Vol. 9/1 (2009) 74 - 78

JOURNAL OF NATURAL REMEDIES

Evaluation of anti-inflammatory and membrane stabilizing property of ethanol root extract of *Rubus ellipticus* Smith in Albino rats

R. Vadivelan*, S. Bhadra, AVS. Ravi, K.Singh A. Shanish, K. Elango and B. Suresh

Department of Pharmacology, J.S.S. College of Pharmacy, Rocklands, Nilgiris - 643 001, Tamilnadu.

Abstract

This study reports the anti-inflammatory and membrane stabilizing property of an ethanolic root extract of *Rubus ellipticus* Smith in rats. The carregeenin-induced rat paw edema was utilized as a model for acute inflammatory and the probable mode by which *Rubus ellipticus* mediates its effects on inflammatory conditions was studied on rat blood cells exposed to hypotonic solution. The results of the study revealed that the extract possesses anti-inflammatory activity. *Rubus ellipticus* significantly ($p<0.01$, $p<0.001$) reduced the oedema swelling induced by carrageenin in rats at both the dose levels of 250 and 500 mg/kg, while 125 mg/kg did not show any significant activity. However, the extract did not exhibit membrane stabilizing property, as it failed to significantly ($p<0.05$) reduced the levels of haemolysis of RBC exposed to hypotonic solution. The acute toxicity studies of oral doses of ethanolic root extract in rats revealed that it has a high safety profile, as the extract was well tolerated by the animals. The results of the study suggest that the anti-inflammatory activity demonstrated by *Rubus ellipticus* may not be related to membrane stabilization.

Key words: *Rubus ellipticus*, Anti-inflammatory, Membrane stabilizing property.

1. Introduction

Rubus ellipticus Smith (Rosacea) [1] is a stout evergreen shrub grows abundantly in the forests at high altitudes like Himalaya and Nilgiris region. Traditionally it used for gastralgia, wound healing [2] dysentery, antifertility, antimicrobial, analgesic, antiepileptic [3]. Rubus species are used in folk medicine such as diabetes mellitus, inflammatory disorders and ulcers. The literature survey revealed the presence of triterpenes in various Rubus species [4]. The presence of anti-inflammatory activity in triterpenes has remarkably less side effects [5]. However, to the best of our knowledge, this is the first report on pharmacological studies of this plant and based on the citations of the use, the purpose of present study was to evaluate anti-inflammatory and membrane stabilizing properties of the ethanolic root extract of *Rubus ellipticus* Smith in rats.

* Corresponding author
Email: rv_sofia@rediffmail.com

2. Materials and Methods

2.1 Plant material

The root of *Rubus ellipticus* was collected from Nilgiri district in Tamilnadu and authenticated by Mr. D. Suresh Baburaj (Survey officer), and voucher specimens deposited in the herbarium, Survey of medicinal plant and collection unit, ministry of health and family welfare, Govt. of India.

2.2 Laboratory Animals

Healthy Wistar albino rats of either sex (150-200 g) were selected for present study. Animals were obtained from J.S.S. College of Pharmacy, Animal House, Ooty, India, maintained under standard laboratory condition and the experimental protocol was approved from Institutional Animal Ethical Committee (IAEC). The animal experiments were carried out as per CPCSEA guidelines and after the IAEC approval.

2.3 Preparation of extract

The dried root of *Rubus ellipticus* pulverized to coarse powder (800 g) was subjected to hot continuous extraction in Soxhlet apparatus using ethyl alcohol (90%) under controlled temperature (50-60°C). The extract was concentrated below 60°C and further drying was carried out under reduced pressure to obtain a semisolid blackish brown mass (140.15 g). The dried extract was stored in a vacuum dessicator for further evaluation.

2.4 Preliminary Phytochemical studies

The preliminary phytochemical screening of extract was performed to identify the presence of sterols, triterpenoids, flavonoids and tannins. [6]

2.5 Acute toxicity study

The acute toxicity study was carried out as per OECD guideline No. 423. The procedure was divided into two phases, Phase I (observation made on day one), and Phase II (observed the animals since next 14 days). Animals were fasted for 18 hours prior to the administration of REE 2000 mg/kg dose orally and individually animals were observed for 4 hours to observe any clinical symptoms, any change in behaviour or mortality and 6 hours post dosing again body weights recorded. From the next day onwards, each day 1 hour the behavioural change, clinical symptoms or mortality was observed in the same animals for next 14 days and animal body weights were recorded on 8^{th} and 14^{th} day. The same procedure was repeated with another set of animals to nullify the errors. [7]

2.6 Evaluation of anti-inflammatory activity

The rats were divided into five groups of six animals each as follows.

Group I - Solvent control 1ml/kg 0.3% CMC orally

Group II - Animals received Indomethacin 10 mg/kg in 0.3% CMC orally

Group III - Animals received *Rubus ellipticus* 125 mg/kg in 0.3% CMC orally.

Group IV - Animals received *Rubus ellipticus* 250 mg/kg in 0.3% CMC orally.

Group V - Animals received *Rubus ellipticus* 500 mg/kg in 0.3% CMC orally.

Acute paw edema was produced by injecting carragennin 1% w/w (0.1ml) into the sub plantar region of the left hind paw in the rats. [8] The *Rubus ellipticus* 125, 250 and 500 mg/kg and Indomethacin 10 mg/kg administered orally one hour before testing. The control group received vehicle 0.1 ml/100gm. The paw volume was measured by using digital plethysmometer (UGO Basile. Italy) at 0, 1, 2, 3, 4, and 6 hrs after carrageenin challenge. The percent increase in the edema (paw volume) was calculated by comparing it with zero minute reading. The percentage inhibition of edema was calculated

at 4th hour assuming 100% inflammation in vehicle group. [8]

2.7 Membrane stabilizing activity

Preparation of erythrocyte suspension:

Whole blood was obtained with heparinized syringes from rats through cardiac puncture. The blood was washed three times with isotonic buffered solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4).The blood was centrifuged each time for 10 minutes at 3000 g.

Hypotonic solution-induced rat erythrocyte haemolysis:

Membrane stabilizing activity of the extract was assessed using hypotonic solution-induced rat erythrocyte haemolysis. [9] The test sample consisted of stock erythrocyte (RBC) suspension (0.50 ml) mixed with 5 ml of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing the extract (0.25- 2.0 mg/ml) or Indomethacin (0.1 mg/ml). The control sample consisted of 0.5 ml of RBC mixed with hypotonic -buffered saline solution alone. The mixtures were incubated for 10 min at room temperature and centrifuged for 10 min at 3000 g and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of haemolysis or membrane stabilization was calculated. % Inhibition of haemolysis = 100 x {OD1-OD2/OD1}

Where:

OD1 = Optical density of hypotonic-buffered saline solution alone

OD2 = Optical density of test sample in hypotonic solution

2.8 Statistical analysis

Data obtained from this study were expressed as mean±SEM. Statistical analysis was performed using one-way ANOVA followed by Dunnets multiple comparison test. $P<0.05$ implies significance.

3. Results

Acute toxicity showed that the extract possessed high safety profile as no death and behavioural changes observed at oral doses of 2 g/kg in rats. The phytochemical study showed the presence of triterpenoids, flavonoids and tannins in ethanolic root extract. The anti-inflammatory activity of *Rubus ellipticus* ethanolic root extract on carrageenin (0.1 ml) induced paw oedema indicated that at both dose levels (250 and 500 mg/kg) of extract had significant ($p<0.01$, $p<0.001$) anti-inflammatory activity from 3rd hour onwards and it was maintained up to 6th hour, while 125 mg/kg did not show any significant ($p>0.05$) anti-inflammatory activity up to 6th hour compared to control group. 500 mg/kg was more active than 250 mg/kg, and equipotent as that of Indomethacin 10 mg/kg treated group ($p<0.001$). (Table. 1)

The extract at concentration range of 0.50-2.0 mg/ml did not significantly protect the rat erythrocyte membrane against lysis induced by hypotonic solution. In contrast, Indomethacin (0.10 mg/ml) offered a significant protection of the rat RBC against the damaging effect of hypotonic solution. At a concentration of 2.0 mg/ml, the extract produced 24.24% inhibition of RBC haemolysis as compared with 53.03% produced by indomethacin1. (Table.2)

4. Discussion

The results of the study showed that *Rubus ellipticus* root extract possesses anti-inflammatory property, as it significantly inhibited oedema induced by carrageenin in rats. However, the extract did not show membrane stabilizing effect as it failed to offer significant protection of the erythrocyte against lysis induced by hypotonic solution.

Table. 1: Inhibitory effects of various concentrations of *Rubus ellipticus* on carrageenan-induced edema of the hind paw in rats.

Sl. No.	Group	Treatment	Swelling volume (mL)				
			1hr	2hr	3hr	4hr	6hr
1.	Control	1mL/kg 0.3%CMC	1.43±0.07	2.23±0.09	3.21±0.05	3.55±0.06	3.38±0.07
2.	Indomethacin	10 mg/kg	1.44±0.05	1.76±0.10	2.03±0.08***	2.05±0.12***	1.89±0.06***
3.	*Rubus ellipticus*	125 mg/kg	1.46±0.21	2.19±0.21	2.98±0.11	3.49±0.43	3.46±0.04
4.	*Rubus ellipticus*	250 mg/kg	1.42±0.19	2.04±0.06	2.48±0.44**	2.69±0.07**	2.43±0.25**
5.	*Rubus ellipticus*	500 mg/kg	1.47±0.78	1.88±0.23	2.06±0.51***	2.09± 0.14***	1.93±0.08***

Values are expressed in terms of mean ± S.E.M, ***P<0.0001, **p<0.01, *p<0.05 Vs control group,

Table 2. Effect of ethanolic root extracts of *Rubus ellipticus* on rat erythrocytes to hemolysis.

Drug	Concentration	Optical density	% Inhibition of haemolysis
Hypotonic medium	50 mM	0.66±0.04	-
Rubus ellipticus	0.25 mg/ml	0.59±0.03	10.60
Rubus ellipticus	0.50 mg/ml	0.58±0.05	12.12
Rubus ellipticus	1.0 mg/ml	0.57±0.04	13.63
Rubus ellipticus	2.0 mg/ml	0.50±0.07	24.24
Indomethacin	0.10 mg/ml	0.31±0.20*	53.03

Values are mean±SEM of 6 experiments,*p<0.05 compared with hypotonic medium.

The inflammatory condition induced by carrageenin involves step-wise release of vasoactive substances such as histamine, bradykinin and serotonin in the early phase and prostaglandins in the acute phase [10, 11]. These chemical substances produce increase in vascular permeability, thereby promoting accumulation of fluid in tissues that accounts for oedema [12, 13]. The ability of the extract to reduce the size of oedema produced by carrageenin suggests it contained chemical components that may be active against inflammatory conditions.

It is well known that the vitality of cell depends on the integrity of their membrane [14]. Exposure of red blood cell to injurious substances, hypotonic medium and phenyl hydrazine results in lysis of its membrane accompanied by haemolysis and oxidation of haemoglobin [15]. The haemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane such as injury to RBC membrane will further render the cell more susceptible to secondary damage through the free radical induces lipid peroxidation. The progression of the bone destruction seen in rheumatoid patients for example, has been shown to be due to increased free radical activity [16, 17]. It is therefore expected that compounds with membrane-stabilizing properties should offer significant protection of cell membrane against injurious substances.

Compounds with membrane stabilizing properties are well known for their ability to interfere with the early phase of inflammatory reactions, namely prevention of the release of phospholipases that trigger the formation of inflammatory mediators [18]. However the extract of this plant did not demonstrate significant membrane stabilizing property which suggests that its anti-inflammatory activity observed in this study may be related to the inhibition of the late phase of inflammatory events, namely the release of chemical mediators.

In conclusion, the results of the study suggest that the *Rubus ellipticus* root extract may offer some beneficial effects in the management of inflammatory conditions. Further studies are essential to elucidate the detailed mechanism of action of action for anti-inflammatory activity.

5. Acknowledgement

The authors are grateful to the management, J.S.S. College of Pharmacy, Ooty – 643001, India for providing the necessary infrastructure to carry out this research work in successful manner.

References

1. Parmar C, Kaushal MK (1982) *Wild Fruits*, Kalyani publishers: New Delhi; 84-87.
2. www.himvikas.org/aug2004/herbs.htm.
3. Erdemoglu N, Kupeli, Yesilada E. (2003) *J. Ethnopharmacol*. 89:123-129.
4. Lien TP, Kamperdick C, Sung TV, Adama G. (1999) *Phytochemistry*. 50:463-465.
5. Gupta MB, Bhalla TN, Gupta GP, Mitra CR, Bhargava KP. (1969) *E. J. Pharmacol*. 6:67-70.
6. Clarke EGC. (1975) *Isolation and Identification of Drugs*, II Ed, Pharmaceutical press: London; 905.
7. "Guidance document on acute oral toxicity testing"series on testing and assessment no.24, organization for economic co-operation and development, OECDenvironment, health and safety publication, Paris 2001 (www.oecd.org/ehs).
8. Ramanathan M, Jaswanth A, Kumar EP, Elango K, Suresh B. (2001) *Advances in Pharmacology and Toxicology*. 2:33-39.
9. Shinde UA, Phadke AS, Nair AM, Mungintiwar AA, Dikshit VJ, Saro and VO. (1991) *Fitoterapia*. 70:251-257.
10. Dirosa M, Giround JP, Willoughby DA. (1971) *Journal of Pathology*. 104:15-29.
11. Heller A, Koch T, Schmerk J and Acker VK. (1998) *Advances in Pharmacology and Toxicology*. 55:487-496.
12. Williams TJ, Morlay J. (1973) *Nature*. 246:215-217.
13. White M. (1999) *Immunology*. 103:5378-5381.
14. Ferrali M, Signorni C, Ciccoli L, Comporti M. (1992) *Biochemical journal*. 285:295-301.
15. Augusto O, Kunze KL, Montellano PR. (1982) *J.Bio.Chem*. 257:6231-6241.
16. Cotran RS, Kumar V, Collins T. (1999) In: *Robbins pathologic basis of diseases*, VI Edn, Saunders company: New York; 50-88.
17. Pattison DJ, Silman AJ, Goodson NJ, Lunt M, Bunn D, Weich A, Bingham S, Khaw KT, Day N, Symmon DPM.(2004) *Ann. Rheum. Dis*. 63:843-847.
18. Aitadafoun M, Mounier C, Heyman SF, Binistic C, Bon C, Godhold J. (1996) *Biochem. Pharmacol*. 51:737-742.