Original Article

Evaluation of Synergistic Activity of *Hemidesmus indicus* and *Terminalia catappa* on Rheumatoid Arthritis in Rats

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ABSTRACT

In this study synergistic activity on the root of *Hemidesmus indicus* (family: Apocynaceae) and leaf of *Terminalia catappa* (family: combretaceae) on rheumatoid arthiritis model were studied. Investigations was performed using the phlogistic agent such as Carrageenan-induced paw edema in rats. Ethanolic extracts on root of *Hemidesmus indicus* and leaves of *Terminalia catappa* at a dose of 200 mg/kg and 400 mg/kg orally were tested. Diclofenac sodium at the dose of 100mg/kg was used as standard. Both the extracts showed significant activity (*p<0.05 & **p<0.01, ***p<0.001) compared with the control and showed a significant % of reduction (80%) in Carrageenan induced rat paw edema model. Thus it is revealed from the screening model that the two different plants extract possess synergistic anti-inflammatory activity.

Keywords: *Hemidesmus indicus* and *Terminalia catappa* synergistic anti-inflammatory activity.

INTRODUCTION

The herbal medicines are getting more importance in the treatment of inflammation because of the toxic effect of the current therapy used to treat that inflammation using synthetic drugs[1]. Herbal medicines are less toxic and less costly when compared to the synthetic drugs. *Hemidesmus indicus* Linn (family: Apocynaceae) roots of this taxon have been used in folk medicine as well as in ayurvedic and unani preparations. They have been prescribed against the diseases of blood, inflammation, diarrhea, respiratory disorders, skin diseases, syphilis, fever, bronchitis, asthma, eye diseases, epileptic fits in children, kidney and urinary disorders, loss of appetite, burning sensation and rheumatism[2,3]. It is also reported for antioxidant and anti-thrombotic property[4] and the root powder or its water extract increased the water and electrolyte absorption from rat intestine[5] and antienterobacterial activity[6]. The leaves, bark and fruit of the tree *Terminalia catappa*

L. (family: Combretaceae) have been commonly used as a folk medicine foranti diarrhea, antipyretic and haemostatic purposes[7]**.** The leaves of *Terminalia catappa* have been used for the prevention and treatment of hepatitis and liver-related diseases[8]. So, the present research work has been undertaken with the ethanolic root extract of *Hemidesmus indicus* and leaves extract of *Terminlia catappa* combination toinvestigate synergistic anti-inflammatory activity.

MATERIALS AND METHODS

Collection of plant and authentication

The roots of *Hemidesmus indicus* was collected from suvarnapuram, area of Khammam District of Andhra Pradesh and the leaves of *Terminalia catappa* was collected from Jangaon institute of Pharmaceutical Sciences in Jangaon, Warangal district. The botanical identity of the plant material was confirmed with the help of Mrs. P. Krishnaveni, junior lecturer in botany, A.S.R, Government Junior College, Shanthinagar, Khammam, Andhra Pradesh, India.

Preparation of Extraction

The dried powdered roots and leaves were mixed with petroleum ether to defat the fatty material and mixed with twice the amount of ethanol and macerated for 72 hours. It was filtered three times and boiled on a hot plate to get a crude extract. Then crude extract was subjected to phytochemical screening.

Phyto chemical analysis of the extract

The extract was screened for the presence of various constituents employing standard screening tests[9]. Conventional protocols for detecting the presence of secondary metabolites such as glycosides, saponins, flavonoids, tannins were used. Several phytoconstituents like flavonoids, terpenoids and tannins were present which is known to promote anti-inflammatory process due to their antioxidant activities.

Experimental animal

All the experiments were carried out according to the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), (1322/ac/10/CPCSEA/2010).

Acute Oral Toxicity study

The EEHI and EETC treated animals were observed continuously for the initial 2 hrs for its general behavior, intermittently up to 24 hrs for its mortality (short term toxicity) and up to 14 days for long term toxicity[10-12]. The animals did not show any mortality up to the dose level of 2000 mg/kg body weight in any of the groups and were considered as safe. Hence 2000mg/kg body weight was considered as MTD (Maximum Tolerated Dose), $1/10^{th}$and $1/5^{th}$of the value of MTD were taken as treatment dose for further studies (200mg/kg and 400mg/kg).

Determination of anti inflammatory activity

Acute inflammation is provided by injection of 0.1ml of 1% carrageenan into the sub plantar surface of rat hind paw.

Group I: Served as control.
Group II: Rats were received 0.1 ml of 1% carrageenan.
Group III: Rats were received diclofenac (100mg/kg/p.o)
Group IV: Rats were received EEHI (200 mg/kg/p.o) and 0.1ml of carrageenan.
Group V: Rats were received EEHI (400mg/kg/p.o) and 0.1 ml of carrageenan.
Group VI**:** Rats were received EETC (200 mg/kg/p.o) and 0.1ml of carrageenan.
Group VII: Rats were received EETC (400mg/kg/p.o) and 0.1 ml of carrageenan.
Group VIII: Rats were received EEHI (100 mg/kg/p.o) + EETC (100 mg/kg/p.o)and 0.1ml of carrageenan.

Group IX: Rats were received EEHI (200mg/kg/p.o) + EETC (200 mg/kg/p.o)and 0.1 ml of carrageenan.

The paw volume up to the tibiotarsal articulation was measured at 0, 1, 2, 3, 4 and 5 hours. Reduction in the paw volume is compared with the vehicle treated controlled animals with that of the test groups and the anti-inflammatory activity was carried on the basis of the percentage (%) of inhibition of edema. The percentage of inhibition of edema was calculated by using the formula % inhibition of edema = (Vc-Vt/Vc) x100 Where Vt = Paw volume in test group animals and Vc = Paw volume in control group.

Statistical Analysis

The statistical analysis of the evaluation of the anti-inflammatory activity of ethanolic extract of *Hemidesmus indicus* roots and *terminalila catappa* leaves against the carrageenan induced paw oedema in Wistar rats were analyzed using ANOVA followed by kruskal walis test and expressed as mean ± SEM.

RESULTS

The effects of EEHI, EETC and EEHI/EETC combination on Carrageenan induced oedema in rats is shown in table 1. Sub-plantar injection of carrageenan in rat resulted in a time-dependant increase in paw volume; this increase was observed at 1 h and was maximal at 5 h after administration of carrageenan. The results obtained indicate that the high dose EEHI/EETC combination had significant ($p<0.05$) anti-inflammatory activity in rats. Mean increase in paw volume was significantly ($p<0.05$) decreased in EEHI alone, low dose combination of EEHI/EETC and high dose combination of EEHI/EETC.

High dose combination of EEHI/EETC showed significant anti-inflammatory activity peaked at 5h where it caused 80.00% inhibition in paw volume. The low dose combination EEHI/EETC showed 79.2% reduction in inflammation. However low dose EEHI and EETC given alone like 59% and 66.2% respectively. Moreover high dose EEHI and EETC were given alone like 72.3% and 70.0% respectively.

DISCUSSION

In living animal tissues, inflammatory processes involve the release of several mediators, including prostaglandins, histamine, thermo-attractants, cytokines, and protease and so on; as well as substances that regulate adhesion of molecules and the processes of cell migration, activation and degranulation[13].

NSAIDs are the most commonly prescribed analgesics worldwide for the management of acute and inflammatory pain, *Hemidesmus indicus* and *Terminalia catappa* has been used for centuries in acute gouty arthritis[14].

`The present study established the synergistic anti-inflammatory activity of EEHI in combination with EETC. Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1 – 2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages[15].

Table1 & fig 2 showed better result in high dose combination of EEHI/EETC significantly reduces the inflammation as compared to control than their individual extracts up to 5h.

The results of the present study clearly indicate that the high dose combination of EEHI with EETC has significant anti-

inflammatory effect in Carrageenan induced rat paw edema.

CONCLUSION

The combination of EEHI and EETC showed profound increase in anti-inflammatory activity as compared to their individual components, which may open new avenues in the treatment of arthritis, gout and other inflammatory disorders. Hence anti-inflammatory activity of this combination needs to be explored further.

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Table 1. Effect of EEHI, EETC and their combination on carrageenan induced paw edema in rats

Treatment	Dose (mg/kg)	Increase in paw volume at time in (ml)						% inhibition
		0hr	1hr	2hr	3hr	4hr	5hr	
Control (tween 80)	1ml	1.60±0.40	1.60±0.40	1.60±0.40	1.60±0.40	1.60±0.40	1.60±0.40	-
Disease control (carrageenan)	0.05ml	***4.49±0.53	***5.90±0.60	***6.65±0.13	***7.28±0.08	***8.89±0.33	***9.69±0.04	-
Standard diclofenac	100mg/kg	***4.36±0.54	***5.65±0.13	***4.20±0.20	**3.75±0.32	**3.96±0.46	**4.03±0.27	58.0%
EEHI – 200	200mg/kg	***4.71±0.57	***5.37±0.12	**4.49±0.62	**3.96±0.46	**3.30±0.20	**3.95±0.02	59.0%
EEHI – 400	400mg/kg	***4.21±0.20	***5.35±0.15	**4.71±0.57	**3.61±0.90	*3.07±0.20	*2.73±0.03	72.3%
EETC – 200	200mg/kg	***4.48±0.30	***5.17±0.07	***4.37±0.12	***3.95±0.32	**3.30±0.20	**3.30±0.20	66.2%
EETC – 400	400mg/kg	***4.37±0.23	***5.37±0.12	***4.68±0.22	***3.70±0.25	**3.10±0.18	**2.88±0.08	70.0%
EEHITC –200	200mg/kg	***4.68±0.02	***5.45±0.35	***4.04±0.32	**3.60±0.18	*2.88±0.08	*2.02±0.10	79.2%
EEHITC –400	400mg/kg	***4.46±0.08	***5.35±0.15	***4.64±0.20	**3.39±0.07	*2.49±0.08	*1.90±0.17	80.0%

*P < 0.05 when compared to control. ** P < 0.01. *** P < 0.001 when compared to control. Number of animal / in each group = 6 Data expressed in mean ± SEM;EEHI-Ethanolic extract of *Hemidesmus indicus*, EETC - Ethanolic extract of *Terminalia catappa*, EEHITC- Ethanolic extract of *Hemidesmus indicus* and *Terminalia catappa*.

Figure.1. Carrageenan induced paw edema.

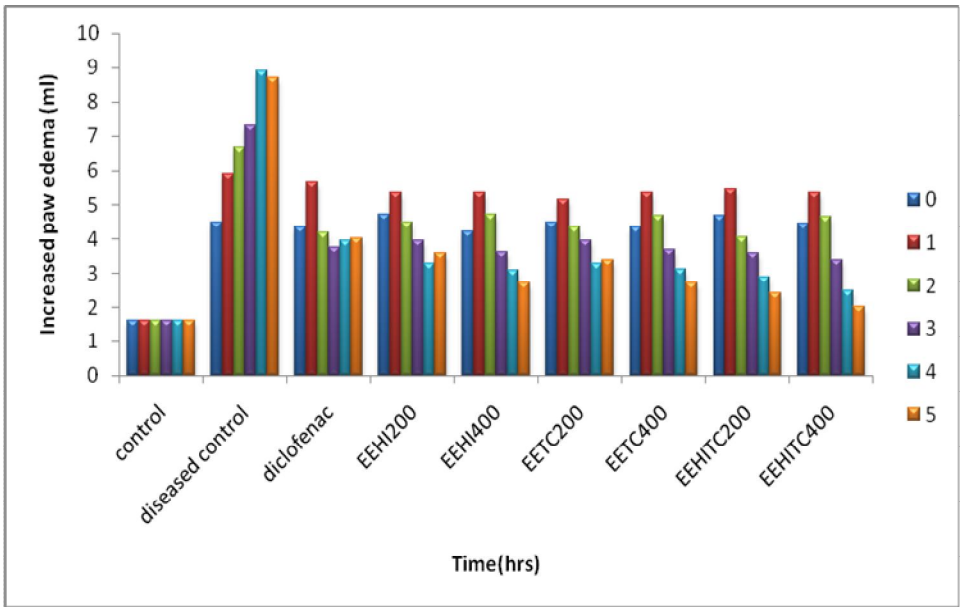

Figure.2. Effect of EEHI, EETC and their combination on carrageenan induced paw edema in rats.