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Protective Effects of *Garcinia talbotii* bark extract against Induced Oxidative Damage on Human Erythrocytes

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ABSTRACT: *Garcinia talbotii* is a tree belonging to the family Clusiaceae, found in evergreen forests of Western Ghats. Reactive oxygen species induces cell and tissue injury and contribute to chronic inflammation underlying many neurodegenerative, cardiovascular, and metabolic diseases. Shade dried *Garcinia talbotii* bark was subjected to ethanol extraction using soxhlet apparatus and *Garcinia talbotii* bark ethanol extract was screened for protective effects against H_2O_2 induced oxidative damage on human erythrocytes by HRBC membrane stabilization assay, inhibition of albumin denaturation assay and also inhibition of H_2O_2 induced oxidative damage on erythrocyte ghost membrane proteins by SDS-PAGE. The results showed that *Garcinia talbotii* bark extract (GBE) at a concentration of 50-250µg/ml, significantly protected the heat induced protein denaturation. At the concentration of 100 and 200 µg/ml, GBE significantly ($p<0.01$) inhibited H_2O_2 induced haemolysis and also protected the erythrocyte morphology, which was further confirmed by performing SDS-PAGE for erythrocyte ghost membrane proteins. The present study for the first time demonstrates the *in vitro* anti-inflammatory effects of *Garcinia talbotii.*

Keywords: *Garcinia talbotii*, Erythrocytes, Inflammation, Oxidative damage, Ghost membrane.

INTRODUCTION

Since ages, plants have been used for medicinal purposes. When synthetic medications were not available, the plant world made a significant contribution to human wellbeing. Around the globe, a variety of plant species are used to treat human illness. Garcinia genus is a member of the Clusiaceae family and is found in equatorial Asia, Africa, and Brazil. In India, they are found in Western Ghats. The majority of these plant species contain active ingredients like alkaloids, phenols, flavonoids, tannins, steroids, glycosides, and terpenoids (Idu, 2009). The biologically active metabolites found in the genus Garcinia have drawn a lot of interest in recent years due to the chemical composition of their extracts, which are high in derivatives of polyisoprenylated benzophenones, polyphenols, bioflavonoids, and xanthones (Williams *et al.,* 2003; Nguyen *et al.,* 2019). Garcinia Genus has shown antioxidant, anti-inflammatory, anticancer, leishmanicidal, and antiprotozoal properties (Martins *et al.,* 2008; Sõukand *et al.,* 2015). Garcinia extracts from the pericarp, epicarp, and seeds have been used to treat cuts, ulcers, and dysentery (Acuna *et al.*, 2012).

Normal metabolism generates reactive species that play a prominent role in physiological functions, including gene expression, cell signalling, and immune responses. Oxidative stress is brought in biological systems by an imbalance between ROS production and antioxidant action. This imbalance can cause cellular dysfunction by altering the stability of proteins, gene expression, and membrane fluidity, which can result in cell damage and mortality (Edeas, 2011). Antioxidants neutralize reactive species when they exceed normal level which prevents chelation of redox active metal ions, modulation of gene expression and interaction with the cell signalling pathways (Edeas, 2011; Halliwell and Gutteridge 2015). Inflammation is an intricate biological reaction on exposure to variety of elements such as free radicals, heavy metals, medications, natural toxins, and environmental chemicals (Naraki *et al.,* 2021). Inflammation initiates the healing process, which involves recruitment of immune cells and chemical mediators which act together at the damaged site and repairs tissues and also eliminates infectious pathogens. Tissue inflammation results from the body's reaction to stress and it is a protective reaction characterised by swelling, redness, pain, heat, and loss of function in the injured region (Chen *et al.,* 2017). Plant metabolites can alter cellular responses and the produced chemical mediators involved in inflammatory processes (Ginwala *et al.,* 2019).

This research study was conducted to investigate the anti-inflammatory and protective effects of *Garcinia talbotii* extract using human red blood cells against H_2O_2 induced oxidative damage.

MATERIALS AND METHODOLOGY

Materials. All chemicals, reagents and solvents used in the present study were of analytical grade. Hydrogen Peroxide, Bovine Serum Albumin (BSA), EDTA, Acrylamide, Bis-acrylamide, SDS, Tris-Base, Quercetin, Ammonium persulphate, TEMED, Sample loading

buffer were procured from HiMedia Laboratories, Mumbai.

Plant material collection, extraction and phytochemical screening. The bark of *Garcinia talbotii* was collected from Sirsi forest of Uttara Kannada district, Karnataka. The plant material was authenticated by Dr. Srikanth Gunaga, Taxonomist, Department of Forest Biology and Tree Improvement, University of Agricultural sciences (Dharwad), Karnataka. The Bark of *Garcinia talbotii* was shade dried, grinded to course powder. The powdered material with 96% ethanol was subjected to hot extraction using Soxhlet apparatus. Extract was filtered and concentrated using rotary flash evaporator (Buchi Rotavapor R-3, Switzerland). The preliminary group tests were conducted to screen the presence of both primary and secondary metabolites in bark ethanol extract.

Inhibition of albumin denaturation assay. The inhibition of albumin denaturation assay was done by the method of Mizushima and Kobayashi (1968); Sakat *et al.* (2010). 1 ml of GBE extract (50-250 µg/ml) with 1% bovine albumin fraction of aqueous solution were taken in a test tube and a small amount of 1N HCl was added to the mixture and pH was adjusted to 7.5. Samples were incubated at 370ºC for 2 minutes and cooled to room temperature and again heated at 51 ºC for 20min. The Samples were cooled and the turbidity was recorded at 660 nm. Quercetin was used as a standard drug.

Effect of plant extracts on H_2O_2-induced oxidative stress in human erythrocytes

Preparation of erythrocytes. Fresh blood was drawn from healthy individuals and collected in EDTA-coated tubes. The plasma and buffy coat were removed by centrifuging at 3000 rpm for 10 minutes, and the blood was then washed with 10 mM phosphate buffer saline and cells were suspended in Phosphate buffered saline (pH 7.4) to get a final quantity of 1×10^8 erythrocytes/ml.

HRBC membrane stabilization assay. An aliquot of 500µl of erythrocytes was incubated with 1 mM H_2O_2 and two different concentrations of GBE (100 and 200 µg/ml) for 3 hours at 37°C with periodic shaking. The oxidative stress on erythrocytes was assessed by haemolysis test (Hilary *et al*., 2017). After incubation, the mixtures of erythrocytes were centrifuged for 10 min at 3000 rpm, supernatant was collected and absorbance was measured at 540 nm. Haemolysis in treated and untreated erythrocytes was represented as a percentage of complete haemolysis, which was the amount of haemolysis of erythrocytes in pure water.

Evaluation of inhibition of oxidative damage on erythrocyte ghost membrane proteins by SDS-PAGE. Erythrocyte ghost membrane was prepared by treating with 10 volumes of hypotonic lysis buffer comprising 5 mM phosphate buffer (pH 8.0) and 1 mM EDTA, followed by a 10-minute incubation on ice bath and the hemolysate prepared was centrifuged at 12,000 rpm for 30 minutes. Fresh lysis buffer was added each time until a light pink erythrocyte ghost membrane was visible at the bottom (Fairbanks *et al.,* 1971; Tulipani *et al*., 2014). The reaction mixture containing 50 µg of ghost membrane protein was determined by lowry's technique (Pomory, 2008). Oxidative damage to ghost membrane proteins was initiated by adding 200 µM of hydrogen peroxide. GBE extract of 100 and 200 µg/ml with 100 µl of 1mM H_2O_2 were added to ghost membrane protein samples and incubated for one hour on ice bath. Ghost membrane proteins from the treated and control groups were separated using a 10% SDS-PAGE with a constant voltage of 50 V. Coomassie brilliant blue was used to stain the gels, and bands were visualized using Biorad (GelDoc Go, USA) gel documentation system.

Statistical analysis. All experiments were performed in triplicates. Data are presented as mean ± SD. A one-way analysis of variance (ANOVA) was employed to assess data using Graphpad prism version 8. Significance in results were inferred with * if $p < 0.05$, ** if $p < 0.01$, and *** if $p < 0.001$.

RESULTS AND DISCUSSION

Preliminary phytochemical screening revealed the presence of carbohydrates, alkaloids, phenols, flavonoids, glycosides, lactones, tannins, oils and fats. These are important and commonly present secondary metabolites which play key role in several pharmacological activities. In Garcinia species, xanthones are the major class of compounds followed by benzophenones and bioflavonoids (Aravind *et al.,* 2016). The genus Garcinia is an abode to several secondary metabolites such as hydroxycitric acid (HCA), flavonoids, terpenes, and polyisoprenylated benzophenones, procyanidines and guttiferone derivatives. The HCA has been known for its hypolipidemic property and used in several commercial preparations as an anti-obesity agent (Hemshekhar *et al*., 2011). Several polyisoprenylated benzophenone and xanthones are proved to have antioxidant, anti-inflammatory, anti-cancer, anti-microbial and HAT inhibiting properties (Espirito Santo *et al*., 2020).

Inhibition of albumin denaturation assay. GBE treated at different concentrations prevented denaturation of the protein albumin. Results show that with the increasing (50-250µg/ml) concentrations, GBE consistently inhibited albumin denaturation which is shown in Table 1. At 250µg/ml, the bark extract exhibited a maximum inhibition of 96.66±2.26% which is comparable to the standard drug quercetin which showed inhibition of 87.67±0.66% at 100 µg/ml concentration. This could be due to the presence of several phytochemicals present in *Garcinia talbotii* such as xanthones, phenols, flavonoids etc.

Protein denaturation is a well-known contributor to inflammation. When denatured, the majority of biological proteins cease to operate biologically (Chandra *et al.,* 2012). The assay is based on the principle that at high temperature albumin gets denatured and co-treatment with the extract prevents the protein denaturation. Previous reports have showed that several plant extracts prevent the protein denaturation owing to the presence of secondary metabolites. *Garcinia kola* extracts showed about 69.42% inhibition in albumin

denaturation at a concentration of 300µg/ml (Dairou *et al.,* 2021).

Table 1: Denaturation of albumin Inhibition using different concentration of *Garcinia talbotii* bark ethanol extracts.

Sr. No.	Concentration of GBE(µg/ml)	Inhibition (%)
1.	50	27.16± 0.70
2.	100	43.06± 4.32
3.	150	71.66± 2.35
4.	200	83.47± 4.31
5	250	96.66± 2.26
6	Quercetin (100)	87.67± 0.66

Effect of GBE on H_2O_2-induced oxidative stress in erythrocytes. From data shown in Fig. 1, *Garcinia talbotii* bark ethanol extract at the doses of 100 and 200µg/ml protected the human erythrocyte membrane against haemolysis induced by H_2O_2. The percentage of inhibition of haemolysis shown by the GBE was dose dependent. GBE showed 43.06% inhibition of haemolysis at 100 µg/ml and 83.47% inhibition of haemolysis at 200µg/ml ($p < 0.001$) which is almost near to the haemolysis inhibition ability of quercetin (92.88%) at 100 µg/ml when compared with the negative control (29.64%).

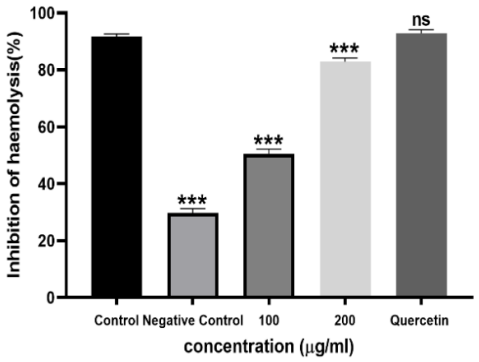

Fig. 1. Percentage inhibition of erythrocyte hemolysis by *Garcinia talbotii* bark ethanol extract.

Excess accumulation of reactive oxygen species is capable of damaging cellular components by lipid peroxidation, membrane damage and destruction of proteins which ultimately leads to cell death. HRBC membrane has been used as a method to investigate the *in vitro* anti-inflammatory activity. The erythrocyte membrane is similar to the lysosomal membrane and its stabilisation suggests that the extract may stabilise lysosomal membranes (Gandhidasan *et al.,* 1991; Shenoy *et al.,* 2010). Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophils which upon extracellular release results in additional tissue inflammation and injury (Chippada *et al*., 2011).

The phytochemical findings of the present study demonstrated that GBE is incredibly rich source of flavonoids, alkaloids, phenols, and tannins. Plant flavonoids have been proved in numerous studies to have strong anti-inflammatory and antioxidant effects (Middleton Jr and Kandaswami 1992; Read, 1995; Halliwell *et al.*, 2005). The leaves of *Garcinia gummi-gutta* and *Garcinia cambogia* plant showed strong *in vivo* anti-inflammatory activity in rats and also significant *in vitro* anti-inflammatory action in HRBC membrane stabilization method (Prasanth *et al.,* 2013) Other Garcinia species have also been reported to stabilize the erythrocyte membrane. Shakya *et al.* (2020) showed that *Garcinia zeylanica* leaf extract exhibited membrane stabilization potential at concentrations ranging from 100 -1000 µg/ml.

Evaluation of inhibition of oxidative damage on erythrocyte ghost membrane proteins by SDS-PAGE. Erythrocytes were hypotonically lysed to produce the erythrocyte ghost membrane and then they were treated with H_2O_2 alone and with GBE extracts. The pattern of erythrocyte membrane proteins after induced oxidative damage is showed in Fig. 2. The protein band observed after Coomassie stain shows that the integrity of the membrane protein was preserved in lanes 3, 4 and 5 compared to control as a result of extracts' ability to scavenge free radicals, compared to lane 2, which served as a negative control that has exposed to H_2O_2, and the protein band appeared to be distorted.

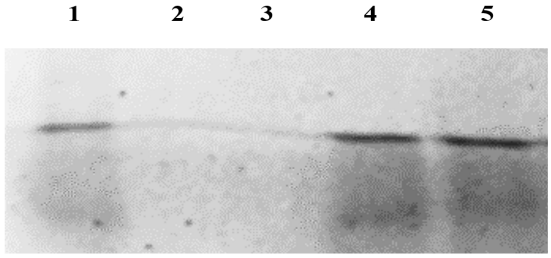

Fig. 2. Inhibition of oxidative damage on erythrocyte ghost membrane proteins by *Garcinia talbotii* bark extract. Lane 1: ghost membrane alone. Lane 2: ghost membrane with 200 µM H_2O_2. Lane 3: Ghost membrane with 200 µM H_2O_2 and 100 µg/ml GBE. Lane 4: ghost membrane with 200 µM H_2O_2 and 200 µg/ml GBE. Lane 5: Ghost membrane with 200 µM H_2O_2 and 50 µg/ml quercetin.

The secondary metabolites present in plant extract prevent oxidation of protein sample from H_2O_2. Phenols and xanthones which are abundantly found in Garcinia species are predominantly responsible for its antioxidant and protective effects. Ajila and Rao (2008) reported that the *Mangifera indica* peel extracts displayed protective effects to counter the H_2O_2 instigated oxidative stress in ghost membrane of rat erythrocytes. Other reports also demonstrated that extracts of *Hypericum retusum, Messua ferrea* bark, *Hypericum scabrum* and Pearl powder presented comparable defensive effects on induced oxidative damage of erythrocyte membranes (Kızıl *et al.,* 2011; Rajesh *et al.*, 2013; Yang *et al.,* 2017). Plant secondary metabolites present in the plant extracts could interact with the membranes which are major hotspots of lipid peroxidation (Parthiban *et al.,* 2019).

CONCLUSIONS

The presence of bioactive compounds in *Garcinia talbotii* bark extract contributed to the protection of proteins and HRBC membrane, oxidative stress in erythrocytes has been significantly reduced by *Garcinia talbotii* bark extract. The results obtained in the present study indicate that ethanol extract of *Garcinia talbotii* bark can be a potential antioxidant and anti-inflammatory agent.

FUTURE SCOPE

Garcinia talbotii is an endemic plant native to Western Ghats and the present work is the first study on the anti-inflammatory effects of the plant. Since other plants of the Genus Garcinia such as *Garcinia indica, Garcinia cambogia, Garcinia mangostana, Garcinia gummigutta* etc. have showed tremendous pharmacological activity, *Garcinia talbotii* could also be studied for various pharmaceutical effects owing to the presence of a wide array of secondary metabolites. Also, isolation of bioactive compounds from this plant could provide new vistas in drug discovery as potential pharmacological agents.

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Conflict of Interest. None.

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