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RBC MEMBRANE STABILIZATION PROPERTY & ANTIOXIDANT ACTIVITY OF MYRICETIN 3-O-GLUCOSIDE - AN *IN VITRO* METHOD

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ABSTRACT

The membrane stabilizing property and anti-oxidation property of myricetin 3-O-glucoside on the human red blood cells (HRBC) and sheep red blood cells (SRBC) reported in this study. The probable mode by which myricetin 3-O-glucoside mediates its effects on inflammatory conditions was studied on HRBC & SRBC exposed to hypotonic solution. The results of the study revealed that the myricetin 3-O-glucoside possesses anti-inflammatory property. However, the extract did not have the membrane stabilizing property. The results of the study suggest that the anti-inflammatory activity may not be related to membrane stabilization. It failed to show inhibition nature even in the higher concentration during the estimation of antioxidizing property.

KEYWORDS : *Myricetin 3-O-glucoside, Hibiscus cannabinus, Membrane stabilizing property, HRBC, SRBC, antioxidizing property*

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INTRODUCTION

Phytochemistry involves the study of flavonoids, alkaloids etc., the research on plants of medicinal importance is growing phenomenally at the international level. Recent estimates suggest that several thousands of plants have been identified with medicinal applications in various cultures [1]. Flavonoids are group of natural substances with variable phenolic structures and are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine [2]. These natural products were known for their beneficial effects on health long before flavonoids were isolated as the effective compounds. Flavonoids are responsible for the attractive colours of flowers, fruit and leaves [3]. However their occurrence is not restricted to flowers but includes all parts of plant. The widespread distribution of flavonoids, their variety and their relatively low toxicity compared to other active compounds mean that many animals, including humans, ingest significant amount of flavonoids in their diet. Flavanoids have been referred to as "nature's biological response modifiers" because of strong experimental evidence of their inherent ability to modify the body's reaction to allergens, viruses and

carcinogens. They show antiallergic, anti-inflammatory, antimicrobial and anticancer activity [4]. Phytochemical investigation carried out on the gynaceum parts of *Hibiscus Cannabinus* flowers by using various solvents. The solvents with increasing polarity like EtOH, benzene, peroxide free Et_2O and EtOAc have been employed to isolate the active ingredient from the ethyl acetate fraction of ethanolic extract. The results obtained from Wilson's Boric acid, Gibb's, Shinoda and Molisch's tests, Horammer-Hansel test and ^{13}C-NMR and ^{1}H-NMR spectral studies showed that the newly isolated compound is said to be Myricetin-3-O-glucoside [5,6].

Erythrocytes have been used as a model system by a number of workers for the study of interaction of drugs with membranes [7,8,9]. Drugs like anesthetics transquilisers and non-steroidal anti-inflamatories stabilize erythrocytes against hypotonic haemolysis at low concentration [10]. When the RBC is subjected to hypotonic stress the release of hemoglobin (Hb) from RBC is prevented by anti-inflammatory agents because of membrane stabilization. So, the stabilization of HRBC membrane by drugs against hypotonicity induced haemolysis serves as a useful in vitro method for assessing the anti-inflammatory activity of various compounds [11].

Oxidative stress (OS) is a state of imbalance between generation of Reactive Oxygen species (ROS) like hydroxyl and superoxide radicals and the level of antioxidant defence system [12]. The consequences OS involve damage of biomolecules including nucleic acids, proteins, polyunsaturated fatty acids and carbohydrates abnormality in calcium metabolism, destruction of thiol group containing enzymes and inactivation of membrane-bound receptors [13,14]. OS and free radical medicated processes have been implicated in the pathogenesis of a variety of diseases like atherosclerosis, cancer, liver damage, rheumatoid arthritis, immunological incompetence [15] neuro-degenerative disorders [16].

The endogenous antioxidant defence includes enzymatic (e.g. Superoxide dismutase, catalase, peroxidase etc.,) and non-enzymatic (e.g. *C.indicum*, α-tocopherol, *T.peruviana* etc.,) systems [17]. Nutritional antioxidant deficiency may lead to OS [18]. Neuro degeneration results from prolonged deficiency of vitamin E in patients unable to handle fat property, [18] low plasma concentrations of *C.indicum,* α-tocopherol may be associated with higher incidence of myocardial incidence of myocardial infraction and cancer [18]. Low concentration of reduced *T.peruviana* has been found in the lymphocytes of AIDS patients [19]. Administration of vitamin E has been used to treat retrotental fibroplasia and haemolytic syndrome of premature babies. Both of which are condition characterized by increased oxidative stress [20]. Protective role of antioxidants against free-radical mediator toxicities is now well established.

Lipid peroxidation (LP) is oxidative deterioration of polyunsaturated lipids and it involves ROS and transition metal ions [21]. It is a molecular mechanism of cell injury leading to generation of peroxides as lipid hydroperoxides which can decompose to yield a wide range of cytotoxic products most of which are aldehydes, as exemplified by malondialdehyde (MDA), 4-hydroxynonenal etc. [22]. The stimulation of LP as a consequence of tissue injury can sometimes make significant contribution to worsening of injury. There is good evidence that LP occurs within atherosclerotic lesion and also in case of traumatic injury to brain and spinal cord [18]. Many drugs and medicinal substances like adriamycin, menadione, paraquat, alloxan, etc., have capacity to produce peroxides [18]. LP induction capacity of drugs may be related to their toxic potential adriamycin induced cardiotoxicity is mediated through free-radical mediated process [23]. Thus evaluation of antioxidant as suppressors of drug induced LP provides a scope to select free-radical scavengers which on co-administration in vivo, in case of reduced endogenous antioxidant defence may reduce toxic effects of drugs used for therapeutic purpose.

The present study deals with Lipid Peroxidation induced by a drug Ceftizoxime Sodium (CZX), a third generation cephalosporin antibiotic an antiviral agents and *in vitro* evaluation of myricetin

3-O-glucoside which is a component of endogenous antioxidant defence mechanism, as inhibitors drug induced Lipid Peroxidation.

MATERIALS AND METHODS

Membrane Stabilization:

Fresh blood was collected from healthy sheep and healthy human volunteer and mixed with equal volume of sterilized Alsever solution (containing 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride) and stored at 4^{o}C and used within 5 hrs. Saline at two different concentrations were prepared (isosaline 0.85% and hyposaline 0.25%).

RBC suspension: The blood samples were centrifuged at 300 RPM and the packed cells obtained were washed with isosaline (pH 7.2) 3 times and 10% (v/v) suspension was made with isosaline.

Solutions of different concentrations of the Myricetin 3-O-glucoside were prepared. Assay mixture contained the active drug, 1 ml of phosphate buffer (0.15 m pH 7.4) 2 ml of hyposaline and 0.5 ml of 10% RBC suspension. In another tube instead of 2 ml of distilled water was taken and this served as the control. All the tubes were incubated at 37^{o}C for 30 min. They were centrifuged and the haemoglobin content in the supernatant was estimated using photoelectric colorimeter at 560 nm. The percentage inhibition of heamolysis or membrane stabilization was calculated [24].

% inhibition of heamolysis = 100 X {OD1-OD_2/OD_1}

where;

OD1 = Optical density of hypotonic-buffered saline solution alone

OD_2 = Optical density of test sample in hypotonic solution

Antioxidant Activity:

Blood sample was collected from goat and it was used as the lipid source. Blood being the transporting tissue may be considered as close stimulator of more complex biological system. Goat blood was selected because of its easy availability and close similarity to human blood. Collection, pre-treatment and preservation of sample blood and incubated blood samples were done as in the case of membrane stabilization process. Different portions of the blood were treated with drug (CZX) and or myricetin 3-O-glucoside. A portion of blood not treated with drug or antioxidant served as control. CZX was treated as solution in saline and the effective concentration was 40 mg%. The antioxidant also was treated as solutions in saline in effective concentrations 10µg, 50 µg, and 100 µg of myricetin-3-O-glucoside solution.

Lipid peroxidation of blood samples was measured in terms of Malondialdehyde (MDA) content following the thiobarbituric acid (TBA) method [25,26]. Different sets of experiments were performed for each drug-antioxidant part and it was repeated. In CZX measurement of MDA content of blood samples were done at 3, 6, 8 and 24 hrs of incubation. The mean MDA content of 0 hr of the control sample served as the reference for comparison in all cases.

The method of measurement of MDA content involved precipitation of the protein part of the blood by treating with 10% trichloroacetic acid (TCA) solution and centrifugation at 3000 RPM for 30 min. followed by filtration of the supernatant. The filtrate was then treated with 0.002 M TBA solution and boiled for 30 min. The resultant mixture was cooled to room temperature and its absorbance was estimated at 530 nm against TBA blank by using EC digital spectrocolorimeter GS5700 B. The standard curve was prepared using tetraethoxy propane and TBA according to the method and the corresponding best fit equation was found out using the method of least squares.

The percent changes in MDA content of different samples were calculated with respect to the corresponding control 0 hr. and change in MDA level was considered as an indicator of the extend of LP.

RESULTS AND DISCUSSION

The Myricetin 3-O-glucoside at concentration range of 10 µg – 150 µg did not significantly protect the human and sheep erythrocyte membrane against haemolysis induced by hypotonic solution. At a concentration of 150 µg, the test sample produced

25.0 % and 12.5 % inhibition of RBC membrane haemolysis in human and sheep bloods respectively. The test sample partially capable of inhibiting the infer percentage of release enhances with the increase in concentration. At higher concentration i.e. at 100 µg the extract was found to be protecting the cells around 63 %. The results of the study showed that Myricetin 3-O-glucoside isolated from the gynaceum part of *Hibiscus cannabinus* possesses anti-inflammatory property, however the extract did not show membrane stabilizing effect even at the higher concentration to both the HRBC and SRBC also failed to protect the human and sheep erythrocyte membrane against haemolysis induced by hypotonic solution. The results of the antioxidant activity study showed that the Myricetin 3-O-glucoside is capable of protecting the cells and it decreases the percentage of release thereby it is capable of protecting the cells.

Table 1: Effect of Myricetin 3-O-glucoside on human erythrocyte haemolysis

Sample	Concentration (µg)	Optical Density (OD)	% Inhibition of Haemolysis
Hypotonic Medium	-	0.40	-
Myricetin 3-O-glucoside	10	0.34	15.0
Myricetin 3-O-glucoside	25	0.33	17.5
Myricetin 3-O-glucoside	50	0.30	25.0
Myricetin 3-O-glucoside	75	0.30	25.0
Myricetin 3-O-glucoside	100	0.30	25.0
Myricetin 3-O-glucoside	125	0.30	25.0
Myricetin 3-O-glucoside	150	0.30	25.0

Figure 1: Effect of Myricetin 3-O-glucoside on human erythrocyte haemolysis

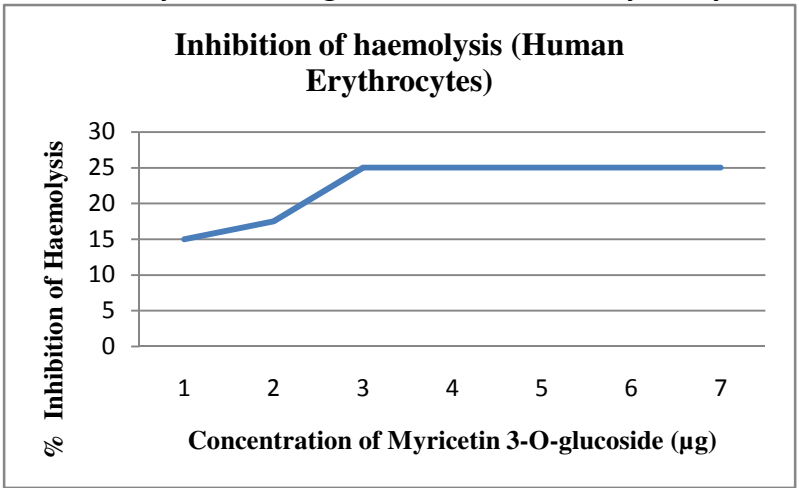

Table 2: Effect of Myricetin 3-O-glucoside on sheep erythrocyte haemolysis

Sample	Concentration (µg)	Optical Density (OD)	% Inhibition of Haemolysis
Hypotonic Medium	-	0.80	-
Myricetin 3-O-glucoside	10	0.75	6.25
Myricetin 3-O-glucoside	25	0.70	12.5
Myricetin 3-O-glucoside	50	0.72	10.0
Myricetin 3-O-glucoside	75	0.72	10.0
Myricetin 3-O-glucoside	100	0.72	10.0
Myricetin 3-O-glucoside	125	0.72	10.0

Myricetin 3-O-glucoside	150	0.70	12.5

Figure 2: Effect of Myricetin 3-O-glucoside on sheep erythrocyte haemolysis

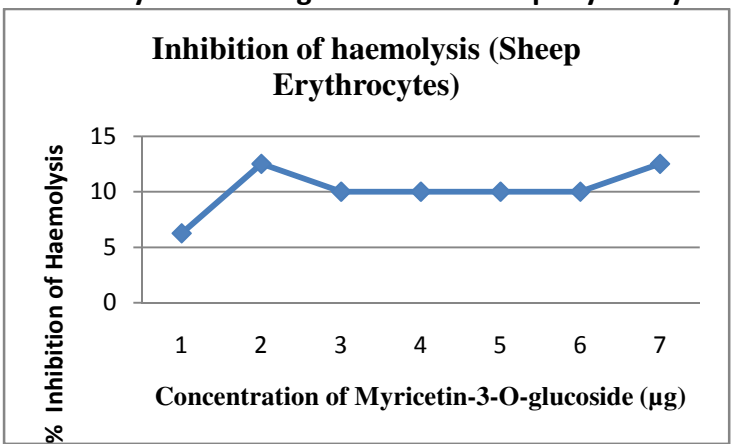

Table 3: Antioxidant effect of Myricetin 3-O-glucoside against induced Lipid Peroxidation

Dose in µg	% of Release	% of Inhibition
10	67.48	32.51
50	61.35	38.65
100	36.80	63.19

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