ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 13, Issue 8, 2020

Online - 2455-3891
Print - 0974-2441

Research Article

EVALUATION OF *IN VITRO* ANTI-INFLAMMATORY ACTIVITY OF *CITRUS MACROPTERA* MONTR

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Received: 28 April 2020, Revised and Accepted: 09 June 2020

ABSTRACT

Objective: The use of naturally occurring medicines dependent on essential oils (EOs) is nowadays of great interest. In addition, within the human body, EO shows high efficacy as antioxidants and anti-inflammatory drugs. The present experiment was conducted to access the anti-inflammatory activity of EO obtained from the fruit peels of *Citrus macroptera* Montr. (Rutaceae) against the denaturation of protein *in vitro* model.

Methods: The test sample (EO) was incubated under controlled laboratory conditions at varying concentrations with egg albumin and was subjected to absorbance determination for the anti-inflammatory property analysis. Diclofenac sodium was used as the standard reference drug for the experiment.

Results: The results show a concentration-dependent inhibition of protein (albumin) denaturation by the test oil. This was concluded by comparing their IC_{50} average values. *Citrus macroptera* Montr. EO possessed IC_{50} average value 54.6+0.07 µg/mL, whereas that of diclofenac sodium was found to be 52.89+0.06 µg/ml. The result shows that the test oil is more effective than the standard drug.

Conclusion: From the above experimental finding, it can be concluded that *Citrus macroptera* Montr. EO has significance anti-inflammatory effect against the denaturation of the protein *in vitro* model. The activity may be due to the presence of terpene polyphenolic component or some other active compound present in the oil. The provided information was first of its kind of knowledge to keep the scientific data for future reference.

Key words: Anti-inflammatory, essential oil, *Citrus macroptera*, Protein denaturation, Polyphenolic compounds.

 DOI: http://dx.doi.org/10.22159/ajpcr.2020.v13i8.38063

INTRODUCTION

Inflammation is a complex process often associated with pain and involves occurrences such as increased vascular permeability, enhanced protein denaturation, and rearrangement of the membrane. When cells in the body are damaged by microbes, physical agents, or chemical agents, the injury is in the form of stress. Inflammation of tissue is due to response to stress. It is a defensive response that is characterized by redness, pain, heat, and swelling and loss of function in the injured area. Loss of function depends on the area and severity of the condition occurring. Since inflammation is one of the non-specific intelligences which is based on mechanisms of the body, a tissue response to an unintentional cut is similar to that of other forms of tissue damage caused by heat, radiation, bacterial, or viral [1]. An organism or tissue elicits the inflammatory responses as a defensive mechanism; however, prolonged inflammation can result in undesired health effects as a result of interplaying various biomolecules that are secreted during the inflammation phase. Inflammation in many diseases has been documented including cancer [2]. Chronic pain induced by inflammatory processes is a major clinical problem worldwide, steroidal and nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely used treatments in these chronic pain states. NSAIDs, such as diclofenac, aspirin, and indometacin, block the biosynthesis pathway of prostaglandins by inhibiting the cyclooxygenase (COX) enzymes, producing anti-inflammatory, analgesic, and antipyretic effects [3]. At present, used medications for pain control and inflammatory disorders pose dangerous side effects on chronic administration. Attempts are, therefore, being made to research promising plants which can lead to the production of newer or safer drugs [4].

MATERIALS AND METHODS

Collection, identification, and authentication of plant

The fruits of the plant *Citrus macroptera* were collected locally from East Khasi Hills, Meghalaya. The plant was identified, confirmed, and authenticated by Botanical Survey of India, Shillong, Meghalaya.

Extraction of EO

The fruit peels of *Citrus macroptera* were thoroughly washed with distilled water, cut into small pieces and about 200 g in four fractions were subjected to hydrodistillation using a Clevenger apparatus for about 4 h. The steamed and vaporized oil were condensed into liquid by a vertical condenser and collected in a measuring cylinder. Being immiscible and lighter than water, the volatile oil is separated out as an upper layer. The oil from the fruit peels were collected by following the standard procedure (Chutia *et al*., 2009). Finally, it was dried over anhydrous sodium sulfate and kept in an airtight container at 4–8°C until further analysis (Isman, 2000).

Standard drugs

Diclofenac sodium was purchased from Sigma-Aldrich Co., and purity was labeled to be 98%, respectively.

Preparation of crystallized egg albumin

The crystallized albumin obtained from 24 eggs was dissolved in about 500 ml of distilled water and poured into 5 L of boiling distilled water with rapid stirring so that the coagulated albumin was finely divided. It was then poured on several large filters and washed until the washings were entirely free of sulfates. The substance was then allowed to drain thoroughly, transferred to a flask containing about 10 volumes of 95% boiling alcohol for an hour, and filtered. This was repeated once with five volumes of 95% alcohol and once with five volumes of absolute alcohol. The material was filtered and then stirred with three volumes of a good grade of ether and again filtered. This process was repeated twice and the protein was finally dried in a vacuum desiccator over sulfuric acid. This gave a dry white product which could easily be reduced to a powder. The yield was about 1 g/egg [5-7].

Anti-inflammatory bioassay *in vitro*

Inhibition of albumin denaturation

The total 5 ml reaction mix consists of 0.2 ml of egg's albumin (fresh hen's egg), 2.8 ml of solution containing phosphate-buffered saline (PBS, pH 6. 4), and 2 ml of different concentrations of *Citrus macroptera* Montr. EO. Therefore, the final result concentrations (100µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, and 500 µg/ml) were made as per the standard method [8-12]. An equal volume of distilled water was used as regulation. The mixtures were then incubated in an incubator at 37±2°C for 15 min, then heated for 5 min at 70°C. Using vehicles as blanks, their absorbance was assessed at 660 nm after cooling down. The standard drug used as a control in this experiment was diclofenac sodium at the final concentration of 100–500 µg/ml and treated the same way for absorbance determination [13-17]. The percentage inhibition of protein denaturation was calculated using the following formula:

$$\% \text{ inhibition} = 100 \times [\text{abst}/\text{absc} - 1]$$

Where, abst = absorbance of test sample and absc = absorbance of control.

The oil/drug concentration for 50% inhibition (IC_{50}) was determined from the dose–response curve by plotting percentage inhibition with respect to control against treatment concentration (Sangita Chandra *et al*., 2012).

Statistical analysis

Statistical analysis was done using one-way analysis using ANOVA where $p<0.05$ was considered as statistically significant. Values are expressed as mean+SD (n=3).

RESULTS

The present investigation reports the *in vitro* bioassay of anti-inflammatory effect of EO obtained from the fruit peels of *Citrus macroptera* against denaturation of egg albumin. The results are given in Table 1 and IC_{50} values are summarized in Table 2.

RESULTS AND DISCUSSION

The present investigation reports the *in vitro* bioassay of anti-inflammatory effect of EO obtained from the fruit peels of *Citrus macroptera* against denaturation of egg albumin. The results are given in Table 1 and IC_{50} values are summarized in Table 2. As per the results, the test sample (EO) was found to be effective and comparable to the standard drug, that is, diclofenac sodium. This was concluded by comparing their IC_{50} average values. *Citrus macroptera* Montr. EO possessed IC_{50} average value 54.6+0.07 µg/mL, whereas that of diclofenac sodium was found to be 52.89+0.06 µg/ml.

Since ancient times, essential (volatile) oils from aromatic and medicinal plants have had biological activity, especially antibacterial and antifungal (Adel *et al.*, 2017). Waikedre *et al.*, 2010, Rana *et al.*, 2012, Bhutia *et al.*, 2017, and Singh *et al.*, 2017, reported almost 35–57 phytoconstituents in the EO. The EO study of GC/MS showed that the oil is composed of limonene (22.69%), α-terpineol (10.27%), α-myrcene (7.69%), terpinene (4.48%), cyclohexanol (3.88%), and carene (1.48%) and showed antimicrobial activity. According to Nizam Uddin *et al.*, 2014, the fruit extracts consist of saponins, steroid, and terpenoids and also report potent hypoglycemic activity (doses – 500 mg/kg and 1000 mg/kg). Based on latest study (Singh *et al.*, 2017), in albumin denaturation, the fruit peels and leaves EO found significant anti-inflammatory activity (IC_{50} at 73.91 µg/ml and that of leaves showed IC_{50} at 87.48 µg/ml) and as per present result, we have taken the fruit peels and significant result (IC_{50} average value 54.6+0.07 µg/mL, whereas that of diclofenac sodium was found to be 52.89+0.06 µg/ml) (Fig. 1). The result differences may be due to demography or geographical variations or due to the methodology followed during the *in vitro* experiment.

Table 1: *In vitro* anti-inflammatory activity of *Citrus macroptera* Montr. EO (egg albumin)

S. No.	Concentration (µg/ml)	*Citrus macroptera* essential oil	Standard (diclofenac sodium)
1.	100	19.5±0.9	14.77±0.6
2.	200	26.3±1.2	32.78±2.5
3.	300	44.7±1.9	41.88±3.8
4.	400	87.6±2.1	75.43±4.3
5.	500	95.1±3.4	99.60±5.30
IC_{50} average	300	54.6±0.07	52.89±0.06

IC_{50}: Inhibitory concentration, µg/ml=Microgram/ml

Table 2: Average IC_{50} of *Citrus macroptera* Montr. essential oil and diclofenac sodium against protein denaturation

S. No.	Treatment	IC_{50} values
1.	*Citrus macroptera* essential oil	240
2.	Standard (diclofenac sodium)	580

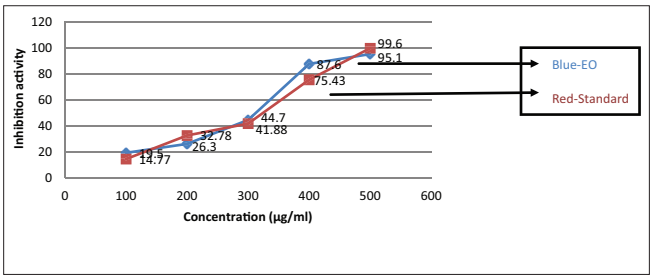

Fig. 1: *In vitro* anti-inflammatory activity of *Citrus macroptera* Montr. (egg albumin)

CONCLUSION

Eventually, the findings of this investigation concluded that extracts of EO derived from *Citrus macroptera* Montr. were determined and have anti-inflammatory function and show prominent results as opposed to normal diclofenac sodium. As we all know, the protein denaturation is a well-documented cause of inflammation. As a part of the investigation into the anti-inflammation activity mechanism, EO has been successful in inhibiting heat-induced denaturation of albumin. The EO tested in this study could practically be used as a natural medication or as a candidate for pharmaceutical and medical use.

ACKNOWLEDGMENT

The authors would like to thank the entire staffs (teaching and non-teaching) of Government Pharmacy College, Sajong, and Himalayan Pharmacy Institute, Majitar, for providing the scientific platform and the resources to perform the experimental procedure without any obstacles.

AUTHOR'S CONTRIBUTIONS

S.B, conceived, reviewed, performed the sophisticated steps during the trial errors concepts, interpretation, drafted the manuscript, and was responsible for data analyses and communication to reputed scientific journal.

CONFLICTS OF INTEREST

Author declared that there are no conflicts of interest to disclose.

AUTHOR'S FUNDING

The study was self-funded.

REFERENCES

1. Leelaprakash G, Dass SM. *In vitro* anti-inflammatory activity of methanol extract of *Enicostemma axillare*. Int J Drug Dev Res 2011;3:189-96.
2. Lalrinzuali K, Vabeiryureilai M, Jagetia GC. Investigation of the anti-inflammatory and analgesic activities of ethanol extract of stem bark of sonapatha *Oroxylum indicum in vivo*. Int J Inflamm 2016.
3. De Almeida Barros TA, De Freitas LA, Filho JM, Nunes XP, Giulietti AM, De Souza GE, *et al*. Antinociceptive and anti-inflammatory properties of 7-hydroxycoumarin in experimental animal models: Potential therapeutic for the control of inflammatory chronic pain. J Pharm Pharmacol 2010;62:205-13.
4. Divya TS, Latha PG, Usha K, Anuja GI, Suja SR, Shyamal S, *et al*. Anti-inflammatory, analgesic and anti-lipid peroxidative properties of *Wattakaka volubilis* (Linn. F.) Stapf. Natl Prod Rad 2009;8:137-41.
5. Heidelberger M, Kendall FE. A quantitative theory of the precipitin reaction: III. The reaction between crystalline egg albumin and its homologous antibody. J Exp Med 1935;62:697-720.
6. Vinueza D, Lopez E, Acosta K, Abdo S. Assessment of anti-inflammatory activity and cytotoxicity of freeze dried hydroalcoholic extract of *Bidens andicola* on isolated neutrophils. Asian J Pharma Clin Res 2017;10:160-3.
7. Fraternale D, Flamini G, Ascrizzi R. *In vitro* anticollagenase and antielastase activities of essential oil of *Helichrysum italicum* subsp. Italicum (Roth) G. Don. J Med Food 2019;22:1041-6.
8. Williams LA, O'Connar A, Latore L, Dennis O, Ringer S, Whittaker JA, *et al*. The *in vitro* anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. West Indian Med J 2008;57:327-31.
9. Biradar S, Kangralkar VA, Mandavkar Y, Thakur M, Chougule N. Antiinflammatory, antiarthritic, analgesic and anticonvulsant activity of *Cyperus* essential oils. Int J Pharm Pharm Sci 2010;2:112-5.
10. Perrone MR, Artesani MC, Viola M, Gaeta F, Caringi M, Quaratino D, *et al*. Tolerability of rofecoxib in patients with adverse reactions to nonsteroidal anti-inflammatory drugs: A study of 216 patients and literature review. Int Arch Allergy Immunol 2003;132:82-6.
11. Lourens AC, Reddy D, Başer KH, Viljoen AM, Van Vuuren SF. *In vitro* biological activity and essential oil composition of four indigenous South African *Helichrysum* species. J Ethnopharmacol 2004;95:253-8.
12. Chou ST, Lai CP, Lin CC, Shih Y. Study of the chemical composition, antioxidant activity and anti-inflammatory activity of essential oil from *Vetiveria zizanioides*. Food Chem 2012;134:262-8.
13. Siani AC, Ramos MD, Menezes-de-Lima O Jr., Ribeiro-dos-Santos R, Fernadez-Ferreira E, Soares RO, *et al*. Evaluation of anti-inflammatory-related activity of essential oils from the leaves and resin of species of *Protium*. J Ethnopharmacol 1999;66:57-69.
14. Nongalleima KH, Ajungla T, Singh CB. GC-MS based metabolic profiling of essential oil of *Citrus macroptera* montruz leaves and peel, assessment of *in vitro* antioxidant and anti-inflammatory activity. Int J Pharm Pharm Sci 2017;9:107-4.
15. Manjamalai A, Valavil S, Grace V. Evaluation of essential oil of *Tridax procumbens* L. For anti-microbial and antiinflammatory activity. Int J Pharm Pharm Sci 2012;4:356-63.
16. Al-Reza SM, Yoon JI, Kim HJ, Kim JS, Kang SC. Anti-inflammatory activity of seed essential oil from *Ziziphus jujuba*. Food Chem Toxicol 2010;48:639-43.
17. Baricevic D, Sosa S, Loggia RD, Tubaro A, Simonovska B, Krasna A, *et al*. Topical anti-inflammatory activity of *Salvia officinalis* L. leaves: The relevance of ursolic acid. J Ethnopharmacol 2001;75:125-32.