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Original Article

HRBC Membrane Stabilization as a study tool to explore the Anti Inflammatory activity of Alliumcepa Linn. –Relevance for 3R

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***ABSTRACT*:**
Introduction: Many countries, including the United States, Canada, European Union member states, and others, require that alternatives to animal experimentation for pharmacological research. Hence, attention needs to be given to assess the medicinal value of herbs using in vitro methods. **Objective:** The present study was carried out to evaluate the anti inflammatory activity of ethanolicextractof Allium cepa peel. **Methods:** The present research work had carried out on laboratory level assay to avoid the use of different animal models. Hence, ethanolic extract of Allium cepa (EEAC) peels were investigated for anti-inflammatory activity by simple, reliable, less toxic and less time consuming HRBC membrane stabilization method. The presentation of hypo tonicity induced HRBC membrane lysis was taken a measure of anti-inflammatory activity. Their activities were compared with standard drug diclofenac sodium. **Results:** The results of the study demonstrated that Allium cepa contains active constituents, which possess anti-inflammatory activity which is probably related to the inhibition of prostaglandin synthesis. **Conclusion:** The result obtained from this study was indicating that ethanolic extract of onion skin possesses significant anti-inflammatory activity and this is a possible rationale for its folkloric use as an anti-inflammatory agent.
Key words: HRBC membrane stabilization, Anti-inflammatory, Diclofenac, Aliumcepa, ,ethanolic extract.

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INTRODUCTION:

An 'alternative' is a new research technique that either (a) Replaces the use of animals altogether, (b) Reduces the number of animals used or (c) Refines the study design to cause less distress to the animals – these are commonly referred to as the '3R' of animal alternatives [1].Non-animal approaches are now considered as advanced methods that can overcome many of the limitations of animal experiments. In testing medicines and chemicals, in vitro assays have spared hundreds of thousands of animals [2].

Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli [3]. Lysosomal enzymes released during inflammation produce a variety of disorders which leads to the tissue injury by damaging the macromolecules and lipid peroxidation of membranes [4]. Extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. An uncontrolled and persistent inflammation may act as an etiologic factor for several chronic illnesses such as heart attacks, septic shocks and rheumatoid arthritis etc [3].

Stabilization of lysosomal membrane is important in limiting the inflammatory response by inhibiting 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 or by stabilizing the lysosomal membrane [5]. HRBC or erythrocyte membrane is analogous to the lysosomal membrane [6] 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.

Maximum number of clinically important drugs belongs to steroidal or non-steroidal anti-inflammatory chemical therapeutics for treatment of inflammation-related diseases. Though these have potent activity, long-term administration is required for treatment of chronic disease. Furthermore, these drugs have various and severe adverse effects. Therefore, naturally originated agents with very little side effects are desirable to substitute chemical therapeutics [7]. Currently, the use of natural antioxidants present in food and other biological materials has attracted considerable interest due to their presumed safety, nutritional and therapeutic value. Although several plants have gained importance for the nutritional and therapeutic values, many remain to be scientifically investigated [8]. Allium cepa is highly valued for its therapeutic properties. It has been used as a food remedy from time immemorial.

Allium cepa Linn.is a well-known traditional nutraceutical and medicinal plant that is cultivated and used around the world. It has great health significance and is consumed for its putative nutritional and health benefits for centuries. Allium cepa L.belongs to Family: Liliaceae (lilies); and commonly known as Onion (english) and Pyaj (hindi). It is an easily digestible aromatic vegetable which is usedas traditional Indian spices.As a foodstuff they are usually cooked, as a vegetable or part of a prepared savory dish, but can also be eaten raw or used to make pickles or chutneys. They are pungent when chopped and contain certain chemical substances which irritate the eyes [1]. The main properties of onion include antimicrobial activity, cardiovascular support, hypoglycemic action, antioxidant/anticancer effect, and asthma protection [2].Phytochemical analysis of Onion peel extract revealed the presence of various biochemical compounds such as flavonoids, tannins, anthocyanes, triterpenoids, sterols and/or terpenes, quinons and saponin compounds.

The phenolics and flavonoids compounds have potential anti-inflammatory, Anti platelet, anti-cholesterol, anticancer, and antioxidant properties [11-13].Since triterpenoids and flavonoids have remarkable anti inflammatory activity so that, the proposed study was to investigate the anti inflammatory activity of Allium cepa Linn using in vitro HRBC membrane stabilization.

METHODS:

Plant materials &extraction

The bulbs of Allium cepaLinn.were collected from the local vegetable market of Udaipur and authenticated by Botanist. Voucher specimens were deposited for future reference.The preparation of the extract of the Onion peel was done in the Department of Pharmacology, Geetanjali Medical College, Udaipur using Soxhlet apparatus with 50% ethanol at 60°C for 24 hrs.The percentage of yield (w/w) is 24% respectively. Extract was stored as dried powder at 4°C.

Preliminary Phytochemical screening

The ethanolic extract of Allium cepa (EEAC) was subjected to phytochemical screening for the detection of major chemical constituents [14].

HRBC Membrane Stabilization Method

The human red blood cell membrane stabilization method (HRBC) has been used as a method to study the invitro anti-inflammatory activity [15].

Blood was collected from healthy human volunteer who was not taken any NSAIDS for 2 weeks prior to the experiment. Study was commenced with due permission of the Institutional ethics committee and written consent from the subjects. The collected blood was mixed with equal volume of sterilized Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.05% citric acid and 0.42 % sodium chloride in water). The blood was centrifuged at 3000 rpm for 10 min and packed cells were washed three times with isosaline (0.85%, pH 7.2). The volume of the blood was measured and reconstituted as 10% v/v suspension with isosaline [16, 17].

The assay mixture contains 1ml phosphate buffer [P^H 7.4, 0.15 M], 2 ml hypo saline [0.36 %], 0.5 ml HRBC suspension [10 % v/v] with 0.5 ml of plant extracts and standard drug diclofenac sodium of various concentrations (50, 100, 250, 500, 1000, 2000 µg/ml) and control (distilled water instead of hypo saline to produce 100 % hemolysis) were incubated at 37^{o}C for 30 min and centrifuged respectively. The hemoglobin content in the suspension was estimated using spectrophotometer at 560 nm [6].

The percentage of hemolysis of HRBC membrane can be calculated as follows:

% Hemolysis = (Optical density of Test sample / Optical density of Control) X 100

The percentage of HRBC membrane stabilisation can be calculated as follows:

% Protection = 100 – [(Optical density of Test sample / Optical density of Control) X 100]

RESULTS:

Phytochemical Study: The preliminary phytochemical investigation of the EEAC revealed the presence of flavonoids, glycosides, tannins, treterpenoids saponins, and phenolic compounds.

Anti-inflammatory activity: Ethanolic extract Allium cepa was exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization indicates that the extract may also well stabilize lysosomal membranes membrane is shown in table-1. It possesses significant activity comparable with that of the standard diclofenac sodium [18, 19].

In vitro anti inflammatory activity of ethanol extract of Allium cepa (EEAC) were concentration dependent, the maximum protection of 95.18% was seen at the concentration of 2000 µg/ml. All results were compared with standard diclofenac sodium which showed 97.25% protection at the concentration of 2000 µg/ml is shown in Figure 1 and 2.

Table-1: Effect of EEAC and Standard drug on HRBC membrane hemolysis and membrane stabilization

Conc. (µg/ml)	% Hemolysis of Allium cepa L.	% Stabilisation of Allium cepa L.	% Hemolysis of Diclofenac sodium	% Stabilisation of Diclofenac sodium
50	38.44	61.48	34.65	65.12
100	27.12	72.64	20.82	78.64
250	21.55	79.15	16.17	83.65
500	16.21	83.76	11.45	88.36
1000	11.26	88.85	8.12	91.73
2000	4.76	95.18	2.86	97.25

Figure – 1: Effect of Allium cepa L. on HRBC membrane hemolysis.

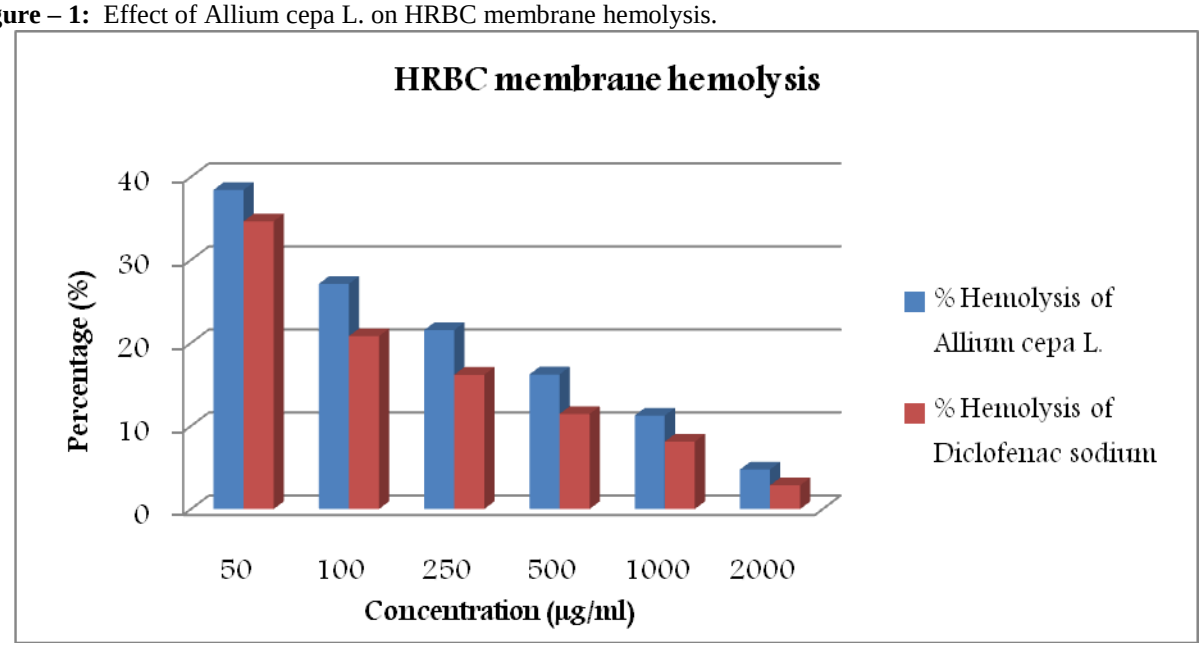
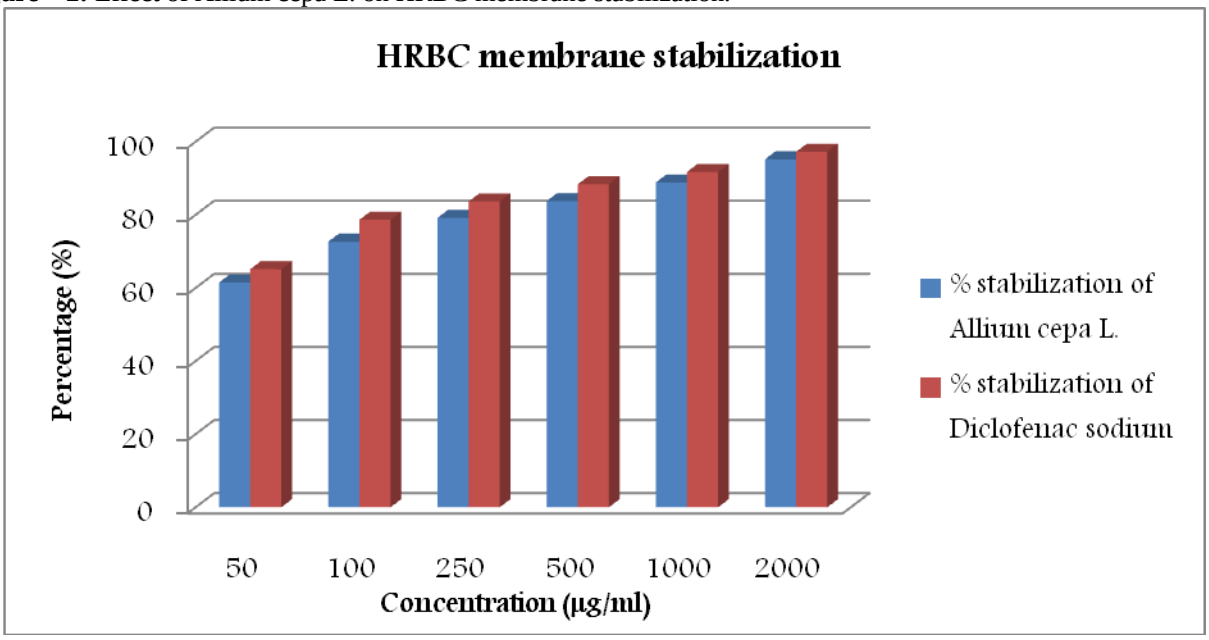


Figure – 2: Effect of Allium cepa L. on HRBC membrane stabilization.



DISCUSSION:

The human red blood corpuscular membrane is similar to lysosomal membranes that influence inflammatory process. The lysosomal enzymes released during inflammation produce a variety of disorders. 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 [4].

The non-steroidal drugs (NSAIDs) act either by inhibiting these lysosomal enzymes or by stabilizing 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 extra cellular release or by stabilizing the lysosomal membrane [20]. Furthermore, chronic use of these drugs have various and severe adverse effects.

Therefore, naturally originated agents with very little side effects are desirable to substitute chemical therapeutics. The investigation is based on the need for newer anti-inflammatory agents from natural source with potent activity and lesser side effects as substitutes for chemical therapeutics. Realizing the fact this study was carried out to evaluate the in vitro anti inflammatory activity of ethanolic extract of Allium cepa peel (EEAC) in this direction.

Results of the study is obtained that ethanolic extract of Allium cepa was exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane in concentration dependent manner. It is due to the presence of active principles such as flavonoids and tritrepenoids may responsible for this activity.

In the previous study, quercetin has been reported to inhibit allergy and inflammation. Also, it is known that outer skin of onion contains much higher levels of quercetin than its bulb [21, 22].Hence, Onions can be used as a potent anti inflammatory agent. The suggested model allows studying particle effects at a cellular and molecular level. It may be used in different areas such as medicinal, pharmaceutical and ayurvedic research works.

CONCLUSION:

In conclusion, the present study has shown that Allium cepa Linn. have membrane stabilization effect by inhibition of hypo tonicity induced lysis of erythrocyte membrane. Hence, it implies the anti inflammatory and analgesic properties mediated by prostaglandin synthesis inhibition. Membrane stabilization may contribute to the anti-inflammatory effect. A successful introduction of the proposed model in industry may reduce a substantial number of painful animal experiments, replace animal experiments and refine in vitro model systems used today to study basic particle-host interactions.

No agent can be initiate or considered for clinical trial or for animal model induction, because it is quite harmful and offensive as well so if the agent have in vitro good result then these steps can be considered.

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