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Cognitive enhancement effect of *Ginkgo biloba* extract on memory and learning impairments induced by fluoride neurotoxicity

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ABSTRACT

The present work evaluated the neuroprotective functions of G*inkgo* biloba *extract* (GBE) on cognitive and behavioural functions in fluoride intoxicated rats. Thirty male Wistar rats were randomly divided into 5 Groups (n=6 in each Group). Group 1 was Control that received water, Groups2 to 5 were treated with 100 ppm of sodium fluoride for 30 days while the Groups 3, 4, and 5 were drug treated at 50 mg/kg, 100mg/kg and 200 mg/kg body weight of GBE for 15 days. After 45 days of treatment protocol various behavioural tests (Spatial learning (Y maze, T maze and Passive avoidance test) performed. The data were compared between the groups. The fluoride administered rats that received the only fluoride showed significant impairment in spatial learning and memory as assessed by behavioural tests; the GBE treated animals showed significantly improved learning and memory in a dose-dependent manner. The present study concludes the dose-dependent protective role of GBE in sodium fluoride toxicity induced learning and memory deficits.

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INTRODUCTION

Fluorine is an electronegative constituent and scattered universally as fluorides in the environment. World health organization (WHO) has declared that 1.5 ppm of the fluoride is safe in the drinking water. Fluoride is one of the micronutrients that improve enamel to prevent dental caries. Dental and skeletal fluorosis is due to prolonged exposure to high concentration of fluoride. Extreme exposure to fluoride may generate central nervous system dysfunctions (Spittle, 1994). The incidence of learning and memory deficits were typically due to exposure of 100 ppm of sodium fluoride in 30 days of period (Pereira *et al.,* 2011). Prolonged exposure to fluoride was the reason for neuronal activity instability and also the unusual behavioural patterns (Mullenix *et al.,* 1995). Fluoride can cross the blood-brain barrier especially at the time of pregnancy period leading to develop neural deterioration of offspring which promotes learning and memory impairments (Madhusudhan, Piler, 2009; Shivarajashankara *et al.,* 2002).

Administration of high fluoride causes decreased acetylcholine esterase and antioxidant enzymes like glutathione- S- transferase, superoxide dismutase and catalase in the hippocampus (Bhatnagar *et al.,* 2006). Fluoride affects the neuronal differentiation, migration which resulted in

the motor, sensory, learning and memory disturbances (Madhusudhan *et al.,* 2010). Fluoride exposure is considered as neurotoxic because it is associated with intellectual deficits (Choi *et al.,* 2012). Animals exposed to fluoride toxicity showed poor performance when subjected to perform behaviour tests such as maze tests (Basha, Sujitha, 2012). Fluoride has a role in memory and learning impairments because it releases oxygen free radicals by disrupting oxygen metabolism (Chirumari, Reddy, 2007). Fluoride alters the neurotransmitters levels that can reduce spontaneous motor activity (Paul, Ekambaram, Jayakumar, 1998).

Ginkgo biloba is the commonly cultivated Chinese medicinal plant. It has neuroprotective, antioxidant, free radical scavenging, anti-apoptotic, memory enhancing properties. The *Ginkgo biloba* leaf extract is composed of flavone glycosides 24% (quercetin, kaempferol, isorhamnetin) and terpene lactones 6% (ginkgolides and bilobalide) (O'Reilly J, 1993; Mahadevan, Park, 2008). GBE helps in the formation of memory consolidation and storage in the brain (Stoll *et al.,* 1996). GBE modulates the memory by acting antagonist to GABA receptor (Nooshinfar, 2006). GBE reverses the memory deficit caused by intraventricular administration of beta-amyloid by influencing the cholinergic system (Tang *et al.,* 2002). Spatial learning and memory impairments were reduced by GBE by enhancing the synaptic plasticity in the hippocampus (Wang *et al.,* 2006). In the present work, the potentials of *Ginkgo biloba* leaf extract on cognitive and behavioural parameters were evaluated in fluoride intoxicated rats.

MATERIALS AND METHODS

Chemicals: Sodium fluoride was obtained from the Madras Fluorine Private Ltd (MFPL: Batch. No.038P011), Chennai India. *Ginkgo biloba* leaf extract was obtained from Kshipra Biotech Ltd, (Batch. No. KBPL/GBE/140101) Indore, Madhya Pradesh, India. All other reagents and chemicals employed in this study were high pure analytical grade.

Animals: Thirty adult male Wistar rats, weighing (120- 160 g) were procured from Center for Laboratory and Animal Research (CLAR), Saveetha Institute of Medical and Technical Sciences, Chennai, India. Animals were housed in polypropylene cages and supplied with regular diet and filtered water *ad libitum* and maintained at natural light, and dark cycle, 40–70% humidity at room temperature of 22–24°C. The animal experiments were approved by the Institutional Animal Ethics Committee (SU/CLAR/RD/019/2016) and the work involving rats strictly followed the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) India.

Experimental Design

Animals were divided into 5 experimental groups, and each group had 6 animals (n=6).

Group I control (C): Received water *ad libitum*
Group II (Fluoride): Provided water containing 100 ppm of fluoride for 30 days *ad libitum*
Group III (Fluoride+50 mg GBE): Provided 100 ppm of fluoride water for 30 days *ad libitum* followed by *Ginkgo biloba Extract* (50mg/kg b.w) for 15 days.
Group IV (Fluoride+100 mg GBE): Provided 100 ppm of fluoride water for 30 days *ad libitum* followed by *Ginkgo biloba Extract* (100mg/kg b.w) for 15 days.
Group V (Fluoride+200 mg GBE): Received 100 ppm of fluoride water for 30 days *ad libitum* followed by *Ginkgo Biloba Extract* (200mg/kg b.w) for 15 days.

Behavioural tests (Y Maze, T Maze, Passive avoidance test) were conducted to all groups at the end of 45 days.

Dose Selection: Prepared 100 ppm of fluoride, 221 mg of sodium fluoride was weighed and mixed well in one litre of filtered water (Raghu Jetti, Raghuveer, Mallikarjuna Rao, 2016). Fluoride was administered to animals through drinking water (*ad libitum*) in water feeding bottles. *Ginkgo biloba Extract* was given at doses of 50 or 100 or 200 mg/kg body weight orally with oral gavage needle fixed to the syringe.

Behavioural tests

Y Maze test: The method was followed to evaluate the percentage of spontaneous alternations (Nitta *et al.,* 2002).
T Maze test: The method was employed to evaluate spontaneous alternation test and reward alternation test. This method described by Dunnett *et al.* (1982) was followed.
Passive avoidance test: This test employed a method described by (Bures J, Buresova O, Huston JP, 1983) which includes exploration test, acquisition and retention test.
Statistical analysis: The data were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's post-test using Graph Pad Prism, version 7.04 (Graph Pad Prism Software Inc., USA). P value ($P<0.05$) was considered as statistically significant.

RESULTS AND DISCUSSION

Y-maze test: Fluoride intoxicated rats given high dose (200mg/kg b.w) of GBE ($P< 0.001$) showed significantly high percentage of alternations when

compared with other doses such as 100mg/kg body weight (P< 0.01) and 50 mg/kg body weight (P< 0.05). Rats from fluoride group alone showed statistically less percentage of alternations when compared with drug-treated groups (50, 100 and 200 mg/kg body weight). Results showed that fluoride intoxicated group had a significant deficit in spatial learning in comparison with control. (The results of the Y-maze test are depicted in Figure 1).

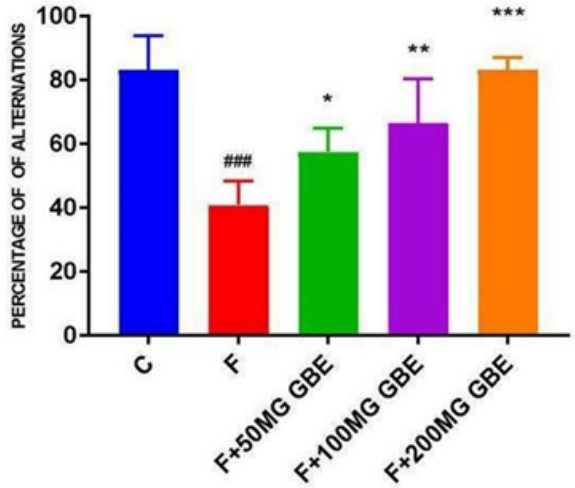

Figure 1: Y- Maze Test to assess the spontaneous alternations in rats

Graph showing the percentage of spontaneous alternation in Y MazeTest. Each bar express Mean ± SD. C vs F: ### P<0.001, F vs F + 50MG GBE: * P<0.05, F vs F + 100MG GBE: ** P<0.01, F vs F + 200MG GBE: *** P<0.001 (One way ANOVA, Bonferroni's test) : C: Control, F: Fluoride, F+GBE: Fluoride+ Ginkgo Biloba Extract.

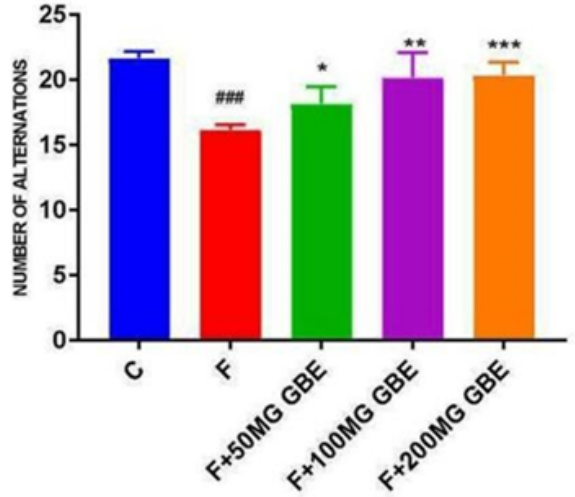

Figure 2: T- Maze Test to assess the spontaneous alternations in rats

Graph showing the number of spontaneous alternation in T maze. Each bar express Mean ± SD. C vs F: ### P<0.001, F vs F + 50MG GBE: * P<0.05, F vs F + 100MG GBE: ** P<0.01, F vs F + 200MG GBE: *** P<0.001 (One way ANOVA, Bonferroni's test) : C: Control, F: Fluoride, F+GBE: Fluoride+ Ginkgo Biloba Extract.

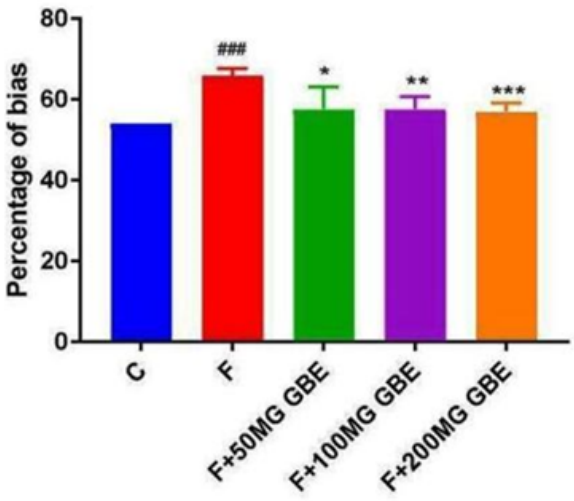

Figure 3: T- Maze Test showing percentage of bias

Graph showing the percentage of bias in T - maze spontaneous alternation test. Each bar represents Mean ± SD. C vs F: ### P<0.001, F vs F + 50MG GBE: $$ P<0.01, F vs F + 100MG GBE: ** P<0.01, F vs F + 200MG GBE: *** P<0.001 (One way ANOVA, Bonferroni's test) : C: Control, F: Fluoride, F+GBE: Fluoride+ Ginkgo Biloba Extract.

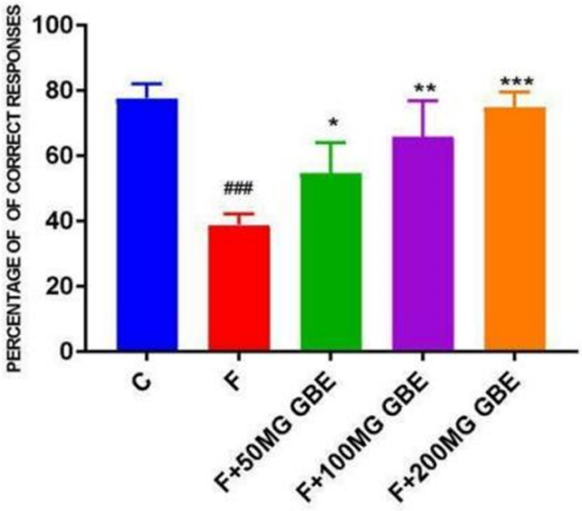

Figure 4: Rewarded alternation test in T- Maze

Graph showing the percentage of correct responses in T maze rewarded alternation test. Each bar represents Mean ± SD. C vs F: ### P<0.001, F vs F + 50MG GBE: * P<0.05, F vs F + 100MG GBE: ** P<0.01, F vs F + 200MG GBE: *** P<0.001 (One way ANOVA, Bonferroni's test) : C: Control, F: Fluoride, F+GBE: Fluoride+ Ginkgo Biloba Extract.

T-maze test: Fluoride intoxicated Rats treated with high dose of GBE, 200mg/kg b.w (P< 0.001) revealed significantly high number of spontaneous alternations than that seen with medium dose, 100mg/kg b.w (P< 0.01) and lower dose, 50 mg/kg b.w (P< 0.05). Rats from fluoride group showed statistically less number of alternations when compared with various doses of GBE treated groups.

Results revealed that fluoride group of rats showed spatial learning, which was evidenced in the form of less alternations and more percentage of bias and less percentage of correct responses in comparison with the control group in T maze test. (The results of the T-maze test were shown in Figure 2, 3, 4).

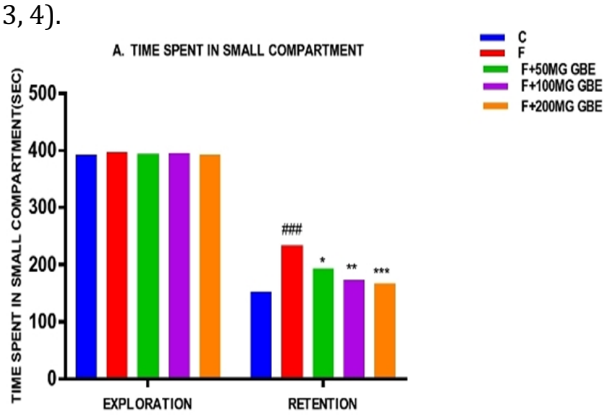

Figure 5: Passive Avoidance Test Results

Graph showing the Time spent in dark compartment in Passive Avoidance Test. Each bar represents Mean ± SD. C vs F: ### P<0.001, F vs F + 50MG GBE: * P<0.05, F vs F + 100MG GBE: ** P<0.01, F vs F + 200MG GBE: *** P<0.001 (One way ANOVA, Bonferroni's test) : C: Control, F: Fluoride, F+GBE: Fluoride+ Ginkgo Biloba Extract.

Passive avoidance test

Exploration: In an exploration test, no significant differences were noticed between the groups during exploration for the time spent in the dark compartment in passive avoidance test.

Retention: Retention of memory was significantly high in fluoride intoxicated rats treated with high dose of GBE 200mg/kg body weight (P< 0.001) as compared to GBE such as 100mg/kg body weight (P< 0.01) and 50 mg/kg body weight (P< 0.05). Treatment with drugs either with 50 or 100 or 200 mg/kg body weight showed significant high memory retention compared to the fluoride group rats. In fluoride, intoxicated group rats reduced retention of memory and more time spent in the dark compartment was seen as compared to the control (The results of passive avoidance tests are showed in Figure 5).

The present study explored the ameliorative effect of different doses of GBE on fluoride-induced memory and learning deficits. The findings revealed a dose-dependent protective role of GBE that reduced deficits in spatial learning and memory of rats caused due to sodium fluoride neurotoxicity by assessment of behavioural tests for the first time. Spatial learning and memory impairments observed in group2 (Fluoride alone treated) rats noticed in present work indicate the role of fluoride toxicity. In Y maze test, group2 rats revealed significant impairments of learning and memory as compared to the control group, while GBE treated groups showed considerable significant reduction of impairments compared to fluoride group. Rats subjected to treatment with high dose GBE were comparatively more effective than the medium and low dose treated groups. In the T-maze test, the fluoride alone treated group depicted deficits of significant learning and memory as compared to the control group. These impairments were reduced in GBE treated groups. The effect was more pronounced in a high dose of GBE treated groups.

Based on Passive avoidance test, during the retention phase, animals treated with fluoride spent more time in a small compartment representing memory impairment, but *Ginkgo biloba* extract treated groups spent less time. In passive avoidance test during retention phase, fluoride intoxicated rats spent more time in dark compartment, that indicates the memory impairment, time spent in the dark compartment is significantly less in a high dose of *Ginkgo biloba extract* treated groups than medium and low dose groups. In passive avoidance test, during the exploration phase, the time spent in dark compartment revealed no significant difference between all the groups.

In the present study, it was observed that a high dose of GBE (200 mg/kg body weight) showed more beneficial effects in recovering fluoride induced memory and learning impairments. Therefore, *Ginkgo biloba extract* may have a protective role in fluoride toxicity. Adult mice treated with high doses of *Ginkgo biloba extract* promoted acquisition and long-term retention of operant conditioning (Winter, 1991). Administration of 240 mg/kg body weight of GBE in humans for 3 months showed improvement in cognition in very mild cognitive impairment condition (Grass-Kapanke *et al.,* 2011). Working memory deficits caused by yohimbine were found to be reversed by a single dose of different GBE (50, 100 and 200 mg/kg body weight) by oral administration in rats (Zhang, Cai, 2005). Notably, the highest dose of GBE (200 mg/kg) had a better effect in improving the ability of spatial learning and memory of aluminium-intoxicated rats by decreasing the production of insoluble fragments of beta-amyloid (QH Gong *et al.,* 2005). The ability of GBE (200 mg/kg) in antagonising the aluminium-induced neurotoxicity had already proved its anti-amnestic property (Rasha *et al.,* 2013). Long-term ingestion of GBE at 50, 100 and 150 mg/kg body weight per day improved the spatial memory (Kamilla Blecharz-Klin *et al.,* 2009). It is evident that repeated administration of GBE at 50 and 100 mg/kg body weight reduced the

deficits caused by unavoidable shock (Porsolt *et al.,* 1990). GBE when administered 150 mg/kg body weight in adult rats for two months resulted in protection against rotenone-induced neurotoxicity (Ahmed *et al.,* 2009). Fluoride toxicity could have resulted in learning and memory deficits due to a decrease in the nicotinic acetylcholine receptors (Shan *et al.,* 2004). The drug GBE might have enhanced the spatial memory in rats by modulating the neurotransmitters levels in various regions of the brain (Blecharz-Klin *et al.,* 2009). The present study findings suggest that administration of high dose of GBE can overall improve the memory and learning abilities by enhancing the synaptic plasticity of the hippocampus by attenuating fluoride toxicity.

CONCLUSION

Chronic consumption of fluoride causes cognitive impairments and skeletal, dental and other soft tissue lesions. The present study showed a considerable reduction of cognitive impairments in rats caused by fluoride intoxication after treatment with three different doses of GBE. Fluoride-induced learning and memory disturbances were ameliorated by GBE supplementation in a dose-dependent fashion. Taken all together, a high dose of GBE showed more beneficial neuroprotective effects on fluoride toxicity.

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Conflict of Interest

Authors do not have any conflicts of interest.

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