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## **IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF COMBINED EXTRACTS OF OPUNTIA DILLENII, BERBERIS ARISTATA AND TINOSPORA CORDIFOLIA PLANTS**

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### **ABSTRACT**

Inflammation is a vital response provided by the immune system that ensures survival during infection and tissue injury. The aim of the present study was to study the *in-vitro* anti-inflammatory activity of combined extracts of *Opuntia Dillenii*, *Berberis Aristata* and *Tinospora Cordifolia* Plants. Non-Steroidal Anti-Inflammatory drugs (NSAIDs) are the most common medication used to treat inflammation. But these drugs may cause moderate to severe adverse effects upon long term use. Here, an attempt was made to use the herbal drug combinations to treat inflammation and to evaluate best combination to treat inflammation. HRBC method was used to study the anti-inflammatory activity. The *in-vitro* results suggest that, the combination of *Opuntia dillenii* and *Berberis aristata* inhibits the haemolysis of erythrocytes; so the combination can be used to treat inflammation.

### **KEYWORDS**

Anti-inflammatory activity, *Opuntia dillenii*, *Berberis aristata* and HRBC method.

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### **INTRODUCTON**

Inflammation is a vital response provided by the immune system that ensures survival during infection and tissue injury. Inflammatory responses are essential for the upkeep of characteristic tissue homeostasis. The molecular mechanism of inflammation is fairly an intricate process which is begun by the identification of precise molecular patterns linked with either infection or tissue injury. The complete process of the inflammatory response is interceded by several crucial regulators involved

in the selective expression of pro-inflammatory molecules. Prolonged inflammations are often associated with severe serious side effects on health<sup>1</sup>. Non-Steroidal Anti-Inflammatory drugs (NSAIDs) are the most common medication used to treat inflammation. The most common side effects of NSAIDs are gastrointestinal and may include nausea, vomiting, diarrhoea, constipation, heartburn and stomach pain or cramps. Other common side effects of NSAIDs include dizziness, tiredness, headache, tinnitus and increased blood pressure. NSAIDs cause fluid retention called oedema, which leads to heart failure or kidney failure<sup>2</sup>. *Opuntia Dillenii*, *Berberis Aristata* and *Tinospora Cordifolia* Plants are selected for the present study. Here, an attempt was made to use the herbal drug combinations to treat inflammation and to evaluate best combination to treat inflammation.

#### Plan of the study

1. Evaluation of *in-vitro* anti-inflammatory activity of *Opuntia dillenii*.
2. Evaluation of *in-vitro* anti-inflammatory activity of *Berberis aristata*.
3. Evaluation of *in-vitro* anti-inflammatory activity of *Tinospora cordifolia*.
4. Evaluation of *in-vitro* anti-inflammatory activity of combination of plant extracts.

## MATERIAL AND METHODS

### Plant Collection

*Opuntia dillenii* leaves, *Berberis aristata* leaves, *Tinospora cordifolia* leaves were collected from different areas of Telangana region with proper authentication. The collected plant materials were washed dried for two weeks under shade. The dried plant materials were powdered mechanically; the powdered was sieved through mesh number 44 and stored in air tight container. Other chemicals procured were analytical grade.

### Method

About 50mg of coarsely powdered crude drugs were placed separately in a stoppered container with the ethanol and allowed to stand at room temperature for 5 days with frequent agitation until the soluble matter has dissolved, then filtered to get the extract. The extract was evaporated to dryness

under room temperature for 4 days to get the desired product. The dried product used for phytochemical evaluation and *in-vitro* anti-inflammatory activity by HRBC method<sup>3</sup>.

### HRBC Method<sup>4</sup>

The blood was collected from healthy volunteer, mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.4% sodium chloride in water). The blood was centrifuged at 300rpm and packed cells were washed with iso-saline (0.85%, pH7.4) and 10% v/v suspension was made with iso-saline. The assay mixture contained the drug. 1ml phosphate buffer (0.15M, pH7.4), 2ml of hyposaline (0.36%) 0.5ml HRBC suspension. Diclofenac was used as the reference drug. Instead of hypo-saline, 2ml of distilled water was used as control. All the assay mixtures were collected at 37°C for 30minutes and centrifuged. The haemoglobin content in the supernatant solution was estimated using colorimeter at 560nm. The percentage haemolysis was calculated by assuming the haemolysis produced in the presence of distilled water as 100%. The percentage of HRBC membrane stabilization or protection was calculated using the following formula. % Inhibition of haemolysis =  $\{(\text{OD control} - \text{OD test}) / \text{OD control}\} * 100$ .

## RESULTS AND DISCUSSION

### Phytochemical screening

#### *Opuntia dillenii*

The phytochemical evaluation of *Opuntia dillenii* shows the presence of alkaloids, tannins, saponins, glycosides and terpenoids; which matches with the previous literature<sup>5,6</sup>.

#### *Berberis aristata*

The phytochemical evaluation of *Berberis aristata* shows the presence of alkaloids, tannins, glycosides and carbohydrates; which matches with the previous literature<sup>7,8</sup>.

#### *Tinospora cordifolia*

The phytochemical evaluation of *Tinospora cordifolia* shows the presence of alkaloids, tannins, saponins, glycosides and terpenoids; which matches with the previous literature<sup>9,10</sup>.

The percentage inhibition of haemolysis was calculated in UV-Visible spectrometry method at 560nm. The standard drug diclofenac, showed 33.78% haemolysis inhibition at 100mg and 60.13% haemolysis inhibition at 200mg dose which was significant at p<0.005.

*Opuntia dillenii* extract, showed 12.83% haemolysis inhibition at 200mg and 21.62% haemolysis inhibition at 400mg concentration, which was significant at p<0.05.

*Berberis aristata* extract, showed 10.81% haemolysis inhibition at 200mg and 18.24% haemolysis inhibition at 400mg concentration.

*Tinospora cordifolia* extract, showed 9.45% haemolysis inhibition at 200mg and 14.86% haemolysis inhibition at 400mg concentration.

Combination of *Opuntia dillenii* and *Berberis aristata* extracts, showed 24.32% haemolysis inhibition at 200mg, significant at p<0.05, and 59.45% haemolysis inhibition at 400mg concentration, which was near to standard and also significant at p<0.005.

Combination of *Opuntia dillenii* and *Tinospora cordifolia* extracts, showed 19.59% haemolysis inhibition at 200mg, significant at p<0.05 and 31.75% haemolysis inhibition at 400mg concentration, which was significant at p<0.05.

Combination of *Berberis aristata* and *Tinospora cordifolia*, showed 18.24% haemolysis inhibition at 200mg, significant at p<0.05, and 31.08% haemolysis inhibition at 400mg concentration, which was significant at p<0.005.

**Table No.1: Preliminary phytochemical screening of the various extracts of *Opuntia dillenii*, *Berberis aristata* and *Tinospora cordifolia***

S.No	Contents	<i>Opuntia dillenii</i>	<i>Berberis aristata</i>	<i>Tinospora cordifolia</i>
1	Alkaloids	+	+	+
2	Tannins	+	+	+
3	Saponins	+	-	+
4	Glycosides	+	+	+
5	Carbohydrates	-	+	-
6	Steroids	-	-	-
7	Terpenoids	+	-	+

+ = Present, - = Absent

**Table No.2: Effect of ethanolic extract of various plants on % inhibition of haemolysis**

S.No	Treatment	Concentration (mg)	Absorbance at 560nm	% Inhibition of haemolysis
1	Control	-	0.148±0.003	0
2	Standard (Diclofenac)	100	0.098±0.022	33.78
		200	0.059±0.005**	60.13
3	<i>Opuntia dillenii</i>	200	0.129±0.006	12.83
		400	0.116±0.006*	21.62
4	<i>Berberis aristata</i>	200	0.132±0.003	10.81
		400	0.121±0.022	18.24
5	<i>Tinospora cordifolia</i>	200	0.134±0.009	9.45
		400	0.126±0.007	14.86
6	<i>Opuntia dillenii</i> + <i>Berberis aristata</i>	200	0.112±0.004*	24.32
		400	0.06±0.008**	59.45
7	<i>Opuntia dillenii</i> + <i>Tinospora cordifolia</i>	200	0.119±0.008*	19.59
		400	0.101±0.005*	31.75
8	<i>Berberis aristata</i> + <i>Tinospora cordifolia</i>	200	0.121±0.004*	18.24
		400	0.102±0.003**	31.08

\*p<0.05, \*\*p<0.005

## CONCLUSION

The present study states that, the combination of *Opuntia dillenii* and *Berberis aristata* at the concentration of 400mg inhibits the haemolysis of erythrocytes significantly; so the combination can be used to treat inflammation effectively.

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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