ejpmr, 2015,2(3), 568-574

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

HYDROGEN PEROXIDE INDUCED DNA DAMAGE: PROTECTION BY PIPPALI (*PIPER LONGUM*) AQUEOUS EXTRACT

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Article Received on 22/03/2015 **Article Revised on 15/04/2015** **Article Accepted on 08/05/2015**

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ABSTRACT

Reactive oxygen species causes oxidative damage to biological molecules like DNA which leads the formation of cancer and other chronic diseases. Dietary antioxidants are easily available, inexpensive, nontoxic in nature and well known to prevent the oxidative damage. The aqueous extract of Pippali (*Piper longum*) was investigated for its protective activity against Hydrogen peroxide induced DNA damage by using a sensitive DNA submarine gel electrophoresis method. The antioxidants present in the extract prevent the DNA damage very effectively. The water extract at 15µg concentration was used against H_2O_2 hydrogen peroxide induced DNA fragmentation in agarose gel electrophoresis which provides same protection like other standard antioxidant BHA (400µM). The cytotoxicity studies showed that, the extract and BHA provides a protection of 75% and 71% respectively. In other words, the dietary spice source Pippali (*Piper longum*) extract showed a better DNA protectant activity against Hydrogen peroxide induced DNA damage.

KEYWORDS: Reactive oxygen species, DNA damage, Dietary antioxidants, lymphocytes, Hydrogen peroxide.

INTRODUCTION

Reactive Oxygen Species (ROS) induced oxidative damage plays a key role in DNA damage (Ray et al., 2012). The term ROS is often used by biologists to include oxygen radicals and certain non-radicals that are either oxidizing agents and /or converted into radicals, like HOCl, ozone, peroxynitrite, singlet oxygen and Hydrogen peroxide (Wiseman H and

Halliwell B, 1996). ROS have been implicated in the pathophysiology of various states, including ischemia reperfusion injury, atherosclerosis, heart failure, acute hypertension, cancer etc. The free radicals, nitric oxide (NO) and $O_2^{\cdot-}$ react to form peroxynitrite (ONOO-) known as potent cytotoxic oxidant (Hemnani T and Parihar MS, 1998). During oxidative stress, the over produced Hydrogen peroxide can damage proteins, nucleic acids, cell membranes.[4] It is reported that, Hydrogen peroxide cause oxidative DNA damage primarily through the hydroxyl radical that results from the Fenton reaction (Siddique et al., 2009, Imlay et al., 1988, Halliwell B and Gutteridge JM., 1992, Josephson et al., 1991), reported that, Hydrogen peroxide associated with the induction of cancer in animals, mutagenic and carcinogenic (Andreoli et al., 1999, Pryor WA, 1986). It has been reported that, which may cause DNA damage in the form of chromosomal aberrations, sister chromatid exchanges, single strand breaks and double strand breaks (Sofni T, and Ishidate M, 1984, Tsuda H., 1981, Prise et al., 1989, Thibodeau PA and Paquette B, 1999, Namrah anwar et al., 2014). In the present study, we have examined the DNA damage protectant activity of aqueous extract of *Piper longum*.

MATERIALS AND METHODS

Calf thymus DNA (CT DNA), BHA, Agarose, Ethidium bromide, SDS, H_2O_2, Thiobarbutaric acid, Diphenylamine (DPA) was from Sigma Chemical company USA. Ascorbic acid was from HIMEDIA, India. EDTA, H_2O_2 were from s.d. fine Chem. Ltd. India. All the other chemicals were of Anal. R grade. All organic solvents were distilled prior to use.

The fresh *Piper longum seeds* were collected from local market, homogenized to fine powder and stored in air tight glass container. The extraction was done according to the method of Dinesha and Chikkanna (Dinesha R and Chikkanna D, 2014) using 10g of powder in 100ml of double distilled water. The mixture was vortexed for 2 hours and centrifuged at 10000 rpm. The obtained supernatant was concentrated to require volume using hot water bath and the preliminary qualitative phytochemical studies was done as explained by Dinesha and Chikkanna, 2014.

Isolation of lymphocytes

The lymphocytes were isolated from human blood according to Phillips HJ, 1973 method. 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 **(**Kotzé, 1997). 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 60 minutes of incubation. Viability was tested by Tryphan blue exclusion method. The percentage viability was calculated by using the following formula

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

Time course study of the effect of H_2O_2 on the viability of lymphocytes. Lymphocytes cells (1X 10^6) were treated with H_2O_2 (144µM) 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.

DNA damage and its protection by Agarose gel electrophoresis

The DNA damage studies using agarose gel electrophoresis was done according the method of Sultan et al., 1995. 10µg of Calf thymus DNA was pretreated with Pippali (*Piper longum*) extract (10µg), or BHA (400µM) in 0.5 ml HBSS, pH 7. At 37°C for 20 minutes, then H_2O_2 (144µM) was added and the final volume was made to 1ml with HBSS, pH 7.4 and incubated at 37°C for 60 min, then centrifuged at 1200 rpm, 20 minutes at 40°C. Then DNA samples were run on 1% Agarose prepared in TBE buffer and the Ethidium bromide was incorporated into the gel at a concentration of 1µg/ml, 2µg of DNA was loaded on to the wells and run in TBE buffer (10mM Tris, Boric acid, EDTA, pH 8.0) at 60 volts. The bands were visualized and photographed under transilluminator.

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 significance of the experimental observation was checked by student's test and the value of p value

RESULTS

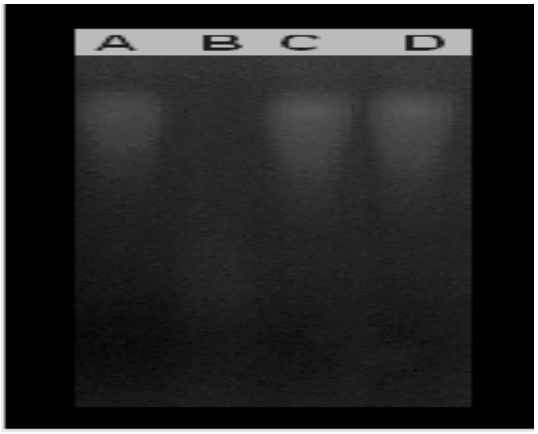

Fig.1: H_2O_2 (144µM) induced DNA damage and its prevention by Pippali (*Piper longum*) aqueous extract.

Lane A: Calf thymus DNA (10µg)

Lane B: Calf thymus DNA (10µg) + H_2O_2 (144µM)

Lane C: H_2O_2 (144µM) + Pippali *(Piper longum)* aqueous extract (25µg)

Lane D: H_2O_2 (144µM) + BHA (400µM)

in 100 µl TBE (10 mM Tris–boric acid–EDTA, pH 7.4), incubated at 37°C for 30min. Electrophoresis was carried out at 80 V. Bands were visualized using UV transilluminator.

Table -1: Study of cell toxicity induced by H_2O_2 and protection by antioxidants

Lymphocytes	% viability
Lymphocytes alone (10µl)	88± 3
Lymphocytes (10µl) + H_2O_2 (144µM)	43± 1
Lymphocytes + H_2O_2 (144µM) + BHA (400µM)	71± 4
Lymphocytes + H_2O_2 (144µM) + Pippali *(Piper longum)* (25µg)	75± 3

Lymphocytes (10^6cells) pretreated with or without antioxidants at indicated concentrations in 0.5ml HBSS pH 7.4, incubated at 37°C for 20min., then H_2O_2 (144µM) was added, incubated at 37°C 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.

DISCUSSION

The extent of H_2O_2 (144µM) induced DNA damage was considerable in the lymphocytes as measured by diphenylamine method. As shown in Fig.1., the *Piper longum* extract offered effective protection at 25µg concentration against H_2O_2 (144µM) induced DNA fragmentation in lymphocytes where as *Piper longum* aqueous extract at 25µg and BHA at 400 µM dosage inhibited DNA fragmentation respectively. The above result indicated that *Piper longum* aqueous extract is effective in preventing DNA fragmentation like standard antioxidant BHA. We also investigated the protective effects of *Piper longum* extract against H_2O_2 induced lymphocyte cell death. The viability of lymphocytes on simultaneous pre treatment of H_2O_2, a time course study was done. As shown in the Table-1, the decrease in viability brought about by H_2O_2 after 60 mins of incubation and the viability was found to be 43 ± 1% from 88 ± 3% and it was vastly improved by the presence of BHA at 400 µM and *Piper longum* (25µg) which showed 71 ± 4 and 75 ± 3% viability, respectively. These results indicate that the efficiency of the each antioxidant tested exhibits efficient protection against H_2O_2. Thus the protective mechanism against oxidative DNA damage by *Piper longum* extract is probably due to quenching the free radicals mainly hydroxyl radical or ROS. In summary, the protective effect of aqueous extract of *Piper longum* was equally efficient to BHA and more efficient on pro-oxidants induced lymphocyte cell damage and oxidative DNA damage in lymphocytes at a lower dose of 25µg concentration.

CONCLUSION

This preliminary results showed that, the protective effect of an aqueous extract of *Piper longum* against pro-oxidant induced DNA damage and proved that, it is non toxic to cells.

ACKNOWLEDGMENT

The authors gratefully acknowledged Adichunchanagiri Institute of Medical Sciences and Adichunchanagiri Biotechnology and Cancer Research Institute, B.G.Nagara for providing the facilities for carrying out this research work.

REFERENCE

1. Andreoli C, Leopardi P, Rossi S and Crebelli R. Processing of DNA damage induced by hydrogen peroxide and methyl methane sulfonate in human lymphocytes: analysis by alkaline single gel electrophoresis and cytogenetic methods. *Mutagenesis*, 1999; 14: 497-503.

2. Daroui P, Desai SO, Li TK, Liu AA and Liu LF. Hydrogen peroxide induces topoisomerase in mediated DNA Damage and cell death. *J Biol Chem.*, 2004; 279: 14587-95.
3. Dinesha R and Chikkanna D, Antioxidant activities of Pippali *(Piper longum)* proteins, *Int. J. Pharm Drug Anal*, 2014; 2(11): 811-814.
4. Halliwell B and Gutteridge JM. Biologically relevant metal ion dependent hydroxyl radical generation. An update. *FEBS Lett*, 1992; 307: 108-12.
5. Hemnani T and Parihar MS, Reactive Oxygen Species and oxidative DNA damage-Review article, *Indian J Physiol Pharmacol*., 1998; 42(4): 440-452.
6. Imlay JA, Chin SM, and Linn S. DNA damage and oxygen radical toxicity. *Science,* 240, 1988, 640-42.
7. Josephson RA, Silverman HS, Lakatta EG, Stern MD and Zweier JL. Study of the mechanisms of Hydrogen peroxide and hydroxyl free radical induced cellular injury and calcium overload in cardiac myocytes. *J Biol Chem*, 1991; 266: 2354-61.
8. Kotzé, Awie R, Henrik L, Lueβen, Bas J. de Leeuw, J. Coos Verhoef and Hans E. Junginger. N-trimethyl chitosan chloride as a potential absorption enhancer across mucosal surfaces: in vitro evaluation in intestinal epithelial cells (Caco-2), *Pharmaceutical research.*, 1997; 14(9): 1197-1202.
9. Phillips HJ, Dye exlusion test for cell viability. In: Tissue culture methods (Krus, P.F., Pa tterson, M.K. eds)., 1973; 406-408.
10. Prise KM, Davies S, Michael BD. Cell killing and DNA damage in Chinese hamster V79 cells treated with hydrogen peroxide. *Int J Radiat Biol*, 1989; 55: 583-92.
11. Pryor WA. Oxy radicals and related species, their formation, life times and reactions. *Annu Rev Physiol*, 1986; 48: 657-67.
12. Ray, Paul D, Huang BW and Tsuji Y, Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling, *Cellular signaling.*, 2012; 24(5): 981-990.
13. Siddique YH., Beg T., and Afzal M., Protective effect of ascorbic acid against oxidative damage induced by hydrogen peroxide in cultured human peripheral blood lymphocytes, *Indian Journal of Clinical Biochemistry*, 2009; 24(3): 294-300.
14. Sofni T, and Ishidate M. Induction of chromosomal aberrations in cultured Chinese hamsters cells superoxide generation system. *Mutat Res*, 1984; 140: 27-31.
15. Sultan S, Perwaiz S, Iqbal M and Athar M. Crude extracts of hepatoprotective plants, *Solanum nigrum* and *Cichrium intybus* inhibit free radical mediated DNA damage. *Journal of Ethanopharmacology*, 1995; 45: 189-192.

16. Thibodeau PA and Paquette B., DNA damage induced by catechol estrogens in the presence of copper (II): generation of reactive oxygen species and enhancement by NADH Free. *Radic Biol Med*, 1999; 27: 1367-77.
17. Tsuda H. Chromosomal aberrations induced by hydrogen peroxide in cultured mammalian cells. *Japanese J Genet*, 1981; 58: 1-8.
18. Wiseman H and Halliwell B, Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer - Review article, *Biochem. J.*, 1996; 313: 17–29.