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IN VITRO ANTI-INFLAMMATORY COMPOUND QUERCIMERITRIN ISOLATED FROM *TITHONIA DIVERSIFOLIA* FLOWERS BY HRBC MEMBRANE STABILIZATION

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ABSTRACT

The aqueous solutions of the flavanoid glycoside isolated from the fresh flowers of the *Tithonia diversifolia* was screened for anti-inflammatory activity by human red blood cell (HRBC) membrane stabilization method. The prevention of hypotonicity-induced HRBC membrane lysis was taken as a measure of the anti-inflammatory activity. All the fractions showed a biphasic effect on the membrane stabilization. However their activities decreased with time.

Key words: *Tithonia diversifolia,* Quercimeritrin, Anti-inflammatory, Human Red Blood Cell (HRBC), Membrane stabilization.

INTRODUCTION

Inflammation was described as " the succession of changes which occurs in a living tissue when it is injured provided that the injury is not of such a degree as to at once destroy its structure and vitality" (Sanderson, 1871) , or " the reaction to injury of the living microcirculation and related tissues" (Spector *et al.*, 1963). Inflammatory response to tissue injury involves a complex array of enzyme activation, mediated release, fluid extravasations, cell migration, tissue breakdown and repair (Vane *et al.*, 1995) which are aimed at host defense and usually activated in most disease conditions.

HRBC or erythrocyte membrane is analogous to the lysosomal membrane (Chou, 1997) and its stabilization implies that the extract may as well stabilize lysosomal membranes.

Stabilization of human red blood cell membrane (HRBC) by hypo tonicity induced membrane lysis can be taken as an *in vitro* measure of anti-inflammatory activity of the drugs or plant extracts.

The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed (UNESCO, 1996). Interest in medicinal plants as a re-emerging health and has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well being and the bioprospecting of new plant-derived drugs (Lucy and Edgar, 1999). Furthermore an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies (UNESCO, 1998).

Tithonia diversifolia(HEMSL.) A. GRAY (Compositae) is native to Mexico and also grows in parts of Africa, Australia, Asia and other countries of North America and is commonly called Mexican sunflower or tree marigold (Pereira *et al.*,1997). An extract of *Tithonia diversifolia* has been traditionally used for the treatment of diabetes, diarrhoea, menstrual pain, malaria, hematomas, hepatitis, hepatomas and wound healing (Tona *et al.*, 2000; Rungeler *et al.*, 1998; Lin *et al.*, 1993; Kuo *et al.*, 1990). Pharmacological studies of *Tithonia diversifolia* showed that it has anti-diabetic (Miura *et al.*, 2002; Miura *et al.*, 2005), anti-malarial (Elufioyc *et al.*, 2004), anti-inflammatory (Owoyclc *et al.*, 2004), analgesic and cancer chemopreventive activity, some of which account for the folkloric claims of this medicinal plant. Several sesquiterpenoids were isolated from *Tithonia diversifolia* (Gu *et al.*, 2002; Kuo *et al.*, 1997).

MATERIALS AND METHODS

HRBC Membrane Stabilization Method

The human red blood cell membrane stabilization method (HRBC) has been used as a method to study the *in vitro* anti-inflammatory activity (Gandhisan *et al.*, 1991). Blood was collected from healthy human volunteer who was not taken any NSAIDS for 2 weeks prior to the experiment. The collected blood was mixed with equal volume of sterilised Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% NaCl in water) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline (0.85%, pH 7.2) and a 10% (v/v) suspension was made with isosaline.

Solutions of different concentrations of flavanoid were prepared (10, 25, 50, 75, 100 and 200µg/ml) using distilled water and to each concentration 1ml of phosphate buffer (0.15M, pH 7.4), 2 ml of hyposaline (0.36%) and 0.5ml of HRBC suspension were added. It is incubated at 37°C for 30 minutes and centrifuged at 3,000 rpm for 20 minutes. The haemoglobin content in the supernatant solution was estimated spectrophotometrically at 560nm.

A control (distilled water) was prepared omitting the extracts. The percentage hemolysis was calculated by assuming the hemolysis produced in presence of distilled water of as 100%. The percentage of HRBC membrane stabilization or hemolysis was calculated using the formula

% inhibition of Hemolysis = 100 × OD1-OD2/ OD1

Where OD_1 and OD_2 are absorbance of prednisolone and test extracts respectively.

RESULTS AND DISCUSSION

HRBC membranes are similar to lysosomal membrane components, the prevention of hypotonicity induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity of drugs. The results are reported in Table 1. All the fractions of ethanolic extract of the *Tithonia diversifolia* flowers showed biphasic effects on HRBC membrane stabilization. They showed increasing activity at low concentration levels but decreasing activity with higher concentrations. They have a critical concentration (50 µg/ml) at which their activities are maximum. The activities of the various fractions are comparable to that of prednisolone at the concentration of 50 and 100µg/ml. Hence anti- inflammatory activity of the extracts was concentration dependent.

The HRBC membrane stabilization on the yellow pigments of *Tithonia diversifolia* shows a biphasic property. It shows a dose-dependent activity. The drug has a maximum stabilization at 25 µg and further increase in concentration, the stabilizing effect once again occur at 100 µg. Beyond this, the curve declines. Thus the drug has the maximum efficiency at two concentrations such as at 25 and 100 µg.

The lysosomal enzymes released during inflammation produce a variety of disorders. The extracellular activity of these enzymes is said to be released to acute or chronic inflammation. The extracellular activity of these enzymes is said to be related to acute or 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) (Arun Shirwaikar *et al.*, 2011).

The non-steroidal drugs (NSAIDS) act either by inhibiting these lysosomal enzymes or by stabilising the lysosomal membranes by means of inhibiting the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes (cyclooxygenase) and proteases, which cause further tissue inflammation and damage upon extracellular release or by stabilizing the lysosomal membrane (Seema *et al.*, 2011).

Table1: *In vitro* anti-inflammatory activity of *Tithonia diversifolia* flowers by HRBC membrane stabilization

S.No	Concentration of Drug (µg)	Optical density
1.	10	0.08
2.	25	0.10
3.	50	0.07
4.	75	0.08
5.	100	0.11
6.	200	0.08

CONCLUSION

The results of this study have shown that the flowers of *Tithonia diversifolia* possess anti-inflammatory mediated by prostaglandin synthesis inhibition. Membrane stabilization may contribute to the anti-inflammatory effect. The study also provides empirical evidence for the use of the flowers of *Tithonia diversifolia* in folkloric treatment of inflammatory disorders.

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