

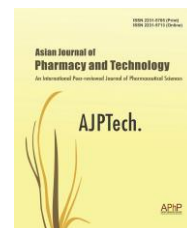
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## **RESEARCH ARTICLE**

# ***In-Vitro* Anti-inflammatory Activity of Traditionally reported Poly Herbal Combination**

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## **ABSTRACT:**

Inflammatory diseases are still one of the most important health-problems in the world. Most of anti-inflammatory drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) have number of adverse effects hence there are a need to develop safe and new anti-inflammatory formulation with minimum side effects. Present study focused on investigating and comparing anti-inflammatory potential of hydroalcoholic extracts of *Tinospora cordifolia*, *Boerhavia diffusa*, *Tribulus terrestris* and their combinations. Inhibition of heat induced HRBC membrane lysis was taken as a measure of the anti-inflammatory activity. The combination ratio of hydroalcoholic extracts of *Tinospora cordifolia*, *Boerhavia diffusa*, *Tribulus terrestris* (2:5:3) showed the most significant membrane stabilizing action on HRBC membrane as compare to hydroalcoholic extracts of individual drugs and their other combinations. The present study revealed that the proper combination of *Tinospora cordifolia*, *Boerhavia diffusa*, *Tribulus terrestris* gives more effective anti-inflammatory activity than any of the individual drug.

**KEYWORDS:** Anti-inflammatory, *Tinospora cordifolia*, *Boerhavia diffusa*, *Tribulus terrestris*, Poly Herbal.

## **INTRODUCTION:**

Inflammation is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. It is characterized by redness, swollen joints, joint pain, its stiffness and loss of joint function. Most of anti-inflammatory drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) have number of adverse effects hence there are a need to develop safe and new anti-inflammatory formulation with minimum side effects. Present study focus on combination of three plant drugs *Boerhavia diffusa*, *Tinospora cordifolia* and *Tribulus terrestris* which are traditionally reported to have better anti-inflammatory activity<sup>1</sup>.

*Boerhavia diffusa* (family Nyctaginaceae) commonly known as punarnava in Sanskrit, an important medicinal plant in Ayurveda and Unani. The root of *B. diffusa* is mainly used to treat gonorrhoea, internal inflammation of all kinds, dyspepsia, oedema, jaundice, menstrual disorders, anaemia, liver, gallbladder and kidney disorders, enlargement of spleen, abdominal pain, abdominal tumours, and cancers<sup>2</sup>. The root of *B. diffusa* contains alkaloids (punarnavine), rotenoids (boeravinones A-F), flavonoids, amino acids, lignans (liriodendrons),  $\beta$ - sitosterols and tetracosanoic, esacosanoic, stearic and ursolic acids<sup>3,4</sup>.

*Tinospora cordifolia* (family Menispermaceae) is commonly known as Guduchi. *T. cordifolia* is widely used medicinal plant in Ayurvedic system for its general tonic, antiperiodic, anti-spasmodic, anti-inflammatory, antipyretic, anti-arthritic, anti-lepritic, anti-allergic and anti-diabetic properties<sup>5</sup>. The plant mainly contains alkaloids, glycosides, steroids, sesquiterpenoids,

aliphatic compounds, essential oils, mixture of fatty acids and polysaccharides<sup>6</sup>. The alkaloids include berberine, bitter gilonin, non-glycoside gilonin gilosterol<sup>6</sup>.

*Tribulus terrestris* (family Zygophyllaceae) commonly known as *Gokshur* or *Gokharu*. It is used in the traditional medicine of many countries for the treatment of cardiac diseases, edema, eye trouble, skin itch and impotency<sup>7</sup>. *T. terrestris* contains different types of constituents such as steroidal saponins, flavonoids, glycosides, phytosterols, tannins, terpenoids, amide derivatives, amino acids and proteins<sup>8</sup>.

## MATERIALS AND METHODS:

### Plant material:

The plants roots of *Boerhavia diffusa*, leaves of *Tinospora cordifolia* and fruits of *Tribulus terrestris* were collected from Rajkot district of Gujarat, India. The plant material was identified by Faculty in botany, Biology Department, Gyanyagna College of Science and Management, Rajkot and a voucher specimen (Voucher No. SPAU/19/02, SPAU/19/03 and SPAU/19/04 respectively) has been retained in Department of Pharmacognosy, School of Pharmaceutical Sciences, Atmiya University, Rajkot, Gujarat.

### Reagents and chemicals:

All the reagents were of analytical grades. Indomethacin capsules (25mg) non-steroidal anti-inflammatory drug was purchased from a pharmaceutical shop at Rajkot, Gujarat, India.

### Preparation of plant extracts:

The dried plant materials were ground into powder using an electric grinder separately. Each dried powder material macerated with 80% for 24 hours separately. Filtered all the extracts and wash three times with 80% alcohol separately. The collected individual hydroalcoholic extracts were dried up to constant weight and subjected for *in-vitro* anti-inflammatory study.

### Preparation of test samples:

Dried hydroalcoholic extracts of *Boerhavia diffusa*, *Tinospora cordifolia* and *Tribulus terrestris* were dissolved separately in 80% alcohol to make concentration 300µg/ml of each. Then made different combinations of the prepared test samples (300µg/ml of each) of *Boerhavia diffusa* (BD), *Tinospora cordifolia* (TC) and *Tribulus terrestris* (TT) in ratio of 2:3:5, 3:2:5, 5:3:2, 5:2:3, 2:5:3 and 3:5:2. Indomethacin 100µg/ml in distilled water was taken as standard sample.

### Anti-inflammatory activity:

#### Membrane stabilizing activity assay:

The reaction mixture (2ml) consisted of 1ml of test sample and 1ml of 10% human red blood cells (HRBCs)

suspension, instead of test sample only saline was added to the control test tube. Indomethacin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated at 56°C for 30min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500rpm for 5 min and the absorbance of the supernatants was taken at 560nm. The experiment was performed in triplicates for all the test samples. The percentage inhibition of haemolysis was calculated as follows: % membrane stabilization =  $[(Ac - At)/Ac] \times 100$ . Where, Ac is the absorbance of control, At is the absorbance of test sample<sup>9</sup>.

## RESULT AND DISCUSSION:

The results of membrane stabilization activity of hydroalcoholic extracts of *Boerhavia diffusa*, *Tinospora cordifolia*, *Tribulus terrestris* and their combinations shown in Table 1.

**Table 1** Effects of hydroalcoholic extracts of *Boerhavia diffusa*, *Tinospora cordifolia*, *Tribulus terrestris* and their combinations in membrane stabilizing activity assay

Test samples and Concentration	Combination ratio	Mean absorbance	% Membrane Stabilization (mean±S.E.M.)
Control	-	0.341	-
Indomethacin (100µg/ml)	-	0.092	73.02±0.13
TC (300µg/ml)	-	0.142	58.36±0.11
BD (300µg/ml)	-	0.202	40.76±0.18
TT (300µg/ml)	-	0.163	52.20±0.14
TC (300µg/ml): BD (300µg/ml): TT (300µg/ml)	2: 3: 5	0.184	46.04±0.09
	3: 2: 5	0.143	58.06±0.12
	5: 3: 2	0.122	64.22±0.15
	5: 2: 3	0.171	49.85±0.18
	2: 5: 3	0.103	69.79±0.13
	3: 5: 2	0.132	61.13±0.11

Values are expressed as mean± S.E.M., (N=3). \*Significantly different from control ( $P < 0.01$ )

Membrane stabilization is a process of maintaining the integrity of biological membrane such as erythrocyte and lysosomal membranes against heat induced lyses<sup>10,11</sup>. The neutrophil lysosomal constituents upon extracellular release cause further tissue inflammation and damage<sup>12</sup>. Stabilization of lysosomal membrane is important in limiting the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophil. The erythrocyte membrane is analogous to the lysosomal membrane so, the effect of drug to stabilize erythrocyte membrane could be extrapolated to stabilize lysosomal membrane. The hydroalcoholic extracts of *Boerhavia diffusa*, *Tinospora cordifolia*, *Tribulus terrestris* and their combinations may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation.

There are three combinations ratios 5: 3: 2, 2: 5: 3 and 3: 5: 2 of *Tinospora cordifolia*, *Boerhavia diffusa*, *Tribulus terrestris* showed more activity compare to individual hydroalcoholic extracts of them. The combination ratio of hydroalcoholic extracts of *Tinospora cordifolia*, *Boerhavia diffusa*, *Tribulus terrestris* (2:5:3) showed the most significant membrane stabilizing action on HRBC membrane as compare to hydroalcoholic extracts of individual drug and other combinations of the hydroalcoholic extracts.

The present study reveals that as compare to the individual drug extracts or any random combinations, the proper combination of *Tinospora cordifolia*, *Boerhavia diffusa*, *Tribulus terrestris* gives more effective anti-inflammatory activity.

### CONCLUSION:

The present study revealed that the proper combination of *Tinospora cordifolia*, *Boerhavia diffusa*, *Tribulus terrestris* gives more effective anti-inflammatory activity than any of the individual drug. Thus there is need to do further research on these drugs combinations to find out the synergistic effect of them and to develop combined formation so that can be beneficial to the society.

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