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IN-VITRO ANTI- INFLAMMATORY ACTIVITY OF COMBINED METHANOLIC EXTRACTS OF *TERMINALIA CHEBULA* AND *ALLIUM SATIVUM*

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ABSTRACT

The combine bi- herbal methanolic extract made up of equal quantities of *Terminalia chebula* and *Allium sativum.* The combined bi- herbal methanolic extract was evaluated for its individual methanolic extracts of *Terminalia chebula* and *Allium sativum.* The extracts were screened for anti- inflammatory activity by Human red blood cell membrane stabilization method (HRBC). Paracetamol was taken as reference drug. Results revealed that the bi-herbal methanolic extract possesses significant anti- inflammatory activity at 200µg/ml. The individual methanolic extract of *Allium sativum* showed more activity when compared to that of the *Terminalia chebula*. The plant extracts showed dose dependent activity.

KEY WORDS: *Terminalia chebula*, Anti- inflammatory, *Allium sativum*, Combined, Human red blood cell membrane stabilization.

INTRODUCTION

Inflammation is defined as the local response of living mammalian tissues to injury from any agent. It is a body protective response in order to eliminate or suppress the spread of injurious agent, followed by removal of necrosed cells and tissues. The injurious agents causing inflammation may be as infective agents, immunological agents, physical agents, chemical

agents and inert materials.[1] Inflammation can be classified as acute and chronic inflammation depending upon the capacity of host cells and duration of response. Acute inflammation is of short duration involved in vascular changes which are associated with increased vascular permeability, capillary infiltration and migration of leukocyte. Chronic inflammation is of long-term which is associated with infiltration of mononuclear immune cells, macrophages, monocytes, neutrophils, fibroblast activation proliferation and fibrosis.[1, 2] Non-steroidal drugs (NSAIDs) are usually prescribed which help in relieving pain and inflammation.[3] *Terminalia chebula* which belongs to the family combretaceae. It is found in the sub – Himalayan traces and in all deciduous forests of India. It grows an altitude of 1800m, 15-25m height and 1.5-2.5m in diameter. The fruits are fully matured and yellow to brownish colure. Terminalia chebula also called as "king of medicine" it is used in herbal formulation of triphala.[8] The pharmacological effects are anti- diabetic activity, cardio tonic activity, anti- microbial, anti- ulcerative and anti- oxidant properties.[4, 5] The garlic consists of bulbs of the plant known as *Allium sativumlinn* which belongs to the family liliaceaae. It is easily available in almost all the states. Garlic is a hard perennial with narrow flat leaves and bears white small flowers and bulbils.

The pharmacological uses of garlic are vermifuge, diuretic, antioxidant, antimicrobial, carminative, expectorant and stimulant.[4, 6] Natural products which are derived from plants impart compulsive optimistic etymology for isolating and evolve therapeutic molecules which are used to cure various diseases. Natural products have less side effects and more beneficial uses.[7]

MATERIALS AND METHODS

Drugs and chemicals

Paracetamol, Sodium chloride, Potassium chloride, Potassium di- hydrogen phosphate, di-sodium hydrogen phosphate, Alsever solution and methanol.

Collection of plant material

The fresh bulbs of *Allium sativum* and seeds of *Terminalia chebula* were purchased from local market in Gopalpatnam, Visakhapatnam.

Extraction of plant material

Fresh bulbs of *Allium sativum* and seeds of *Terminalia chebula* were taken and thoroughly cleaned with water and dried under shade for five to ten days until it become grindable. The

dried materials were grounded into moderate powder with the help of electric grinder. The powders were taken in two separate round bottomed flask in equal ratio to this methanol is added and they were subjected to maceration process for two to three days and then followed by soxhelation. Solvent is separated from the extract by simple distillation method. The obtained extract is taken in test tubes and allowed for dryness, weighed and kept in refrigerator for further analysis.

Evaluation of In-vitro anti-inflammatory activity

Human red blood cell (HRBC) membrane stabilization method

This is done as per the method of Althaf Faimum D et.al with major modifications.[9] The HRBC membrane stabilization method has been used to study in-vitro anti- inflammatory activity. Blood was taken from healthy human volunteer who was not taken any NSAIDs for 2weeks prior to the experiment. The collected blood was mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride in water) and centrifuged at 3000rpm. The bottom settled cells were washed with isosaline and a 10% suspension was made. Various concentrations of bi-herbal extracts& individual methanolic extracts (100,200,300µg/ml) were prepared using distilled water and to each concentration add 1ml of Phosphate buffer solution (PBS) of pH 7.4, 2ml of hyposaline and 0.5ml of HRBC suspension were added. The concentrations were incubated at 37ºc for 30min and centrifuged at 3000rpm for 20min. the hemoglobin content in the supernatant solution was estimated by using UV- spectrophotometer at 560nm. Paracetamol was taken as the reference drug and control was prepared by omitting the extracts. The experiment was performed in triplicates. Percent inhibition of HRBC membrane stabilization was calculated by using the following formula.

$$\%\text{inhibition} = 100 \times OD1 - OD2 / OD1$$

Where OD1 & OD2 are absorbance of reference drugs and test extracts respectively

RESULTS AND DISCUSSION

The inhibition of HRBC membrane stabilization method was taken as a measure of the anti-inflammatory activity. The percent inhibition of membrane stabilization of bi- herbal and individual methanolic extracts of *Terminalia chebula* and *Allium sativum* were done at different concentrations (100,200,300µg/ml). Reference drug is taken at a concentration of 200µg/ml. The bi-herbal methanolic extract showed its optimum activity at a concentration

200µg/ml as shown in table 1. By increasing the concentration of the extract it shows better activity. The plant extracts showed dose dependent activity.

Table1 1: In-vitro anti – inflammatory activity of combined methanolic extracts of *Terminalia chebula* and Allium *sativum*.

EXTRACTS	CONCENTRATIONS(µg/ml)	(%) PERCENT OF INHIBITION
GARLIC	100 200 300	41 52 53
MYROBALAN	100 200 300	18 27 30
BI-HERBAL METHANOLIC EXTRACT	100 200 300	48 67 60
REFERENCE DRUG	100	74

CONCLUSION

On the basis of the above principals it can be concluded that the bi- herbal methanolic extract possess better anti-inflammatory activity which may be due to the presence of active constituents present in it. Further studies are required for isolation of bioactive compounds and in-vivo activity of the bi-herbal extracts needs to be evaluated.

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