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Anti-inflammatory potential of flavonoids from *Hemigraphis colorata*

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ABSTRACT

Agents that inhibit inflammatory mediators are highly recommended in the development of anti-inflammatory drugs. Flavonoids are large class of secondary metabolites around 10,000 structures, possess anti-inflammatory and various other properties which are valuable to human beings. In this study, different fractions of *Hemigraphis colorata* were collected based on increasing polarity and quantified the flavonoid content. Anti-inflammatory property was evaluated both in vitro and in vivo method. The chloroform fraction possess highest amount of flavonoid, 6.66 µg/100 µg sample. In the in vitro experiment acetone fraction possess maximum activity. In the in vivo experiment, acetone extract showed 43% and 48% of inhibition at 250 & 500 mg/kg b.wt. respectively against carrageenan induced paw oedema, while standard anti-inflammatory Diclophenac(10mg/Kg) exhibit 52.5 % inhibition.

Key words : *Hemigraphis colorata*, flavonoids, anti-inflammatory, diclophenac

INTRODUCTION

Inflammation is a normal protective response to tissue injury and it involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair (Vane *et al*. 1995). It is a complex process, which is frequently associated with pain and involves occurrences such as: the increase in vascular permeability, increase of protein denaturation and membrane alterations (Umapathy *et al*. 2010). Harmful stimuli including pathogens, irritants or damaged cells initiate response of vascular tissue as inflammation. Inflammation is a protective attempt by the organism to remove injurious stimuli as well as initiate the healing process for the tissue (Denko1992). However, if inflammation is not treated it leads to onset of diseases like vasomotor rhinorrhoea, rheumatoid arthritis and atherosclerosis (Henson *et al*. 1989).

Prostaglandin endoperoxide synthase, commonly called cyclooxygenase (COX), is the key enzyme required for the conversion of arachidonic acid to prostaglandins. Prostaglandins are the important mediators of inflammation (Marco E. Turini and Raymond N. DuBois 2002). COX 1 & COX 2 are the two isoforms of COX. COXs are the target of the nonsteroidal anti-inflammatory drugs (NSAID), which play a primary therapeutic role in treatment of pain,fever and inflammation (Vane, J. R. 1971). The discovery and characterization of COX-2 have answered some long-standing puzzles and created new and fascinating problems in biology. They have also solved one problem in therapeutics, how to suppress inflammation without the side effects of the present range of NSAIDs. These side effects such as gastrointestinal ulceration and bleeding, renal damage, and platelet dysfunction were accepted as inevitable consequences of the inhibition of COX activity. Selective inhibitors of COX-2 offered the possibility of inhibition of inflammatory PGs without affecting PGs generated by COX-1. This possibility has generated a great deal of effort and a considerable degree of success in pharmaceutical research.

Flavonoids are a large class of secondary metabolites encompassing more than 10,000 structures (Giovanni Agati et al.,2012). Flavonids possess antioxidant, anti-inflammatory and various other properties which are beneficial to human beings. Inflammation, the first physiological defense system in the human body, can protect against injuries caused by physical wounds, poisons, etc. This defense system, also called short-term inflammation, can destroy infectious microorganisms, eliminate irritants, and maintain normal physiological functions. However, long-term over-inflammation might cause dysfunctions of the regular physiology, i.e., asthma and rheumatic arthritis. (Chia-Jung Lee2010).Various medicinal plants possess wide variety of biological properties.

Hence an attempt was made to evaluate new anti-inflammatory sources among the plant kingdom. On the basis of literature survey *Hemigraphis colorata* was selected for the study. *Hemigraphis colorata* (Figure 1) belong to Acanthaceae, an exotic plant adapted to India, is a versatile tropical low-creeping perennial herb. It holds wound healing, antidiabetic and antibacterial activities (Devi priya 2013). It is an exotic plant adapted to India. Literally, Hemigraphis means 'half writing' because the filament of the outer stamen bear brushes (Gledhill D. 2008). The plant is known by several names such as Aluminium plant, Cemetary plant, Metal leaf, Red flame Ivy, Waffle plant, Java Ivy etc. In Kerala, the plant is popular in the name 'murikootti' or 'murian pacha' because of its incredible potency to heal wounds. The leaf has metallic purple luster on upper surface and a solid dark purple on ventral side. The leaves are opposite, ovate to cordate, serrate-crenate, about 2 to 8 cm long and 4 to 6 cm wide, bearing well-defined veins

Figure 1. *Hemigraphis colorata*

The phytoconstituents present in *H. colorata* are phenols, saponins, flavonoids, terpenoids (Sheu J *et al*. 2012), coumarins, carbohydrates, carboxylic acid, xanthoproteins, tannins, proteins, alkaloids, steroids and sterol (Saravanan *et al*.2010). The leaves of the plant contain flavonoids, polyphenols, tannins along with high potassium and low sodium levels; stem contains saponins and tannins, while roots contain flavonoids and polyphenols. These phytochemicals provide curative properties. Benzene extract of *H. colorata* leaves has showed its activity against *Acinetobacter* species and *Streptococcus aureus* (Anitha *et al*.2012). Phenolic compounds found in the extract are responsible for the activity. The steroids and coumarins present in this plant provide anti-diabetes activity (Bourdy 1992). The crude leaf paste promotes excision wound healing (Bhargavi *et al*. 2011, Pawar & Toppo 2012). In mice, the leaf paste provides faster wound contraction and epithelialisation but oral administration is seen ineffective (Subramoniam *et al*. 2001). The excision and incision wound model studies revealed that methanolic extract is comparable to standard reference Vokadine (Saravanan *et al*.2012). The herbal scaffold made from chitosan was highly haemostatic and can be effectively applied for infectious wounds (Annapoorna et *al*. 2013). Phenolic compounds are effective hydrogen donor which makes them a good antioxidant. The phenolic acids such as chlorogenate, cinnamate,

coumarate, gallate and ferulate present in the plant acts as pro-oxidants and exhibits free radical scavenging activity (Deepak *et al.*2007).

In this study, different fractions of *Hemigraphis colorata* were collected based on increasing polarity and quantified the flavonoid content. Anti-inflammatory property was evaluated both *in vitro* and *in vivo* method.

MATERIAL AND METHODS

2.1 Plant

The experimental plant *Hemigraphis colorata* was collected from herbal garden , Kerala Forest Research Institute (KFRI), Peechi, Thrissur, Kerala and botanically authenticated by Dr.N.Sasidharan, Scientist KFRI. The voucher specimen was kept in the herbarium (No 28495) of KFRI. The whole plant was used for the study. The collected plant material was washed with water and dried under shade. The dried material was chopped and powdered using mixer grinder.

2.2 Chemicals

All biochemicals were purchased from Sigma-Aldrich. Chemicals used were procured from Merck India.

2.3Extraction

Hemigraphis colorata was subjected to successive extraction with increasing polarity such as Petroleum ether, Chloroform, Acetone and Methanol in soxhlet extraction system. 50gms of *Hemigraphis colorata* was taken for Thimble preparation.300ml of solvent were used. After extraction each fraction were evaporated to dryness. From which samples were taken and dissolved in DMSO.

2.4 Total flavonoid estimation

Total flavonoid was estimated quantitatively by Aluminium Chloride Method (Chang C *et al.*2002). Quercetin was used to make the caliberation curve. 10 mg of quercetin was dissolved in 80 % ethanol and then diluted to 25,50 and 100 µg/ml. The diluted standard solution (0.5ml) were separatly mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1.0 molar potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a Shimadzu UV-1700 Spectrophotometer. The amount of 10 % aluminium chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5 ml of ethanol extracts were reacted with aluminium chloride for determination of flavonoid content as described above.

2.5 *In vitro* Anti-inflammatory activity

In vitro anti inflammatory activity was done by methods of Mizushima and Kobayashi (1968) followed with minor modifications. The reaction mixture was consisting of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount at 37^0C HCl. The sample extracts were incubated at 37^0C for 20 min and then heated to 51^0C for 20 min. after cooling the samples the turbidity was measured spectrophotometrically at 660nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as fallows,

$$\% \text{ inhibition} = \frac{\text{Abs control- Abs sample}}{\text{Abs control}} \times 100$$

Where Abs control is the absorbance without sample, Abs sample is the absorbance of sample extract/standard.

2.6 *In vivo* anti-inflammatory activity

2.6.1 Animals

The experimental animals (Balb/c mice, 20-24g weight) were purchased from Small animal Breeding Station, Kerala veterinary and Animal Science University, Mannuthy. Animals were maintained under standardized environmental conditions(22-28°C, 60-79% relative humidity, 12 hour dark/light cycle) and fed with standard rat feed (Sai durga Feeds, Banglore,India) and water. All the animal experiment conducted at Amala Cancer Research Centre, Amala Nagar, Thrissur, Kerala with prior permission from Institutional Animal Ethics Committee (IAEC) and strictly followed the guidelines of Animal Ethics Committee, Government of India.

2.6.2 Grouping of animals

Female Baib/c mice were divided into five groups comprising five animals in each group.

Group I	Control
Group II	Vehicle control – Propylene glycol
Group III	Diclophenac– Standard anti-inflammatory drug
Group IV	Drug 250mg/Kg Body weight
Group V	Drug 500mg/Kg Body weight

Group IV & V were treated with 250 & 500mg/Kg body weight of drugs and Group II treated with propylene glycol orally for five consecutive days. Group III was administered Diclophenac (10 mg/Kg body weight) intraperitoneally as standard reference drug. On fifth day acute inflammation was induced by sub-plantar injection of freshly prepared carrageenan (0.02 ml freshly prepared 1% suspension of carrageenan in 0.1% Carboxy methyl cellulose) on right hind paw in the entire group. The inflammation was measured using plethysmometer one hour before and four hours after carrageenan injection. The percentage of inhibition was calculated according to the following formula [(Vt-Vo) Control – (Vt-Vo) Treated group/(Vt-Vo) Control] x 100, were Vt is the paw oedema at various time intervals and Vo is the initial paw oedema.

3. Statistical Analysis

The values presented as mean ±SD .

RESULTS AND DISCUSSION

It is believed that current drugs available such as opoids and non-steroidal anti-inflammatory drugs (NSAIDs) are not useful in all cases of inflammatory disorders, because of their side effects and potency (Ahmadiani *et al*. 1998). As a result, a search for other alternatives seems necessary and beneficial. The study of plants that have been used traditionally for curing inflammation is still fruitful and logical research strategy in the source of new anti-inflammatory drugs (Kumarappan *et al*. 2006). Research on the biological activities of plants during the past two centuries has yielded compounds for the development of modern drugs (Arivazhagan *et* al.2000). Medicinal plants have a wide variety of chemicals from which novel anti-inflammatory agents can be discovered.

3.1 Amount of total flavonoid.

The flavonoid concentration is more in the chloroform extract in the experimental plant (Table 1). Results are expressed in 100 µg of sample.

Table 1:

Fractions	Flavonoid concentration
Petroleum ether	1.25 µg
Chloroform	6.66 µg
Acetone	2.9 µg
Methanol	0.73 µg

Table 2. *In vitro* anti-inflammatory activity

Fractions	
Petroleum ether	11 fold higher than control
Chloroform	14.25 fold higher than control
Acetone	35.76 fold higher than control
Methanol	10.44 fold higher than control

Among the fractions studied acetone fraction possess significant *in-vitro* anti-inflammatory activity (Table 2).

3.2 *In vivo* anti-inflammatory activity

Table 3. Effect of *Hemigraphis colorata* on carrageenan induced paw oedema in mice

Groups	Initial paw thickness (mm)	Paw oedema volume after 2nd hour	Increase in paw oedema volume (mm)	Percentage of inhibition (%)
Control	1.98±0.110	3.35±0.249	1.37	------
Diclophenac(10mg/Kg)	2.37±0.193	3.02±0.295	0.65	52.5
250 mg/Kg	1.99±0.082	2.77±0.103	0.78	43.06
500mg/Kg	1.89±0.080	2.6±0.181	0.71	48.1

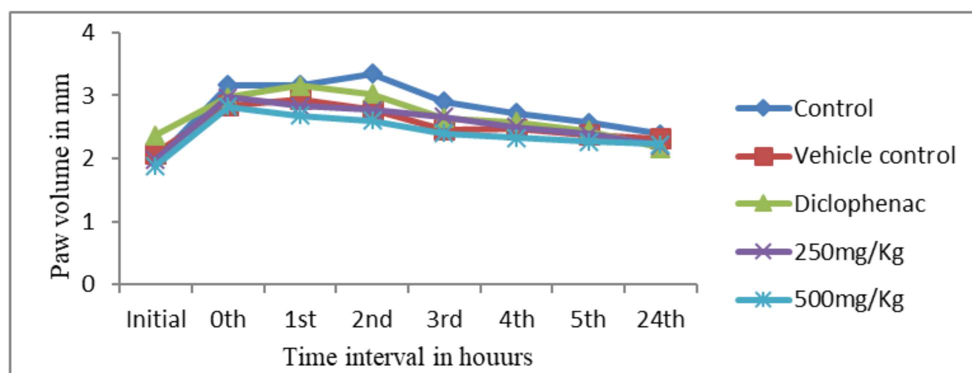

Fig. 2: Effect *of Hemigraphis colorata* on carrageenan induced paw oedema in mice

The sub-plantar injection of carrageenan in to mice elicited an inflammation that was increasing. The inflammatory response was reduced by the administration of acetone extracts of *Hemigraphis colorata* at the dose of 250 and 500 mg/Kg, 2nd hour following the carrageenan injection whereas in control and vehicle control group the decrease was minimal. The *Hemigraphis colorata* acetone extract showed 43% and 48% of inhibition at 250 & 500 mg/kg b.wt. respectively against carrageenan induced paw oedema (Table 3, Fig. 2 1).

CONCLUSION

The agents that reduce the inflammation have prime importance in the current scenario, since most widely prescribed drug worldwide is anti-inflammatory drugs. COX is the important enzyme in the inflammatory pathway and those agents that block the enzyme can be used for the synthesis of anti-inflammatory drug. In addition to COX, LOX play a pivotal role in the inflammatory pathway. Agents that block both COX and LOX are good enough to manufacture anti-inflammatory drug. In this study we conclude that flavonoids from *Hemigraphis colorata* possess significant anti-inflammatory activity. Further study is necessary to find out the mechanism of action of inhibition, the regulated enzyme either COX1, COX 2, LOX or any other inflammatory markers. Elucidate the structure of active component is highly promising.

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Declaration of interest

Authors declare no conflict of interest.

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