ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2012; 3(6):1467-1471

ORIGINAL RESEARCH ARTICLE

Evaluation of Pharmacological Potential of Fruits of *Solanum Xanthocarpum*

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Received 11 Sep 2012; Revised 05 Dec 2012; Accepted 18 Dec 2012

ABSTRACT

The aim of the present study to evaluate the some of the pharmacological activities (RBC membrane stabilizing property and the antioxidant activity) of the methanolic extract of the fruits of *Solanum Xanthocarpum* on red blood cells of both human and sheep. Also the antioxidizing activity of the extract is estimated. The probable mode by which *Solanum Xanthocarpum* mediates its effect on inflammatory conditions was studied on red blood cells exposed to hypotonic solution. The results of the study showed that the extract possess anti-inflammatory property but did not have membrane stabilizing ability. The anti-inflammatory activity may not be related to membrane stabilization.

Key words: *Solanum Xanthocarpum*, anti-inflammation, membrane stabilization, lipid peroxidation.

INTRODUCTION

Solanum Xanthocarpum (Schrad & Wendl.) is a well known traditional medicinal plant belongs to the family solanaceae. It is popularly known as febrifuge plant, bhatakataiya and kandangatri in English, Hindi and Tamil respectively. *S. xanthocarpum* is non toxic and has been reported to be safe for human use. [1] It was already in use and is clinically safe to consume. The whole plant is useful in the treatment of dental caries, inflammations, flatulence, constipation, dyspepsia, anorexia, leprosy, skin diseases, hypertension, fever, cough, asthma, bronchitis, hiccough, lumbago, haemorrhoids and epilepsy. The plant is bitter, acrid, thermogenic, anthelmintic, anti-inflammatory, digestive, carminative, appetizer, stomachic, febrifuge, expectorant, laxative, stimulant, diuretic, rejuvenating, emmenagogue and aphrodisiac. [2]

Many number of chemical constituents like carpesterol, glucoalkaloid, solanocarpine, solanine-S, solasodine, solasonine, solamargine, ß-solamargine, cycloartanol, stigmasterol, campesterol, cholesterol, sitosteryl-glucoside, stigmasteryl glucoside, solasurine, galactoside of ß -sitosterol, methyl ester of 3,4-dihydroxycinnamic acid and 3,4-dihydroxycinnamic acid (caffeic acid), isochlorogenic, neochlorogenic, chlorogenic acids (fruit); flavonal glycoside, quercetin-3-0- ß -D-glucopyranosyl-0- ß -D-mannopyranoside, apigenin, sitosterol (flower); solanocarpine and amino acids (seeds); coumarins, scopolin, scopoletin, esculin and esculetin (leaves, roots and fruits); carpesterol, tomatidenol, norcarpesterol and solasonine were reported to present in various parts of the plant.[3]

The plant is useful in treatment of fever, cough, asthma etc.[4] The stem, flowers and fruits are prescribed for treating burning sensation in the feet accompanied by vesicular eruptions. The hot aqueous extract of dried fruits is used for treating cough, fever and heart diseases.[5] The fruit is known for several medicinal uses like anthelmintic, antipyretic, anti-inflammatory, antitumor, cytotoxic activities, antiasthmatic, antispasmodic and hypotensive. Juice of fruit of *S.xanthocarpum* is used in sore throats and rheumatism. The fruit paste is applied externally to the affected area for treating pimples and swellings. [6] It is also used in the preparation of contraceptive drug. Plant powder is anti-tussive and its effect on patients with bronchial asthma and nonspecific cough has been explained as due to depletion of histamine from lung and its expectorant action as due to inorganic nitrogen content. [7] Root is an expectorant. It is prescribed in cough, asthma, used in the form of electuary. [8] *S.xanthocarpum* is treated as a traditional healer of many ailments and it was revealed by various research works. [9]

Many works have been carried out to find out the constituents present in the various parts of the

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plant by various researchers in different times.[10,11] It was reported that *S.xanthocarpum* is found to possess many number of pharmacological activities like antimicrobial and antioxidant. Its different parts also used as an ayurvedhic medicine for various diseases.[12,13]

Erythrocytes have been used as a model system by a number of workers for the study of interaction of drugs with membranes.[14-16] Drugs like anesthetics transquilisers and non-steroidal anti-inflamatories stabilize erythrocytes against hypotonic haemolysis at low concentration.[17] 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. [18]

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.[19] 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.[20,21] 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[22] neuro-degenerative disorders.[23]

The endogenous antioxidant defence includes enzymatic (e.g. Superoxide dismutase, catalase, peroxidase etc.,) and nonenzymatic (e.g. *C.indicum*, α-tocopherol, *T.peruviana* etc.,) systems.[24] Nutritional antioxidant deficiency may lead to OS.[25] Neuro degeneration results from prolonged deficiency of vitamin E in patients unable to handle fat property,[25] low plasma concentrations of *C.indicum*, α-tocopherol may be associated with higher incidence of myocardial incidence of myocardial infraction and cancer.[25] Low concentration of reduced *T.peruviana* has been found in the lymphocytes of AIDS patients. [26] 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. [27] 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.[28] 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. [29] 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. [25]Many drugs and medicinal substances like adriamycin, menadione, paraquat, alloxan, etc., have capacity to produce peroxides. [25]

LP induction capacity of drugs may be related to their toxic potential adriamycin induced cardiotoxicity is mediated through free-radical mediated process.[30] 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 and antiviral agent and an *in vitro* evaluation of methanolic extract of fruits of *S.xanthocarpum* which is a component of endogenous antioxidant defence mechanism. As inhibitors drug induced Lipid peroxidation and also the evaluation of RBC membrane stabilization property of the methanolic extract was carried out in an *in vitro* condition both for human and sheep RBC.

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 methanolic extract of fruits of *S.xanthocarpum* 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. [31]

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

where;

OD_1 = 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 methanolic extract of fruits of *solanum xanthocarpum.* 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 methanolic extract.

Lipid peroxidation of blood samples was measured in terms of Malondialdehyde (MDA) content following the thiobarbituric acid (TBA) method. [32,33] 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.

Table 1: Effect of Methanolic extract of fruits of *S. xanthocarpum* on human erythrocyte haemolysis

Sample	Concentration (µg)	Optical Density (OD)	% Inhibition of Haemolysis
Hypotonic Medium	-	0.80	-
Methanolic extract	10	0.60	25.0
Methanolic extract	25	0.58	27.5
Methanolic extract	50	0.55	31.3
Methanolic extract	75	0.52	35.0
Methanolic extract	100	0.51	36.3
Methanolic extract	125	0.51	36.3
Methanolic extract	150	0.51	36.3

Table 2: Effect of methanolic extract of fruits of *S. xanthocarpum* on sheep erythrocyte haemolysis

Sample	Concentration (µg)	Optical Density (OD)	% Inhibition of Haemolysis
Hypotonic Medium	-	0.92	-
Methanolic extract	10	0.75	18.5
Methanolic extract	25	0.72	21.7
Methanolic extract	50	0.71	22.8
Methanolic extract	75	0.70	23.9
Methanolic extract	100	0.70	23.9
Methanolic extract	125	0.70	23.9
Methanolic extract	150	0.70	23.9

Table 3: Antioxidant effect of Methanolic extract of fruits of *S. xanthocarpum* against induced Lipid Peroxidation

Dose in µg	% of Release	% of Inhibition
10	72.80	72.20
50	64.35	35.65
100	32.60	67.40

RESULTS AND DISCUSSION

The Methanolic extract of fruits of *S. xanthocarpum* 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 extract produced 36.3 % and 23.9 % inhibition of RBC membrane haemolysis in human and sheep bloods respectively. The results of the study showed that methanolic extract 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 methanolic extract of *S.xanthocarpum* is moderately capable of protecting the cells and it decreases the percentage of release thereby it is capable of protecting the cells.

CONCLUSION

Methanolic extract of the fruits of *S. xanthocarpum* is partially capable of protecting the erythrocyte membrane of both human and sheep blood cells against haemolysis induced by hypotonic solution. It is also capable of protecting the cells by decreasing the percentage of release against induced lipid peroxidation.

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