Research Article

In vitro anti-inflammatory activity of ethanolic bark extract of *Guettarda speciosa* (L.) by human red blood cell membrane stabilization

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ABSTRACT

Introduction: The anti-inflammatory effect of medicinal plants is aimed to be evaluated as inflammation is the clinical condition associated in many disease states. Medicinal plants are widely distributed all over the different regions of India. **Aim:** Thus, the present study was aimed to carry out anti-inflammatory activity of bark ethanolic extracts of *Guettarda speciosa* which is used in ancient traditional medicine. **Materials and Methods:** The suppression of hypotonicity-induced human red blood cell membrane lysis was got as an estimation of the anti-inflammatory activity. **Results:** The percentage of membrane stabilization for methanolic extracts and diclofenac sodium was conducted at different concentrations (50–2000 µg/ml). **Discussion:** Hence, it can be suggested from the present study that possible mechanisms in anti-inflammatory activity were suppressing prostaglandin synthesis. **Conclusion:** In the future, studies are needed to evaluate *in vivo* anti-inflammatory activities of *G. speciosa.*

KEY WORDS: Anti-inflammatory activity, Diclofenac sodium, *Guettarda speciosa*, Human red blood cell membrane

INTRODUCTION

For host defense, inflammation is the major mechanism in most of the diseases which may include tissue break down, repair, cell migration, fluid extravasations, enzyme activation, and mediator release. The use of medicinal plants for their anti-inflammatory effect results in reducing too many side effects by the use of medicines.[1] Thus, the present study was aimed to carry out *in vitro* anti-inflammatory activity of bark ethanolic extracts of *Guettarda speciosa* which is used in ancient traditional medicine. In the previous studies, we have confirmed that antiepileptic activity of *G. speciosa* and it contains loganic acid and secologanin.[2-5]

MATERIALS AND METHODS

Collection of Plant Materials

Plant specimen for the proposed study was collected from Chittoor in December 2018, which was identified and authenticated by Dr. P. Jayaraman, Plant Anatomy Research Centre, Pharmacognosy Institute, Chennai, Tamil Nadu.[6]

Preparation of Plant Extract

Coarse powder of *G. speciosa* bark was prepared by allowing them to air dry at room temperature in shade zone. The coarse powder thus collected was subjected to successive Soxhlet extraction using ethanol and the final drug was subjected to maceration using chloroform water. In a tumble made of Whatman's filter paper, the collected bark extract was packed. The powdered material is air-dried using oven each time before subjecting for extraction. Under controlled temperature and pressure reduced to required volumes, the extract thus obtained was dried in a flash evaporator. The obtained residue appears as sticky and thick paste in yellowish-brown to dark color. The extract was then stored in a refrigerator. The maximum percentage yield of ethanolic extract of *G. speciosa* bark was 16.5% w/w and it will be subjected for administration using intragastric feeding tube, it is subjected to grading using Tween 80.

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Animals

Wistar rats that weigh between 160 and 220 g and aging between 12 and 14 weeks were used. Water *ad libitum* and commercial pellet diet are given to the experimental animals. Ash (9%), fat (5%), fiber (4%), carbohydrate (55%), calcium (0.6%), phosphorous (0.3%), and protein (24%) are contained in diet in appropriate values. The experimental rats were acclimatized to laboratory condition for about a week before allocating them. Not more than 4 rats were kept in one polypropylene cages (38 cm × 23 cm × 10 cm) under standard laboratory conditions of 250°C ± 20°C maintained a relative humidity of 55 ± 10%, alternating 10 h dark/14 h light photoperiod. Based on the approval from the Indian National Science Academy, all ethical requirements were cleared and followed for animal use. As per the guidelines of CPCSEA, the present study was approved and carried out.

Methods

Preparation of human red blood cells (HRBCs) suspension

Sterilized Alsever solution was mixed with equal volumes of fresh whole human blood at proportions of 0.05% citric acid, 2% dextrose, and 0.42% sodium chloride and 0.8% sodium citrate in water. Then, it was subjected to centrifugation at a rate of 3000 rpm for a period of 10 min followed by washing of packed cells for 3 times with isoline at 0.85% with pH 7.2. Using isosaline, the measure volume of blood was reconstituted to 10% v/v suspension.[7-9]

Heat-induced hemolysis

Membrane lysis by hypotonicity is the principle involved in stabilization of HRBCs. Various concentrations such as 50, 100, 250, 500, 1000, and 2000 µg/ml of standard drug diclofenac were added and control group to produce 100% hemolysis, instead of hyposaline distilled water has been used with an assay mixture of 1 ml phosphate buffer at a pH of 7.4, 0.15 M. This was allowed for incubation for about 30 min. Hemoglobin level of the suspension was estimated using spectrophotometer at 560 nm.[10]

The hemoglobin percentage of HRBC was estimated as follows:

$$\% \text{ Hemolysis} = (\text{Optical density of T}/\text{Optical density of C}) \times 100$$

The percentage of HRBC membrane stabilization can be estimated as follows:

$$\% \text{ Protection} = 100 - ([\text{Optical density of T}/\text{Optical density of C}] \times 100)$$

Test sample – T; Control – C.

Statistical Analysis

All results were represented as ± S.E.M. The differences between experimental groups were compared by one-way ANOVA and were suggested statistically significant when $P < 0.005$.

RESULTS AND DISCUSSION

In vitro Anti-inflammatory Activity

The methanolic *G. speciosa* extract was effectively inhibiting hypotonicity-induced HRBC membrane lysis at different concentrations. For measuring anti-inflammatory activity, stabilization of HRBC membrane was used. At 50, 100, 250, 500, 1000, and 2000 µg/ml doses, the membrane stabilization of methanolic *G. speciosa* extract and diclofenac sodium was measured.

At different concentrations such as 50–2000 µg/ml, as shown in Table 1, the ethanolic extract of *G. speciosa* was found to be effective by inhibiting the heat-induced hemolysis of HRBC. At a dose of 2000 µg/ml of ethanolic extract of *G. speciosa* showed maximum inhibitory effect at 95.33%, as shown in Figures 1 and 2, whereas diclofenac sodium showed the maximum inhibition of 98.28% at 2000 µg/ml. In the present study, the *in vitro* anti-inflammatory activity was confirmed with increasing

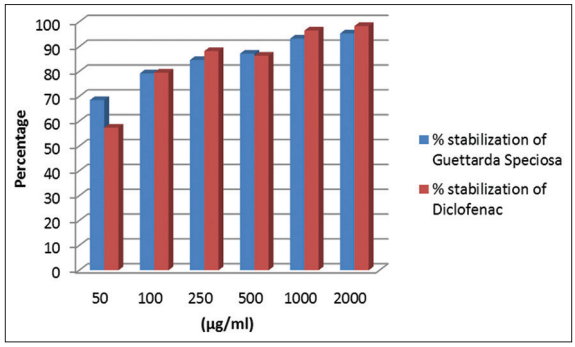

Figure 1: Effect of *Guettarda speciosa* on human red blood cells membrane hemolysis

Table 1: Effect of *Guettarda speciosa* and standard on HRBC membrane hemolysis and membrane stabilization

Conc. (µg/ml)	% hemolysis of *Guettarda speciosa*	% stabilization of *Guettarda speciosa*	% hemolysis of diclofenac sodium	% stabilization of diclofenac sodium
50	35.62	68.52	48.24	57.39
100	23.19	79.24	24.19	79.54
250	16.33	84.62	20.46	88.21
500	12.42	87.19	15.37	86.33
1000	10.21	93.22	8.49	96.47
2000	5.64	95.33	3.19	98.28

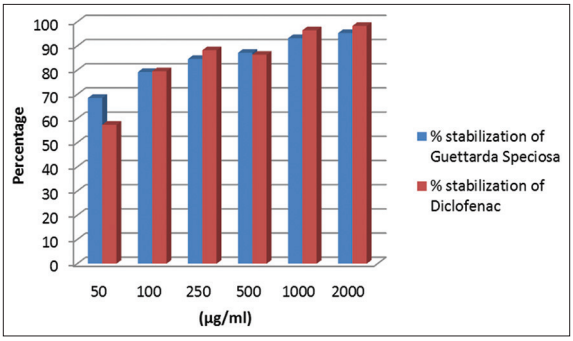

Figure 2: Effect of *Guettarda speciosa* on human red blood cells membrane stabilization

concentration of extract and standard drug inversely decreased the membrane hemolysis and increased the membrane stabilization/protection. Therefore, anti-inflammatory effect was dose dependent. These effects may be the presence of phytoconstituents such as flavonoids, tannins, and steroids which were previously reported. The extract acts as major antioxidants and inhibiting hypotonicity-induced HRBC membrane lysis by stabilized the red blood cells membrane.[11,12]

CONCLUSION

Thus, it can be concluded from the study that possible mechanisms in anti-inflammatory activity were inhibiting prostaglandin synthesis. Further studies are required to elucidate other anti-inflammatory properties of the medicinal plants. It is also proved in the study that the mechanism uses folkloric use for showing anti-inflammatory activity.

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REFERENCES

1. Chandrappa CP, Govindappa M, Kumar NV, Channabasava R. *In vitro* anti-inflammatory activity of *Carmona retusa* (Vahl.). World J Pharm Pharm Sci 2013;2:3991-7.
2. Weiner MA. Ethnomedicine in Tonga. Econ Bot 1971;25:423-50.
3. Weiner MA. Secrets of Fijian Medicine. Suva, Fiji: Government Printer; 1984. p. 93.
4. Saravanakumar A, Amutha P, Gandhimathi R, Dhanapal R. Study on phytochemical profile and antiepileptic activity of inner bark of *Guettarda speciosa* (L.). Iran J Pharmacol Ther 2009;8:73-6.
5. Cambie RC, Ash J. Fijian Medicinal Plants. Australia: CSIRO; 1994. p. 255.
6. Anosike CA, Obidoa O, Ezeanyika LU. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (*Solanum aethiopicum*). Daru 2012;20:76.
7. Umapathy E, Ndebia EJ, Meeme A, Adam B, Menziwa P, Nkeh-Chungag BN, *et al*. An experimental evaluation of *Albuca setosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. J Med Plants Res 2010;4:789-95.
8. Oyedapo OO, Akinpelu BA, Akinwunmi KF, Adeyinka MO, Sipeolu FO. Red blood cell membrane stabilizing potentials of extracts of *Lantana camara* and its fractions. Int J Plant Physiol Biochem 2010;2:46-51.
9. Zhang J, Wang H, Wang T, Chong Y, Yu P, Lu C, *et al.* Anti-inflammatory activity of Yanshu spraying agent in animal models. Exp Ther Med 2013;5:73-6.
10. Oyekachukwu AR, Elijah JP, Eshu OV, Nwodo OF. Anti-inflammatory effects of the chloroform extract of *Annona muricata* leaves on phospholipase A_2 and prostaglandin synthase activities. Transl Biomed 2017;8:137.
11. Seema CC, Sharan SV, Srinivasa RB, Meena V. *In vitro* anti inflammatory activity of ethanolic extract of *Centella asiatica* by HRBC membrane stabilization. Rasayan J Chem 2011;4:457-60.
12. Sakat S, Juvekar AR, Gambhire MN. *In vitro* antioxidant and anti-inflammatory activity ofmethanol extract of *Oxalis corniculata* Linn. Int J Pharm Pharmacol Sci 2010;2:146-55.

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