SE			
		1000 µg	500µg	250µg	125µg
Solvent control		0.024 d ±0.0017	0.024 d ±0.0017	0.024 d ±0.0017	0.024 bc ±0.0017
Positive control (Ibuprofen)		0.045 a ±0.0012***	0.038 a ±0.0017**	0.034 a ±0.017	0.026 bc ±0.0011
Crude	Red form	0.044 ab ±0.0012***	0.038 a ±0.0005**	0.031 ab ±0.0036	0.029 b ±0.0012
	White form	0.039 c ±0.0005**	0.036 ab ±0.0010**	0.032 ab ±0.0026	0.026 bc ±0.0011
Methanol soluble fractions	Red form	0.039 bc ±0.0006**	0.033 b ±0.002	0.025 cd ±0.0012	0.024 c ±0.002
	White form	0.026 d ±0.0015**	0.028 cd ±0.0011*	0.033 ab ±0.003	0.037 a ±0.001**
Methanol insoluble fractions	Red form	0.042 abc ±0.0005***	0.039 a ±0.0015**	0.029 bc ±0.0015	0.026 bc ±0.0005
	White form	0.040 bc ±0.0005**	0.032 bc ±0.0020*	0.030 ab ±0.0015	0.025 bc ±0.0011

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.
P values vs. respective control by Student's t - test *P < 0.05, ** P < 0.01, *** P <0.001.

Table 2. Percentage of inhibition in the anti-inflammatory activity of crude and its fractions of red and white forms of *A. precatorius* seeds by inhibition of bovine serum albumin denaturation.

Compound (Drug)		Percentage of inhibition			
		1000 µg	500µg	250µg	125µg
Positive control		87.50	58.33	41.76	8.33
Crude	Red form	83.33	58.33	29.16	20.83
	White form	62.50	50.00	33.33	8.33
Methanol soluble fractions	Red form	62.50	37.50	4.16	0.0
	White form	8.33	16.66	37.50	54.16
Methanol insoluble fractions	Red form	75.00	62.50	20.83	8.33
	White form	66.00	33.33	25.00	4.16

Table 3. Anti-inflammatory activity of crude extract of red and white forms of *A. precatorius* seeds by carrageenin induced rat paw oedema.

Drug		Dose	Volume displaced in ml studied in minutes				
			0 min	30 min	60 min	120 min	240 min
Solvent control		0.3% w/v	0.25 bcd ±0.022	0.45 a ±0.022	0.65 a ±0.022	0.75 a ±0.022	0.85 a ±0.022
Positive control		100 mg/kg	0.25 bcd ±0.022	0.316 e ±0.016***	0.25 h ±0.022***	0.233 g ±0.021***	0.216 f ±0.031***
Crude	Red form	500 mg/kg	0.333 a ±0.040	0.368 b-e ±0.016***	0.335 g ±0.020***	0.313 ef ±0.024***	0.218 f ±0.015***
		250 mg/kg	0.29 abc ±0.015	0.391 bcd ±0.033	0.428 cd ±0.036***	0.393 cd ±0.032***	0.338cde ±0.030***
		125 mg/kg	0.31 a ±0.016*	0.425 ab ±0.031	0.458 bc ±0.029***	0.493 b ±0.028***	0.433 b ±0.023***
	White form	500 mg/kg	0.288 abc ±0.019	0.353 cde ±0.015**	0.45 bc ±0.022***	0.391 cd ±0.024***	0.231 f ±0.016***
		250 mg/kg	0.21 d ±0.015	0.333 de ±0.016**	0.411 c-f ±0.016***	0.383 cd ±0.015***	0.288 e ±0.017***
		125 mg/kg	0.298 ab ±0.029	0.385 bcd ±0.027	0.468 bc ±0.027***	0.416 c ±0.027***	0.341cde ±0.031***

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT
P values vs. respective control by Student's t - test * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
Results are expressed as Mean ± SE
No. of animal : 06 /group
Average Body weight : 180-220 gms
Route of administration : Oral
Induction of Inflammation : 0.1ml of 1%w/v of carrageenin in normal saline injected into the lateral malleolus of hind paw of rat.

However, the methanol soluble fraction of white form has not showed any significant effect even at a higher dose level of 1000 µg/ml. Quite differently the same form showed good activity at lower concentration (125 µg/ml, which may be due to tachyphylaxic effect and an increase in dose than the required (Tables 1, 2).

Both the varieties at the various dose levels tested showed significant anti-inflammatory activity. On comparison of the anti-inflammatory activity of both the forms at three different dose levels, it was observed that crude red form offered better protection (74.3%) at 500 mg/kg dose levels, when compared to solvent control. But the white form itself exhibited quick, better, sustained anti-inflammatory activity at lower dose of 250 mg/kg and showed a protection of 66.1% at the end of 240 minutes. Further the red form at 500 mg/kg dose level equally potent with that of positive control (Ibuprofen 100 mg/kg) (Tables 3, 4, 5).

Among the three dose levels studied for red form it was clear that significant dose dependent activity was present at the end of 120 minutes and 240 minutes, whereas there was no such a dose dependent activity in the case of white form at various time intervals.

The results clearly indicate that methanol soluble and insoluble fractions of both the varieties showed significant antiinflammatory activity and afforded more than 50% protection at the end of 120 minutes. The methanol insoluble fraction of red form (63.76%) showed better protection at the end of 240 minutes. It was also evident that an increase in dose to 200 mg/kg did not alter the percentage protection, in fact, in certain conditions there was a decrease in the percentage protection with increasing dose level. The methanol soluble fraction of white form (100 mg/kg) offered better protection at the end of 120 minutes (59.06%) and it was maximum protection at 240 minutes (66.12%) and this fraction was more potent in acute model of carrageenin induced inflammation (Table 5).

Both the varieties at the various dose levels tested exhibited significant anti-inflammatory activity. The

Table 4. Anti-inflammatory activity of methanol soluble and insoluble fractions of red and white forms of *A. precatorius seeds* by carrageenin induced rat paw oedema.

Drug		Dose	Volume displaced in ml studied in minutes ±SE				
			0 min	30 min	60 min	120 min	240 min
Methanol soluble franctions	Red form	100 mg/kg	0.237 cd ± 0.006	0.403abc ± 0.006	0.488 b ± 0.005***	0.358 c-f ± 0.007***	0.383 bc ± 0.007***
		200 mg/kg	0.237 cd ± 0.005	0.393bcd ± 0.008*	0.437 bc ± 0.007***	0.367cde ± 0.009***	0.372 c ± 0.013***
	White form	100 mg/kg	0.235 cd ± 0.008	0.383bcd ± 0.010	0.377 d-g ± 0.008	0.307 f ± 0.008	0.288 e ± 0.010
		200 mg/kg	0.235 cd ± 0.008	0.380bcd ± 0.008*	0.372 d-g ± 0.005***	0.367cde ± 0.012***	0.340cde ± 0.011***
Methanol insoluble franctions	Red form	100 mg/kg	0.238 cd ± 0.005	0.383bcd ± 0.013*	0.350 g ± 0.009***	0.363 c-f ± 0.011***	0.308 de ± 0.010***
		200 mg/kg	0.227 d ± 0.008	0.377bcd ± 0.010*	0.355 fg ± 0.011***	0.317 ef ± 0.009***	0.332cde ± 0.010***
	White form	100 mg/kg	0.232 cd ± 0.009	0.393bcd ± 0.009*	0.413cde ± 0.010***	0.343 def ± 0.010***	0.357 cd ± 0.008***
		200 mg/kg	0.235 cd ± 0.008	0.388bcd ± 0.011*	0.365efg ± 0.013***	0.335 def ± 0.008***	0.353 cd ± 0.011***

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.
P values vs. respective control by Student's t - test $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P<0.001$.
No. of animal : 06/ group
Average Body weight : 180-220 gms
Route of administration : Oral
Induction of Inflammation of : 0.1ml of 1%w/v of carrageenin in normal saline injected into the lateral malleolus hind paw of rat.

Table 5. Percentage of inhibition in the anti-inflammatory activity of crude and its fractions red and white forms of *A. precatorius seeds* by carrageenin induced rat paw oedema.

Drug		Dose	Percentage of inhibition			
			30 min	60 min	120 min	240 min
Positive control		100 mg/kg	29.70	61.50	65.30	76.40
Crude	Red form	500 mg/kg	22.20	48.40	58.20	74.30
		250 mg/kg	13.10	34.10	47.10	60.20
		125mg/kg	5.50	29.50	34.20	49.40
	White form	500 mg/kg	21.50	30.70	47.80	72.80
		250 mg/kg	26.60	36.90	48.90	66.10
		125mg/kg	14.40	29.20	44.50	59.80
Methanol soluble franctions	Red form	100 mg/kg	10.40	24.92	52.26	54.71
		200 mg/kg	12.60	32.76	51.06	56.23
	White form	100 mg/kg	14.88	42.00	59.06	66.12
		200 mg/kg	15.56	42.76	51.06	60.00
Methanol insoluble franctions	Red form	100 mg/kg	14.88	46.15	51.60	63.76
		200 mg/kg	16.22	45.38	57.73	60.94
	White form	100 mg/kg	12.67	36.46	54.27	58.00
		200 mg/kg	13.78	43.85	55.33	58.47

Table 6. Data showing the anti-inflammatory activity of crude extract of red and white forms of *A. precatorius* seeds by cotton pellet granuloma method in rats.

Drug		Dose	Granuloma dry weight (mg)	Percentage of inhibition
Solvent control		0.3% w/v	0.0622 g ±0.003	-
Positive control		100 mg/kg	0.0206 ab ±0.0004***	66.8%
Crude	Red form	500 mg/kg	0.0229 bc ±0.0006***	63.1%
		250 mg/kg	0.0315 d ±0.0001***	49.4%
		125mg/kg	0.0391 e ±0.0009***	37.1%
	White form	500 mg/kg	0.0224 abc ±0.0002***	63.9%
		250 mg/kg	0.0254 c ±0.0003***	59.1%
		125mg/kg	0.0302 d ±0.0005***	51.4%

Table 7. Data showing the anti-inflammatory activity of methanol soluble and insoluble fractions red and white forms of *A. precatorius* seeds by cotton pellet granuloma method in rats.

Drug		Dose	Granuloma dry weight (mg)	Percentage of inhibition
Methanol soluble fractions	Red form	100 mg/kg	0.0296 d ±0.0009***	52.36%
		200 mg/kg	0.0259 c ±0.0003***	58.36%
	White form	100 mg/kg	0.0435 c ±0.0028	30.03%
		200 mg/kg	0.0385 bc ±0.0007***	38.26%
Methanol soluble fractions	Red form	100 mg/kg	0.0255 f ±0.0003	59.03%
		200 mg/kg	0.0235 e ±0.0004***	62.16%
	White form	100 mg/kg	0.0241 bc ±0.0008***	61.25%
		200 mg/kg	0.0192 a ±0.0005***	69.18%

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT
P values vs. respective control by Student's t - test $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P<0.001$.
Results are expressed as Mean ± SE
No. of animal : 06 / group
Average Body weight : 180-220 gms
Route of administration : Oral
Weight of sterilized cotton pellets implanted : 10 mg, 2 implants /animal/ sub-cutaneously

anti-inflammatory activity of crude red form was significant and dose dependent with 37.1%, 49.4% and 63.1% protection for 125, 250 and 500 mg/kg respectively. But for white form all the three doses showed percentage protection above 50% but among 250 and 500 mg/kg dose levels there was no significant change in the activity as in red form. In both forms the 500 mg/kg dose level was comparable to that of Ibuprofen positive control (66.8%) (Tables 6, 7).

The white form at 125 mg/kg showed better activity than red form at that particular dose level. But the increasing dose up to 500 mg/kg in white form did not increase the percentage protection. Hence it was known that the lower dose of 250 mg/kg itself was sufficient for better protection.

Of all the three dose levels, studied in the case of red form, there was significant dose dependent anti-inflammatory activity at 5 % level at DMRT. In the white form there was significant dose dependent activity with 125, 250 mg/kg anti-inflammatory activity at 5% level at DMRT.

The chronic anti-inflammatory activity of red form of crude extract showed around 50% and more protection at 250 mg and 500 mg/kg. Similar results are observed with the white form at the same dose levels. But, only with red form at 500 mg/kg dose level produced a significant increase in the percentage protection, whereas the white form did not show any significant increase in the percentage protection by increasing the concentration to 500 mg/kg from 125 mg/kg.

The anti-inflammatory activity - cotton pellet granuloma of methanol soluble and insoluble fractions of both red and white forms showed significant protection at 100 mg/kg and 200 mg/kg. In white form methanol insoluble fraction at 200 mg/kg offered better protection than red form, and in the insoluble fraction itself there was a dose dependent protection. The methanol soluble portion of white form offered better protection than the red form.

Although both the fractions of red form at two

dose levels offered better protection, no significant change in the activity was evident an increased dose of 200 mg/kg. Hence lower dose (100 mg/kg) was sufficient for anti-inflammatory activity.

In comparison with the crude extracts and the fractions from both forms, it was observed that red form in the crude extract at 500 mg/kg showed better activity, whereas a similar activity in white form occurred at 250 mg/kg. In the methanol insoluble fractions of white form offered protection at 200 mg/kg than the other fractions including red form.

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