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ORIGINAL ARTICLE

Ginkgo biloba Ameliorates Fluoride Toxicity in Rats by Altering Histopathology, Serum Enzymes of Heme Metabolism and Oxidative Stress without Affecting Brain mGluR5 Gene

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ABSTRACT

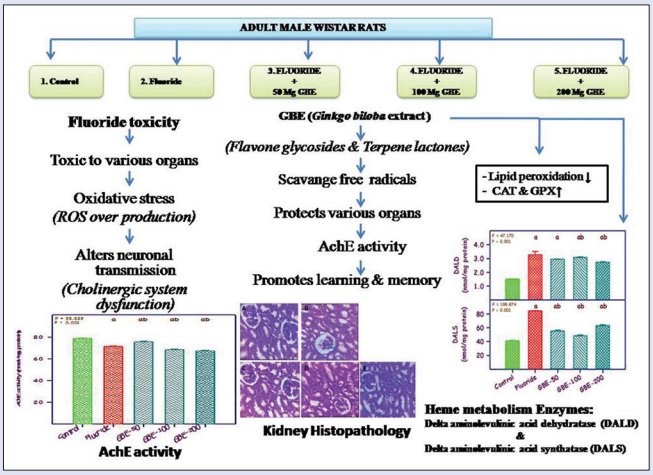
Background: To evaluate the therapeutic potential of *Ginkgo biloba* extract (GBE) in experimental model of fluorosis. **Objectives:** To study the protective effect of GBE in fluoride toxicity by assessment of oxidative stress, serum biochemical parameters, acetylcholinesterase (AChE) activity, histopathology and brain mGluR5 gene expression. **Materials and Methods:** Fifteen adult male Wistar rats were randomly assigned to 5 groups (n = 3 rats in each group). Group 1 (control) received water, Groups 2–5 were treated with 100 ppm of sodium fluoride for 30 days, while the Groups 3, 4 and 5 were GBE treated with 50 mg/kg, 100 mg/kg and 200 mg/kg body weight for 15 days, after sodium fluoride treatment for 30 days. **Results:** Elevated serum delta aminolevulinic acid dehydratase and delta aminolevulinic acid synthatase levels in fluoride intoxicated rats were ameliorated by various doses of GBE treatment. Elevated serum glutathione and decreased oxidized gluatathione levels observed in fluoride intoxicated rats were also ameliorated by GBE treatment but effectively at 100 mg/kg dose. Reduced AChE activity of hippocampus in fluoride-induced toxicity was reverted by 50 mg/kg of GBE whereas other doses (100 and 200 mg/kg) caused significant inhibition of AChE activity in comparison with fluoride group. Fluoride group rats showed significant reduction of mGluR5 gene expression levels whereas in all GBE treatment groups those changes Were not significantly reverted. GBE treatment to fluoride intoxicated rats almost reverted the degenerative changes in liver and kidney caused by fluorosis. **Conclusion:** The present study concluded beneficial effects of GBE in experimental model of fluorosis.

Key words: Acetylcholineesterase activity, Fluoride, *Ginkgo biloba* extract, glutathione, heme metabolism enzymes,reduced glutathione

SUMMARY

- To summarize the findings, the current investigation provides evidence on the beneficial effects of *Ginkgo biloba* extract in experimental model of fluorosis through assessment of serum biochemical, hippocampal neurotransmitter activity, and histopathological studies.

Abbreviations used: AChE: Acetylcholinesterase; AD: Alzheimer's disease; ALA: Aminolevulinic acid; DALD: Delta aminolevulinic acid dehydratase; DALS: Delta aminolevulinic acid synthetase; GBE: *Ginkgo biloba* extract; GSH: Glutathione; GSSG: Oxidized glutathione; mGluR5: Metabotropic glutamate receptor5; MDA: Malondialdehyde; NAF: Sodium fluoride; ROS: Reactive oxygen species; RT-PCR: Reverse transcription polymerase chain reaction.



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INTRODUCTION

Prolonged intake of fluoride leads to fluorosis. Fluoride consumption in optimal amounts through drinking water can protect against the development of dental caries.[1] According to World Health Organization, 1.5 part per million of fluoride in drinking water is safe. The principal source of fluoride to the human body is drinking water.[2] Dental fluorosis causes mottling of enamel of the teeth, whereas skeletal fluorosis is characterized by swollen, deformed joints and enlarged bones. Chronic fluorosis can lead to neurodegenerative related problems such as Alzheimer's dementia with learning and memory impairments.[3-5] Chronic fluoride toxicity decreased the number of nicotinic acetylcholine receptors in rat brain[6] and also affected the expression of M1 and M3 muscarinic acetylcholine receptors.[4] Reactive oxygen species (ROS) plays a significant role in the

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pathogenesis of chronic fluoride toxicity that increases oxidative stress in tissues.[7] Delta aminolevulinic acid dehydratase (DALD) is an enzyme that plays important role in hematopoiesis.[8] DALD, a rate limiting enzyme in heme biosynthesis has been found altered in people living in fluoride affected areas implicating a link between heme metabolism and fluorosis.[9] A recent study finding connected a link between iron overload and fluoride-induced hepatic oxidative stress.[10]

Fluoride alters the serum enzyme antioxidant biochemical markers like glutathione (GSH), oxidized glutathione (GSSG) levels. GSH is well known to protect the cellular system against harmful effects of lipid peroxidation.[11,12] Elevated GSH levels, increased lipid peroxidation and altered antioxidant system were found in rats that received 100 ppm fluoride in drinking water for 4 months.[13] The hepatic content of GSH is increased with aging when rats were treated with fluoride.[14] Acetylcholinesterase (AChE) activity is essential to maintain normal brain physiological functions and necessary in the processing of learning and memory. Fluoride crosses the blood-brain barrier and accumulates in rat hippocampus that causes inhibition of the activity of cholinesterase. Fluoride penetrates the blood brain barrier, interacts with AChE located on cell membranes and hamper with their physiological functions and thus induce neurotoxicity.[15] mGluR5 is the subtype receptor of group I metabotropic glutamate receptor and plays a vital role in modulation of synaptic plasticity. The function of mGluRs in synaptic plasticity and synaptic transmission are responsible for the source of learning and memory, characterized in the hippocampus.[16] Evidence support reduced mGluR5 gene and protein expression in the hippocampus and cortex of fluoride intoxicated rats.[17] However, no significant changes in hippocampal mGluR5 gene expression levels were seen in mouse pups following maternal exposure of fluoride during gestation and lactation period which is contrasting.[18] Fluoride toxicity associated pathological alterations in the glomeruli and the proximal and distal collecting tubules of nephrons were reported earlier.[19]

Toxic effects of fluoride can be ameliorated by supplementation of phytochemical agents with potent antioxidant activity.[10,20,21] *Ginkgo biloba* belongs to ginkgoacece family and its leaf extract is composed of flavone glycosides, 24% (quercetin, kaempferol, isorhamnetin) and terpene lactones, 6% (ginkgolides and bilobalide).[22] Treatment with GBE reduced intracellular ROS accumulation, apoptosis and mitochondrial dysfunction and increased the cell viability.[23] In our previous published reports, we have shown the therapeutic efficacy of GBE in rat model of fluorosis by evaluating various serum biochemical markers, hematological parameters and neurobehavioral tests assessment.[24,25] In the present work, we studied the histopathology, serum enzymes of heme metabolism and oxidative stress and brain mGluR5 gene expression levels.

MATERIALS AND METHODS

Chemicals

Sodium fluoride was purchased from Madras Fluorine Private Limited, India (Batch. No. 038P011, 99% pure). *G. biloba* leaves extract powder (Kshipra Biotech Private Limited, India Batch. No. KBPL/GBE/140101) and enzyme standards (Sigma Aldrich, USA) were procured as indicated. All other reagents and chemicals used in this study were of high pure analytical grade.

Animals

Fifteen adult male Wistar rats, weighing (120–160 g) were procured from Biogen Laboratory (CPCSEA Reg. No. 971/Po/RcBibt/S.2006), Bangalore, India and maintained in Centre for Laboratory and Animal Research (CLAR), Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, India. Animals were housed in polypropylene cages and supplied with pellet feed and filtered water as *ad libitum* and maintained at room temperature of 22°C–24°C, 40%–60% humidity and natural light and dark cycle. The animal experiment was approved by the Institutional Animal Ethics Committee (Reference No: SU/CLAR/RD/019/2016) and the work involving rats were strictly followed as per guidelines of the CPCSEA, Government of India.

Experimental design

Fifteen male Wistar rats were randomly divided into 5 groups and each group had 3 rats. Group 1 control rats received water *ad libitum*, Group II (Fluoride) rats received water containing 100 ppm of fluoride for a period of 30 days *ad libitum*, Group III (fluoride + 50 mg GBE) rats received 100 ppm of fluoride water for 30 days *ad libitum* followed by GBE (50 mg/kg b. w) for 15 days, Group IV (fluoride + 100 mg GBE) rats received 100 ppm of fluoride water for 30 days *ad libitum* followed by GBE (100 mg/kg b. w) for 15 days and Group V (fluoride + 200 mg GBE) rats received 100 ppm of fluoride water for 30 days *ad libitum* followed by GBE (200 mg/kg b. w) for 15 days. Estimation of serum enzymes, reverse transcription polymerase chain reaction (RT-PCR) analysis of brain mGluR5 gene expression, AChE activity in hippocapmpus, and histopathology of liver and kidney were performed at the end of 45 days of experimental period.

Dose selection

Two hundred and twenty one milligram of sodium fluoride was dissolved in one liter of water to accomplish 100 ppm of fluoride.[26] Fluoride was administered to animals through drinking water (*ad libitum*) in water feeding bottles. GBE was administered at the doses of 50 or 100 or 200 mg/kg orally using oral gavage needle fixed to syringe. The method of preparation of the *G. biloba* was water extract method according to the manufacturer instructions and the extract contained total flavone glycosides >24% and total terpene lactones >6%.

Serum enzyme assay

DALD: DALD activity and index of reactivation, was measured in serum by spectrophotometry, according to Sassa.[27]

Determination of delta-aminolevulinic acid synthetase (DALS): DALS activity was determined using the method of Marver *et al*.[28]

Determination of GSSG and reduced GSH: GSSG and GSH were measured in serum by spectrophotometry and results are expressed as nmol/mL of serum.

Determination of AChE activity in rat hippocampus: The activity of AChE in the hippocampus region was determined by the method described by Ellman *et al*.[29] as modified by Srikumar *et al*.[30] The activity is expressed as nmol Ach hydrolysed/min/mg protein.

Determination of gene expression by real-time PCR: Total RNA was isolated from the brain hippocampus regions of control and experimental rats using Trizol reagent (Thermo Fischer Scientifc, India). RNA concentration was determined by measuring absorbance at 260 nm using Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., USA). cDNA was prepared from 0.5 µg total RNA by reverse transcription using Roche PCR Kit. TaqMan based detection was used RT-PCR analysis. The primers used for amplification of mGLUR5 and β-actin were as follows:

- mGluR5
  forward, 5‘-CACTCTTGCCCAACATCAC-3‘
  reverse, 5‘-CACAGCGTACCAAACCTTC-3‘.
- β-actin

forward, 5'-AGCCATGTACGTAGCCATCC-3'
reverse, 5'-ACCCTCATAGATGGGCACAG-3'.

Histopathology of liver and kidney

To assess histoarchitectural changes, small sections of liver and kidney from each of the experimental animals were taken and subjected to H and E staining. The pathological changes were viewed under the Microscope (Olympus CX23).

Statistical analysis

The data was analyzed by one-way analysis of variance followed by Student Newman Keuls's multiple comparison test (Sigma Plot 13 Software Inc., USA). *P* value ($P < 0.05$) was considered as statistically significant.

RESULTS

Results of serum enzyme parameters

Effect of Ginkgo biloba extract on delta aminolevulinic acid dehydratase in fluoride-induced toxicity

The fluoride group showed an elevated levels of DALD compared to control. It was found to be statistically significant ($P < 0.001$). GBE 50 mg group showed reduced levels of DLAD compared to fluoride group and it was not statistically significant. GBE 100 mg/kg group showed reduced levels of DALD compared to fluoride group and it was not statistically significant. GBE 200 mg/kg group showed further reduced levels of DALD compared to fluoride and it was statistically significant. Treatment with GBE 200 mg/kg showed improvement in DALD levels in fluoride-induced toxicity. 200 mg/kg GBE provided effective protection in fluoride toxicity in the present study [Figure 1: Top panel].

Effect of Ginkgo biloba extract on delta aminolevulinic acid synthatase in fluoride-induced toxicity

The fluoride group showed an elevated levels of DALS compared to control. It was found to be statistically significant ($P < 0.001$). GBE 50 mg reduced the DALS level compared to fluoride, and it was found to be statistically significant. GBE 100 mg/kg further reduced the DALS levels compared to fluoride, and it was found to be statistically significant. GBE 200 mg/kg reduced the DALS level compared to fluoride, it was found to be statistically significant. 100 mg/kg GBE provided effective protection in fluoride toxicity in the present study [Figure 1: Bottom panel].

Effect of Ginkgo biloba extract on gluatathione in fluoride-induced toxicity

The fluoride group showed elevated levels of GSH compared to control. It was found to be statistically significant ($P < 0.001$). In all GBE treatment groups (50, 100, and 200 mg/kg), the GSH levels were significantly reduced than that of fluoride group. However, it was found that GBE 100 mg/kg had better protective and antioxidant effect than other doses tested [Figure 2: Top panel].

Effect of GBE on oxidized gluatathione in fluoride-induced toxicity

The fluoride group showed reduced levels of GSSG as compared to control and it was statistically significant ($P < 0.001$). GBE drug treatments (50 and 100 mg/kg) significantly elevated GSSG levels as compared to fluoride group. However, GBE 200 mg/kg although elevated the GSSG levels compared to fluoride, it was statistically not significant. Results showed GBE 100 mg/kg had rendered better protective effects from fluoride toxicity [Figure 2: Bottom panel].

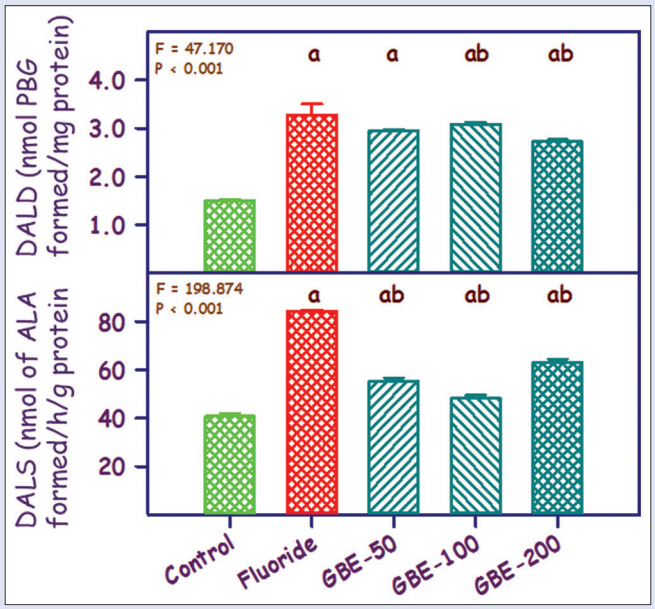

Figure 1: The effect of GBE on serum delta aminolevulinic acid dehydratase and delta-aminolevulinic acid synthetase in fluoride intoxicated rats. GBE-50, GBE-100 and GBE-200 are the doses (mg/kg, p. o). The values are mean ± standard error ($n = 3$ each). The 'F' and '*P*' values are by one way analysis of variance with Student Newman Keuls's multiple comparison test, [a]Significantly different from the control group, [b]Significantly different from the fluoride group. GBE: *Gingko biloba* extract

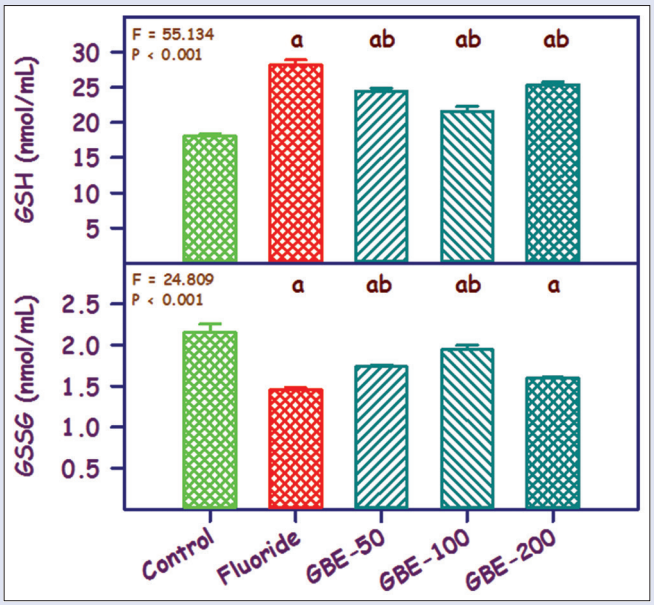

Figure 2: The effect of GBE on serum gluatathione and oxidized gluatathione in fluoride intoxicated rats. GBE-50, GBE-100 and GBE-200 are the doses (mg/kg, p. o). The values are mean ± standard error ($n = 3$ each). The 'F' and '*P*' values are by one-way analysis of variance with Student Newman Keuls's multiple comparison test, [a]Significantly different from the control group, [b]Significantly different from the fluoride group. GBE: *Gingko biloba* extract

Effect of Ginkgo biloba extract on acetylcholinesterase activity in fluoride-induced toxicity of hippocampus

The fluoride group showed reduced levels of AChE activity compared to control. It was found to be statistically significant ($P < 0.001$). GBE 50 mg elevated the AChE levels compared to fluoride group and it was found to be statistically significant. Notably, treatment with GBE at 100 mg/kg and 200 mg/kg significantly decreased the AChE activity than those of the fluoride intoxicated group [Figure 3] which is interesting.

Effect of Ginkgo biloba extract on reverse transcription polymerase chain reaction mGluR5 gene expression in fluoride-induced toxicity

The fluoride group showed a decreased gene expression compared to control. It was found to be statistically significant ($P < 0.001$). GBE 50 and GBE 200 mg/kg groups showed decreased mGluR5 gene expression when compared to fluoride, it was found to be statistically significant. GBE 100 mg/kg showed decreased gene expression when compared to fluoride and it was not statistically significant. GBE drug treatment at various dose levels (50, 100, and 200 mg/kg) to fluoride exposed rats did not revert back the considerable loss in the expression levels of mGluR5. The present findings indicate that GBE did not ameliorate the fluoride toxicity through group I metabotropic glutamate receptor. In fact, higher dose GBE 200 mg treatment caused further reduction of mGluR5 expression levels [Figure 4a and b].

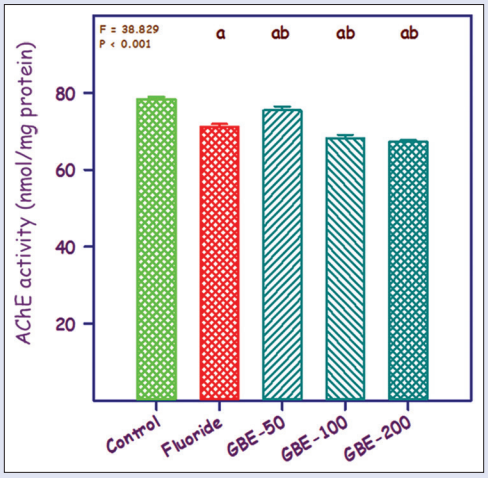

Figure 3: The effect of GBE on hippocampal acetylcholinesterase activity in fluoride intoxicated rats. GBE-50, GBE-100 and GBE-200 are the doses (mg/kg, p. o). The values are mean ± standard error ($n = 3$ each). The 'F' and '*P*' values are by one way analysis of variance with Student Newman Keuls's multiple comparison test, [a]Significantly different from the control group, [b]Significantly different from the fluoride group. GBE: *Gingko biloba* extract

Effect of Ginkgo biloba extract on histopathology of liver and kidney in fluoride-induced toxicity

Histopathological assessment of the liver of rats treated with fluoride showed hepatocellular necrosis, degenerative changes, hepatic hyperplasia and alterations in liver architecture [Figure 5]. Fluoride-induced rat kidney showed glomerular necrosis and atrophy, glomerular capsule tubules dilatation, shrunken glomeruli and vacuolation of cytoplasm in renal tubules. But GBE treatment to fluoride intoxicated rats reverted all those changes and reduced the damage considerably [Figure 6].

DISCUSSION

Several lines of research have shown the importance of testing plant based drugs to overcome fluoride toxicity. In the present work, the efficacy of GBE was tested in a rat model of fluorosis by the assessment of serum biochemical parameters, histopathology, brain AChE activity and mGluR5 gene expression. Increased serum DALS activity was noticed in NAF toxicity and in toxicity caused by other metals.[31-34] DALD activity was found elevated in heavy metals toxicity like iron[35] and lead.[36-38] In addition, plasma delta-aminolevulinic acid concentrations were also found increased in lead poisoned cattle.[39] The present study results showed significantly elevated levels of both DALD and DALS in fluoride treated rats compared with the control group. GBE drug treatment ameliorated the fluoride-induced toxicity by significantly altering the DALD and DALS levels. Increased DALS activity after arsenic and fluoride co-exposure implicates increased oxidative stress.[31] While several studies depicted decreased delta-aminolevulinate dehydratase

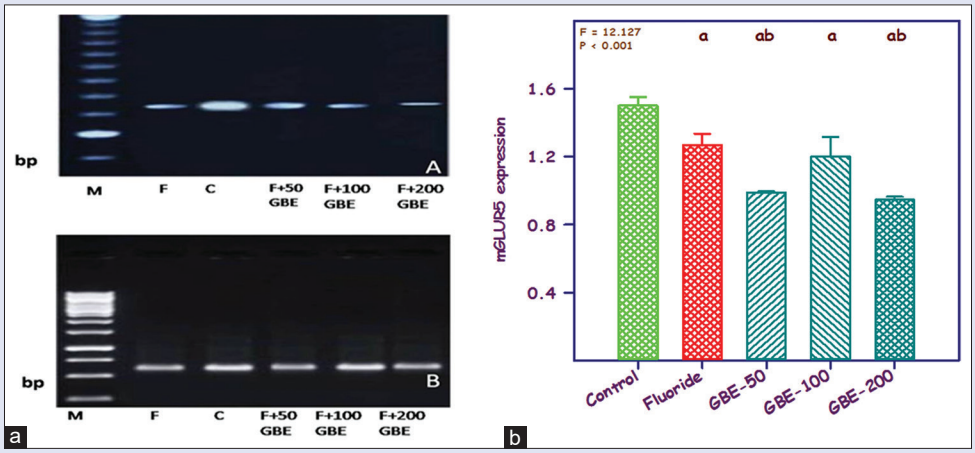

Figure 4: (a) The effect of *Gingko biloba* extract on mGluR5 in fluoride intoxicated rats ($n = 3$ rats each group) by reverse transcription polymerase chain reaction technique. Panels (A: MGluR B: β-actin) represents mGluR5 and β-actin mRNA expression in the hippocampus of rats (M: Marker, F: Fluoride, C: Control, F + 50 GBE: Fluoride + 50 mg GBE, F + 100 GBE: Fluoride + 100 mg GBE, F + 200 GBE: Fluoride + 200 mg GBE). (b) Representative bar graph showing the fold-change expression of mGluR5 gene in various groups. GBE-50, GBE-100 and GBE-200 are the doses (mg/kg, p. o). The values are mean ± standard error ($n = 3$ each). The 'F' and '*P*' values are by one way analysis of variance with Student Newman Keuls's multiple comparison test, [a]Significantly different from the control group, [b]Significantly different from the fluoride group. GBE: *Gingko biloba* extract

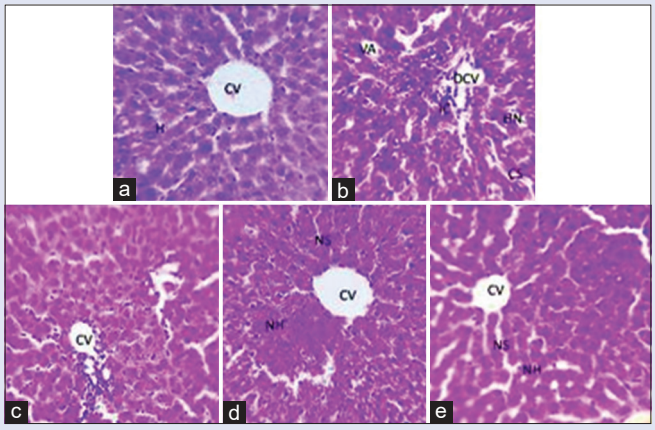

Figure 5: The effect of GBE on liver histopathology in fluoride intoxicated rats (*n* = 3 each group). Photomicrograph showing rat liver. (a) Control, (b) Fluoride, (c) F +50 mg GBE, (d) F +100 mg GBE, (e) F +200 mg GBE (H and E, ×100) CV: Central vein, H: Hepatocyte, HN: DCV: Dilated central vein, Hepatocyte necrosis, IC: Inflammatory cells, VA: Vacuolization, CS: Congested Sinusoids, NS: Normal hepatocyte, NS: Normal sinusoids, GBE: *Gingko biloba* extract

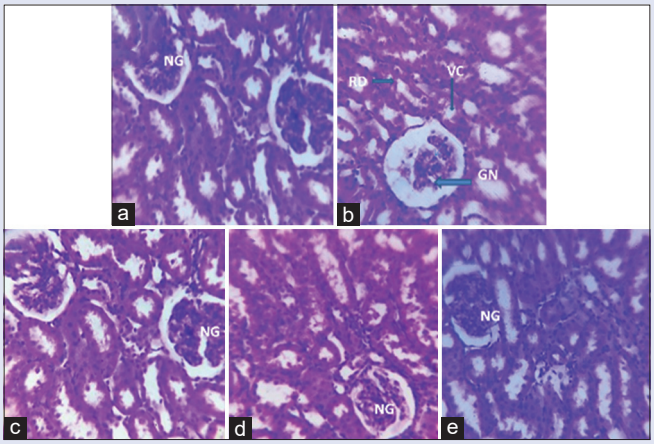

Figure 6: The effect of *Gingko biloba* extract on kidney histopathology in fluoride intoxicated rats (*n* = 3 each group). Photomicrograph showing rat kidney: (a) Control, (b) Fluoride, (c) F +50 mg GBE, (d) F +100 mg GBE, (e) F +200 mg GBE (H and E, ×100) GN: Glomerular necrosis and atrophy, RD: Renal tubules dilatation, VC: Vacuolation, NG: Normal glomerulus

activity during metal and fluoride toxicity associated oxidative stress condition,[40-43] we indent to report elevated delta-aminolevulinate dehydratase activity from the present study findings. Quite interestingly, elevated δ-aminolevulinic acid dehydratase was reported in rat erythrocytes due to lead poisoning.[38] It was suggested that increased activity of DALD in blood could be due to the counter effect of anaemia implicating heme synthesis.[44] As fluorosis is connected with anemia based on some studies[45,46] our findings on elevated serum DALD levels could be justified clearly. However, it is interesting to notice that the striatal DALD activity was increased in MPTP-induced PD mice implicating the role of heme groups.[47]

Reduced GSH is one of the main scavengers of ROS and along with oxidised glutathione (GSSG) ratio it may be considered as a good marker of oxidative stress. GSH is one of the most abundant non-enzymatic antioxidant bio-molecules present in tissues.[48] The ratio between GSH: GSSG is considered as main marker to diagnose the cellular toxicity.[49] Previous studies conducted on GSH levels indicate the severity of lipid peroxidation. Study results showed increased GSH levels,[50] decreased GSH levels[13] and also unaltered GSH levels in fluoride exposure.[51] Chronic fluoride toxicity in sheep resulted in increased levels of serum MDA, red blood cells and levels of GSH due to free radical mediated oxidative stress and increased lipid peroxidation.[52] Female mice treated with 50 mg/L fluoride for 30 days showed significant elevation in brain GSH levels, which was reversed and ameliorated by treatment with 20 mg/kg GBE.[53] Present study results revealed elevated GSH levels in rats exposed to 100 ppm of fluoride and showed decreased levels of GSSG. The present results were found to be similar to previous works showing increased GSH levels after fluoride exposure. The antioxidant functions of GBE have been reported suggesting its effect against fluoride-induced oxidative stress and lipid peroxidation.[54] Antioxidant functions of GBE against hydrogen peroxide-induced lipoperoxidation were also noticed in normal human erythrocyte membrane samples.[55]

AChE is an enzyme that takes part in cholinergic neurotransmission. AChE activity is inhibited by several compounds which include drugs, pesticides and some chemical toxins[56,57] although many studies support beneficial neuroprotective functions following AChE activity inhibition.[58] In fact, reduced AChE activity was reported in several neurological and neurodegenerative disorders.[59-62] Similar to present findings, inhibition of brain AChE activities in fluoride ingested rats were reported by several works.[63,64] By contrast, GBE treatment at 50 mg/kg to fluoride intoxicated rats increased the brain AChE activity whereas other doses (100 and 200 mg/kg) caused greater inhibition of AChE activity than the fluoride group. While reduced brain AChE activity in hypercholesterolemic mice was accounted for inflammation and mitochondrial dysfunctions *per se* leading to cognitive dysfunctions in neurodegenerative diseases.[65] Mice treated with sodium fluoride for 30 days showed reduced brain AChE enzyme activity.[66] Female rats treated with 500 ppm of sodium fluoride in drinking water for 60 days revealed decreased AChE activity.[67] Taken together, the present study results are in agreement with previous works showing fluoride mediated reduction of AChE activity in brain tissues. Previous research findings revealed that administration of certain phytochemical drugs could alter the activity of AChE in brain to ameliorate the toxicity of several agents including metals.[68-70] But it is important to notice that inhibition of AChE activity after GBE treatment has proven neuroprotective effects in animal models of neurotoxicity.[71,72] Therefore, it suggested that studies that would explore in details the mechanistic insights of inhibition of AChE activity during fluorosis would better indicate the sequel of brain associated pathogenesis.

In earlier research work, GB treatment in AD patients along with ChEIs provided additional cognitive benefits.[73] *G. biloba* leaf extract rich in flavonol compounds promoted dopaminergic and cholinergic neurotransmission in the prefrontal cortex of rats.[74]

G. biloba protected the brain from beta-amyloid neurotoxicity and reversed the memory deficits through its effect on the cholinergic system.[75] Ginkgolides A and B administration inhibited the effect of β-amyloid on the release of acetylcholine from hippocampal neurons.[76] It was reported that *G. biloba* improved the cognitive ability through interactions with the antioxidant and cholinergic systems.[77] In the present work, treatment with various doses of GBE (50, 100, and 200 mg/kg) ameliorated fluoride toxicity by altering hippocampal AChE activity.

Glutamate is the primary excitatory neurotransmitter in the brain and activates both ionotropic glutamate receptors and G protein coupled metabotropic glutamate receptors mGluRs.[78] mGluR5 signaling changes were noticed in various neurodegenerative diseases like Parkinson's disease, Huntington's disease, and Alzheimer's disease.[79] mGluR5 are found in the neurons and glia cells all over the CNS including the cortex and the hippocampus.[80,81] Inhibition of mGluR5 expression impaired

the spatial learning in experimental rats[82] Present study showed inhibition of mGluR5 expression in fluoride-induced toxicity. However, treatment of GBE at various dose levels (50, 100, and 200 mg/kg) to fluoride exposed rats did not reveal any considerable changes in the mGluR5 expression levels when compared with fluoride group. The present findings indicate that GBE did not ameliorate the fluoride toxicity through group I metabotropic glutamate receptor.

Based on previous study, accumulation of fluoride diminished the aerobic metabolism and distorted the free radical metabolism in liver and kidney.[83] Chronic fluoride exposure leads to critical degenerative changes in rat kidney like glomerular necrosis and vascular congestion.[84] Hepatoprotective activity of *G. biloba* may be due to presence of compounds like flavonoid (ginkgo-flavone glycosides) and terpenoid (ginkgolides and bilobalides) which have antioxidant effect.[85] Present study results confirmed that rats treated with GBE showed amelioration in structure of liver and kidney against fluoride toxicity.

CONCLUSION

The present study investigated the therapeutic efficacy of GBE in experimental model of fluorosis. Fluoride-induced serum enzyme parameter alterations, AChE activity, liver and kidney histopathological changes were reversed by GBE. Results of the present study concluded beneficial effects of GBE in experimental model of fluorosis.

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Conflict of interest

There are no conflict of interest.

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