Short Communication

Anti-inflammatory and immunomodulation activity of detox water in experimental animals

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Abstract

Objective: Detox water is now days essential to detoxify the body and boosting immune system. The present study was carried out to evaluate the anti-inflammatory effect and immunomodulation activity of detox water in experimental animals. **Material and methods:** The animals were randomized into four groups consist of six animals per group. The control group- saline, standard group-diclofenac (10 mg/kg, p.o.), treatment group received detox water single dose and twice a dose respectively in carrageenan induced inflammation and carbon clearance test in rats. **Results and conclusion**: Study indicates detox water twice a dose shows significant ($p>0.01$) anti-inflammatory activity by reducing paw edema and significant ($p>0.01$) phagocytic index (Macrophage activation) than control and single dose of detox water group.

Keyword: Anti-inflammatory, immunomodulation, edema, detox water

Introduction

Herbal therapy, although still an unwritten science, is well established in some countries and traditions and has become a way of life in almost 80% of population in rural areas. Chronic inflammatory diseases including rheumatoid arthritis are still one of the main health problems of the world's population (Yesilada *et al.,* 1997). At present, although synthetic drugs are dominating the market but element of toxicity that these drugs, cannot be ruled out. Their prolonged use may cause severe adverse effects on chronic administration. the most common being gastrointestinal bleeding and peptic ulcers. Consequently there is a need to develop a new anti-inflammatory therapy with minimum side effects (Corley *et al.,* 2003). Search for safe and effective anti-inflammatory activity have been given priority in scientific research in herbal system of medicine.

Immunomodulators are the substances which modify the activity of the immune system. Immunomodulators have biphasic effects, some tend to stimulate immune system which is low while others inhibit host parameters which are normal or already activated (Deharo, *et al.,* 2004) Immunostimulation and immunosuppression both need to be tackled in order to regulate the normal immunological functioning. Hence, both immunostimulating and immunosuppressing agents have their own standing and search for better agents exerting these activities is becoming the field of major interest all over the world (Patwardhan *et al.,*1990) Immunomodulation using plant material can provide an alternative to conventional chemotherapy for a variety of diseases, especially when the host defense mechanism has to be activated under the condition of impaired immune response (Srikumar *et al.,* 2006) Traditional and folklore medicines play an important role in health services around the globe. Therefore, the present study we planned to evaluate detox water for anti-inflammatory and immunomodulation activities.

Materials and methods

Experimental animals

Male Swiss albino rats (150-200 g) and mice (20-25 g) were procured from Biogen Laboratory Animal Facility Bengaluru. The animals were housed in polypropylene

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cages under standard conditions of temperature (22±2°C) and relative humidity (30-50%) with a 12:12 light: dark cycle. The animals were fed with standard pellet diet (Champaka Feeds and Foods, Bengaluru) and water *ad libitum*. The Institutional Animal Ethics Committee (IAEC) of ABMRCP (IAEC/ABMRCP/2019/16) approved the proposal.

Preparation of Detox water

The detox water was prepared freshly every day using the half piece of cucumber, half lemon, 10-12 mint leaves soaked in 200 ml purified water and kept overnight. Next morning just warmed for 5 min and administered to animals.

Anti-inflammatory activity

Carrageenan-induced rat paw edema

The animals were randomized into four groups consist of six animals per group. The control group- saline, standard group-Diclofenac (10 mg/kg, p.o), treatment group received detox water single dose and twice a dose respectively. Animals were fasted for 24 h before the experiment with free access to water. Approximately 0.1 ml of a 1% suspension of carrageenan in saline was prepared l hour before each experiment and injected into the plantar surface of right hind paw of rat. Paw volume was measured immediately after carrageenan injection and at l, 2, 4 and 6 h intervals after the administration of the noxious agent by using a plethysmometer (Sumana, 2016)

Immunomodulatory activity

Carbon clearance tests

The mice were randomized into four groups consist of six animals per group. The control group- saline, standard group-Levamisole (25 mg/kg, p.o.), treatment group received detox water single dose and twice a dose respectively for 5 days. After 48 h of the last dose of the drug, animals were injected with 0.1 ml of Indian ink via the tail vein. Blood samples were withdrawn at 0 and l5 min after injection. A 50 µL blood sample was mixed with 4 ml of 0.1% sodium carbonate solution and the absorbance of this solution was determined at 660 nm (Phatru *et al*.,2010)

The phagocytic index K was calculated using the following equation:

K: (Log OD1-Log OD2)/l5

Where OD1 and OD2 are the optical densities at 0 and 15 min respectively

Statistical analysis

Data were expressed as mean ± standard error of mean. Statistical comparisons were made by

using one-way ANOVA followed by Dunnet multiple comparison test. The results were considered statistically significant if $P < 0.01$.

Results

The data revealed that with all the treatments the rate of paw volume was decreased in mice when compared to vehicle control. The paw volume decreased significant ($p< 0.01$) with twice dose of detox water and Diclofenac (10 mg/kg, p.o.) at 4^{th} and 6^{th} hour and results were represented in table 1. This indicates inhibition of inflammation mediators.

The results reveal that with all the treatments the rate of clearance of carbon particles was increased in mice when compared to vehicle control but the Phagocytic Index was increased significant ($p< 0.01$) with twice dose of detox water and Levamisole (25 mg/kg, p.o.) and results depicted in table 2. This indicates stimulation of the reticuloendothelial system.

Discussion

Carrageenan-induced acute inflammation is one of the most suitable methods to screen anti-inflammatory agents. The time course of edema development in carrageenan-induced paw edema model in rats is generally represented by a biphasic curve (Vinegar et al., 1969). The first phase of

Table 1. Anti-inflammatory activity of detox water by carrageenan induced paw edema in rats

Groups	Paw volume (ml)				
	0 h	1 h	2 h	4 h	6 h
Vehicle control (2 ml/kg, p.o.)	0.72±0.04	1.30±0.06	1.53±0.05	1.58±0.04	1.72±0.06
Diclofenac (10 mg/kg, p.o.)	0.82±0.02	1.12±0.04	1.02±0.03*	1.08±0.04**	1.00±0.03**
Detox water (2.5 ml /kg, p.o.) per day	0.75±0.05	1.05±0.08	1.32±0.04	1.26±0.06*	1.24±0.06*
Detox water (2.5 ml /kg, p.o.) twice day	0.86±0.05	0.92±0.06*	1.09±0.09*	1.12±0.04**	1.06±0.03**

All values are expressed in Mean ± SEM; The results were analyzed using Prism, version-5. One way analysis of variance (ANOVA) test followed by Dunnett's test was used to analyze the results, * $p< 0.05$ ** $p< 0.01$ Vs control.

Table 2. Immunomodulatory activity of detox water by carbon clearance test

Groups	Phagocytic Index
Vehicle control (2 ml/kg, p.o.)	0.011± 0.025
Levamisole (25 mg/kg, p.o.)	0.019± 0.023**
Detox water (2.5 ml/kg, p.o.) per day	0.014± 0.052*
Detox water (2.5 ml/kg, p.o.) twice day	0.017± 0.042**

All values are expressed in Mean ± SEM; The results were analyzed using Prism, version-5. One way analysis of variance (ANOVA) test followed by Dunnett's test was used to analyze the results, * $p< 0.01$, ** $p< 0.01$ Vs control.

inflammation occurs within an hour of carrageenan injection and is partly due to the trauma of injection and also due to other inflammatory mediators like histamine and serotonin component. Detox water treatment shows inhibition of paw edema. The carbon clearance test was done to evaluate the effect of drugs on the reticulo-endothelial system. The reticulo-endothelial system (RES) is a diffuse system consisting of phagocytic cells. Cells of the RES play a vital role in the clearance of particles from the bloodstream. When colloidal carbon particles in the form of ink are injected directly into the systemic circulation, the rate of clearance of carbon from the blood by macrophage is governed by an exponential equation (Gokhale et al.,2003). Since Detox water treatment showed remarkable augmentation in the phagocytic index, it is speculated that it might be due to increase in the activity of the reticulo-endothelial system by prior treatment of animals with detox water.

Conclusion

Study indicates that twice dose of Detox water treatment shows the significant anti-inflammatory activity by carrageenan induced inflammation than other groups. Also it shows significant phagocytic index (Macrophage activation) than vehicle control and single dose of detox water treatment group.

Conflict of interest

None

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