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IN VITRO ANTI–INFLAMMATORY ACTIVITY OF TANKANA CHURNA

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ABSTRACT: Tankan churna is an ayurvedic medicine which is used as anti-inflammatory agent. Therefore, our investigation was aimed to screen anti–inflammatory activity of Tankan churna by membrane stabilizing and protein inhibitory methods. The prevention of hypotonicity induced human red blood cells (HRBC) membrane lysis and protein inhibition was taken as a measure of the anti-inflammatory activity. The potency of the Tankana churna was compared with standard diclofenac sodium. Tankan churna showed significant membrane stabilizing activity of 72.22% and protein inhibition activity of 94.65% at concentration of 200 µg/mL.

Key words: *Tankan churna, membrane stabilizing activity, protein inhibitory activity, diclofenac sodium*

INTRODUCTION

Inflammation is the reaction of living tissues to injury, infection or irritation. Bacterial infections cause an increased number of neutrophils, which produce an oxidative burst at the site of microbial invasion. The uncontrolled relase of reactive oxygen species is assumed to be responsible for certain pathological conditions as heart attacks, septic shocks and rheumatoid arthritis (Vane et al., 1995).

Tankan churna, an ayurvedic formulation, consists of Borax Ash. Tankana chruna is traditionally used as anti–inflammatory agent in a form of dusting powder to treat ulcers, as it acts antiseptic and astringent. Internally it is used with honey to heal cough, acidity, diarrhea and chronic bronchitis (Anonymous, 2000). In Ayurveda, the aconite toxicity is treated by giving Tankan (Borax) and ghee or mixture of turmeric juice, borax and ghee to the patient (Sajan Shyaula, 2011).

Erythrocytes have been used as a model system by a number of scientists to investtigate interaction of drugs with membranes (Sessa and Weisman, 1968; Litman et al., 1976; Oyedepo and Famurewa, 1995).

Drugs, like anesthetics, tranquilizers and non–steroidal anti–inflammatories, stabilize erythrocytes against hypotonic–induced stress haemolysis. Therefore, they prevent the release of haemoglobin as a result of their membrane stabilizing activity (Seeman, 1972). This membrane stabilizing activity of red blood cells (RBC) that are exhibited by some drugs is used for *in vitro* method for assessing the anti–inflammatory activity of various compounds (Naibi et al., 1985).

There is no scientific evidence that Tankan churna may act as an anti–inflammatory agent. Therefore, the objective of this work was to provide the scientific proof by carrying out preliminary anti–inflammatory

screening using membrane stabilizing and protein inhibitory methods.

MATERIALS AND METHODS

Dose: 200–500 mg, two or three doses a day with honey for internal use.

Preparation of Red Blood Cells (RBCs) Suspension

Fresh whole human blood (10 mL) was collected and transferred to the heparinnized centrifuged tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline (Sadique et al., 1989).

Membrane Stabilizing Activity (Heat Induced Haemolysis)

The reaction mixture contained aqueous solution of Tankan churna (20–200 µg/mL) and standard diclofenac sodium (50–100 µg/mL) and 1 mL of 10% RBCs suspension. Instead of drug only saline was added to the control test tube. Tubes containing reaction mixture were incubated in a water bath at 56 ^{o}C for 30 min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was measured at 560 nm. Membrane stabilizing activity (in %) was calculated by the following formula (Shinde et al., 1999).

Inhibition (%) = [100 - (Optical Density of control - Optical Density of test) / Optical Density of control] x 100

Protein Inhibitory Activity

The reaction mixtures contained 0.5 mL trypsin and chymotrypsin (8.000 Armour units of enzyme activity), 1.0 mL 25 mM tris–HCl buffer (pH 7.4) and 1.0 mL aqueous solution of Tankan churna (20–200 µg/mL) and standard diclofenac sodium (50–100 µg/mL). The mixtures were incubated at 37 °C for 5 minutes. Then 1.0 mL of 0.8% (w/v) casein was added. The mixtures were incubated for the additional 20 minutes. 2.0 mL of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged. Absorbance of the supernatant was measured at 280 nm against buffer as a blank (Chatterjee & Das,1996). Protein inhibitory activity (in %) is calculated as follows:

Protein inhibitory activity (%) = [100 – (O.D. of control – O.D. of test) / O.D. of control] x 100

where O.D. is optical density.

RESULTS AND DISCUSSION

The aqueous solution of Tankan churna exhibited membrane stabilization effect by inhibiting hypotonic induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane (Chou, 1997) and its stabilization implies that the extract may also well stabilize lysosomal membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bacterial enzymes and proteases which cause further tissue inflammation and damage (Murugasan et al., 1981). From the obtained results (table 1) it was concluded that the Tankan churna aqueous extract has significant membrane stabilizing activity which was comparable to the standard diclofenac sodium. The aqueous solution of Tankan churna was effecttive in inhibiting the heat induced hemolysis of erythrocyte membrane and its effectiveness was dose–dependent. This fact provides an evidence for membrane stabilization as an additional mechanism of its anti–inflammatory effect. This extract may possibly inhibit the release of lysosomal content of neutronphils at the site of inflammation. The neutronphil lysosomal constituents include bactericidal enzymes and proteases, which upon extracellular release cause further tissue inflammation and damage. It showed the maximum inhibition of 72.22% at 200 µg/mL. Diclofenac sodium, standard drug showed the maximum inhibition of 82.14% at 100 µg/mL. Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a rich source of proteinases.

Table 1.
In vitro anti–inflammatory activity of Tankan churna

Treatment	Concentration (µg/mL)	Membrane stabilizing activity (%)	Protein inhibition (%)
Tankan churna	20	16.66	67.927
	40	25.00	68.86
	60	32.01	70.44
	80	47.22	72.32
	100	54.16	82.07
	200	72.22	94.64
Diclofenac sodium	50	73.43	94.65
	100	82.14	99.71

They contain many neutral serine proteinases in their lysosomal granules. Leukocyte proteinases play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors (Das & Chatterjee, 1995). Tankan churna at the concentration of 200 µg/mL exhibited significant anti–proteinase activity (94.64%) (Table 1).

CONCLUSION

Tankan churna is used as an anti–inflammatory agent in ayurvedic treatment. The results obtained using *in vitro* studies confirm Tankan churna's capacity as a traditionnal drug.

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IN VITRO АНТИ–ИНФЛАМАТОРНА АКТИВНОСТ БИЉКЕ TANKANA CHURNA

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Сажетак: Tankan churna је ајурведски лек који се користи као анти–инфламаторно средство. Стога је циљ нашег истраживања био испитивање анти–инфламаторне активности Tankan churna, коришћењем метода за одређивање стабилизације мембране и инхибиције протеина. Спречавање хипотонично изазване разградње мембране хуманих црвених крвних зрнаца и инхибиција протеина, представљали су меру анти–инфламаторне активности. Потенцијал Tankan churne је упоређен са стандардом, натријум дихлорфенаком. Tankan churna је показала значајну активност стабилизације мембране од 72,22% и активност инхибиције протеина од 94,65% при концентрацији од 200 µg/ml.

Кључне речи: *Tankan churna, активност стабилизације мембране, активност инхибиције протеина, натријум дихлорфенак*

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