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RESEARCH ARTICLE

IN VITRO ANTIINFLAMMATORY ACTIVITY OF *JUGLANS REGIA* BARK

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ABSTRACT

To evaluate the anti-inflammatory property of the different extract of *bark* of *Juglans regia*, family Juglandaceae is a deciduous tree. Its fruits are consumed as food, which are rich in unsaturated fatty acids. Walnut leaf has been widely used in traditional medicine for the treatment of skin inflammations and ulcers and for its antidiarrheic, antihelmintic, antiseptic and astringent properties. The present study aimed at the evaluation of anti-inflammatory property of the aqueous, chloroform and alcoholic extracts of the *bark* by in vitro methods. In vitro method was estimated by human red blood cell membrane stabilization (HRBC) method. Results showed significant anti-inflammatory property of the different extracts tested. The aqueous extract at a concentration of 200 mg/ml. showed potent activity on comparing with the standard drug diclofenac sodium.

Key words: *Juglans regia*, HRBC, Inflammation and dicloenac.

INTRODUCTION

Inflammation is a reaction of living tissues towards injury and it comprises systemic and local responses[1]. In spite of our dependence on modern medicine and the tremendous advances in synthetic drugs, a large number of the world populations (80% of people) cannot afford the products of the pharmaceutical industry and have to rely upon the use of traditional medicines, which are mainly derived from plant material. The fact is well recognized by the WHO which has recently compiled an inventory of medicinal plants listing over 20 000 species. There are several important medicinal plants with wide range of pharmacological, biological activities and interesting phyto chemical constituents. The main action of anti-inflammatory agents is the inhibition of Cyclooxegenase enzymes which are responsible for the conversion of Arachidonic acid to prostaglandins. Since human red blood cell (HRBC) membranes are similar to these lysosomal membrane components, the prevention of hypotonicity induced HRBC membrane lysis was taken as a measure in estimating the anti-inflammatory property of various extracts of *Juglans regia*. Thus, Human red blood cell membrane stabilization (HRBC method)[2,3] has been used as a method in estimating the anti-inflammatory property. *Bark*s of *Juglans regia* consist of several constituents like Gallic acid, Quercetin, Coumarin,etc but the most active constituent isan aromatic phytochemical Juglone ($C_{10}H_6O_3$; 1,4– napthaquinone, 5 hydroxy –8Cl) and juglanin A,B,C and the volatile constituents are given as Alpha-thujene, sabinene, p-cymene, 1,8-cineole, alpha-cardinol, Benzyl alcohol, alpha-Bisabalol, Linalol,Isopuegol, Carvacrol, Myretenal, Estrsgol, Pinocarveol, Globulol, Verbenol. In certain parts of India the *bark* of this plant was traditionally used in the treatment of inflammation. The present study aimed to authenticate that traditional information by in vitro antiinflammatory screening.

MATERIALS AND METHODS

Preparation of extracts

Fresh *bark* of *Juglans regia* were collected from FRI, Dehradun and were authenticated by botanist. The *bark* were dried in shade and powdered to a coarse form. It was then successively extracted with methanol, ethanol, water and hydroalcohol using continuous cold maceration process. The extracts were concentrated under reduced pressure and preserved at low temperature.

Chemicals and instruments

All chemicals used in the estimation were of analytical grade. Reference standard diclofenac sodium was obtained as gift sample from Arbro pharmaceutical, New Delhi. Shimadzu 1701 UV Visible spectrophotometer was used for the in vitro study.

In vitro Anti-inflammatory activity

The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution(2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of extracts were prepared (100 and 200 µg/ml) using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension were added. It was incubated at 370C for 30 min and centrifuged at 3,000 rpm for 20 min. and the hemoglobin content of the supernatant solution was estimated on UV spectrophotometer at 560 nm. Diclofenac (100 and 200 g/ml) was used as reference standard and a control was prepared by omitting the extracts.[4,5]

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RESULT

In vitro anti-inflammatory activity

Juglans regia extracts at different concentrations (100, 200 mg/mL) showed significant stabilization towards HRBC membranes. The percentage protection of aqueous extract at concentration 200 mg/mL was higher than that of other concentrations. However the percentage protection was found to be lesser then the reference concentration. The results were tabulated in Table 1.

Table 1: % inhibition of different extracts of the *Juglans regia*

S. No.	Type of extract	Concentration(µg/ml)	Absorbance	% Inhibition of denaturation
1	Control		0.019±1.20	
2	Water	200	0.773±1.20	32.02±0.21
3	Water	100	0.0260±0.023	40.76±1.23
4	Chloroform	200	0.0331±1.20	29.30±0.022
5	Chloroform	100	0.0409±0.24	23.71±1.015
6	Methanol	200	0.0292±0.323	36.30±0.019
7	Methanol	100	0.0405±0.021	23.70±1.03
8	Diclofenac	100	0.0374±0.121	79.25±1.025
9	Diclofenac	200	0.0207±1.25	88.43±0.029

DISCUSSION

Inflammation is a common phenomenon and it is a reaction of living tissues towards injury. Steroidal anti-inflammatory agents will lyse and possibly induce the redistribution of lymphocytes, which cause rapid and transient decrease in peripheral blood lymphocyte counts to affect longer term response. Phytochemical evaluation of the various extracts of *Juglans regia* reveals the presence of flavonoids, glycosides, saponins, steroids, tannins and polyphenols. Here anti-inflammatory activity was performed based on the folk lore information using two methods. HRBC method was selected for the in vitro evaluation of anti-nflammatory property because the erythrocyte membrane is analogous to the lysosomal membrane[6] and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release. The result indicted that the *bark* extract of *Juglans regia* at various concentrations has significant anti-inflammatory property. The present result indicates the efficacy of *Juglans regia* as an effective therapeutic agent in the treatment of acute inflammations. The result of present study authentifies the folk lore information on the anti-inflammatory property of the *bark* extract of *Juglans regia* . Further and detailed studies are in process for the isolation of active constituent responsible for this property and to identification of the possible mechanism of its anti inflammatory property.[7]

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