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IN-VITRO AND *IN-VIVO* ANTI-INFLAMMATORY POTENTIAL OF *TABERNAEMONTANA DIVARICATA* LEAVES

Sumithra M[*1], Chitra.V[1], Anbu J[2], Arijit Chakraborty[2]

[1]SRM College of Pharmacy, SRM University, Kattangulathur.

[2]Vels University, School of Pharmaceutical Sciences, Pallavaram.

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***Correspondence for Author:**

Mrs. M.Sumithra,

M.Pharmacology, Lecturer

Dept of Pharmacology,

SRM College of Pharmacy,

SRM University.

sumi26379@gmail.com

ABSTRACT

Tabernaemontana divaricata is an evergreen shrub traditionally used as Anti-inflammatory, analgesics and sedative effect. In the current study, an attempt has been made to prove the anti-inflammatory potential of *Tabernaemontana divaricata* leaves in in-vitro Human red blood cell membrane stabilization method, and in-vivo carrageenin induced anti-inflammatory model. The doses were fixed after performing acute toxicity study according to the OECD guideline 423. Diclofenac sodium was administered as standard drug for both in-vitro and in-vivo study. In case of in-vitro study blood samples were collected and centrifuged. Then supernatant fluid was collected and mixed with standard and test drugs. Then the reaction mixture was centrifuged and collected supernatant was subjected for UV analysis at 560 nm and percentage inhibition of Haemolysis was calculated. In in-vivo method carrageenin induced paw edema model was evaluated using rats. 200mg/kg and 400mg/kg dose were orally given to the test groups once daily for a month. Edema was produced injection of carrageenin by sub-plantar route in the right hind paw of each rat one hour after the administration of corresponding drugs. The paw volume was measured by using the plethysmometer. Mean increase in the volume of oedema was measured and the percentage inhibition was calculated. After performing those two models, we can conclude that the ethanolic extract of *Tabernaemontana divaricata* leaves was reduced the inflammation as compared with the standard drug diclofenac sodium.

Keywords: Carrageenin, Diclofenac, Tabernaemontana, Human RBC membrane stabilization, Polymorph nuclear cells.

INTRODUCTION

Plants are well known as a major source of modern medicines. From ancient times, humans have utilized plants for the treatment or prevention of diseases, leading to the dawn of traditional medicine. Tabernaemontana is one of the genera that are used in Chinese, Ayurvedic and Thai traditional medicine for the treatment of fever, pain and dysentery. Tabernaemontana plants are widely distributed in Thailand. Species found in Thailand are T. bufalina, T. crispa, T. divaricata, T. pandacaqui, T. pauciflora and T. rostrata. One of the most interesting species is Tabernaemontana divaricata (L). Growing evidence suggests that this plant has medicinal benefits and its extracts could possibly be used as pharmacological interventions in various diseases. In this review, information regarding ethno botany, ethno pharmacology and therapeutic benefits of T. divaricata is discussed [1-6]. Inflammation is a local, protective response to microbial invasion or injury. It must be fine-tuned and regulated precisely, because deficiencies or excesses of the inflammatory response cause morbidity and shorten lifespan. Inflammation spreads into the bloodstream, as occurs in septic shock syndrome, sepsis, meningitis and severe trauma; the inflammatory responses can be more dangerous than the original inciting stimulus. Homeostasis and health are restored when inflammations limited by anti-inflammatory responses that are redundant, rapid, reversible, localized, adaptive to changes in input and integrated by the nervous system.

Description and taxonomy of T. divaricata

T. divaricata belongs to the Apocynaceae family, Plumeroidae subfamily, Tabermontanae tribe and Tabernaemontana genus. The generic synonym, Ervatamia, is widely distributed in tropical countries as a garden plant, which usually has sweet-scented double flowers [7].

Phytochemistry of T. divaricata

T. divaricata has been used in traditional medicine and for other purposes. The phytochemistry and a number of chemical constituents from the leaves, stems, and roots have been reported previously. Constituents studied include alkaloids and non-alkaloid constituents such as terpenoids, steroids, flavonoids, phenyl propanoids, phenolic acids and enzymes, 66 different alkaloids of T. divaricata have been identified. The phytochemical data for each alkaloid provide information about its biosynthesis. Such information can assist in the search for new, medically interesting compounds that may be useful against diseases [8-16].

MATERIAL & METHODS

Plant material: Leaves of T. divaricata collected from the local area. Dry those leaves for 2 weeks.

In-vitro Anti – Inflammatory Activity

The Human Red Blood Cells (HRBC) membrane stabilization has been used as a method to study the anti-inflammatory activity.

Preparation of alsever solution

Alsever solution prepared by 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride dissolved in distilled water and sterilized.

Procedure

Blood was collected from healthy rats. The collected blood was mixed with equal volume of sterilized alsever solution. The blood was centrifuged at 3000 rpm and packed cells were washed with Isosaline and a 10% (v/v) suspension was made with isosaline. The two extracts taken separately (concentration as mentioned in table), to each extract added 1 ml phosphate buffer, 2 ml of hypo saline and 0.5 ml HRBC suspension. Diclofenac sodium was used as the reference drug. Instead of hypo saline, 2 ml of distilled water was used in the control. The assay mixtures were incubated at 37^0C for 30 minutes and centrifuged. The haemoglobin content in the supernatant solution was estimated using UV analysis at 560 nm. The percentage haemolysis was calculated by assuming the haemolysis produced in the presence of distilled water as 100%. [17]

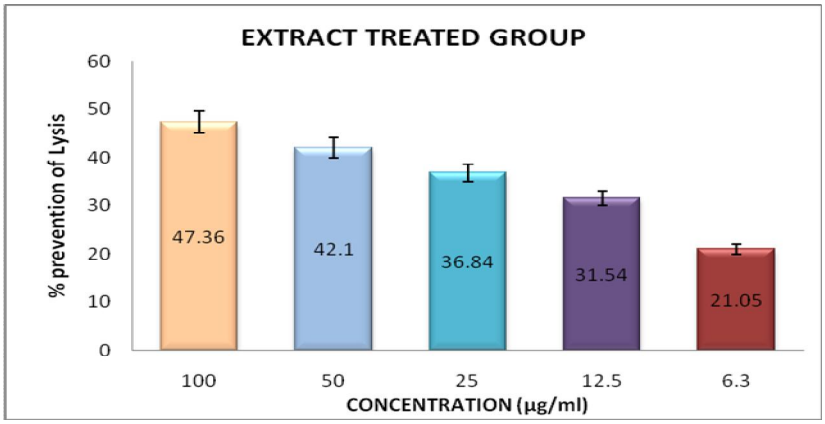
The percentage of HRBC membrane stabilization or protection was calculated using the formula

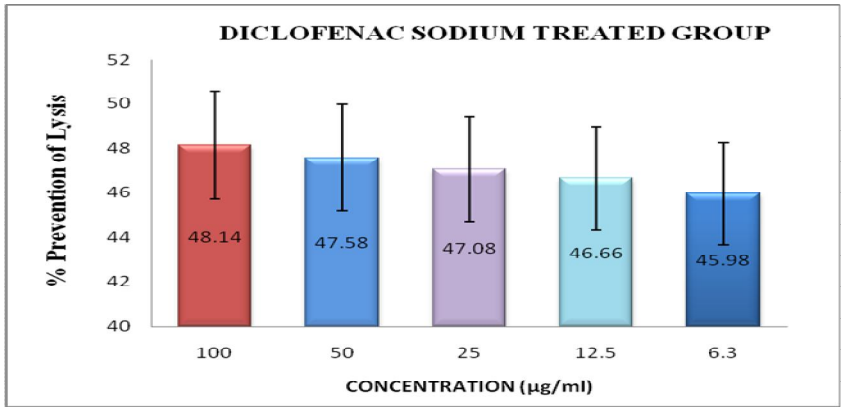
$$\text{Percentage Inhibition of Haemolysis} = 100 - \frac{\text{O.D of Test Solution} - \text{O.D of Product Control}}{\text{O.D of Test Control}} \times 100$$

Table: 1 Effect of Ethanolic Extract of Tabernaemontana divaricata on Human erythrocyte haemolysis by in-vitro model.

Concentration (µg/ml)	% Prevention of Lysis	
	Extracts	Diclofenac Sodium
100	47.36	48.14
50	42.1	47.58
25	36.84	47.08
15.5	31.54	46.66
6.3	21.05	45.98

Graph between % prevention of Lysis vs. drug concentration of extract treated group and control drug treated group:





IN-VIVO ANTI-INFLAMMATORY ACTIVITY

ACUTE TOXICITY STUDY

According to OECD guideline-423, acute toxicity study was done. The total extract of Tabernaemontana divaricata leaves were administered orally to the different group of rats. No deviation was observed from any of the group within span of 24 hrs. There is no science of toxicity occurred up to the dose of 2000 mg/kg body weight. Based on the result obtained from this study, the dose for anti-inflammatory was fixed to be 200 mg/kg and 400 mg/kg for dose dependent study.

METHOD

Carrageenin induced paw edema method has been used to study the anti-inflammatory activity.

ANIMALS

Swiss albino rats (150-200g) were used. They were housed in standard microloan boxes and were given standard laboratory diet and water.

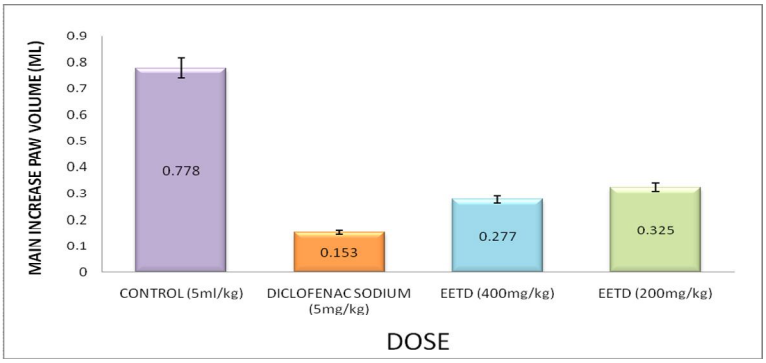
METHOD

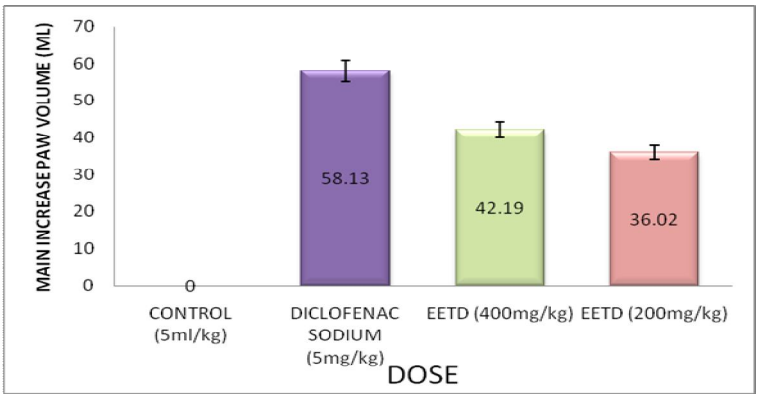
The rats were divided in to three groups (n=6) and the first group treated with saline (5ml/kg), second group treated with diclofenac sodium (mg/kg) and third group treated with Extract (400mg/kg) orally. Edema was produced by sub-plantar injection of carrageen an in the right hind paw of each rat one hour after the administration of corresponding drugs. The paw volume was measured by using the plethysmometer. Mean increase in the volume of oedema was measured and the percentage inhibition was calculated [18].

Table: 2 Effect of Ethanolic Extract of Tabernaemontana divaricata by Paw edema method.

TREATMENT	DOSE (mg/kg)	MEAN INCREASE IN PAW VOLUME(ml)	PERCENTAGE INHIBITION
Control	5ml/kg	0.778+.056	-
Diclofenac sodium	5mg/kg	0.153+0.034	58.13
EETD	400mg/kg	0.277+0.023	42.19
EETD	200 mg/kg	0.325+0.081	36.02

Graph between mean increases in paw volume (ml) vs. dose (mg/kg) & percentage reduction vs. dose:





DISCUSSION

The main action of anti-inflammatory agents was to inhibit the Cyclooxygenase enzyme. Cyclooxygenase enzyme is responsible for conversion of arachidonic acid to prostaglandin (PG). The cyclooxgenase enzymes are bifunction the main action conversion of PGG_2 to PGH_2 and also peroxidase action both COX-1 and COX-2 enzymes which are associated with membranes, consist of long channels. These channels opening occur due to release of chemical mediations and so arachidonic acid released from membrane and converted to prostaglandin. The extracellular activity of this enzyme is said to be related to acute and chronic inflammation.NSAID'S act either by inhibiting these lysosomal enzymes (cyclooxygenase) (or) by stabilizing the lysosomal membrane.[19]

The extract at concentration range of 6 -100 mg/ml protect the human erythrocyte membrane against lysis induced by hypotonic solution. At concentration of 100 mg/ml, the extract produced 47.36 %inhibition of RBC haemolysis as compared with 48.14% produced by diclofenac sodium (Table 1).

Since HRBC membranes are similar to lysosomal membrane components, the prevention of hypotonicity – induced HRBC membrane lysis was taken as a measure of anti-inflammatory activity of drugs. It is well known that vitality of cells depends on the integrity of their membranes. Exposure of RBC to injurious substances such as hypotonic medium, methyl salicylate, phenylhydrazine results in the lysis of its membrane accompanied by haemolysis and oxidation of hemoglobin. The hemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Since injury to RBC membrane will further render the cell more susceptible to secondary damage through free radical – induced lipid per oxidation [20-22].

This action is consistent with the observation that breakdown of bimolecules leads to the formation of free radicals which in turn enhance cellular damage. The progression of bone destruction seen in rheumatoid arthritis has been shown due to increased free radical activity. It is therefore expected that compounds of cell membrane against injurious substances [23-26].

Extract with membrane stabilizing properties are well known for their activity to interfere with the early phase of the release of inflammatory reactions, namely the prevention of the release of phospholipases that trigger the formation of inflammatory mediators. The

development of carrageenin induced oedema is biphasic; the first phase is attributed to the release of histamine, 5HT & kinins, while the second phase is related to the release of prostaglandins. Carrageenin induces paw edema by inducing protein exudates containing a large number of neutrophils [27-30].

CONCLUSION

The result suggests that the extract becomes significant within three hour, during the phagocyte phase of carrageenan induced inflammation, when the mast cells release cytoplasmic enzymes and serotonin. Superoxide is known to participate in the formation of chemotactic factors and the recruitment of polymorph nuclear cells (PMNs). Hence the studies indicated that *Tabernemontana divaricata* leaf extract which coule scavenge the superoxide anion, might inhibit the recruitment of PMNs and thereby reduce inflammation.

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