CODEN (USA): IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com **Research Article**

t-BOOH INDUCED DNA DAMAGE: PROTECTION BY GLYCOPROTEIN BGS-HARIDRIN FROM TURMERIC (*CURCUMA LONGA*)

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Abstract:
The main objective of the study is to find a non toxic dietary spice towards preventing oxidative DNA damage induced by t-BOOH. Standard antioxidant Butylated Hydroxy Anisole, Curcumin, BGS-Haridrin, Tertiary butyl hydroperoxide, calf thymus DNA, Tryphan blue. The sheared DNA was used in submarine agarose gel electrophoresis where DNA damage was induced by using tertiary butyl hydroperoxide. Similarly, the cytotoxicity study was also done using Tryphan blue to know that the antioxidant itself is not toxic to cells by using lymphocytes isolated from fresh human blood. The submarine gel electrophoresis bands were visualized in Transilluminator where, it was confirmed that, tertiary butyl hydroperoxide induced DNA damaged was successfully prevented by BHA (400µM), Curcumin (400µM) and BGS-Haridrin (0.1µM). In cell cytotoxicity studies, Curcumin, alpha-tocopherol and BGS-Haridrin inhibits the cell damage 55%, 63% and 68% respectively. The results confirm that, BGS-Haridrin extract can inhibit tertiary butyl hydroperoxide induced DNA damage and is nontoxic to cells.

Keywords: *Reactive oxygen species, DNA damage, Dietary antioxidant, lymphocytes, t-BOOH, BGS-Haridrin, Curcumin*

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Please cite this article in press as Leela Srinivas et al , ***t-Booh Induced DNA Damage: Protection By Glycoprotein Bgs-Haridrin From Turmeric (Curcuma Longa),*** *Indo Am. J. Pharm. Sci, 2015;2(7).*

INTRODUCTION:

Free radicals are highly reactive because of having unpaired electrons. Free radicals try to steal an electron from neighbour atom and hence, there by create another free radical. These excessive free radicals can damage the body, if it is not stopped or removed [1, 2, 3]. Oxygen species is harmful to the DNA is the key macromolecule of human cell [4]. Chemicals, organic smoke, exposure to radiation, nutrient deficiency, chronic disease and environmental prooxidants will also helps in the production of free radicals and also normal cellular metabolism produces free radicals [5]. Reactive Oxygen Species (ROS) cause a spectrum of DNA lesions, including single-strand breaks, double-strand breaks, crosslinking of DNA and damage to bases and the deoxyribose moiety [6]. The highly reactive hydroxyl radical is responsible for DNA damage and the organic hydroperoxide, tertiary-butylhydroperoxide (t-BOOH), causes oxidative damage in a number of cell types [7, 8]. Antioxidants thus are known to protect cellular macromolecules like DNA against oxidative injury [9] and may either act as scavenging or as chain breaking antioxidants [10]. Given the above possibility of dietary modulation of oxidative stress induced DNA damage, it was pertinent to understand whether BGS-Haridrin, as an antioxidant is able to prevent the damage induced by ROS to DNA. Herein we reporting that, BGS-Haridrin is an efficient antioxidant and protects DNA against Lipid peroxide

MATERIALS AND METHODS:

Calf thymus DNA (CT DNA), BHA, α-tocopherol, Curcumin, Agarose, Ethidium bromide was from Sigma Chemical company USA. Ascorbic acid was from HIMEDIA, India. Ethylene diamine tetra acetic acid (EDTA), Tertiary Butyl hydroperoxide (t-BOOH) was procured from s.d. fine Chem. Ltd. India. All the other chemicals were of Anal. R grade. Organic solvents were distilled prior to use.

Isolation of BGS-Haridrin glycoprotein from Turmeric (*Curcuma longa* L)

The BGS-Haridrin was isolated according to the method of Dinesha and Leela Srinivas [11]. In brief, the procured Turmeric tubers were washed, shade dried and powdered. Five grams of Turmeric powder mixed with boiling double distilled water, vortexed, centrifuged and the supernatant brought to 65% saturation with ammonium sulphate. These proteins were purified by column chromatography and followed with ion exchange chromatography using DEAE A25. Protein elution was monitored at 280 nm using a spectrophotometer. The homogenecity and molecular weight was confirmed with HPLC and MSMALDI.

t-BOOH induced DNA damage: Protection by BGS-Haridrin and other antioxidants

Oxidative damage of DNA damage resulted in various degenerative diseases [12]. Calf thymus DNA was sheared 60 times using 21gauze needle. The Sheared DNA (12µg) was treated with t-BOOH (125µM) in 100µl of PBS (20mM, pH-7.4). The same reaction was carried out in the presence of antioxidants using t-BOOH (125µM) in 100µl of PBS (20mM, pH-7.4) incubated at 37ºC for 30min. The DNA protectant activity of BGS-Haridrin (0.1µM) against t-BOOH was studied in comparison with BHA and Curcumin (400µM). These antioxidants were added prior to the addition of t-BOOH. Protection offered by antioxidant to DNA was detected on Submarine agarose gel electrophoresis. DNA bands were visualized under U.V transilluminator.

Isolation of Lymphocytes:

The lymphocytes were isolated from human blood according to Phillips HJ, method [13]. Human peripheral lymphocytes were isolated from 10 ml of venous blood drawn from healthy donors. Blood was collected in ACD (85mM citric acid, 71mM trisodium citrate, 165mM D-glucose) in the ratio of 5:1. Four volumes of hemolyzing buffer (0.85% NH_4Cl in 10mM tris buffer, pH 7.4) were added, mixed well, incubated at 40°C for 30 min. Centrifuged at 1200rpm for 12 min, pellet was washed again with 5ml of hemolysing buffer and the pellet containing cells were washed thrice with 10ml of Hank's Balanced salt solution (HBSS - 250mM m - insositol in 10mM phosphate buffer, pH 7.4) and suspended in same solution. The cell viability test was determined by tryphan blue exclusion method. To 10µl of lymphocyte sample 10µl of tryphan blue (0.02%) added and the cells were charged to Neuber's chamber and the cell number was counted. The survival rate lymphocytes were determined at sixty minutes of incubation. Viability was tested by Tryphan blue exclusion and exceeded 96% in each isolation. The percentage viability was calculated by using the following formula

$$\%\ \text{viability} = \frac{\text{Total no. of viable cells}}{\text{Total no. of viable cells + dead cells}} \times 100$$

Time course study of the effect of t-BOOH on the viability of lymphocytes was done. Lymphocytes cells (1X 10^6) were treated with t-

BOOH (10µg) in the presence or absence of antioxidants in 1ml of HBSS, pH 7.4 at 37^0C.
The simultaneous, post and pre treatment of antioxidants were carried out and after the desired incubation time up to 6 hours, the viability of the cells was determined by Tryphan blue exclusion analysis and the percentage of the viable cells was calculated.

Statistical Analysis:
The data were expressed as means ± standard deviations (SD). All the experiments were repeated at least six times and the values are expressed as Mean ± SD. The significant of the experimental observations was checked by Student's t-test and the value of p.

RESULTS AND DISCUSSION:

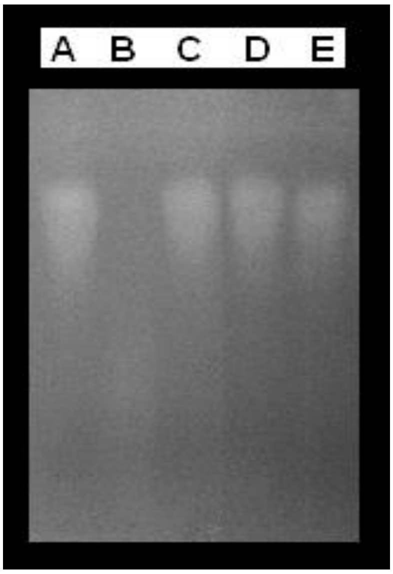

Fig.1: t-BOOH (125µM) Induced DNA Damage and Its Prevention by BGS-Haridrin (0.1µM) and other Antioxidants (400µM)

Lane A: Calf thymus DNA sheared (10 µg)
Lane B: DNA + t-BOOH (125µM)
Lane C: DNA + t-BOOH + BGS-Haridrin (0.1µM)
Lane D : DNA + t-BOOH + BHA (400µM)
Lane E: DNA + t-BOOH + Curcumin (400µM)

Sheared Calf Thymus DNA (10µg) ± t-BOOH (125µM) ± BGS-Haridrin (0.1 µM) / BHA (400µM) / Curcumin (400µM) in 100µl of 20mM PBS pH 7.4, 37°C for 30min. Reaction mixture of 4µg DNA loaded on to 0.8% agarose gel.

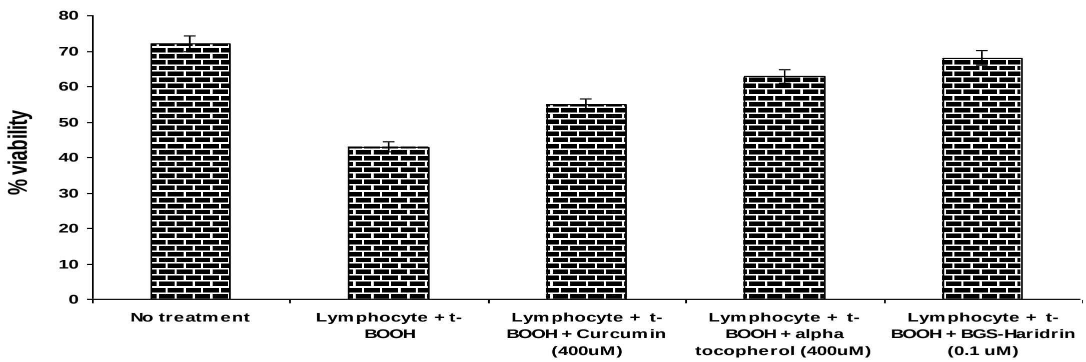

Fig 2: Study of Cell Toxicity Induced By t-Booh and Protection by Antioxidants
Lymphocytes (10^6cells) pretreated with or without antioxidants at indicated concentrations in 0.5ml HBSS pH 7.4, incubated at 37℃ for 20min., then t-BOOH (125µM) was added, incubated at 37℃ for 60 minutes in final volume of 1ml HBSS, pH 7.4. After the desired incubation time (60 minutes), viability of the cells was determined by tryphan blue exclusion and the percentage of viable cells was calculated as mentioned in methods.

The integrity of DNA is essential for the survival of cells. ROS are well known inducers of DNA damage and these reactive molecules include H_2O_2, $O_2^{\cdot-}$, $RO^{\cdot}$, $ROO^{\cdot}$ and $OH^{\cdot}$. DNA damage is also caused by transition metal ion induced reactions involving hydrogen peroxide which is weak ROS [14, 15, 16, 17]. When cells are incubated with t-BOOH causes damage to DNA and membrane disruption, leads to damage the DNA. In the present study, the antioxidant glycoprotein BGS-Haridrin was tested for its DNA protective activity using t-BOOH as a damaging agent in our present study Calf thymus DNA was used as a model system [18, 19].

Tertiary butyl hydroperoxide (t-BOOH) causes oxidative DNA damage [20, 21, 22]. Earlier it is reported that, BGS-Haridrin effectively inhibited the DNA damage induced by Hydrogen peroxide (H_2O_2) [23]. It is also reported that, proteins from *Piper longum* protects DNA damage induced by H_2O_2. **Fig-1** shows the damage caused by t-BOOH to calf thymus DNA. **Lane A** shows the sheared calf thymus DNA without any treatment, **Lane B** shows damage caused by t-BOOH (125µM) to sheared calf thymus DNA resulting in low molecular weight species of DNA and hence it is moved down. **Lane C** shows that BGS-Haridrin (0.1µM) offer protection to DNA against the damage induced by t-BOOH. The **Lane D** and **Lane E** show that, BHA provided same amount of protection as much as of BGS-Haridrin.. When compared to BGS-Haridrin and BHA, Curcumin is little less effective at a dosage of 400 µM. **Fig. 2** shows that, the decrease in viability induced by t-BOOH after one hour incubation and the cells were survived the presence of Curcumin at 400µM, α-tocopherol at 400µM and BGS-Haridrin at 0.1µM. Curcumin, alpha-tocopherol and BGS-Haridrin inhibits the cell damage 55%, 63% and 68% respectively. These results indicate that the efficiency of the each individual antioxidant tested exhibits efficient protection against t-BOOH. This protective role of antioxidants is probably resulted from the scavenging of free radicals.

CONCLUSION:

The protective effect of BGS-Haridrin – a 28kDa glycoprotein isolated, purified and characterized for its antioxidant activity and other biological activity herein showed that, it was more effective when compared to BHA, alpha tocopherol and a compound Curcumin isolated from the same source Turmeric.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Adichunchanagiri Mahasamstana Mutt and Shikshana Trust for providing facilities in AIMS-Central Research Laboratory, ABCRI for carrying out this work.

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