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Ameliorative Effects of *Boerhaavia diffusa* L. on Fluoride Induced Oxidative Stress and Antioxidant Enzymes in Testis and Seminal Plasma of Male Rats

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Abstract:

The present study was carried out to investigate the toxic effects of fluoride on the oxidative stress parameters in male albino rats and also to evaluate the ameliorative effect of Boerhaavia diffusa L. (250mg and 500mg /kg bw/day) after fluoride intoxication. The animals were divided into four groups. Group I received no treatment served as control. Group II, III and IV were orally administered with 100, 200 and 300 ppm of NaF for 20 and 40 days. Rats treated with NaF for 40 days were administrated with Boerhaavia diffusa L extract for next 20 days. Alterations in the antioxidant indices in the testis and seminal plasma were confirmed by significant decrements ($p<0.05$) in the activity of antioxidant enzymes superoxide dismutase (SOD) & catalase (CAT), alongwith significant increase ($p<0.05$) in the level of malondialdehyde (MDA). However, with the supplementation of the NaF treated rats by plant leaf extract manifested the improvement in the recovery and mitigation of devastating effects of fluoride toxicity on the concerned parameters in male albino rats.

Key words: Antioxidant enzymes, *Boerhaavia diffusa* L., Fluoride, Oxidative stress, seminal plasma and testis

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INTRODUCTION

Male reproductive health has significantly declined in the recent years. Environmental toxins are considered as the underlying source of reduced male fertility. Fluoride, commonly found in drinking water and nutrients, threatens the human life.

Several studies have demonstrated that fluoride causes structural, metabolic, and functional damage in tissues. Fluoride also stimulates the rate of protein accumulation, gene expression and intracellular oxidation (Orta and Erkan, 2014). A variety of mechanisms have been proposed to explain fluoride toxicity, including oxidative stress. Fluoride may alter the normal oxygen metabolism and cause reactive oxygen species (ROS) production. Fluoride elevates the formation of superoxide anion (O2-) and mediates the formation of hydrogen peroxide, and peroxinitric and hydroxyl radicals (Izquierdo *et al*., 2008). Moreover, fluoride may alter glutathione levels, and cause excessive ROS production in mitochondria, leading to toxicity, which damages cellular components (Barbier *et al*., 2010). Several cellular defense mechanisms are there that prevent the formation of ROS. Antioxidant enzymes represent such a defense mechanism, and are responsible for detoxification of ROS. Due to exposure to physical stress and environmental pollutants, the rate of male infertility has been increasing, which has become a serious social problem (Meeker *et al*., 2008). Therefore, it is vital to investigate the mechanism of reproductive toxicity and explore the proper antidote.

Medicinal plants form the backbone of traditional system of medicine in India. These plants are rich source of novel drugs that form the ingredients in tradition systems of medicine, bioactive principles, pharmaceutical intermediates, modern medicines, food supplements and lead compounds in synthetic drugs. *Boerhaavia diffusa* L. is a herbaceous plant of

the family Nyctaginaceae distributed in tropics and subtropics and is widely used for the treatment of dyspepsis, jaundice, abdominal pain (Kirtikar and Basu, 1956) and enlagement of spleen and as antistress agent. Pharmacological studies have shown that *B.diffusa* possesses diuretic (Gaitonde *et al*., 1974), anti-inflammatory (Bhalla *et al*., 1968), anti-fibrinolytic (Jain and Khanna, 1989), antibacterial (Olukoya *et al*., 1993) and antihepatotoxic (Chandan *et al*., 1991) properties. This plant is known to rejuvenate male reproductive system, quality and quantity of semen; and other organ systems (Mudgal, 1975).

The present study investigates the relationship between fluoride induced oxidative stress in testis and seminal plasma and ameliorative effects of *Boerhaavia diffusa* L.

MATERIALS AND METHODS

Male Wistar rats (100-150 g) were maintained in groups six per cage on a 12h/12h light/dark cycle at constant temperature (22 ± 2ºC) and humidity (50%). They were given standard rat pellet diet obtained from Hindustan lever Ltd., Mumbai and water was supplied *ad libitum*. The experimental protocol was approved by Instituitional animal ethics committee, Punjabi University Patiala, India (Approval number 107/99/CPCSEA-2012-11).

Preparation of plant extract

The leaf extract of *Boerhaavia diffusa* L. was prepared by the method of Narendhirakannan *et al*., 2006.

Experimental Design

After acclimatization to environment for one week, the male rats were divided randomly into twelve equal groups with six rats in each group: control group (C1) was administered deionized water for 20 and 40 days; the rats in experimental

groups received 100, 200 and 300 ppm sodium fluoride (NaF)/kg bw/day, for the same days by oral gavage. Rats treated with NaF for 40 days were post treated with 250 and 500 mg/kg bw/day of *B. diffusa* extract for next 20 days. 250 and 500 mg/kg bw/day of leaf extract of *B. diffusa* to positive control (C2) and (C3) respectively. At the end of the experimental period, rats were fasted overnight and sacrificed under ether anesthesia.

Isolation of seminal plasma

Epididymis was minced in phosphate buffer saline (pH 7.4) and centrifuged at 3000 rpm for 10 minutes to collect the seminal plasma.

Fluoride analysis

Fluoride concentration was analyzed in testis and seminal plasma of fluoride exposed and antidote treated rats with a potentiometric method using the ion selective electrode Harwood (1969).

Malondialdehyde

Malondialdehyde (MDA) level in the testis and seminal plasma of control, fluoride and plant leaf extract treated rats was determined by the method of Ohkawa *et al*. (1979).

Superoxide dismutase and Catalase activity

The activity of superoxide dismutase (SOD) in testis and seminal plasma was determined by the method of Das *et al*. (2000). The activity of catalase (CAT) was determined by the method of Aebi (1983).

Statistical analysis

Results are expressed as mean ± standard deviation (SD). All analyses were performed using SPSS 17.0 statistical software

(IBM). Statistical significance of difference between the experimental groups was evaluated by one way ANOVA followed by Bonferroni and Dunnetts t (2 sided) multiple comparison test. The correlation between two variables was analyzed by STATISTICA 7 software. A two tailed p value < 0.05 was considered statistically significant.

RESULTS

Malondialdehyde (MDA)

a. Testis

The level of MDA in testis of test rat showed a significant ($F= 63.223$, $p<0.0001$) increase after 20 days of fluoride treatment but more significant ($F= 327.422$, $p<0.0001$) increase was observed after 40 days. More prominent increase (711.75%) was registered in highest dose group (300 ppm NaF/kg b.w./day) **(Fig. 1)**.

Bonferroni multiple comparison test after ANOVA showed a significant ($p<0.05$) increase in the level of MDA in testis (95%CI= -0.0293 to -0.0135; mean difference= -0.0158 to -0.0270) as compared to control as well as among fluoride treated groups after 20 days. Furthermore significant ($p<0.01$) increase was observed (95%Cl= -0.0681 to -0.0848; mean difference= -0.0457 to -0.1071) among all fluoride treated groups as well as compared to control after 40 days of fluoride treatment.

Dunnetts t (2 sided) multiple comparison test revealed that testicular testosterone level was significantly ($p< 0.01$) increased in all NaF + leaf extract treated groups as compared to NaF treated groups with mean difference= -0.0180 to -0.1092 (95%Cl= -0.0297 to -0.0907) treated with 250 mg/kgbw/day and with mean difference= -0.0361 to -0.1706 ($p< 0.0001$, 95%Cl= -0.0478 to -0.1521) after treatment with 500 mg/kgbw/day of extract **(Fig. 2)**.

Correlation analysis

Pearson's bivariate correlation revealed a significant, ($p<0.0001$) positive relationship between concentration of fluoride and level of MDA in testis of test rats after 20days ($r = 0.833$) **(Fig. 3a)** and 40 days ($r = 0.950$) **(Fig. 3b)** of fluoride treatment.

Furthermore, pearson's bivariate correlation revealed, ($p<0.0001$) positive relationship between concentration of fluoride and level of MDA in testis of test rats after 250mg/kgbw ($r = 0.937$) **(Fig. 4a)** and 500mg/kgbw ($r = 0.769$) **(Fig. 4b)** of leaf extract treatment.

b. Seminal plasma:

MDA level in seminal plasma of test rat showed a significant ($p<0.0001$) increase after 20 days ($F=235.387$) and 40 days ($F=282.163$) fluoride treatment. More prominent increase (944.57%) was registered in G3 **(Fig. 5)**.

Bonferroni multiple comparison test after ANOVA showed a significant ($p<0.0001$) increase in the level of MDA as compared to control as well as among fluoride treated groups after 20 days (95%CI= -0.0247 to -0.0201; mean difference= -0.0171 to -0.0277) and 40 days (95%Cl= -0.0613 to -0.0639; mean difference= -0.0439 to -0.0812) of fluoride treatment in seminal plasma of test rats.

After mitigation with antidote there was a significant $p<0.0001$ decrease in level of fluoride in all leaf extract treated groups when compared to groups exposed to fluoride for 40 days. The maximum reversal (59.96%) was observed in 300 ppm NaF group, treated with 500 mg/kg b.w. leaf extract. Dunnetts t (2 sided) multiple comparison test revealed that testicular testosterone level was significantly ($p< 0.0001$) increased in all NaF + leaf extract treated groups as compared to NaF treated groups with mean difference= -0.0246 to -0.0855 (95%Cl= -0.0357 to -0.0654) treated with 250 mg/kgbw/day and with

mean difference= -0.0331 to -0.1097 (95%Cl= -0.0443 to -0.0895) after treatment with 500 mg/kgbw/day of extract **(Fig. 6)**.

Correlation analysis

Pearson's bivariate correlation revealed a significant ($p<0.0001$) positive relationship between concentration of fluoride and level of MDA in seminal plasma of test rats after 20 ($r = 0.859$) **(Fig. 7a)** and 40 days ($r = 0.976$) **(Fig. 7b)** of fluoride treatment.

Furthermore, correlation analyses showed a ($p<0.01$) positive relationship between concentration of fluoride and level of MDA in seminal plasma of test rats after 250mg/kgbw ($r = 0.948$) **(Fig. 8a)** and 500mg/kgbw ($r = 0.493$) **(Fig. 8b)** of leaf extract treatment.

Superoxide dismutase (SOD):

a. Testis

The activity of SOD in testis of test rat showed a significant ($F= 23.357$, $p<0.0001$) increase after 40 days of fluoride treatment but non-significant ($F= 1.286$, $p<0.306$) increase was observed after 20 days. More prominent increase (68.33%) was registered in G3 (300 ppm NaF/kg b.w./day) **(Fig. 9)**.

Bonferroni multiple comparison test after ANOVA showed a significant ($p<0.05$) decrease in the SOD activity in testis (95%CI= 0.2932 to 1.9362; mean difference= 0.8123 to 1.4170) as compared to control after 20 days of fluoride intoxication and (95%CI= 0.0855 to 1.1238; mean difference= 0.6047) among test groups treated with fluoride for 40 days.

Dunnetts t (2 sided) multiple comparison test revealed that SOD activity was significantly increased in 200 and 300 ppm NaF treated groups with mean difference 0.8177 ($p< 0.05$; 95% CI= 0.1253 to 1.5100) and mean difference 0.8750 ($p< 0.0001$, 95% CI= 0.5069 to 1.2431) respectively treated with 500 mg/kgbw/day of leaf extract Recovery was maximum (133.24%) in highest dose group **(Fig. 10)**.

Correlation analysis

Pearson's bivariate correlation showed a non-significant, ($p<0.093$) negative relationship between concentration of fluoride and total SOD activity in testis of test rats after 20 days ($r = -0.351$) **(Fig. 11a)**. But a significant, $p< 0.0001$ negative relationship between concentration of fluoride and total SOD activity in testis of test rats after 40 days ($r = -0.790$) **(Fig. 11b)** of fluoride treatment.

Furthermore, pearson's bivariate correlation revealed, negative relationship between concentration of fluoride and activity of SOD in testis of test rats after 250mg/kgbw ($r = -0.560$, $p<0.01$) **(Fig. 12a)** and 500mg/kgbw ($r = -0.298$, $p<0.157$) **(Fig. 12b)** of leaf extract treatment.

b. Seminal plasma

The activity of superoxide dismutase (SOD) in seminal plasma of test rat showed a non significant ($F = 2.741$, $p<0.070$) decrease after 20 days of fluoride treatment but a significant ($F =22.131$, $p<0.0001$) decline was observed after 40 days. Maximum decrease (61.39%) was registered in highest dose group **(Fig. 13)**.

Bonferroni multiple comparison test after ANOVA showed a significant ($p<0.05$) decrease in the activity of SOD in seminal plasma (95%CI= 0.0691 to 1.7279; mean difference= 0.5527 to 1.2443) as compared to control after 20 days of fluoride intoxication and (95%CI= 0.2081 to 1.1752; mean difference= 0.6917) among test groups treated with fluoride for 40 days.

Dunnetts t (2 sided) multiple comparison test revealed that SOD activity was significantly ($p< 0.01$) increased in 200 and 300 ppm NaF treated groups with mean difference 0.6585 (95% CI= 0.1576 to 1.1594) and mean difference 0.6730 (95% CI= 0.3625 to 0.9835) respectively, treated with 500

mg/kgbw/day of antidote. Maximum reversal (85.98%) was observed in highest dose group **(Fig. 14)**.

Correlation analysis

Pearson's bivariate correlation showed a significant, ($p<0.001$) negative relationship between concentration of fluoride and total SOD activity in seminal plasma of test rats after 20days ($r = -0.591$) **(Fig. 15a)** and 40days ($r = -0.826$) **(Fig. 15b)** of fluoride treatment.

Furthermore, pearson's bivariate correlation revealed, negative relationship between concentration of fluoride and activity of SOD in seminal plasma of test rats after 250mg/kgbw ($r = -0.601$, $p<0.01$) **(Fig. 16a)** and 500mg/kgbw ($r = -0.177$, $p<0.407$) **(Fig. 16b)** of antidote treatment.

Catalase (CAT):

a. Testis

A significant ($p<0.01$) decline was observed in the activity of CAT in testis of test rat after 20 days ($F = 6.246$) and 40 days ($F =16.255$) of fluoride treatment. Maximum decrease (51.83%) was registered in 300 ppm fluoride/kg b.w./day **(Fig. 17)**.

Bonferroni multiple comparison test after ANOVA showed a significant ($p<0.05$) decrease in the CAT activity in testis (95%CI= 0.3834 to 2.2269; mean difference= 1.3052) as compared to control and (95%CI= 0.0093 to 1.8527; mean difference= 0.9310) among test groups treated with fluoride for 20 days and with mean difference= 1.2622 to 2.7020 (95%CI= 0.0495 to 3.9147) as compared to control and (95%CI= 0.2271 to 2.6525; mean difference= 1.4398) among test groups treated with fluoride for 40 days.

Dunnetts t (2 sided) multiple comparison test revealed that activity of CAT was significantly ($p< 0.05$) increased in G6 treated with low dose of leaf extract with mean difference 1.2310 (95% CI= 0.1506 to 2.3114) and ($p<0.01$) 100 ppm NaF

group with mean difference 1.4483 (95% CI= 0.3153 to 2.5813), 200 ppm NaF group with mean difference 1.8758 (95% CI= 0.5104 to 3.2412) and 300 ppm NaF with mean difference 1.7908 (95% CI= 0.7105 to 2.8712) treated with 500 mg/kgbw/day of antidote. Recovery was maximum (71.30%) in 300 ppm NaF group administrated with high dose of antidote **(Fig. 18)**.

Correlation analysis

Pearson's bivariate correlation showed a significant, ($p<0.0001$) negative relationship between concentration of fluoride and total CAT activity in testis of test rats after 20days ($r = -0.717$) **(Fig. 19a)** and 40days ($r = -0.780$) **(Fig. 19b)** of fluoride treatment.

Furthermore, pearson's bivariate correlation revealed, ($p<0.01$) negative relationship between concentration of fluoride and activity of CAT in testis of test rats after 250mg/kgbw ($r = -0.499$) **(Fig. 20a)** and 500mg/kgbw ($r = -0.576$) **(Fig. 20b)** of antidote treatment.

b. Seminal plasma

The activity of CAT in seminal plasma of test rat showed a significant ($p<0.01$) decline after 20 days ($F = 5.716$) and 40 days ($F = 16.612$) of fluoride treatment. Maximum decrease (53.24%) was registered in G3 treated with highest dose of NaF **(Fig. 21)**.

Bonferroni multiple comparison test after ANOVA showed a significant ($p<0.05$) decrease in the activity of CAT in seminal plasma (95%CI= 0.4329 to 2.9155; mean difference= 1.6742) as compared to control after 20 days of fluoride intoxication and with mean difference= 1.6037 to 2.8690 (95% CI= 0.3405 to 4.1321) as compared to control and (95%CI= 0.0022 to 2.5285; mean difference= 1.2653) among groups treated with fluoride for 40 days.

Dunnetts t (2 sided) multiple comparison test revealed that CAT activity was significantly ($p< 0.01$) increased in 100 ppm NaF group with mean difference 1.7188 (95% CI= 0.3719 to 3.0658), 200 ppm NaF group with mean difference 1.9142 (95% CI= 0.4561 to 3.3722) and 300 ppm NaF group with mean difference 2.1093 (95% CI= 0.7797 to 3.4390) treated with high dose of leaf extract. Maximum reversal (83.73%) was observed in group treated with 300 ppm NaF **(Fig. 22)**.

Correlation analysis

Pearson's bivariate correlation showed a significant, ($p<0.0001$) negative relationship between concentration of fluoride and total CAT activity in seminal plasma of test rats after 20days ($r = -0.678$) **(Fig. 23a)** and 40days ($r = -0.816$) **(Fig. 23b)** of fluoride treatment.

Furthermore, pearson's bivariate correlation revealed, negative relationship between concentration of fluoride and activity of CAT in seminal plasma of test rats after 250mg/kgbw ($r = -0.567$, $p<0.01$) **(Fig. 24a)** and 500mg/kgbw ($r = -0.334$, $p<0.111$) **(Fig. 24b)** of antidote treatment.

DISCUSSION

The present investigation was carried out to explore the adverse effects of sodium fluoride and its possible amelioration upon treatment of *Boerhaavia diffusa* L. leaf extract in male albino rats in relation to fluoride induced oxidative stress.

Oxidative stress has been observed in soft tissues such as brain, kidney, testes and liver in animals (Ghosh *et al*., 2002; Krechniak and Inkielewicz, 2005; Mittal and Flora, 2007; Varol *et al*., 2013). Fluoride is also known to inhibit the activity of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase and catalase (CAT). Moreover, fluoride can alter glutathione levels (Chlubek, 2003), often resulting in

excessive production of reactive oxygen species (ROS) at the mitochondrial level, leading to damage of cellular components.

Fluoride intake is known to cause oxidative stress, and its relationship with free-radical generation is well studied in various biological systems (Barbier *et al*., 2010). The present study revealed that there was close relationship between fluoride-induced male testicular toxicity and oxidative stress. Our data also reports that exposure of rats to sodium fluoride significantly elicited elevation of MDA content in testis and seminal plasma after 20 as well as 40 days of NaF treatment. This finding is consistent with those of previous studies (Oncu *et al*., 2007; Huang *et al*., 2007). *Boerhaavia diffusa* L. leaf extract as a supplement significantly reversed the fluoride-induced lipid peroxidation in a dose-dependent manner. Decrease in the level of fluoride after mitigation with leaf extract also resulted in significant decreased in level of MDA as compared with 40 days of fluoride treatment. The level of MDA was more close to that of the control group in rats treated with higher dose of extract.

MDA is an important reactive metabolite and an indicator of lipid peroxidation (LPO). Lipid peroxidation from oxidative stress disturbs the integrity of cellular membranes leading to the leakage of cytoplasmic enzymes (McCord and Fridovich, 1969). LPO represents one of the most frequent reactions caused by free radical attack on biological structures (Krinsky, 1992), as reflected in elevated MDA levels resulting from disturbance of the oxidant/antioxidant balance in the biological system, referred to as oxidative stress (Halliwell, 2007). Lipid peroxidation has been suggested to be important in contributing to mitochondrial dysfunction rather than the inhibition of the mitochondrial electron transport (Sen *et al*., 2006).

Moreover, oxidative stress generated by free radicals and hydrogen peroxide is greater if fluoride hinders the

production of the free radical indices of the defense system (Rzeuski *et al*., 1998). Reduction in these indices has been found in people living in endemic areas as well as in the tissues of experimental animals subjected to fluoride toxicity, thus supporting our observation of higher rather than lower levels of lipid peroxidation (Shanthakumari *et al*., 2004). Yang *et al*. (2014) also examined the effect of oxidative stress on the apoptosis of sertoli cells induced by sodium fluoride (NaF) and reported that compared with the control group, the ROS levels in the NaF-treated groups enhanced significantly with the increase in drug concentration, indicating a dose-dependent manner. In addition, the content of MDA significantly increased in 12 and 24 μg/ml NaF groups, whereas there was a marked decrease in the SOD activity of the NaF-treated groups. Treating cells with 2 mM NAC showed protection against the increase of ROS levels and MDA content when the cells were exposed to NaF. Similarly, the protection of NAC was viewed in the SOD activity also.

Generation of free radicals and reactive oxygen species are a continuous process in the body, and to counteract their devastating effects, mammalian cells are bestowed with considerable antioxidant defense mechanisms consisting enzymatic action by SOD, CAT, GPx, and GST (Rao and Bhatt, 2012; Dubey *et al*., 2012). In this study, it was observed that sodium fluoride exposure in rats caused a significant ($p< 0.0001$) decrease in total activity of SOD and catalase in testis and seminal plasma after 40 days of fluoride treatment. Further correlation analyses have revealed a negative correlation between the level of fluoride and the activity of antioxidant enzymes. As the level of fluoride increases in testis and seminal plasma the activity of SOD and CAT were decreased. Chauhan *et al*. (2013) also investigated the possible impact of fluoride exposure on semen quality in 113 subjects (age 25-40) selected from the high fluoride region of Rajasthan

where fluoride content in ground water was more than 2.0ppm. They found marked changes in all oxidative stress markers in subjects compared with the control and suggested that over production of ROS in male reproductive tract may be potential cause of sperm dysfunctioning. Similar to previous studies that reported significant reduction in antioxidant enzymes is in accordance with previous studies (Sanocka *et al*., 1997; Zini *et al*., 2000; Sarkar *et al*., 2006; Izquierdo *et al*., 2008; Baba *et al*., 2013). While in fluoride-exposed rats both SOD and CAT activities were reduced, *Boerhaavia diffusa* L. leaf extract administration significantly ($p< 0.05$) accelerated the activities of both SOD and CAT in testis as well as in seminal plasma. Further regression analysis of leaf extract treated rats, revealed that as the level of fluoride in testis and seminal plasma decreased after administration with extract the activity of SOD and CAT increased as compared to fluoride intoxicated rats. The improved antioxidant status with significant reduction in testis and seminal plasma lipid peroxidation in fluoride-exposed *B. diffusa* L. leaf extract administrated rats could be attributed to the phytoconstituents of *B. diffusa* L. leaf. This contention also derives support from the fact that *B. diffusa* L. leaf is a potential source of antioxidants. In consonance with our study Rao and Bhatt (2012) administered 5 and 10 mg NaF/kg bw orally to male rats for 60 days to evaluate the effect on the testis in relation to oxidative stress. Alterations in the antioxidant indices in the testis were confirmed by increased lipid peroxidation (LPO) along with decrements in antioxidant parameters such as glutathione peroxidase (GPx), glutathione (GSH), total ascorbic acid (TAA), glutathione-S-transferase (GST), glutathione reductase (GR), superoxide dismutase (SOD), and catalase (CAT) levels affecting testis function as indicated by histopathological study. After supplementation of the NaF-treated rats by the antioxidant melatonin (10 mg/kg bw) revealed a significant

protection to the gonadal function, thus indicating a mitigating effect by melatonin against NaFinduced testis toxicity and oxidative stress in a rat model.

SOD is a part of first line of defense mechanism, and catalyzes the reaction between superoxide anion and hydrogen peroxide to form molecular oxygen and water. Decreased activity of SOD in the present study is suggestive of its excessive utilization for neutralizing superoxide generated by the fluoride. The catalase enzyme is important in the removal of hydrogen peroxide generated by SOD. In addition, CAT activity is inhibited by superoxide radicals (Kono and Fridovich, 1982). Decreased CAT activity in the present study is suggestive of excess production of H_2O_2 and other hydro peroxide radicals, owing to its immoderate utilization.

Thus, the present study clearly indicates the potential of *B. diffusa* L. leaves as an antiperoxidative and antioxidant agent in fluoride-induced toxicity. The improvement in lipid and antioxidant metabolisms could be due to the multi-factorial effects of secondary metabolites present in *B. diffusa* L. leaves. On the other hand, these metabolites could also have acted individually or synergistically to reduce the oxidative stress caused by consumption of fluoride. It is pertinent to note here that, traditionally, tender *B. diffusa* L. leaves are used in food preparations in India with no known toxic effects. Therefore, in light of our observations, we conclude that *B. diffusa* L. leaves could be used as a component in foods to promote the health of people living in endemic fluorotic areas as a means to ameliorate fluoride-induced ailments.

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Conflict of Interests

The authors declare that they have no conflicts of interests.

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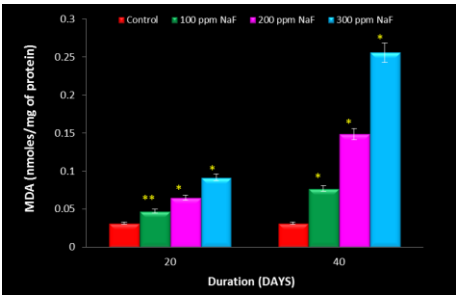


Fig. 1 Level of MDA in testis of control and NaF treated rats. Values are expressed as Mean ± SD. * p<0.0001 and ** p<0.05 NaF treated groups compared with control

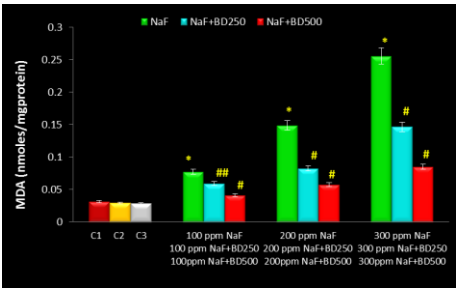
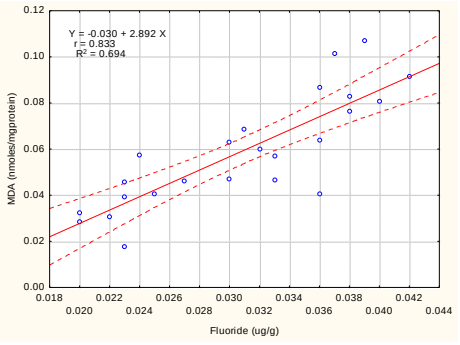
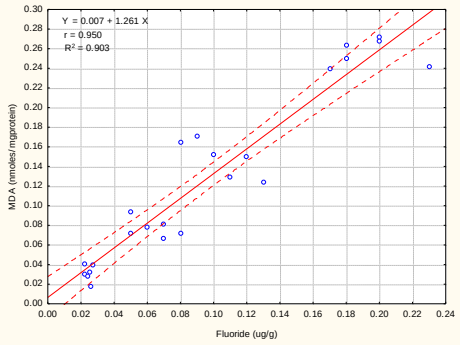


Fig. 2 Level of MDA in testis of control (C1), positive control (C2 and C3), NaF treated and combination of NaF and leaf extract treated group. Values are represented as mean ± SD. * p<0.0001 compared with control (C1).
# p<0.0001 and ## p<0.01 compared to respective NaF treated group

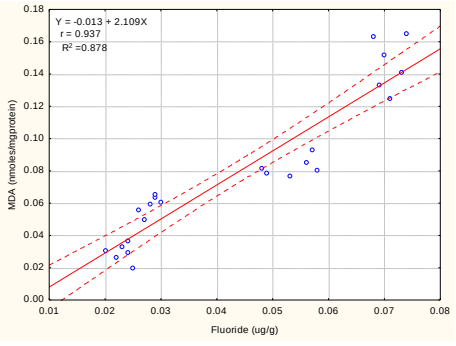


(A)

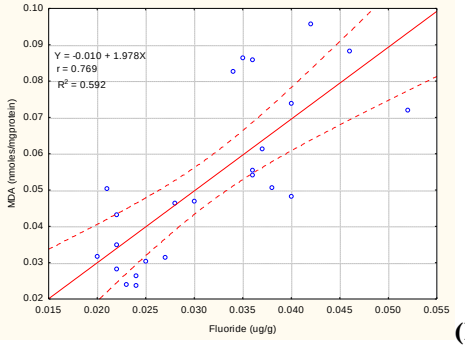


(B)

Fig. 3 Correlation and linear regression between level of fluoride and MDA in testis after (A) 20 days of fluoride intoxication, and (B) after 40 days of fluoride intoxication



(A)



(B)

Fig. 4 Correlation and linear regression between level of fluoride and MDA in testis after administration of *Boerhaavia diffusa* L. leaf extract 250mg/kgbw (A) and 500mg/kgbw (B)

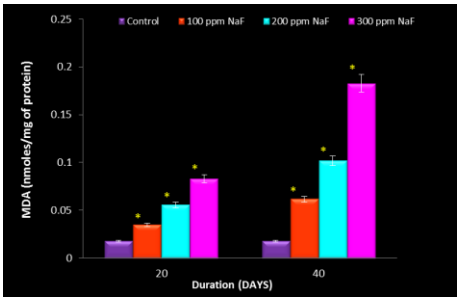


Fig. 5 Level of MDA in seminal plasma of control and NaF treated rats. Values are expressed as Mean ± SD. * p<0.0001 NaF treated groups compared with control

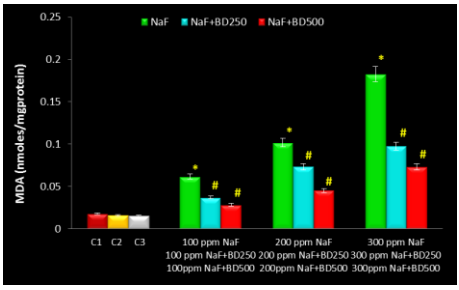
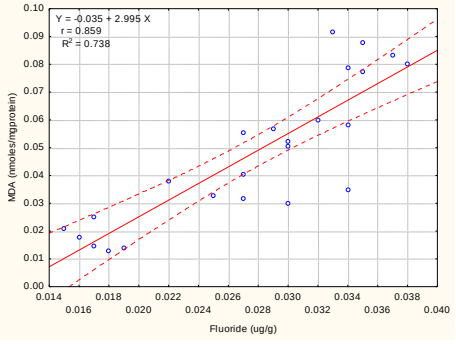
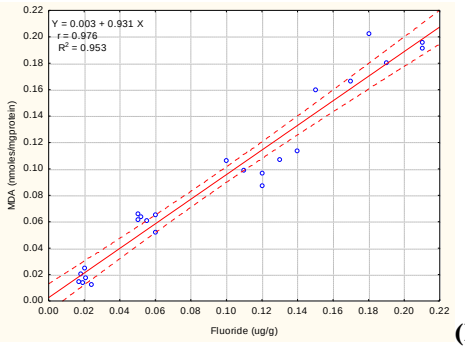


Fig. 6 Level of MDA in seminal plasma of control (C1), positive control (C2 and C3), NaF treated and combination of NaF and leaf extract treated group. Values are represented as mean ± SD. * p<0.0001 compared with control (C1). # p<0.0001 compared to respective NaF treated group

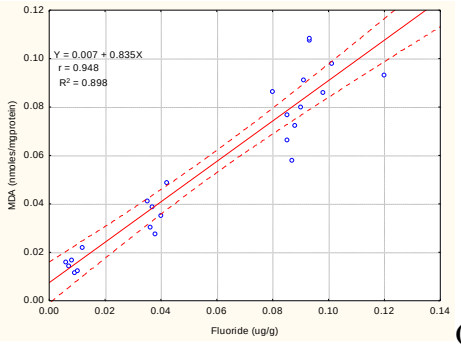


(A)

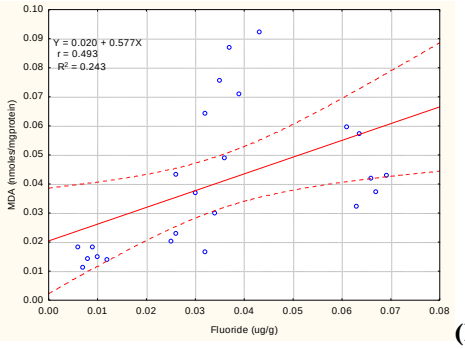


(B)

Fig. 7 Correlation and linear regression between level of fluoride and MDA in seminal plasma after (A) 20 days of fluoride intoxication, and (B) after 40 days of fluoride intoxication



(A)



(B)

Fig. 8 Correlation and linear regression between level of fluoride and MDA in seminal plasma after administration of *Boerhaavia diffusa* L. leaf extract 250mg/kgbw (A) and 500mg/kgbw (B)

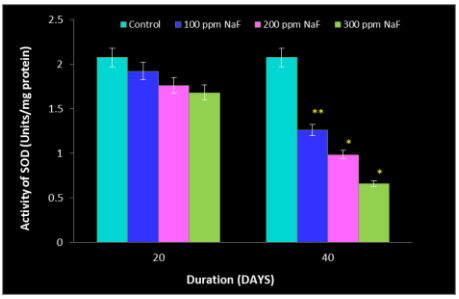


Fig. 9 Activity of SOD in testis of control and NaF treated rats. Values are expressed as Mean ± SD. * p<0.0001 and **p<0.001 NaF treated groups compared with control

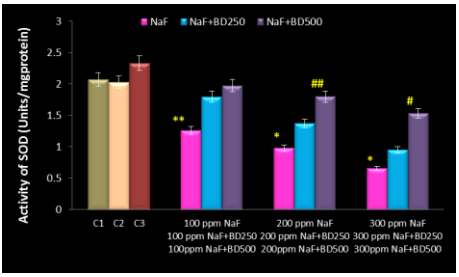
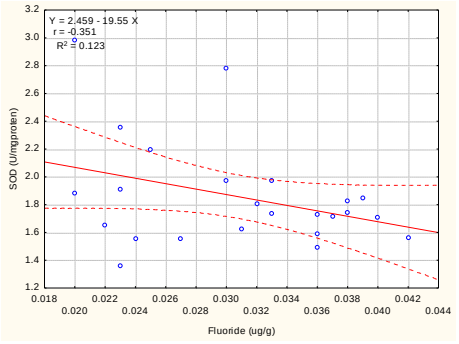


Fig. 10 Activity of SOD in testis of control (C1), positive control (C2 and C3), NaF treated and combination of NaF and leaf extract treated group. Values are represented as mean ± SD. * p<0.0001 and ** p<0.001 compared with control (C1). # p<0.0001 and ##p<0.05 compared to respective NaF treated group



(A)

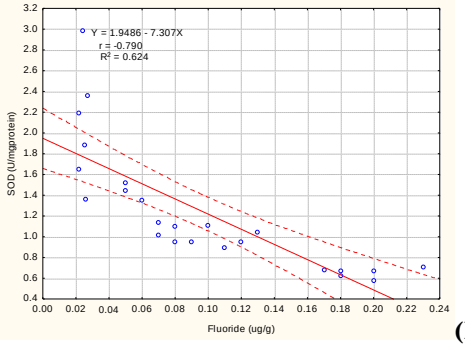


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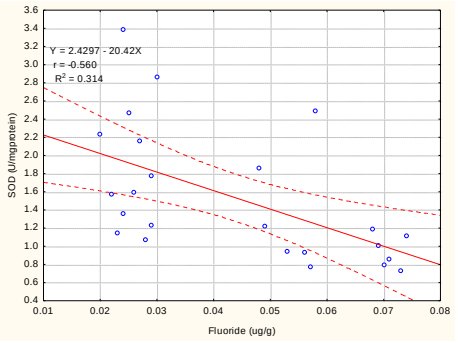
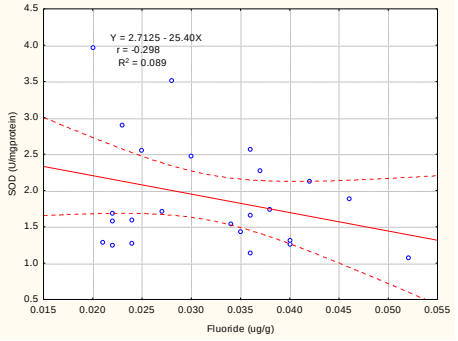
Fig. 11 Correlation and linear regression between fluoride level and activity of SOD in testis after 20 days of fluoride intoxication (A), and after 40 days of fluoride intoxication (B)



(A)



(B)

Fig. 12 Correlation and linear regression between level of fluoride and SOD activity in testis after administration of *Boerhaavia diffusa* L. leaf extract 250mg/kgbw (A) and 500mg/kgbw (B)

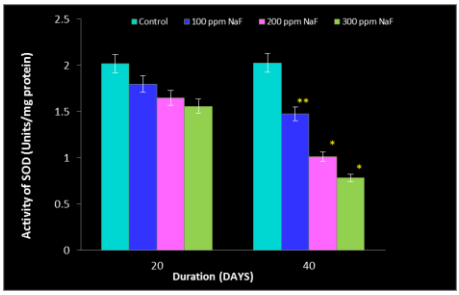


Fig. 13 Activity of SOD in seminal plasma in control and NaF treated rats. Values are expressed as Mean ± SD. * p<0.0001 and **p<0.05 NaF treated groups compared with control

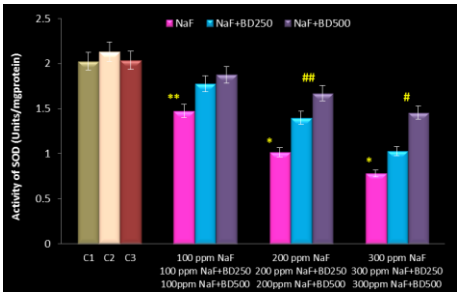
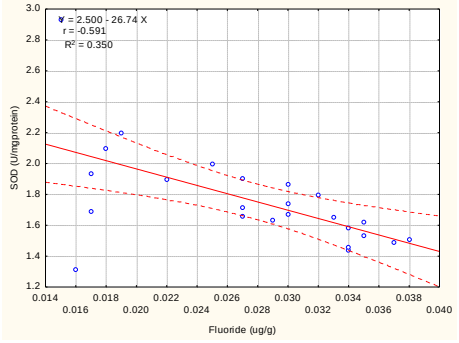
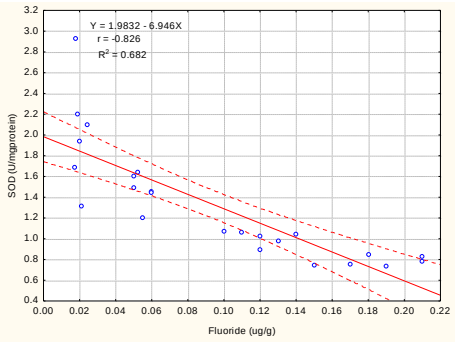


Fig. 14 Activity of SOD in seminal plasma of control (C1), positive control (C2 and C3), NaF treated and combination of NaF and leaf extract treated group. Values are represented as mean ± SD. * p<0.0001 and ** p<0.05 compared with control (C1). # p<0.0001 and ##p<0.01 compared to respective NaF treated group

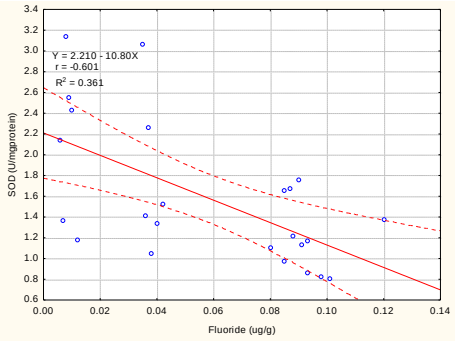


(A)



(B)

Fig. 15 Correlation and linear regression between fluoride level and activity of SOD in seminal plasma after 20 days of fluoride intoxication (A), and after 40 days of fluoride intoxication (B)



(A)

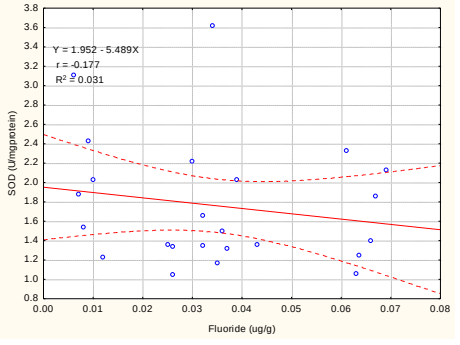


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Fig. 16 Correlation and linear regression between level of fluoride and SOD activity in seminal plasma after administration of *Boerhaavia diffusa* L. leaf extract 250mg/kgbw (A) and 500mg/kgbw (B)

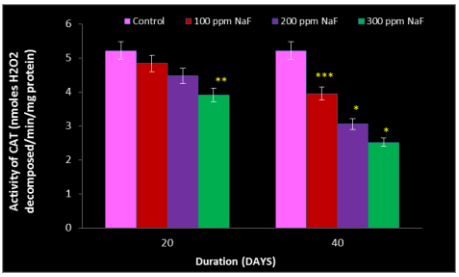


Fig. 17 Activity of CAT in testis of control and NaF treated rats. Values are expressed as Mean ± SD. * p<0.0001, ** p<0.01 and ***p<0.05 NaF treated groups compared with control

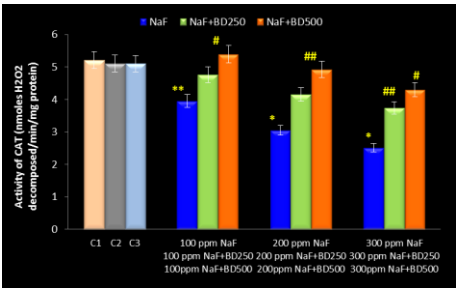
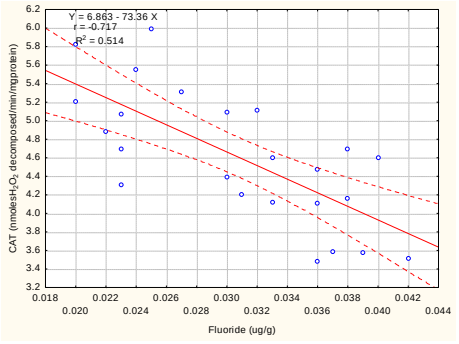
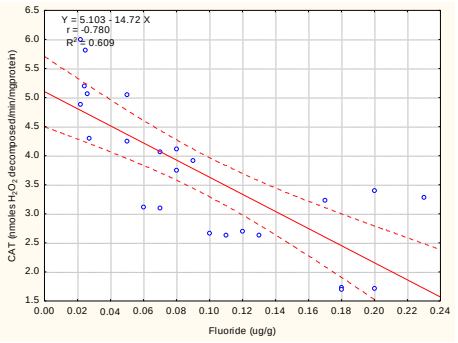


Fig. 18 Activity of CAT in testis of control (C1), positive control (C2 and C3), NaF treated and combination of NaF and leaf extract treated group. Values are represented as mean ± SD. * p<0.0001 and **p<0.05 compared with control (C1). # p<0.01 and ##p<0.05 compared to respective NaF treated group

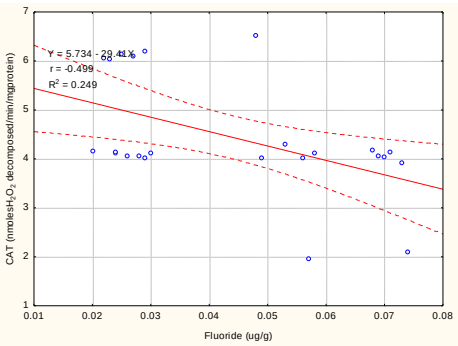


(A)

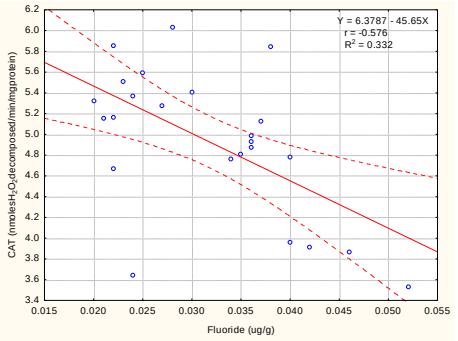


(B)

Fig. 19 Correlation and linear regression between level of fluoride and CAT activity in testis after 20 days of fluoride intoxication (A), and after 40 days of fluoride intoxication (B)



(A)



(B)

Fig. 20 Correlation and linear regression between level of fluoride and CAT activity in testis after curation with *Boerhaavia diffusa* L. leaf extract 250mg/kgbw (A) and 500mg/kgbw (B)

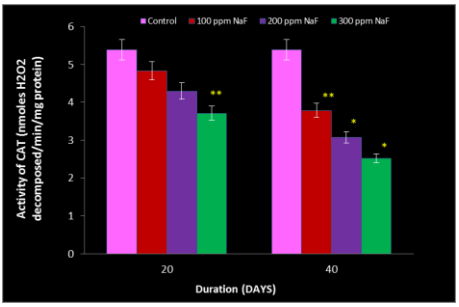


Fig. 21 CAT activity in seminal plasma of control and NaF treated rats. Values are expressed as Mean ± SD. * p<0.0001 and **p<0.01and NaF treated groups compared with control

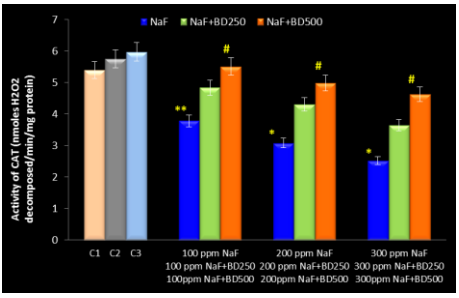
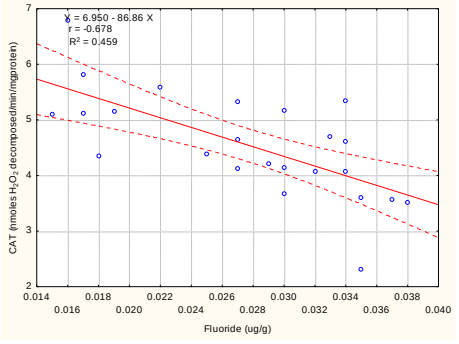


Fig. 22 Activity of CAT in seminal plasma of control (C1), positive control (C2 and C3), NaF treated and combination of NaF and leaf extract treated group. Values are represented as mean ± SD. * p<0.0001 and ** p<0.01 compared with control (C1). # p<0.01 compared to respective NaF treated group



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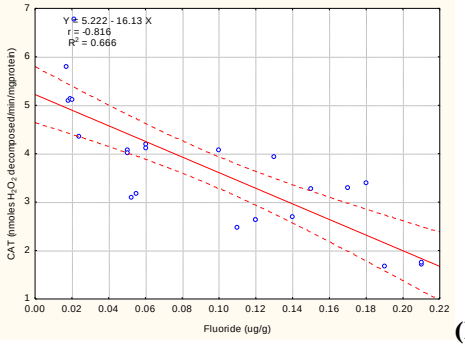
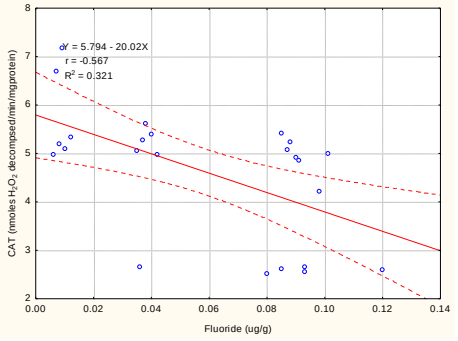
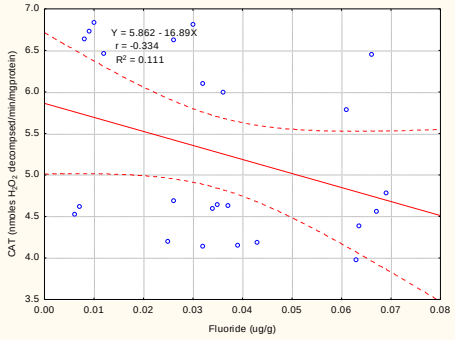


(B)

Fig. 23 Correlation and linear regression between level of fluoride and CAT activity in seminal plasma after 20 days of fluoride intoxication (A), and after 40 days of fluoride intoxication (B)



(A)



(B)

Fig. 24 Correlation and linear regression between level of fluoride and CAT activity in seminal plasma after curation with *Boerhaavia diffusa* L. leaf extract 250mg/kgbw (A) and 500mg/kgbw (B)