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Original article

Protective effect of aqueous and ethanolic extracts of *Tamarindus indica* L. leaf on oxidative stress induced by sodium fluoride in different tissues of rat

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Abstract

The generation of reactive oxygen species (ROS) and lipid peroxidation has been considered to play an important role in the pathogenesis of chronic fluoride toxicity. In the present study, the protective effects of aqueous and ethanolic extracts of tamarind leaf on fluoride induced oxidative stress in liver, kidney, heart and blood were studied. A total number of 36 female healthy albino rats were divided into six groups, each containing six animals. The control group (Gr-I) received normal drinking water and standard diet throughout the study period. The second group (Gr-II) received only 200 µg/ml (ppm) of sodium fluoride (NaF) in their drinking water for 60 days of study period. The third and fourth groups (Gr-III and Gr-IV) were co-administered with 200 ppm of NaF and low (200 mg /kg bwt) and high (300 mg/kg bwt) doses of ethanolic extract of tamarind leaf, respectively. While the fifth and sixth groups (Gr-V and Gr-VI) were similarly co-administered with 200 ppm of NaF in drinking water and low (200 mg /kg bwt) and high (300 mg/kg bwt) doses of aqueous extract of tamarind leaf, respectively for 60 days. Blood was collected at day 0, 30 and 60 of the study period. Liver, kidney and heart were obtained by sacrificing the rats for estimation of antioxidant parameters like super oxide dismutase (SOD), catalase, GSH (reduced glutathione) and (LP) lipid peroxidation. Gr-II showed significant reduction in the activity of catalase, SOD and GSH and significant increase in lipid peroxidation level of liver and kidney when compared with control group. In erythrocyte also, catalase, SOD, and GSH activity declined significantly and lipid peroxidation level increased at day 60 of experiment when compared with day 0. Heart tissue did not show any significant alteration in enzymatic activity. Among the leaf extracts at two different dose levels, aqueous extract at high dose (300 mg/kg bwt) showed better effect in protection of oxidative stress in liver, kidney, heart and erythrocytes of rat because status of oxidative stress which was almost negligible similar to the control group.

Key words: Fluoride, oxidative stress, tamarind leaf, SOD, reactive oxygen species (ROS)

1. Introduction

Prolonged ingestion of fluoride at low dose may result in chronic fluorosis. Cases of fluorosis in man and animals have been reported globally. In India, fluorosis has been encountered due to both natural (Dwivedi *et al*., 1997) and industrial sources (Patra *et al*., 2000). It is well established that ingestion of higher amounts of fluoride causes metabolic disorders, by interacting with various cellular processes such as gene expression, cell cycle, proliferation and migration, respiration, metabolism, ion transport, secretion, endocytosis, apoptosis, necrosis and oxidative stress, and disrupts the antioxidant defense system in the body (Strunecka *et al*., 2007; Barbier *et al*., 2010). In recent decades, extensive information has been accumulated on the role of fluoride in cellular respiratory processes and associated free radical reactions (Rzeuski *et al*.,1998). Fluoride is also known to be an inhibitor/activator of numerous enzymes (Machoy *et al*.,1987). Amelioration of toxic effects of fluoride in man and animal remained unresolved and controversial till date due to lack of safe effective ameliorative agents that can remove fluoride from the body and can ameliorate toxic effects as well (Osweiler, 1999). Chemical compounds such as aluminum sulfate (Radostits *et al*., 2000), ascorbic acid and boron have been reported as effective in experimental fluoride exposure with variable success (Coffey *et al*., 2007). These agents are generally not indicated for prolonged use due to their toxic side effects which underlines need for evaluating a nontoxic-safe agent that can reduce fluoride burden in body and ameliorate toxic effects of fluoride (WHO, 2002). Removal of fluoride from water or any other sources causes dumping in the environment in more concentrate form which is again incorporated with water and other natural resources and find a place in the food chain. Therefore, finding a way to counteract

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the toxic effects of fluoride in animals was aimed in the present study. The tamarind has some protective effects in fluoride poisoning and it has been also reported that tamarind can bind fluoride *in vitro* (Maruthamuthu and Venkatanarayana, 1987). Therefore, the effects of aqueous and ethanolic extracts of tamarind leaf were evaluated by determining SOD, catalase, lipid perxoidation and GSH activity/level following long term exposure of fluoride in albino rats.

2. Materials and Methods

2.1 Plant material

2.1.1 Preparation of crude leaves powder for extraction

Fresh and tender *Tamarindus indica* L. leaves were collected from the tree in the University campus of Mohanpur and authenticated by Botanical survey of India (BSI), Kolkata with a Specimen No. "WBUAFS/LJ/02". The leaves were washed by distilled water, dried under shade and finally dried in a microwave at 45°C for 48 h before grinding. The dried plant materials (leaves) were ground into powder with an electric blender. The coarse powder was passed through Sieve No. 40 and then it was kept in an air tight container for further study.

2.2 Preparation of ethanolic extract

2.2.1 Method of extraction: Continuous hot percolation method

Requirement

Instrument : Soxhlet apparatus

Solvents : Ethanol (95%)

Plant material : Shade dried coarse powder of *T. indica* leaves

The ethanolic extract was prepared by the method described by Rosenthaler (1930). For the preparation of extract, 100 gm of dried coarse leaves powder was charged in Soxhlet apparatus (hot extraction) and extracted with ethanol (95%) at temperature 60°-70°C. The success of extraction with ethanol is directly related to the extent that chlorophyll is removed into the solvent. The extract was filtered and dried under reduced pressure to get a solid mass, free from the solvent and finally stored in desiccators in a cool and dry place.

2.2.2 Preparation of aqueous extract : Decoction method

For the preparation of extract, 100 gm of dried leaves powder was taken in flask to which 1600 ml of distilled water was added. Then flask was kept on heating mental for boiling at 100°C. Heating was done till the content was reduced to one fourth of the total content. The content was cooled and filtered through muslin cloth so as to remove the insoluble material. The filterate was again filtered through Whatman filter paper No. 42 and then poured in a dry, clean and already weighed petridish, placed on hot plate (at 55°C) for complete evaporation. Care was taken to avoid charring. Then the extract was cooled at room temperature and again weighed to calculate the extractability percentage, then stored in cool and dry place. All extracts were stored in dry sterilized container at 4°C until further use. Each extract was reconstituted daily in saline water at room temperature and used for supplementation.

2.3 Chemicals and reagents

Chemicals used in this study were of analytical grade. All others chemicals used in this study were obtained from E. Merck (India), Rankem (India) and Sigma Chemicals co., USA.

2.4 Animals and experimental design

Colony-bred female albino rats (Charles Foster; 3 months, 200-260 g b wt) housed individually in a well-ventilated animal unit (26 ± 2°C, humidity 62 %, 12-h light/dark cycle) were supplied water *ad libitum*. Standard rodent palette diet was given *ad libitum*. The animals were maintained in accordance to the guide line of National Institute of Nutrition, ICMR Hyderabad, India. The research protocol was followed by the guideline of Institutional Animal Ethics Committee (MoEF/CPCSEA/ Reg. 337) and was approved by the Institutional Committee for Animal Research.

2.5 Experimental design

For this study, all total 36 animals were taken which were divided into six groups, all consist of six animals each Group I, Group II……. Group VI. 200 ppm was administered through drinking water (Ranjan *et al*., 2009) to all the groups except experimental control (Group I) for 60 days. Group distribution is given below :

1. Group I : Experimental control
2. Group II : Fluoride control, only fluoride in drinking water for 60 days
3. Group III : Fluoride with tamarind ethanolic extract low dose (200 mg/kg bwt) for 60 days
4. Group IV : Fluoride with tamarind ethanolic extract high dose (300 mg/kg bwt) for 60 days
5. Group V : Fluoride with aqueous extract of tamarind low dose (200 mg/kg bwt) for 60 days
6. Group V : Fluoride with aqueous extract of tamarind high dose (300 mg/kg bwt) for 60 days

2.6 Collection sample

Blood was collected from tail vein at 0, 30 and 60 days for estimation of antioxidant parameters like SOD, catalase, lipid peroxidation and reduced glutathione. At 60th days, all animals were sacrificed under light ether anesthesia. Liver, heart and kidney were excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination, dried by blotting with filter paper and weighed. The tissues were then kept in freezer at –20°C until analysis.

2.6.1 Liver homogenate preparation

Liver was homogenized in chilled potassium chloride (1.17%) using a homogenizer. The homogenates were centrifuged at 800 x G for 5 min at 4°C to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500 x G for 20 min at 4°C to get the post mitochondrial supernatant which was used to assay SOD, CAT, GSH and lipid peroxidation activity (Noori *et al*., 2009).

2.6.2 Kidney homogenate preparation

Kidney homogenates were obtained by using a tissue homogenator, Ultra Taurax T-25 Polytron, at 4°C. The homogenates (1:10 w/v) were prepared by using a 100 mmol KCl buffer (7.0 p H) containing EDTA 0.3 mM. All homogenates were centrifuged at 600 x G for 60 min. at 4°C and the supernatant was used for biochemical assays (Noori *et al*., 2009).

2.6.3 Heart homogenate preparation

Homogenates were prepared on ice in the ratio 4 g tissue for 16 ml of phosphate pH 7.5, containing 1mM/lNa_2 EDTA, 10 ml of 500

mM/l BHT (butylated hydroxytoluene) in acetonitrile was added to prevent formation of new peroxides during the assay. The homogenates were centrifuged at 2000 rpm at 4°C and frozen at -20°C until analysis (Noori *et al*., 2009).

2.6.4 Blood collection and erythrocyte preparation

At the 0, 30 and 60th day of experimental period, blood samples were withdrawn from the animals under light ether anesthesia by puncturing the retro-orbital venous plexus of the animals with a fine sterilized glass capillary and collected in EDTA tubes. Within 10 min of blood collection, the erythrocytes were sedimented by centrifugation at 3500 rpm for 10 min at 4°C. The erythrocytes were washed for three times with cold isotonic saline (5 ml, each) and the buffy coat was discarded. Then, 0.5 ml of the erythrocytes suspension was destroyed by osmotic pressure, using the same volume of deionized water and centrifugation at 10000 × G for 10 min at 4°C. The supernatant was then obtained and stored at – 20°C until measurements within one week. The hemoglobin content was determined by the method of Drabkin *et al*. (1935).

2.6.5 Superoxide dismutase activity (SOD)

SOD activity in blood haemolysate (5%) and tissue homogenate (10%) was measured by the method of Mishra and Fridovich (1972), using UV-VIS Spectrophotometer.

2.6.6 Catalase activity

Catalase activity of blood haemolysate (5%) was measured by the method described by Aebi (1974), using UV- VIS spectrophotometer. The haemolysate was diluted to 1:500 with phosphate buffer.

Catalase activity of tissue homogenate (5%) was measured by the method described by Bergmeyer *et al*. (1984), using UV-VIS spectrophotemer.

2.6.7 Reduced glutathione (GSH)

The reduced glutathione (GSH) level was determined by the method of Beutler *et al*. (1963). Reduced glutathione levels of the haemolysates were calculated, using the GSH calibration curve. The GSH concentration unit is then calculated as micromole per gram of hemoglobin by estimating hemoglobin concentration of original whole blood.

Reduced glutathione level in tissue homogenate was measured by the method described by Griffith (1980), using UV-VIS spectrophotometer at 412 nm wave length.

2.6.8 Measurement of erythrocytes lipid peroxidation

The level of lipid peroxidation in terms of the formation of thiobarbituric acid reactive substances (TBARS) was evaluated in the haemolysate samples by the method of Nabavi *et al.* (2012) and expressed in nmol eq MDA/g Hb.

Estimation of lipid peroxidation of tissue homogenate was carried out according to the method of Nair and Turner (1984), using UV-VIS spectrophotometer.

2.6.9 Protein estimation

Protein concentration was measured in dilute tissue homogenates according to Lowry *et al.* (1951), using bovine serum as a standard. The enzyme activities were expressed as units of enzyme activity per milligram of protein.

2.6.10 Statistical analysis

Each sample was run in duplicate. Data were analyzed by one-way analysis of variance (ANOVA), with post hoc analysis, using LSD multiple comparison tests using SPSS 17.0 software and expressed as mean ± SE with $p < 0.05$ considered statistically significant.

3. Results

3.1 Catalase and serum oxide dismutase (SOD) activity and reduced glutathione (GSH) level

The effect of aqueous and ethanolic extracts of tamarind leaf on various antioxidant parameters like SOD and catalase activity and reduced glutathione (GSH) levels in liver, kidney, heart and erythrocytes of albino rats, following a simultaneous exposure of fluoride (NaF) for consecutive 60 days through drinking water and tamarind (ethanolic and aqueous) extracts daily orally for the same period of 60 days. were presented in the Tables 1, 2 and 3 and Figures 1, 2, 3 and 4.

The GSH level, SOD and catalase activity of both liver and kidney (Tables 1 and 2) had shown almost similar results when compared to the activity of normal control group (Gr-I) and fluoride treated group (Gr-II). There was significant (p<0.05) decrease in the level of GSH and activity of the both SOD and catalase in fluoride treated group when compared to normal control group but no significant changes were found in the activity of both SOD and catalase in the heart of the fluoride treated group compared to the normal control group. Significant (p<0.05) decrease was also observed in the activity of SOD and catalase and GHS level in liver and kidney of rats of Gr-III, Gr-IV and Gr-V when compared to normal control group (Gr-I). However, the animals simultaneously treated with fluoride and high dose aqueous extract (Group VI) during the same period of 60 days did not show any significant changes in the SOD and catalase activity and GSH level in liver and kidney when compared to control group. Groups with tamarind aqueous extract treatment at high dose showed better result, and the antioxidant parameter values of these groups is very near to the control group.

In erythrocyte, administration of fluoride alone causes significant (p<0.05) decrease in the activity of SOD and catalase at 30th and 60th day when compare to day 0 in Gr-II. But there are no significant changes in the activity of SOD and catalase at 30th and 60th days when compared with day 0 of normal control group. Only group co-administered with fluoride and aqueous extract at high dose show similar results with the normal control group, but the other groups (Gr-III, IV and V) showed similarity of results with alone fluoride treated groups. This is indicative of the better performance of the aqueous extract at high dose of tamarind extract in compare with the other dosage forms like ethanolic extract low and high dose and aqueous extract at low dose of tamarind.

In case of liver and kidney GSH level (Tables 1 and 2), it shows similarity in results with SOD and catalase activity in the experimental rats, however, the heart GHS level did not follow the pattern shown by the SOD and catalase activity in heart of the experimental rat. Rather heart responds towards the exposure of fluoride alone in experimental rats in terms of GSH level. There was a significant decrease in the GSH level of alone fluoride treated group (Gr-II) with that of normal control group (Gr-I). Except group co-administered with fluoride and ethanolic extract at low dose (Gr-III), all others showed similarity in GSH level of heart with that of normal control group.

GSH level in erythrocyte (Figure 3) of the experimental rats showed similar results to that of SOD and catalase activity of erythrocyte. No significant changes were seen in the GSH level of erythrocyte at 0, 30th and 60th day in the normal control group but there was a significant (p<0.05) decrease in the 30th and 60th day values of GSH as compared to 0 day in fluoride treated group. Only group VI which was co-administered fluoride and tamarind aqueous extract at high dose showed no significant changes in the GSH level at 30th and 60th days as compared to 0 day. Though ehtanolic extract at high dose also showed good results but better results were observed in the group treated with aqueous extract of tamarind at high dose (300 mg/kg bwt).

3.2 Lipid peroxidation activity

The lipid peroxidation (LPO) activity was measured in the form of malondialdehyde (MDA) level in tissues and thiobarbituric acid reactive substances (TBARS) level in erythrocytes. The MDA content of the liver and kidney (Tables 1 and 2) were significantly increased (p<0.05) in response to treatment of sodium fluoride in normal male rats for 60 days, when compared with normal control groups, but no significant changes were observed in the values of heart MDA level between control group and fluoride treated group. Significant (p<0.05) increase was also noted in MDA level of liver and kidney of groups, given ethanolic extract at low dose, high dose and aqueous extract at low dose along with fluoride (Gr-III, Gr-IV and Gr-V, respectively). Group VI which co-exposed to fluoride and aqueous extract high dose did not show any significant changes in liver and kidney MDA level as compared to control group.

In case of erythrocyte, no significant changes were seen at 30th and 60th days values as compared to the 0 day in TBARS content of normal control group (Figure 1). In fluoride treated group (Gr-II), there was a significant (p<0.05) increase in the TBARS levels of erythrocyte at 30th and 60th days as compared to 0 day. Groups co-exposed to fluoride and ehtanolic extract at low dose (Gr-III) and fluoride with ehanolic extract at high dose (Gr-IV) also exhibited similar results with Group-II. Group co-exposed to F and aqueous extract low dose (Gr-V) only showed significant increase (p<0.05) at 60th when compared with 0 day values. Group VI which was co-exposed to F and aqueous extract high dose showed similar results, to that of control group.

Table1: Estimation of different antioxidant parameters in the tissue of rat liver following daily administration of sodium fluoride (200 ppm) orally for 60 days alone and with the aqueous and ethanolic extracts of tamarind leaf in two different (200 mg and 300 mg/kg bwt) doses (n = 6 ± SE)

Group	SOD (unit/ mg protein)	Catalase (m mole H_2O_2 decomposed/ min/mg protein)	GSH (µ mole/ gm of wet tissue)	Lipid peroxidation (nmoleMDA/gm wet tissue)
Group I	3.68 ± 0.12^{a}	2.62 ± 0.20^{a}	23.33 ± 1.25^{a}	276.8 ± 12.86^{a}
Group II	1.62 ± 0.13^{b}	1.12 ± 0.18^{b}	15.22 ± 1.01^{bcde}	386.11 ± 12.95^{bc}
Group III	2.86 ± 0.17^{c}	1.47 ± 0.17^{bc}	17.39 ± 1.46^{bcde}	358.5 ± 14.80^{c}
Group IV	2.91 ± 0.17^{cd}	1.78 ± 0.21^{cd}	18.93 ± 1.47^{bcde}	344.03 ± 11.39^{cd}
Group V	3.09 ± 0.20^{cd}	2.05 ± 0.18^{de}	18.63 ± 1.28^{bcde}	323.75 ± 12.9^{ce}
Group VI	3.32 ± 0.20^{acd}	2.26 ± 0.16^{ad}	21.29 ± 1.33^{ade}	296.86 ± 11.31^{ae}

Values with the same superscripts are not significant at p<0.05 within the column

Table 2: Estimation of different antioxidant parameters in the tissue of rat kidney following daily administration of sodium fluoride (200 ppm) orally for 60 days alone and with the aqueous and ethanolic extracts of tamarind leaf in two different (200 mg and 300 mg/kg bwt) doses (n = 6 ± SE)

Group	SOD (unit/ mg protein)	Catalase (m mole H_2O_2 decomposed/ min/mg protein)	GSH (µ mole/ gm of wet tissue)	Lipid peroxidation (nmoleMDA/gm wet tissue)
Group I	2.67 ± 0.16^{a}	0.60 ± 0.04^{a}	21.19 ± 1.24^{a}	226.65 ± 9.23^{a}
Group II	1.76 ± 0.22^{b}	0.37 ± 0.03^{b}	13.32 ± 0.67^{b}	313.23 ± 10.62^{b}
Group III	1.91 ± 0.18^{bc}	0.43 ± 0.02^{bc}	16.46 ± 0.90^{cde}	302.87 ± 12.4^{bc}
Group IV	2.18 ± 0.17^{ab}	0.48 ± 0.02^{cd}	17.33 ± 1.09^{cd}	293.48 ± 13.38^{bde}
Group V	1.97 ± 0.16^{bc}	0.47 ± 0.03^{ed}	17.92 ± 1.11^{cde}	279.92 ± 10.08^{ce}
Group VI	2.31 ± 0.15^{ac}	0.53 ± 0.03^{ad}	18.82 ± 0.95^{ac}	242.68 ± 9.45^{a}

Values with the same superscripts are not significant at $p < 0.05$ within the column

Table 3: Estimation of different antioxidant parameters in the tissue of rat heart following daily administration of sodium fluoride (200 ppm) orally for 60 days alone and with the aqueous and ethanolic extracts of tamarind leaf in two different (200 mg and 300 mg/kg bwt) doses (n=6 ± SE)

Group	Lipid peroxidation (nmoleMDA/gm wet tissue)	SOD (unit/ mg protein)	Catalase (m mole H_2O_2 decomposed/ min/mg protein)	GSH (µ mole/ gm of wet tissue)
Group I	209.15 ± 7.4^{a}	1.63 ± 0.09^{a}	0.33 ± 0.01^{a}	19.34 ± 1.04^{ad}
Group II	222.18 ± 7.1^{a}	1.39 ± 0.11^{a}	0.30 ± 0.01^{a}	15.41 ± 0.86^{bc}
Group III	211.87 ± 9.3^{a}	1.49 ± 0.11^{a}	0.31 ± 0.02^{a}	16.43 ± 0.84^{c}
Group IV	216.9 ± 6.2^{a}	1.51 ± 0.11^{a}	0.32 ± 0.01^{a}	16.92 ± 1.04^{abcd}
Group V	219.6 ± 6.6^{a}	1.40 ± 0.08^{a}	0.32 ± 0.01^{a}	16.98 ± 0.98^{abc}
Group VI	223.7 ± 7.2^{a}	1.59 ± 0.10^{a}	0.32 ± 0.02^{a}	17.42 ± 0.94^{abc}

Values with the same superscripts are not significant at $p<0.05$ within the column

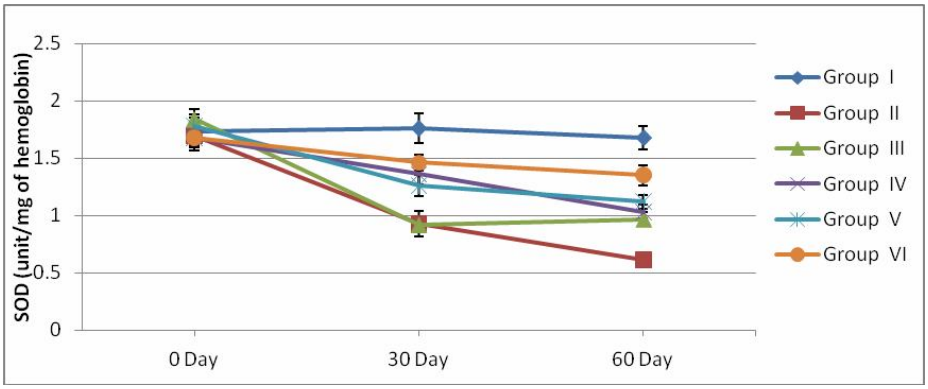

Figure 1: Graphical representation of SOD activity (unit/mg of hemoglobin) of rat erythrocyte at different intervals in the treated experimental groups.

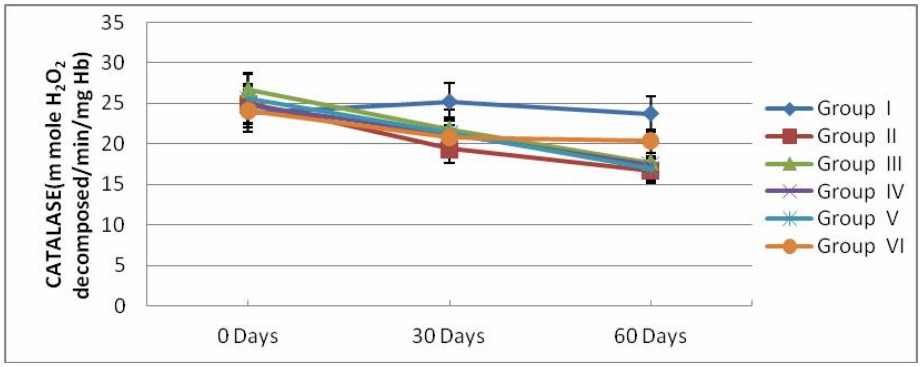

Figure 2: Graphical representation of catalase activity (m mole H_2O_2 decomposed/min/mg hemoglobin) of rat erythrocyte at different intervals in the treated experimental groups.

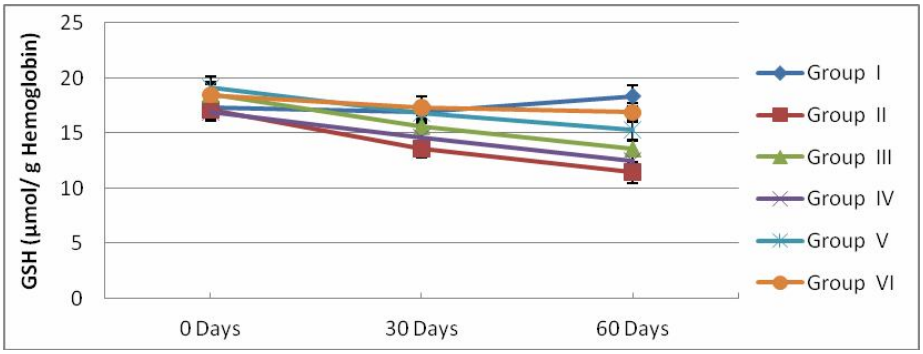

Figure 3: Graphical representation of reduced glutathione (GSH) activity (µmol/g hemoglobin) of rat erythrocyte at different intervals in the treated experimental groups.

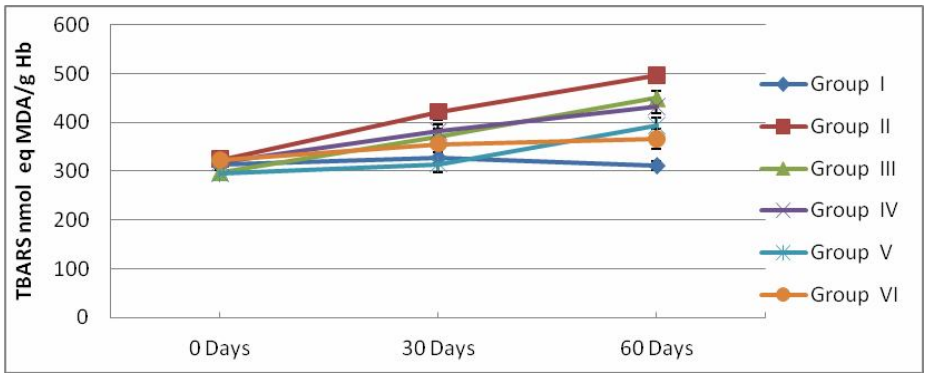

Figure 4: Graphical representation of lipid peroxidation values (TBARS nmol eq MDA/g Hb) of rat erythrocyte at different intervals in the treated experimental groups

4. Discussion

In the present study, the effects of ethanolic and aqueous extracts of *T. indica* leaf on the oxidative stress induced by sodium fluoride in liver, kidney, heart and blood of rats were investigated. It was observed that treatment of fluoride can induce oxidative stress by both inhibiting antioxidant enzymes and stimulating generation of surplus free radicals in experimental animals (Shivarajashankara *et al*., 2001; Shanthakumari *et al*., 2004). In the present study, fluoride treatment caused a decrease in the activity of SOD, catalase and GSH in liver, kidney and erythrocytes of rats. The results were in accordance with the previous works (Shivarajashankara *et al*., 2001; Guo *et al.*, 2003 and Shanthakumari *et al.*, 2004). Fluoride was reported to stimulate respiratory burst and the production of oxygen free radicals inside the neutrophils in a concentration-dependent manner (Della Bianca *et al.*, 1988).

A close connection between chronic fluoride toxicity and increased oxidative stress in both human (Saralakumari and Ramakrishna Rao, 1991; Jeji *et al*., 1985; Li and Cao, 1994) and animals (Zhi-Zhong *et al*.,1989; Sharnia and Chinoy, 1998; Patel and Chinoy, 1998) have been reported. The ROS production is a natural process, and persistent high levels of ROS could damage the cell and its membrane. A variety of enzymatic and non-enzymatic mechanisms were evolved to protect cells against ROS. These enzymatic antioxidants include SOD, catalase and lipid peroxidase whose function is to detoxify peroxides. Studies with experimental models *in vivo* and *in vitro* showed that in different tissues and cells, fluoride induces an excess of ROS production. Fluoride also decreases the biological activity of major antioxidant enzymes such as SOD, catalase, and GSH which play an important role in ROS elimination (Chlubek, 2003; Barbier *et al*., 2010; Mochidome *et al.*, 2013). However, although the mechanism by which fluoride causes these effects is not fully understood, it is thought that the generation of oxidative stress is an important part of the pathological damage mechanism induced by fluoride (Chlubek, 2003; Shanthakumari *et al.*, 2004; Mochidome *et al.*, 2013). In our study, fluoride exposed rats showed enhanced levels of hepatic and renal tissue lipid peroxidation with reduced hepatic and renal antioxidant profiles. The severity of oxidative stress was found to be similar with the study of Vasant *et.al*. (2011) when fluoride toxicity was induced by providing NaF through drinking water continuously with a dose rate of 100 ppm for consecutive 30 days. In the present study, both the extracts were found to have a good antioxidant property when compared to the fluoride control group. Erythrocytes of the rats of only fluoride treated groups also showed significant increase in the TBARS content when day 0 values were compared with day 60 values. Higher dose rates of both the extracts (particularly aqueous extract) showed more beneficial effect than the lower dose rates compared to the fluoride control group to prevent elevation of hepatic and renal tissue lipid peroxidation and to protect lowering of the activities of hepatic and renal antioxidant enzymes, caused by a long term (60 days) administration of sodium fluoride (NaF) when kept untreated. The group given aqueous extract at high dose with simultaneous administration of NaF was found to show the best results in all the parameters among all the treated groups to arrest any further alteration in oxidative stress due to fluoride toxicity when compared to the healthy control. Superoxide dismutase converts superoxide radicals into less harmful products such as hydrogen peroxide and decreases superoxide radical concentration (Robinson, 1998; Brioukhanov and Netrusov, 2004), whereas catalase reduces hydrogen peroxides and provides protection to tissues. While in fluoride-exposed rats both SOD and catalase activities were reduced significantly. However, tamarind leaf extracts (aqueous and ethanolic) treatment at both the dose rates (200 mg/kg bwt and 300 mg/kg bwt) to the diet accelerated the activities of both SOD and catalase in animals in dose dependent manner but the treatment with aqueous extract at high dose (300 mg/kg bwt) only showed significant improvement in the SOD and catalase activity. Similar beneficial effects of tamarind leaf extract on fluoride induced oxidative stress were also shown by Vasant *et al*. (2011). The antioxidant effect could be due to the presence of tannins, alkaloids, flavonoids and ascorbic acid in aqueous leaf extract of tamarind (Kotebagilu *et al*., 2016). Ascorbic acid is an important natural antioxidant that eliminates reactive oxygen species and reduces oxidative stress (Oguntibeju, 2008). No significant alteration in oxidative status was found in tissues of heart. In our study, the aqueous leaf extract of tamarind at high dose (300 mg/kg bwt) was found to be as a good antioxidant to markedly act on the oxidative state during an oxidative stress condition, caused by fluoride toxicity. So, the high antioxidant potency of the leaves of *T. indica* implied the potential of this plant for use as an alternative source of natural antioxidants (Razali *et al.*, 2012). The protective effect on oxidative stress in hepatic, renal and cardiac tissues and blood of fluoride exposed rats confirmed that fluoride is capable of inducing oxidative stress which was reversed variably by co-administration of extracts of tamarind leaf. This beneficial role could be due to various phyto-constituents present in the extract.

5. Conclusion

It was determined that fluoride led to oxidative stress at the indicated dose and period. Supplementation of tamarind leave extract to the fluoride-exposed rats revealed protection against fluoride induced oxidative damage. Moreover, concomitant treatment with aqueous extract of tamarind leaf at the doses of 300 mg/kg body weight with

sodium fluoride administration restored thiobarbituric acid reactive substances (TBARS) levels and catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH) activities in liver and kidney and erythrocyte of adult female rats. The protective effects of extracts of *T. indica* leaf extract may be due to the free radical scavenging activity along with body fluoride reducing ability of its components. The results may be of future therapeutic significance particularly in the areas where man and animal is exposed to fluoride either occupationally or environmentally.

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Conflict of interest

We declare that we have no conflict of interest.

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