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PROTECTIVE ROLE OF PTEROSTILBENE AGAINST SODIUM FLUORIDE INDUCED PLASMA TOXICITY IN RATS: AN *IN-VITRO* AND *IN-VIVO* STUDY

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ABSTRACT

In the present study was to explore the protective effect Pterostilbene (PTSB) on plasma lipid peroxidation, antioxidant status (*In-vivo*) and free radical scavenging property (*In-vitro*) against sodium fluoride (NaF) induced toxicity in rats. In this investigation sodium fluoride (25 mg/kg BW) was administrated intragastrically for 4 weeks to induce toxicity. Pterostilbene (PTSB) was administrated orally (40 mg/ kg BW) for 4 weeks along with sodium fluoride. The toxic effect of fluoride was indicated by the elevated levels of lipid peroxidation and significantly decreased activities of non-enzymatic antioxidants like GSH, Vit.C and E in the plasma of rats. Administration of PTSB revealed a significant ($p<0.05$) decreased in lipid peroxidation with a significant ($p<0.05$) increase antioxidant status in plasma of fluoride treated rats. Using of PTSB with five different concentrations (10, 20, 30, 40, and 50 µM) free radical scavenging activity was evaluated by following *in vitro* assays such as 1,1-diphenyl-2- picrylhydrazyl (DPPH•), 2, 2-azinobis-(3-ethylbenzothiazoline- 6-sulfonic acid) (ABTS), superoxide anion (O•), hydroxyl (OH•) radicals and nitric oxide (NO) assay. In addition to that, gallic acid and butylated hydroxyl toluene was used as the standard antioxidant for radical scavenger compounds. The *in-vitro* assays are confirmed that, the free radical scavenging and antioxidant activity of PTSB has increasing with increasing concentrations. Interestingly, among the different concentrations tested, 40µM of PTSB showed the highest antioxidant and free radical scavenging activities in all in vitro assays. *In-vivo* activity of PTSB also showed elevated antioxidant with decreased lipid oxidation markers. Hence the present study proved that the PTSB has potent In-vitro and In-vivo antioxidant activity with free radicals scavenger, augmenting its therapeutic potential against fluoride induced toxicity in rats.

KEYWORDS

PTSB, Antioxidant, Free radical, *In vivo*, *In vitro*, Sodium fluoride and ROS.

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INTRODUCTION

Fluoride (Fl) is ubiquitous in the environment; therefore, sources of drinking water are likely to contain at least some small amount of fluoride. However, in areas of the world in which endemic fluorosis of the skeleton or teeth has been well documented, the level of fluoride in drinking water

supplies range from 3 to more than 20 mg/l. In areas in which drinking water is fluoridated (i.e., fluoride is intentionally added for the prevention of dental caries), the concentration of fluoride in drinking water generally ranges from 0.7 to 1.2 mg/l. Low intakes of fluoride are associated with an increased incidence of dental caries and addition of fluoride, at 1 mg/kg to water supplies reduces this. The body burden of fluoride is regulated by renal excretion. Fluorosis is excessive deposition of fluoride, particularly in the bones and teeth. It occurs when the daily intake exceeds 20 mg of fluoride (WHO, 2002)[1]. Fl- is a potent inhibitor of many enzymes and was used as an important tool to define certain steps in the glycolytic pathway (Whitford 1996)[2]. Recent studies revealed that Fl induces excessive production of oxygen free radicals, and might cause the depletion in biological activities of some antioxidant enzymes (Chlubek 2003)[3].

Free radicals have been implicated in the causation of several problems like asthma, cancer, cardiovascular disease, cataract, diabetes, gastrointestinal inflammatory disease, liver disease, muscular degeneration and other inflammatory process (Sen, *et al.,* 2008)[4]. Reactive oxygen species (ROS) are continuously produced during cell metabolism and under normal circumstances they are scavenged and renewed to nonreactive species by diverse intracellular enzymatic and non-enzymatic antioxidant system (Shao *et al.,* 2008)[5]. Over production or an ineffective eradication of ROS may induce oxidative stress and cause damage to all types of bimolecular such as proteins, lipids and nucleic acids (Droge 2002)[6]. Antioxidants may act as free radical scavengers, reducing agents, chelating agents for transition metals, quenchers of singlet oxygen molecules and or activators of antioxidative defense enzyme system to suppress the radical damages in biological systems (Murphy *et al.,* 2011[7], Venkatesh *et al.,* 2009)[8]. Antioxidants thus play an important role in the protection of human body against damage by reactive oxygen species (Peng *et al.,* 2011[9], Ling *et al.,* 2011)[10]. Therefore, inhibition of free radical-induced oxidative damage by supplementation of antioxidants has become an attractive therapeutic strategy for reducing the risk of these diseases.

In recent years, it has been investigated that many plant species are serving as source of antioxidants and received therapeutic significance (Pari and Amutha, 2011)[11]. Antioxidants thus play an important role in the protection of human body against damage by reactive oxygen species (Thambiraj *et al.,* 2012)[12]. Therefore, inhibition of free radical-induced oxidative damage by supplementation of antioxidant has become an attractive therapeutic strategy for reducing the risk of these diseases. Pterostilbene (PTSB) is well known to be most abundant in green tea, is an effective scavenger of ROS in vitro and may also function indirectly as antioxidant through its effects on transcription factors and enzyme activities (Rimando *et al*., 2004[13], Kosuru and Singh 2017)[14]. PTSB is known to block many targets in signal transduction pathways, including NF-kB and Nrf2 (Chiou *et al*., 2011)[15]. Therefore the objective of the present investigation was intended to explore the ameliorative potency of Pterostilbene via its *In-vitro* and *In-vivo* against NaF induced toxicity.

MATERIAL AND METHODS

Drug and Chemicals

PTSB was purchased from Sigma Aldrich, USA. Sodium fluoride, reduced glutathione, butylated hydroxytoluene (BHT), 2, 2′- dipyridyl, xylenol orange, 4-dinitrophenylhydrazine (DNPH), 5, 5′-dithiobis-2-nitrobenzoic acid (DTNB), DPPH, ABTS and butylated hydroxytoulene , ascorbic acid were obtained from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals and solvents were of certified analytical grade and purchased from S. D. Fine Chemicals, Mumbai or Hi media Laboratories Pvt. Ltd., Mumbai, India. Reagent kits were obtained from span Diagnostics, Mumbai, India.

Animals

Male albino rats of Wistar strain with a body weight ranging from 160 to 180 g, were procured from Central Animal House, Department of Experimental

Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, and were maintained in an air conditioned room (25±1°C) with a 12 h light/12 h dark cycle. Feed and water were provided ad libitum to all the animals. The study was approved by the Institutional Animal Ethics Committee of Rajah Muthiah Medical College and Hospital (Registration Number: 160/1999/CPCSEA, Proposal number: 1169/2017), Annamalai University, Annamalainagar.

Experimental design:

The animals were randomly divided into four groups of six rats in each group.

Group 1: Control rats treated with normal saline for 28 days.

Group 2: Normal rats orally received PTSB (40mg/kg body weight) (Thangapandiyan and Milton Prabu 2013)[16] dissolved in corn oil for 28 days.

Group 3: Rats received fluoride as sodium fluoride (25 mg/kg body weight) (Chinoy 1991)[17] in normal saline for 28 days.

Group 4: Rats orally received NaF (25mg/kg body weight) with pre-oral administration of PTSB (40 mg/kg body weight) for 28 days.

At the end of the experimental period, animals in different groups were sacrificed by cervical decapitation. Blood samples were collected in heparinised tubes, for plasma. Plasma separated by centrifugation (10000×g for 10min) was used for various biochemical estimations.

Biochemical assays

***In vivo* assays**

Estimation of lipid peroxidation

Lipid peroxidation in plasma was estimated spectrophotometrically by measuring thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (LOOH) by the method of Niehiaus and Samuelsson (1968)[18] and Jiang (1992)[19], respectively. In brief, plasma (0.1 ml) was treated with 2ml of TBA–Trichloroacetic acid (TCA)–HCl reagent (0.37% TBA, 0.25N HCl and 15% TCA, 1:1:1 ratio) placed in a water bath for 15 min and cooled and centrifuged at room temperature, clear supernatant was measured at 535 nm against a reagent blank. A 0.1 ml aliquot of plasma was treated with 0.9 ml of Fox reagent (88 mg of butylated hydroxytoluene, 7.6 mg of xylenol orange and 0.8 mg of ammonium iron sulfate were added to 90 ml methanol and 10 ml of 250mM sulfuric acid) and incubated at 37°C for 30 min. The colour that developed was read at 560nm.

Determination of non-enzymatic antioxidants

Reduced glutathione (GSH) was determined by the method of Ellman (1959)[20]. 1 mL of supernatant was treated with 0.5 mL of Ellman's reagent (19.8 mg of 5, 5-dithiobisnitro benzoic acid in 100mL of 0.1% sodium citrate) and 3.0 mL of phosphate buffer (0.2 M, pH 8.0) was added and the absorbance was read at 412 nm in spectrophotometer. Ascorbic acid (vitamin C) concentration was measured by Omaye (1979)[21]. To 0.5mL of plasma, 1.5mL of 6% TCA was added and centrifuged (3500 ×g, 20 min). To 0.5mL of supernatant, 0.5mL of DNPH reagent (2% DNPH and 4% thiourea in 9N sulfuric acid) was added and incubated for 3 h at room temperature. After incubation, 2.5mL of 85% sulfuric acid was added and colour developed was read at 530 nm after 30 min. Vitamin E was estimated by the method of Desai (1984)[22]. Vitamin E was extracted from plasma by addition of 1.6mL ethanol and 2.0mL petroleum ether to 0.5mL plasma and centrifuged. The supernatant was separated and evaporated on air. To the residue, 0.2mL of 0.2% 2, 2-dipyridyl, 0.2mL of 0.5% ferric chloride was added and kept in dark for 5 min. An intense red colored layer obtained on addition of 4mL butanol was read at 520 nm.

Determination of *In-vitro* activity

Superoxide anion scavenging activity

Superoxide anion scavenging activity of PTSB was determined by the method of Nishmiki *et al.,* (1972)[23] with modification. One ml of NBT (100µMoles of NBT in 100mM phosphate buffer, pH 7.4), 1 ml of NADH solution (14.68µ Moles of NADH in 100m moles phosphate buffer, pH 7.4) and varying concentrations of PTSB (10, 20, 30, 40, and 50µ Moles) were mixed well. The reaction was started by the addition of 100µ Moles of PMS (60µ

Moles) 100m moles of phosphate buffer, pH 7.4). The reaction mixture was incubated at 30ºC for 15 min. The absorbance was measured at 560 nm in a spectrophotometer (Elico- Sl177). Incubation without PTSB was used as blank. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging.

Hydroxyl Radical Scavenging Assay

The hydroxyl radical scavenging activity was determined by the method of Halliwell *et al.,* (1989)[24]. The following reagents were added in the order stated below. The incubation mixture in a total volume of 1 ml contained 0.1 ml of 100m moles of potassium dihydrogen phosphate- KOH buffer, varying concentrations of PTSB (10, 20, 30, 40, and 50µ Moles), 0.2 ml of 500m moles of ferric chloride, 0.1 ml of 1m moles of ascorbic acid, 0.1 ml of 10m moles of H2O2 and 0.2ml of 2-deoxy ribose. The contents were mixed thoroughly and incubated at room temperature for 60 min. Then added, 1 ml of 1% TBA (1 gm in 100 ml of 0.05 N sodium hydroxide) and 1 ml of 28% TCA. All the tubes were kept in a boiling water bath for 30 min. The absorbance was read in a spectrophotometer at 532 nm (Elico- Sl177) with reagent blank containing distilled water in a place of PTSB. The percentage scavenging activity was determined. Decreased absorbance of the reaction mixture indicated increased hydroxyl radical scavenging activity.

Free Radical Scavenging Activity

The ability to scavenging the stable free radical, DPPH was measured as a decrease in absorbance at 517 nm by the method of Mensor *et al.,* (2001)[25] to a methanolic solution of DPPH (90.25m moles), an equal volume of PTSB (10, 20, 30, 40, and 50µ Moles) dissolved in distilled water was added and made up to 1.0 ml with methanolic DPPH. An equal amount of methanol was added to the control. After 20 min, the absorbance was recorded at 517 nm in a Spectrophotometer (Elico-Sl177).

Formula for calculations:

$$\text{RSA activity (\%)} = \frac{\text{Control OD-Sample OD}}{\text{Control OD}} \times 100$$

Determination of nitric oxide (NO) radical scavenging activity

The nitric oxide level was determined by Griess Illosvoy reaction (Sakat *et al.,* 2010)[26]. Various concentrations (1.9-500 mg/mL) of PTSB (10, 20, 30, 40, and 50 µM) (3 mL) and ascorbic acid were dissolved in methanol and incubated at 25◦C for 150 minutes. The prepared sample was reacted with reagents (1% (w/v) sulphanilamide, 2% O-phosphoric acid and 0.1% N-(1-napthyl) ethylenediamine hydrochloride). The absorbance at 546 nm of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylene diamine was measured. The EC_{50} values were determined as the concentration of the test mixture that gave 50% reduction in the absorbance compared to the control blank.

Formula for calculations:

$$\text{NO Scavenging activity (\%)} = \frac{\text{Control OD - Sample OD}}{\text{Control OD}}$$

Statistical Analysis

The values are given as mean ± S.D. for six rats in each group. The data for various biochemical parameters were analyzed using analysis of variance (ANOVA) and the group means were compared by Duncan's Multiple Range Test (DMRT). Values were considered statistically significant when $p<0.05$ (Duncan, 1957)[27].

***In-vitro* Results**

PTSB activity on *In-vitro* superoxide anion scavenge

Figure No.1 shows the superoxide radical scavenging ability of PTSB on *In vitro*. The superoxide radical scavenging activities of PTSB were exhibit dose dependent manner (10, 20, 30, 40, and 50µM). The percentage scavenging activity of PTSB on superoxide radical was increases with increasing concentrations. The maximum free radical scavenging ability of PTSB were observed in the fifty percent of effective concentration (EC_{50}%) at the value of 53.5% at the concentration of 40µM compared with ascorbic acid was observed.

PTSB activity on *In-vitro* Hydroxyl radical scavenge

Figure No.2 shows the hydroxyl radicals scavenging effect of PTSB on *In vitro*. PTSB scavenges hydroxyl radicals scavenging activities in a dose dependent manner (10, 20, 30, 40 and 50µM). The percentage scavenging activity of PTSB on hydroxyl radicals scavenging activities increases with increasing concentration. The maximum activities observed in the highest concentration and the fifty percent of effective concentration (EC_{50}%) value of PTSB is 51.5, at the concentration of 40µM compared with ascorbic acid was observed.

PTSB activity on *In-vitro* Free radical scavenge

Figure No.3 shows the percentage of free radical scavenging effect of PTSB *In vitro* by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH•) assay. The hydrogen atom or electron donation abilities of the compounds were measured from the bleaching of the purple-colored methanol solution of 2, 2′-diphenyl-1-picrylhydrazyl (DPPH). PTSB scavenges DPPH radical in a dose dependent manner (10, 20, 30, 40 and 50µM). The percentage scavenging activity of PTSB on DPPH radical increases with increasing concentration. The maximum activities observed in the highest concentration and the fifty percent of effective concentration (EC_{50}%) value of PTSB is 43.5, at the concentration of 40 µM compared with ascorbic acid was observed.

PTSB activity on *In-vitro* Nitric oxide scavenge

Figure No.4 shows the nitric oxide (NO) scavenging activities of PTSB in *In vitro*. The PTSB nitric oxide (NO) scavenging activities were increased in a dose dependent manner (10, 20, 30, 40 and 50µM). The percentage scavenging activity of PTSB on nitric oxide (NO) increases with increasing concentration. The maximum activities observed in the highest concentration and the fifty percent of effective concentration (EC_{50}%) value of PTSB is 63.2 at the concentration of 40µM compared with ascorbic acid was observed.

***In-vivo* Results**

PTSB activity on plasma lipid peroxidation

Table No.1 shows the changes in plasma lipid peroxidative (LPO) markers (TBARS and LOOH) in control and experimental rats. In rats treated with fluoride, the levels of plasma TBARS and LOOH were significantly increased ($p < 0.05$). Administrations of PTSB significantly decreased fluoride induced LPO compared with fluoride alone treated rats. PTSB alone treated rats did not show any changes in the LPO markers compared with control group.

PTSB activity on antioxidant levels

The levels of non-enzymatic antioxidants (GSH, Vitamin C, and Vitamin E) in plasma of normal and experimental rats have shown in Table No.2. In fluoride treated rats, the non-enzymatic antioxidants were found to be significantly ($p < 0.05$) decreased when compared to control rats. Administration with PTSB to fluoride treated rats showed a significant ($p < 0.05$) increase in non-enzymatic antioxidants levels compared with fluoride alone treated rats. PTSB alone administrated rat's exhibits no changes in their antioxidant levels.

DISCUSSION

Free radicals have been implicated in a number of diseases including asthma, cancer, cardiovascular disease, cataract, diabetes, gastrointestinal inflammatory diseases, liver diseases, muscular degeneration and other inflammatory processes (Wilson, 1998)[28]. Radical scavenging activities are very important due to the deleterious role of free radicals in biological systems. In vitro antioxidant activity of the PTSB was investigated in the present study by DPPH, hydroxyl radical scavenging, superoxide anion assays. These methods have proven the effectiveness of the PTSB in comparison to that of the reference standard antioxidant gallic acid.

Superoxide anion is a precursor to active free radicals that have potential of reacting with biological macromolecules and, thereby, inducing tissue damage. Yen and Duh (1994)[29] have reported that superoxide anions damage biomolecules

directly or indirectly by forming H2O2, OH, and peroxy nitrite or singlet oxygen during aging and pathological events. Perecko *et al.,* (2011)[30] has been reported that Pterostilbene (PTSB), known to be most abundant in blue berries, is an effective scavenger of ROS *in vitro*. In the present study, using different concentration of PTSB shows the highest percentage scavenging effect on superoxide radical scavenging activity with previous findings of Kosuru R and Singh S (2017)[14] was observed. The maximum percentage scavenging effect of PTSB on superoxide radicals was 53.5% observed at the concentration of 40µM. This observable fact due to the presence of PTSB quinone molecules, and they are produced after PTSB molecules are oxidized. Then the PTSB quinone molecules react with PTSB molecules via phenolic coupling reactions (SN2) to form PTSB dimmers (20, 200-gallyl adduct). Each PTSB dimer retains its reactive hydroxyl groups and exhibits a powerful antioxidant capacity (Alosi *et al.,* (2010)[31].

The hydroxyl radical, (•HO) is the neutral form of the hydroxide ion (HO^-). Hydroxyl radicals are highly reactive and consequently short-lived; however, most notably hydroxyl radicals are produced from the decomposition of hydro peroxides. The hydroxyl radical can damage virtually all types of macromolecules: carbohydrates, nucleic acids (mutations), lipids (lipid peroxidation) and amino acids (e.g. conversion of Phe to m-Tyrosine and o-Tyrosine). Hydroxyl radical will react with other elements to produce free radical which is very dangerous to the organism (Rimando *et al.,* 2004)[13]. In the present exploration, we have used different concentration of PTSB and the highest percentage scavenging effect of PTSB on hydroxyl radicals was 51.5% observed at the concentration of 40µM.

Free radical scavenging activity (DPPH) is a very useful method as it is highly sensitive and rapid assay (Zhang *et al.,* 2012)[32]. This assay is independent on substrate polarity where DPPH can accept an electron or hydrogen radical to become a stable diamagnetic molecule (Alosi, *et al*., 2010)[31]. When antioxidant scavenges the free radical by hydrogen donation, the purple color of DPPH in assay solution turns to yellow, which can be monitored spectrophotometrically at 517 nm (Yen *et al.,* 2008)[33]. Chang *et al.,* (2007)[34] proved that Phellinus merrilli extracts shows the effective scavenging activity on DPPH radicals. In the present investigation, our antioxidant PTSB also scavenge DPPH• radical in dose dependent manner. The highest percentage scavenging effect of PTSB 43.5% on 2, 2′-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) radical was observed at the concentration of 40µM. This is due to the prevention of free radical species by PTSB from damaging biomolecules such as lipoproteins, polyunsaturated fatty acids (PUFA), DNA, amino acids, proteins in a biological system by its natural hydrogen donating ability (Mannal *et al*., 2010)[35].

The reduction of the 2, 2'-azinobis (3-ethylbenzothiazoline sulphonate) radical cation (ABTS) has been widely used to measure the antioxidant capacity of natural extracts (Kalpana devi and Mohan, 2012)[36]. ABTS is a relatively stable free radical which involves in the direct generation of ABTS radical monocation without any involvement of intermediary cation. In the present study, the total antioxidant activity of PTSB was investigated in vitro by 2, 2′-azinobis- (3-ethyl-benzothiazoline-6-sulfonic acid) cation which forms the basis of one of the spectrophotometric (734 nm) methods that have been applied to the total antioxidant activities of solutions of pure substances. In this investigation, PTSB scavenges 2, 2′- azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) radical shows dose dependently. A similar result has been reported by Mannal *et al*., (2010)[35] with PTSB. The highest percentage scavenging effect of PTSB (57.8%) on 2, 2′- azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) radical was observed at the concentration of 40µM .This is mainly due to the strong antioxidant property of PTSB and the presence of its vicinal trihydroxy structure in which oxygen atoms act as electron donors to from bonds with electrophilic ions and thereby helps in the recoupment of antioxidant defense system (Higdon and Frei, 2003)[37].

Nitric oxide is a free radical produced in mammalian cells, involved in the regulation of various physiological process including neurotransmission, vascular homeostasis, antimicrobial and antitumor activities. However, excess production of NO is associated with several diseases (Hepsibha *et al.,* 2010)[38]. Rajamanikandan *et al.,* (2011)[39] has reported that administration of ethanolic extracts Mollugo nudicaulis exhibits increasing abrogation of nitric oxide with increasing concentrations. In the present investigation also in agreement with the previous findings that PTSB shows increasing abrogation of nitric oxide with increasing concentrations. The maximum levels of nitric oxide reducing ability of PTSB (63.2%) at the concentration of 40µM. This is due to potent nitric oxide radical scavenging activity of PTSB, which competes with oxygen to react with nitric oxide and thus inhibits the generation of nitrite.

Fluoride is also one of the main environmental and occupational pollutants in industrialized countries and induces a wide array of toxicological effects, biochemical dysfunctions in various organ systems posing a serious threat to health. Fluoride induces oxidative damage by producing reactive oxygen species and decreasing the biological activities of some antioxidant enzymes, such as GSH, vitamins C, and E, which play an important role in antioxidative and elimination of free radicals (Shivarajashankara *et al.,* 2001)[40]. Fluoride has also been reported to cause damage to lipids and by that to generate lipid peroxidation. Cell membranes are phospholipid bilayers with extrinsic proteins and are the direct target of lipid peroxidation which leads to a number of deleterious effects such as increased membrane rigidity, osmotic fragility, cell membrane destruction and cell damage. The observed increase in the level of plasma TBARS and LOOH in fluoride toxicity is generally thought to be the consequence of increased production and liberation of tissue lipid peroxides into circulation due to the pathological changes in tissues. Pei Feng, *et al.,* (2012)[41] also observed that treatment with fluoride increased the lipid peroxide concentration in blood. Increase in lipid peroxidation in plasma and tissues have been implicated in fluoride - induced organ damage and dysfunction. Treatment with PTSB significantly reverted the fluoride induced peroxidative damage in plasma which is evidenced from the lowered levels of TBARS and LOOH. This may be due to the antioxidative effect of PTSB. Vitamin C is a primary antioxidant, water-soluble vitamin that can directly scavenge singlet oxygen, superoxide and hydroxyl radicals. Numerous reports have shown the positive effect of vitamin C as an antioxidant and scavenge of free radicals (Das *et al.,* 2001)[42]. Vitamin E (α-tocopherol) is a well-known antioxidant: it acts as a free radical scavenger, more exclusively within cell membranes by preventing the oxidation of polyunsaturated lipids by free radicals such as the hydroxyl radical. The antioxidant potential of vitamin E is no longer disputed. Most in vivo studies have shown that vitamin E improves various parameters of oxidative stress in both animals and human beings (Golestani, *et al.,* 2006[43], Martin, *et al.,* 1996)[44].

GSH is a tripeptide (L-γ-glutamylcysteinylglycine), an antioxidant and a powerful nucleophile, critical for cellular protection such as detoxification of ROS, conjugation and excretion of toxic molecules and control of inflammatory cytokine cascade (Brown, *et al.,* 2004)[45]. Depletion of GSH in tissues leads to impairment of the cellular defence against ROS and may result in peroxidative injury. In our study we observed decreased concentration of vitamins C, E and GSH in plasma in fluoride-induced rats. Administration with PTSB brought normal levels of these antioxidants. It might be due to excellent antioxidant activity of DATS with mechanism involving both free radical scavenging and metal chelation (Pari and Sathish 2006)[46].

Table No.1: Changes in the levels of plasma TBARS and LOOH of control and experimental rats

S.No	Group	Parameters in plasma	
		TBARS (mM/dl)	LOOH (×10-5 mM/dl)
1	Control	0.13 ± 0.02[a]	13.68 ± 0.73[a]
2	PTSB (40 mg/kg bw)	0.9 ± 0.01[b]	8.06 ± 0.65[b]
3	Fl (25 mg/kg bw)	0.24 ± 0.03[c]	15.70 ± 0.76[c]
4	Fl + PTSB (25 mg/kg bw) + PTSB (40 mg/kg bw)	0.16 ± 0.02[d]	12.32 ± 1.0[d]

Values are given as mean ± S.D. from six rats in each group; Values not sharing a common letter (a-d) differ significantly at p<0.05 (DMRT).

Table No.2: Changes in the levels of vitamin - C, vitamin - E and reduced glutathione (GSH) in plasma of control and experimental rats

S.No	Group	Parameters in Plasma		
		GSH (mg/dl)	Vit.C (mg/dl)	Vit.E (mg/dl)
1	Control	18.15 ± 1.34[a]	1.61 ± 0.18[a]	1.19 ± 0.07[a]
2	PTSB (40 mg/kg bw)	20.17 ± 1.52[b]	1.74± 0.15[a]	1.38 ± 0.08[a]
3	Fl (25 mg/kg bw)	12.64 ± 1.13[c]	1.18 ± 0.10[b]	0.68 ± 0.02[b]
4	Fl + PTSB (25 mg/kg bw)+ PTSB (40 mg/kg bw)	19.43± 1.07[d]	1.50 ± 0.07[c]	1.12 ± 0.17[c]

Values are given as mean ± S.D. from six rats in each group; Values not sharing a common letter (a-d) differ significantly at p<0.05 (DMRT)

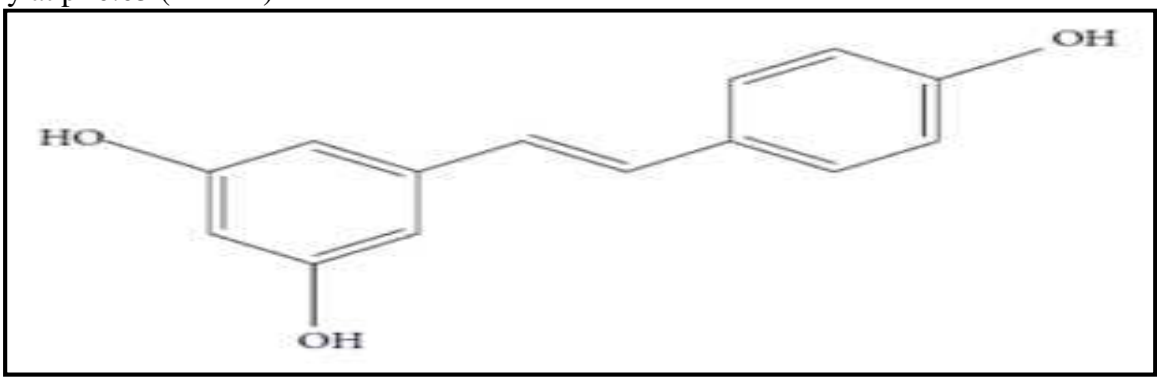

Figure No.1: The structure of Pterostilbene (PTSB)

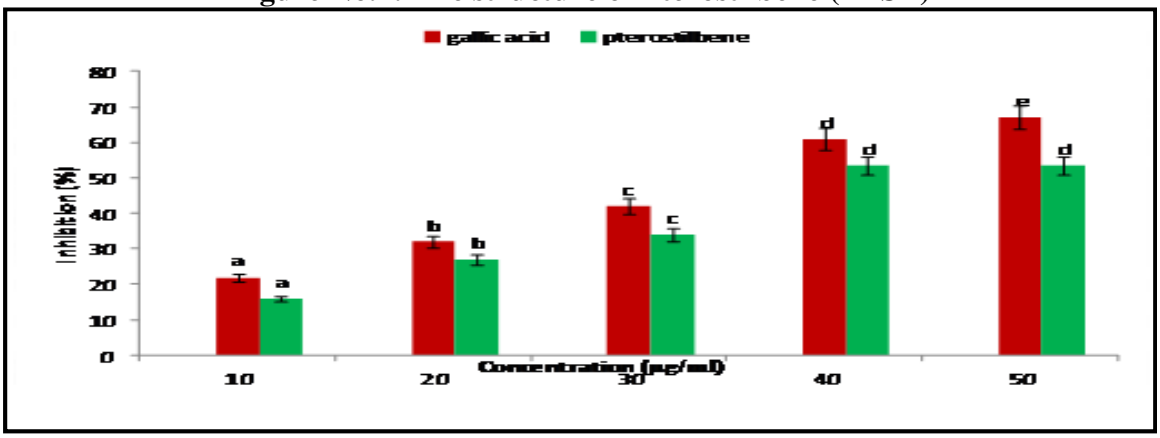

Figure No.2: Superoxide radical scavenging ($O_2^{\cdot}$) activity of PTSB

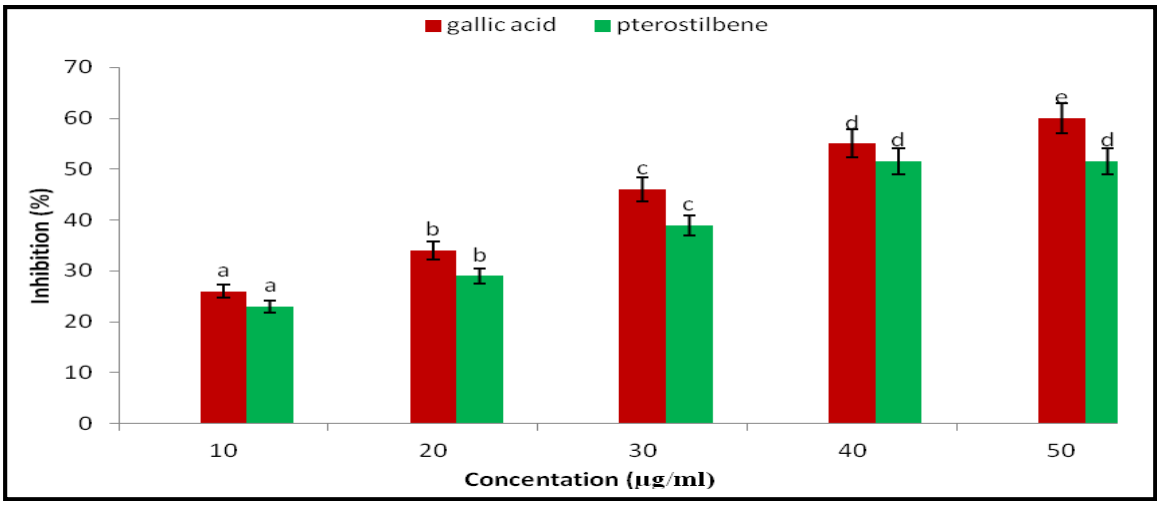

Figure No.3: Hydroxyl radical scavenging (HO·) activity of PTSB

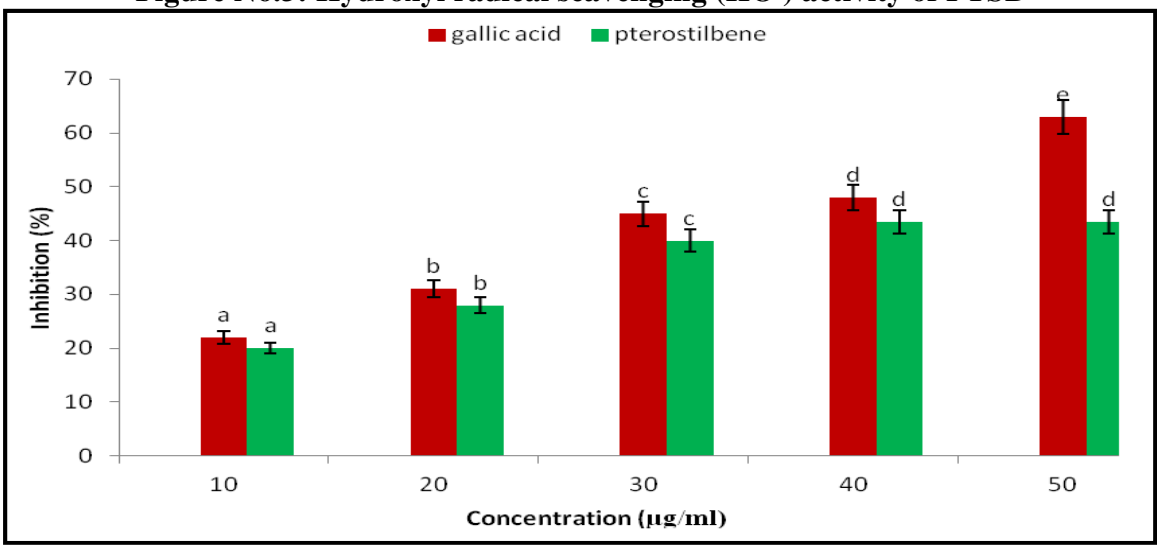

Figure No.4: Free radical scavenging (DPPH·) activity of PTSB

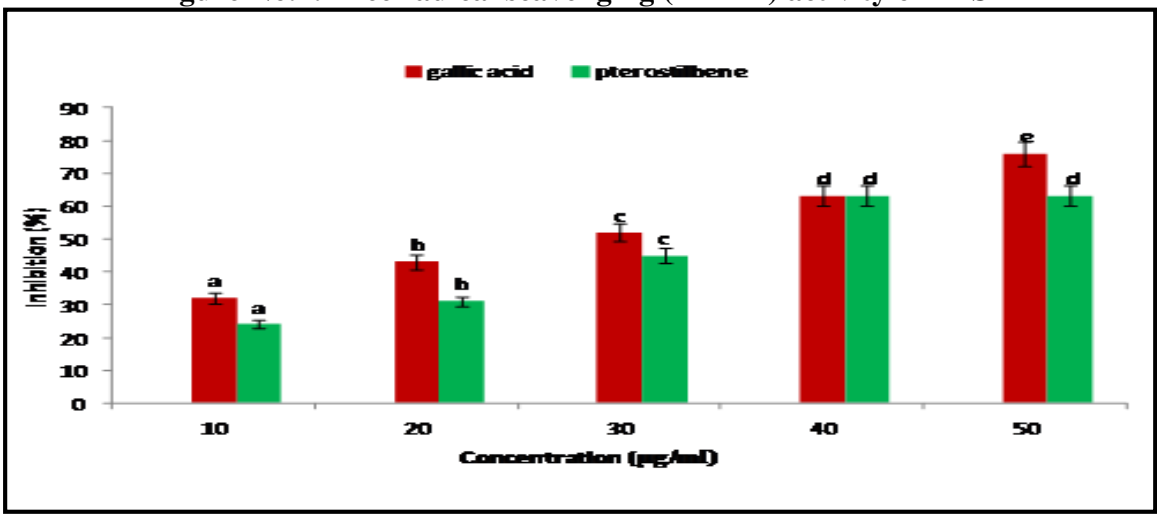

Figure No.5: Nitric oxide scavenging (NO·) activity of PTSB

CONCLUSION

In the present study clearly revealed that, PTSB has potent antioxidant properties against sodium fluoride induced toxicity. Hence significantly improve the enzymatic antioxidant (GSH, Vitamin C and E) and reduce the oxidative stress markers (TBARS, LOOH) through *in vivo* models. Similarly PTSB has been proved good antioxidant compound through *In vitro* study.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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