



ANTI-INFLAMMATORY ACTIVITY OF PIMENTA DIOICA BY MEMBRANE STABILIZATION METHOD

Siju EN^{1*}, Rajalakshmi GR², Vivek D¹, Aiswarya Lakshmi AG¹, Sheena Mathew¹,
Melbin K Joseph¹, Sabita PV¹

¹Department of Pharmacology, Academy of Pharmaceutical Science, Pariyaram Medical College, Kannur, 670503, India.

²Colleges of Pharmaceutical Science, Government Medical College, Kozhikode - 673008, India.

ABSTRACT

The present study aimed to evaluate the anti-inflammatory activity of alcoholic and aqueous extracts of *Pimenta dioica* leaves by simple, reliable, less toxic, less time consuming, HRBC membrane stabilization method. The inhibition of hypotonicity induced by HRBC membrane lysis was taken as a measure of anti-inflammatory activity.

Key words: *Pimenta dioica* L, Myrtaceae, Anti-inflammatory, HRBC membrane stabilization.

INTRODUCTION

Inflammation is the protective mechanism of the local microcirculation to tissue injury which caused by physical trauma, noxious stimuli, by heat, chemical agent, microbial effect, and antigen-antibody reaction (Mahesh G *et al.*, 2011). Nature has provided a complete store house of remedies to cure all ailments of mankind (Ravi V *et al.*, 2009). A large number of population (80% of people) have to rely upon traditional medicines, which are mainly derived from plant material. The fact is recognized by the WHO. NSAIDs act either by stabilizing the lysosomal enzymes or by lysosomal membrane. HRBC membrane is similar to lysosomal membrane, the study was undertaken to check the stability of HRBC membrane by these extracts & thereby predict the anti-inflammatory activity.

Many plants belonging to family Myrtaceae with wide range of pharmacological & biological activity & interesting phytochemical constituents. *Pimenta dioica* is one of the plant of that family. Phytochemical constituents present in this plants are phenyl propanoids and glycosides (kikuizaki *et al.*,

1999) tannins (Marzouk *et al.*, 2007), essential oil constituents like limonene, 1,8 cineole, methyl eugenol (Tucker *et al.*, 1991). It's dried, green mature fruit is the commercial flavourant and curing agent. The essential oils of *P. dioica* leaves and fruits are utilized in food industry mainly meat and tanning industries as well as in perfumery compositions and cosmetic products. In India, it is used as an aromatic stimulant and as an adjuvant to tonics and purgatives (Anonymous, 1969). In Turkey, it is used as an aphrodisiac when taken along with honey (Sekeroglu *et al.*, 2006). The therapeutic properties of the essential allspice oils are antioxidant, antiseptic, acaricidal, carminative, muscle relaxant, rubefacient, stimulant, and tonic (Sharma, 2003; Seidemann, 2005). The present study was undertaken to evaluate its efficiency in pain and inflammatory conditions.

MATERIALS & METHODS

Plant material

The leaves of *Pimenta dioica* (Family: Myrtaceae) were collected from Kannur district in Kerala, India in August 2013. The same were authenticated by Dr. Radhika C, Assistant Professor, Govt Ayurveda College, Pariyaram, Kannur, Kerala. A voucher specimen NOPOCL/01/2013/APSC was submitted at Academy of Pharmaceutical Science Pariyaram Medical College, Kannur for future reference. After collections, the leaves were dried in shade & ground mechanically

Corresponding Author

E. N. Siju

Email: sijuellickal@rediffmail.com

into coarse powder. This is kept into an air tight container.

PREPARATION OF EXTRACTS

Ethyl alcohol extract: The shade dried powdered leaves (500g) were exhaustively extracted with 95% ethanol using a soxhlet apparatus. The ethyl alcohol was concentrated in vacuum to a syrupy consistency. The percentage yield of extract was found to be 4.15 %.

Aqueous extract: The aqueous extract was prepared using fresh powder by maceration process. 100gm of the powdered drug was taken in a 2000ml conical flask with 500ml of distilled water and 10ml chloroform is added as preservative. It was extracted up to 7 days with daily 2 hours stirring with the mechanical stirrer. After 7 days the extract was filtered through the muslin cloth and the marc is discarded and airtight container in its filtrate dried under hot air oven at 45⁰C to semisolid mass. These were stored in refrigerator below 10⁰C. The percentage yield of extract was found to be 6.10 %.

Preliminary phytochemical analysis: The extracts of *Pimenta dioica* were subjected to various qualitative chemical tests for identification of its plant constituents.

METHOD

The HRBC membrane stabilization has been used as method to study the anti-inflammatory

activity (Gandhidasan *et al.*, 1991). Blood was collected from healthy volunteer who was not taken any NSAIDS for two weeks prior to the experiment. The collected blood was mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm and packed cell were washed with isosaline (0.85%.pH 7.2) and a 10 % (v/v) suspension was made with isosaline. The assay mixture contained the drug (concentration as mentioned in the table 2), 1 ml of phosphate buffer (0.15M, pH 7.4), 2 ml of hyposaline (0.36%) and 0.5ml of HRBC suspension. Diclofenac was used as reference drug. Instead of hyposaline 2ml of distilled water was used in the control. All the assay mixture were incubated at 37⁰C for 30 min and centrifuged. The hemoglobin content in the supernatant solution was estimated using spectrophotometer at 560 nm. The percentage hemolysis was calculated by assuming the hemolysis produced in presence of distilled water of as 100%.

RESULTS

The Phytochemical screening subjected to detect the presence of some secondary plant metabolites following standard procedures as shown in Table 1. Phytochemical investigation reveals that ethyl alcohol extracts contains carbohydrates, alkaloids, terpenoids, flavanoids, tannins, polyphenols while aqueous extract contains carbohydrates, flavanoids, tannins, poly phenols.

Table 1. Phytochemical screening of plant material *Pimenta dioica*

Phytochemical constituents	Ethyl Alcohol Extract	Aqueous Extract
Carbohydrates	+	+
Steroids	-	-
Alkaloids	+	-
Saponins	-	-
Terpenoides	+	-
Flavonoids	+	+
Tannins	+	+
Polyphenols	+	+

(+): Present (-): Absent

Table 2. *In vitro* anti-inflammatory activity of ethyl alcohol and aqueous extract of *Pimenta dioica*

Treatment	Conc. (mcg/ml)	Absorbance (560nm)	% inhibition
Control	-----	0.42±0.001	-----
Ethyl alcohol	1000	0.15±0.001	64.02
	500	0.17±0.003	58.89
	250	0.19±0.001	54.54
Aqueous	1000	0.20±.003	53.34
	500	0.23±0.001	45.84
	250	0.27±0.003	37.13
Diclofenac	50	0.13±0.003	69.95

Values are expressed as mean ± SEM. n=6 in each groups

Anti-inflammatory activity: The ethyl alcohol and aqueous extracts of *Pimenta dioica* were studied for in vitro anti-inflammatory activity by HRBC membrane stabilization method. Among the extracts ethanolic extract showed significant anti-inflammatory activity in concentration dependent manner. Ethyl alcohol extract at a concentration of 1000 mcg/ml showed 64% protection of HRBC in hypotonic solution and compared with standard diclofenac which showed 70% of protection.

DISCUSSION

Pain is an unpleasant sensory & emotional experience associated with actual or potential tissue damage. The extracellular activity of lysosomal enzyme released during inflammation is said to be related to acute and chronic inflammation. The main action of anti-inflammatory agents is the inhibition of cyclooxygenase enzyme which is responsible for conversion of arachidonic acid to prostaglandins (PG).

REFERENCES

- Anonymous. Wealth of India (Raw Materials). Publications Informations Division, CSIR Publications. New Delhi, 8, 1969, 58-59.
- Chou CT. The anti-inflammatory effects of *Tripterygium wilfordii* Hook on adjuvant induced paw edema in rats and inflammatory mediators release. *Phytother Res*, 11, 1997, 152.
- Gandhidasan R, Thamarachelvan A and Baburaj. Anti-inflammatory action of *Lannea coromandelica* by HRBC membrane stabilization. *Fitotherapy*, 62, 1991, 82-83.
- Iwueke AV, Nwodo OF and Okoli CO. Evaluation of the anti-inflammatory and analgesic activities of *Vitex doinana* leaves. *African J Biotech.*, 2006.
- Kikiuzaki H, Hara S, Kawai Y and Nakatani N. Antioxidative phenylpropanoids from berries of *Pimenta dioica*. *Phytochemistry*, 52, 1999, 1307-1312.
- Mahesh G, Ramkanth S, Saleem MTS. Anti-inflammatory drugs from medicinal plants- A Comprehensive Review. *Int. J. Rev. Life. Sci*, 1(1), 2011, 1-10.
- Mohamed S, Marzouk A, Fatma A, Moharram M, Mohamed A, Amira M, Gamal-Eldeen and Elsayed AA. Anticancer and Antioxidant Tannins from *Pimenta dioica* Leaves. *Z. Naturforsch*, 62(c), 2007, 526-536.
- Ravi V, Saleem TSM, Patel SS, Raamamurthy J, Gauthaman K. Anti-inflammatory Effect of Methanolic Extract of *Solanum nigrum* Linn Berries. *International Journal of Applied Research in Natural Products*, 2(2), 2009, 33-36.
- Seidemann J. PIMENTA Lindl.-Allspice-Myrtaceae. World Spice Plants, Springer-Verlag, Heidelberg. 2005, 286-287.
- Sekeroglu N, Kaya A and Kirpik M. Essential oil components and ethanopharmacological characteristics of some spices and D., Inan herbal drugs traded in Turkey. *International Journal of Pharmacology*, 2(2), 2006, 256-261.
- Sharma R. Pimenta. Medicinal plants of India –An encyclopedia. Data Publishing House Delhi, New Delhi, 2003.
- Tucker AO and Maciarelo MJ. Volatile Leaf Oils of Caribbean Myrtaceae. II. *Pimenta dioica* (L.) Merr. of Jamaica. *J. Essential Oil Research*, 3, 1991, 195.

Non-steroidal anti-inflammatory drugs (NSAIDs) act either by inhibiting these lysosomal enzymes (Cyclooxygenase) or by stabilizing the lysosomal membrane. The leaf extract of *Pimenta dioica* exhibited membrane stabilization effects by inhibiting hypotonicity induced lysis of erythrocyte membrane. Stabilization of lysosomal membrane is important in preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases(which further tissue inflammation and damage up on extra cellular release) (Chou CT et al., 1997) thereby limiting the inflammatory response.

CONCLUSION

Present investigation reveals that *Pimenta dioica* leaves could be useful resource as biotherapeutic agents. *In vitro* results indicates that it possess anti-inflammatory activity. So these efforts could open up the possibility of finding new clinically useful biotherapeutic agents.