IJAPB International Journal of Advances in Pharmacy and Biotechnology

Journal homepage: http://ijapbjournal.com/

Research Article

# In-Vitro Anti-Inflammatory Activity of Combined Extracts of Selected Plants

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ARTICLE INFO

*Article history:*
Received 10 Jan 2020
Received in revised form 22 Jan 2020
Accepted 30 Jan 2020
doi.org/10.38111/ijapb.20200601001

*Keywords:*
Anti-inflammatory activity, Datura, Hibiscus, Saccharum and HRBC method.

ABSTRACT

Inflammation is a vital response provided by the immune system that ensures survival during infection and tissue injury. 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 the best combination to treat inflammation. HRBC method was used in the present study to evaluate the anti-inflammatory activity. The in-vitro results suggest that, the combination of *Datura stramonium* and *Hibiscus rosasinensis* inhibits the haemolysis of erythrocytes; so, the combination can be used to treat inflammation.

## 1. Introduction

Inflammation is an indispensable reaction given by the immune system that armors survival during infectious diseases and tissue injury. Inflammatory responses are basics for the upkeep of distinctive tissue homeostasis. The molecular mechanism of inflammation is impartially an intricate procedure which is begun by the identification of precise molecular arrangements associated with either infection or tissue injury. The total procedure of the inflammatory response is interceded by few critical regulators associated in the selective expression of pro-inflammatory elements. Sustained inflammations are often interrelated with extreme side effects on health [1]. Non-Steroidal Anti-Inflammatory drugs (NSAIDs) are the most widely used drugs to treat inflammation. The common side effects of NSAIDs are gastrointestinal and may include ulceration, heartburn and stomach pain. Other common side effects of NSAIDs include dizziness, tinnitus and increased blood pressure. NSAIDs cause fluid retention called oedema, which leads to heart failure or kidney failure[2]. Here, an attempt was made to use the herbal drug combinations to treat inflammation and to evaluate best combination to treat inflammation. The study was planned to evaluate anti-inflammatory activity of individual extracts and combination of extracts.

## 2. Materials and Methods

*Plant Collection: Datura stramonium* leaves, *Hibiscus rosasinensis* leaves *Saccharum officinarum* peel 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 powered was sieved through mesh number 44 and stored in airtight container. Other chemicals procured were analytical grade.

*Leaves of Datura stramonium*

*Leaves of Hibiscus rosa sinensis*

*Peel of Saccharum officinarum*

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*Extraction Method:* About 50mgs 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 recurrent agitation until the soluble matter was liquefied, then filtered to get the extract. The extracts were evaporated to dryness under room temperature for 4 days to get the desired products[3].

*HRBC Method:* The blood was obtained from healthy participants, combined with the same amount 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%) and 0.5ml HRBC suspension. Diclofenac was used as the reference. Hypo-saline substituted by 2ml of distilled water in control. Each of the mixture underwent 30min centrifugation. The amount of haemoglobin in the supernatant was measured at 560nm using colorimeter. The percentage of haemolysis was determined by assuming that the haemolysis formed as a cent per cent in the presence of distilled water[4]. The percentage haemolysis inhibition was calculated by the following formula.

% Inhibition of haemolysis = {(OD control – OD test) / OD control}*100

## 3. Results and Discussion

*Phytochemical screening:* The phytochemical evaluation of *Datura stramonium* shows the presence of alkaloids, tannins, saponins, glycosides and terpenoids; which matches with the previous literature[5,6]. The phytochemical evaluation of *Hibiscus rosasinensis* shows the presence of alkaloids, tannins, glycosides and carbohydrates; which matches with the previous literature[7,8] The phytochemical evaluation of *Saccharum officinarum* shows the presence of alkaloids, tannins, saponins, glycosides and terpenoids; which matches with the previous literature[9-11].

**Table 1:** Preliminary phytochemical screening of the various extracts of *D. stramonium*, *H. rosasinensis* and *S. officinarum*

| Contents | *Datura stramonium* | *Hibiscus rosasinensis* | *Saccharum officinarum* |
|---|---|---|---|
| Alkaloids | + | + | + |
| Tannins | + | + | + |
| Saponins | + | - | + |
| Glycosides | + | + | + |
| Carbohydrates | - | + | + |
| Steroids | - | - | - |
| Terpenoids | + | - | + |

+ = Present, - = Absent

*HRBC Method:* The percentage inhibition of haemolysis was calculated in UV-Visible spectrometry method at 560mn. The standard drug diclofenac showed 33.78 % haemolysis inhibition at 100 mg and 60.13 % haemolysis inhibition at 200 mg dose which was significant at p<0.005. *Datura stramonium* extract, showed 12.83% haemolysis inhibition at 200 mg and 21.62 % haemolysis inhibition at 400 mg concentration, which was significant at p<0.05. *Hibiscus rosasinensis* extract, showed 10.81 % haemolysis inhibition at 200 mg and 18.24 % haemolysis inhibition at 400mg concentration. *Saccharum officinarum* extract, showed 9.45 % haemolysis inhibition at 200 mg and 14.86 % haemolysis inhibition at 400mg concentration (**Figure 1 & 2**).

Combination of *Datura stramonium* and *Hibiscus rosasinensis* extracts, showed 24.32 % haemolysis inhibition at 200 mg, significant at p<0.05, and 59.45 % haemolysis inhibition at 400 mg concentration, which was near to standard and significant at p<0.005.

**Table 2:** Effect of ethanolic extract of various plants on % inhibition of haemolysis

| Treatment | Concentration (mg) | Absorbance (560 nm) | % Inhibition |
|---|---|---|---|
| Control | - | 0.148±0.003 | 0 |
| STD | 100 | 0.098±0.022 | 33.78 |
| | 200 | 0.059±0.005** | 60.13 |
| *DS* | 200 | 0.129±0.006 | 12.83 |
| | 400 | 0.116±0.006* | 21.62 |
| *HR* | 200 | 0.132±0.003 | 10.81 |
| | 400 | 0.121±0.022 | 18.24 |
| *SO* | 200 | 0.134±0.009 | 9.45 |
| | 400 | 0.126±0.007 | 14.86 |
| *DS + HR* | 200 | 0.112±0.004* | 24.32 |
| | 400 | 0.06±0.008** | 59.45 |
| *DS + SO* | 200 | 0.119±0.008* | 19.59 |
| | 400 | 0.101±0.005* | 31.75 |
| *HR + SO* | 200 | 0.121±0.004* | 18.24 |
| | 400 | 0.102±0.003** | 31.08 |

DS: *Datura stramonium*; HR: *Hibiscus rosasinensis*; SO: *Saccharum officinarum;* *p<0.05, **p<0.005

Combination of *Datura stramonium* and *Saccharum officinarum* 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 *Hibiscus rosasinensis* and *Saccharum officinarum*, 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.

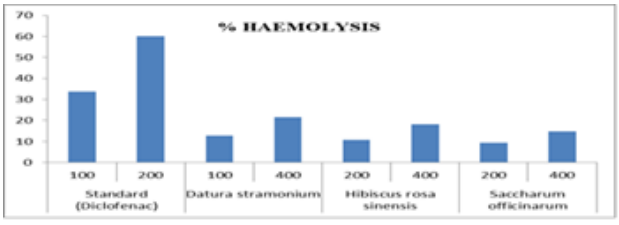


**Fig. 1:** Percentage haemolysis of plant extracts

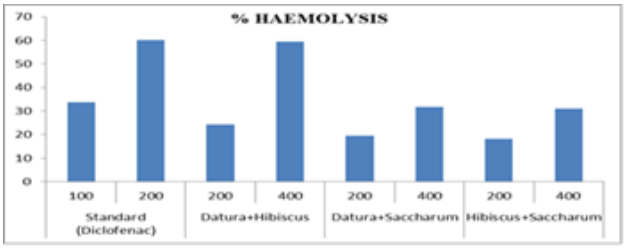


**Fig. 2:** Percentage haemolysis of combined extracts

## 4. Conclusion

The present study states that, the combination of *Datura stramonium* and *Hibiscus rosasinensis* at the concentration of 400mg inhibits the haemolysis of erythrocytes significantly; so the combination can be used to treat inflammation effectively.

## Acknowledgements

Authors are thankful to the Principal and Management, Malla Reddy Pharmacy College, for providing necessary facilities and actions towards the fruitful completion of this research work.

## Conflict of Interest

The author(s) confirm that this article content has no conflict of interest.

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