Original Research Article

Ginkgo biloba Ameliorates?????? Rather reduces Aluminum Induced Neurotoxicity in Rats

Formatted: Highlight

Formatted: Font color: Red

Abstract

Ethnopharmacological relevance

Ginkgo is a large tree with fan-shaped leaves. The leaves are often orally taken by individuals with memory deficits such as Alzheimer's disease and to improve blood flow to the brain in older people.

Aim of the study

We evaluated the protective effects of *Ginkgo biloba* against aluminum chloride ($AlCl_3$)-induced neurotoxicity

Study design

Eighty male albino rats were divided into four main groups (n = 20 per group) and provided with varying doses and combinations of AlCl3 and/or Ginkgo biloba (GB) in drinking water, DW. The treatments were administrated daily for 12 weeks.

Results

Ginkgo biloba extract caused a significant increase in brain neurotransmitters contents [Norepinephrine (NE), Serotonin (5-HT) and Dopamine (DA)] of intoxicated adult male albino rats. The plant extract also improved aluminum induced disruption of tissue architecture and significantly reduced DNA damage as indicated by reduction in different comet assay parameters in the brain of intoxicated rats during the entire experimental period.

Conclusions

Gingko biloba has protective effects against aluminum-induced neurotoxicity. Its mechanisms of action appears to be mediated by increasing monoamine neurotransmitter synthesis, and improving the integrity of DNA and tissue architecture in the brain.

Keywords

Aluminum chloride; Ginkgo biloba; Neurotoxicity; Neurotransmitter

1. Introduction

Aluminum is considered most toxic in its soluble ionic form [1]. It enters the human body at all developmental stages of life [2]. Although, the highest concentrations are found in young rats than old rats [2], aluminum is associated with neurobehavioral changes in mammals. Chronic exposure to aluminum ions leads to mood changes, convulsions, and muscular weakness. The preferred accumulation sites are the bones, spleen, liver and lungs [3] and exposure causes tissue oxidative stress. The latter involves alterations in antioxidant enzymes activity and generation of reactive oxygen species [4, 5] and reduced mRNA expression of endogenous antioxidants [6].

Other pathological effects of aluminum include induction of DNA fragmentation [7], and lesions in the brain, such as neuronal degeneration and hemorrhage [8] and perivascular edema [9]. Aluminum also increases lipid peroxidation and interferes with normal metabolism and distribution of minerals. It displaces biologically important cations such as calcium, iron, zinc, copper and magnesium from their binding sites [10]. The neurotoxic effects of aluminum are well documented in human and experimental animals [11].

The leaves and seeds of *Ginkgo biloba* contain bioactive compounds such as flavonoid and terpenoid that have neuroprotective effects and therapeutic roles

against many neurodegenerative disorders [12]. The organic acid extracts of the plant such as kynurenic, hydroxykynurenic, and vanillic have antioxidant, anti-allergic, anti-inflammatory, anti-tumorigenic, anti-anxiety, anti-carcinogenic effects [13]. *Ginkgo biloba* extract (EGb 761) was viewed as a polyvalent agent with a doable therapeutic use within the treatment of neurodegenerative diseases of complex origin, e.g., Alzheimer's disease (AD) EGb 761 has potential effectiveness against toxicity induced by β-amyloid (Aβ) derived peptides (Aβ25−35, Aβ1−40 and Aβ1−42) on hippocampal primary cultured cells, this space being severely affected in AD. These results recommend that the neuroprotective effects of *EGb 761* [14].??????? The effects of *EGb 761* on the CNS underlie one among its major therapeutic indications i.e., people plagued by deteriorating cerebral mechanisms associated with age-associated impairments of memory, attention and different psychological feature functions. *EGb 761* is presently used as symptomatic treatment for cerebral insufficiency that happens throughout traditional ageing or which can result to chronic degenerative dementia, vascular dementia, and for neurosensory disturbances. Depressive symptoms of patients with illness {Alzheimer's} disease (AD) associated aged non-Alzheimer patients may reply to treatment with *EGb 761* since this extract has an anti-stress result. Basic and clinical studies, conducted each in vitro and in vivo, support these useful neuroprotective effects of *EGb. EGb* 761 has ~~has~~ many major actions ~~it improves.~~ It improves ~~blood~~ Blood natural philosophy and tissue metabolism, and opposes the prejudicial effects of anemia. In animals, *EGb 761* possesses inhibitor and free radical-scavenging activities, it reverses age-related losses in brain alpha1-adrenergic, 5-HT1A and muscarinic receptors, protects against anemia somatic cell death, preserves the work of the hippocampal mossy fiber system, will increase hippocampal high-affinity B-complex vitamin uptake, inhibits the down-regulation of hippocampal corticoid receptors and enhances somatic cell malleability known chemical constituents of *EGb 761* were related to bound actions.(sentence too

Formatted: Highlight

Formatted: Highlight

Formatted: Font: Bold

long. Please subdivide into paragraphs) —Each flavonoid and ginkgolide constituent area unit concerned within the free radical-scavenging and inhibitor effects of *EGb 761* that decrease tissue levels of reactive oxygen species (ROS) [15]. Neuroprotective effects of *Ginkgo biloba* in central nervous system include protection of neurons against ischemia, free-radical-induced apoptosis, and preservation of hippocampal mossy fibers and neural plasticity, and prevention of cognitive deficits subsequent to traumatic brain injury and stress [16, 17]. Administration of *Ginkgo biloba* extract is also associated with improved spatial memory and changes in the neurotransmitter levels in several regions of the brain [18]. The plant is also neuroprotective against several neuronal insults [19], promotes regeneration and survival of neural tissue [20, 21].

The antioxidant activity of *ginkgo biloba* was associated with caspase-3 activation [22]. Its neuroprotective effects were expressed through inhibition of monoamine oxidase (MAO) A and B exist with kaempferol and later increased in the levels of serotonin, noradrenaline (NA), and dopamine (DA) increased in the brain [23, 24, 25]. Additional protective effects of the plant extract against age-related memory impairment might be associated with the inhibition of β-amyloid peptide production, lowering free cholesterol levels, acceleration of acetylcholine release, and modulating neurotransmitter receptors of the central nervous system [12, 26, 27, 28]. Cells are permeable to *Ginkgo biloba* extracts; hence, the extracts have cytoprotective effects at both nuclear and cytoplasmic levels [29].

The Aim of the present study was to determine aspects of the mechanisms of aluminum-induced neurotoxic effects and if such effects could be ameliorated by *Ginkgo biloba.*

2. MATERIALS AND METHODS

A. Chemicals and Diagnostic kits:

Formatted: Font: Bold

Aluminum in the form of anhydrous aluminum chloride ($AlCl_3$) was purchased from Al Gomhuria Company, Egypt. Ginkgo biloba extract in a powder form was obtained from Xiamen Forever Green Source Biochem Tech. Co., Ltd. (FGS). China. All chemicals used for estimation of amine levels were analytical grade.

Full botanical plant names

Family: *Ginkgoaceae* Engl.

Genus: *Ginkgo L.*

The plant list → *Gymnosperms* → *Ginkgoaceae* → *Ginkgo* → *Ginkgo biloba L.*

Product name	*Ginkgo biloba Extract*
Latin Name	*Ginkgo biloba Linn.*
Active ingredients	Ginkgolic Acid, Lactone, Flavone
Appearance	Brown fine powder
Part used	Leaf
Specification	24% Ginkgoflavoglycosides; 6% Terpene lactones; Ginkgolic acid < 5ppm <24/6, Ginkgolic Acid 1ppm max, USP>, <10:1 TLC (Water-soluble)>, <24/6, Ginkgolic Acid 1ppm max, CP2010>, <24/6, Ginkgolic Acid 5ppm max, DAB10>, <Flavone 24%Min,Lactone 6%Min HPLC，USP/EP>, <Flavone 24%Min,Lactone 6%Min HPLC，Ginkgolic Acid 5ppm max，CP05>,< Flavone 24%Min,Lactone 6%Min HPLC，CP05>

Test Method	HPLC, TLC

B. Animals

We used 80 male Wister albino rats (weighting 100-120 gm). Animals were purchased from Al-Zyade experimental animal production center, Giza, Egypt. During the experiment, they were housed in polyethylene cages, with stainless steel wire lids (bedded with wood shavings), and kept at room temperature (20-25 °C) and under 12 h light/dark cycle. Balanced ration diet and water were supplied ad libitum. The study was approved by the Research Ethical Committee of the Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Egypt. The initial 10 days were used to quarantine the animals and as period of acclimatization.

C. Experimental design

Rats were randomly divided into four experimental groups consisting of twenty animals each (n = 20). The specific treatments are:

Group I (Control): Rats were given tap water and feed ad libitum throughout the experiment and kept as a control.

Group II (Aluminum group): Rats received aluminum chloride (AlCl3) in drinking water at a concentration of 1.43 g/L (290 mg/L Al) for 12 weeks. This corresponds to a dose of 40 mg /kg B.W [31].

Group III (Ginkgo group): Rats were supplemented with *Ginkgo biloba* extract at dose of 100 mg/kg body weight [32] dissolved in D. W. daily for 12 weeks.

Group IV (Aluminum-Ginkgo group): Animals were given *Ginkgo biloba* extract at dose of 100 mg/kg (dissolved in D. W.) orally daily, together with aluminum chloride at concentration of 1.43 g/L (290 mg/l Al) in drinking water for 12 weeks.

D. Tissue sampling:

Ten rats were sacrificed from each group after six and twelve weeks. Fresh brain tissues were immediately washed in saline and divided into 3 parts: one part was kept in PBS (phosphate buffered saline) and then stored at -80 C for Comet assay, the second part was stored at -80 C for estimation of monoamine contents (Serotonin, Norepinephrine and Dopamine), and the third part was kept in 10% neutral formalin for the histopathological examination

I-Estimation of brain neurotransmitters

- Brain tissue sample weighing ≤300 mg was homogenized in 3 ml of cold acidified N-*butanol* ; [33]. Dopamine, norepinephrine and serotonin (5-HT) levels in the forebrain were estimated using the fluorometric method [34].

II- Comet assay (Single cell gel electrophoresis)

Slides were prepared by cleaning in methanol and burning over a blue flame. They were then immersed in hot 1.0% normal melting agarose (NMA) and air-dried before storing at room temperature. To isolate cells, a small piece of brain tissue was placed in 1 ml cold HBSS containing 20 mM EDTA and 10% DMSO. The piece was minced into fine pieces, and the Pellet? resuspended in 1% low melting point agarose (LMPA). A 10 µl suspension containing about 10,000 cells was placed on a slide and subjected to cell lysis and electrophoresis. The slides were subsequently stained with Ethidium bromide [35]. The fluorescent stain was

visualized (magnification 400 x) using an automated fluorescence microscope and the images were captured on a computer, equipped with Comet Score software (Komet IV). Three parameters were adopted as indicators of DNA damage: Tail length (TL; length of DNA migration), the percent of DNA in the comet tail (% Tail DNA) and Tail moment (TM) [36].

III- Methods used for histopathological study:

Brain tissue samples intended for histopathological investigation were fixed in 10 % neutral formalin, and then embedded in paraffin. After deparafinnization, tissue sections that were 5-µm in thickness were prepared and stained by Haematoxyline and Eosin staining [37] for subsequent evaluation.

IV- Statistical analysis:

Data were analyzed by using a one-way analysis of variance (ANOVA). Duncan's post hoc test was used to determine the significant differences between treatment means. The differences between means were considered statistically significant at $P \leq 0.05$.

3- Results:

3.1. Brain neurotransmitters:

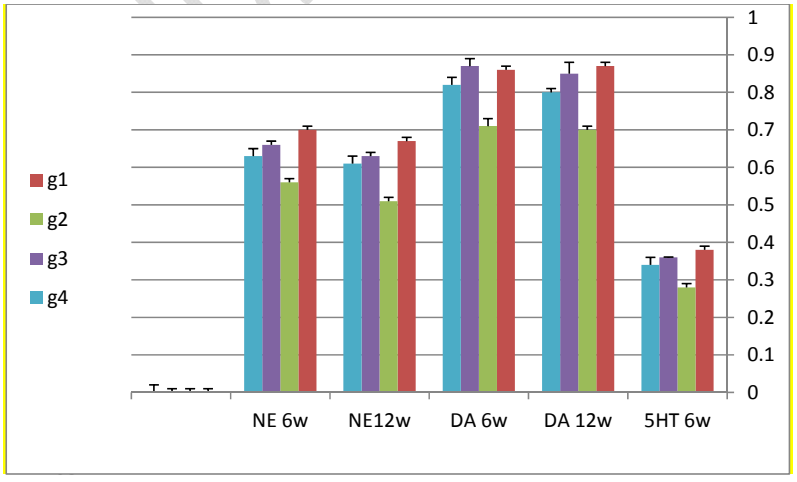
The effects of AlCl3 or/and *Ginkgo biloba* on norepinephrine, serotonin and dopamine levels are shown in Table 1. Levels of norepinephrine, serotonin and dopamine in the forebrain of the rats were significantly decreased ($p<0.05$) in AlCl3 administrated rats (2nd group) as compared with control group (1st group) after 6th and 12th week. Oral administration of *Ginkgo* alone (3rd group) or with AlCl3 (4th group) elevated norepinephrine, serotonin and dopamine levels in the brain of adult male albino rats significantly as compared to aluminum treated rats (2nd group) after 6th and 12th week but still lower than control group (1st group).

Table (1): Effect of $AlCl_3$ and *Ginkgo biloba* on norepinephrine (NE), dopamine (DA) and serotonin (5-HT) level in the brain of four groups of adult male albino rats (n= 10rat/group)

Treatment groups	NE		DA		5-HT	
	6W	12W	6W	12W	6W	12W
I	0.70±0.01^{a}	0.67±0.01a	0.86 ± 0.01a	0.87 ± 0.01^{a}	0.38±0.01^{a}	0.35±0.01^{a}
II	0.56±0.01^{c}	0.51±0.01^{c}	0.71 ± 0.01c	0.70 ± 0.02^{c}	0.28±0.01^{c}	0.26±0.01^{c}
III	0.66±0.01^{ab}	0.63±0.01^{ab}	0.87 ± 0.01a	0.85 ± 0.02^{a}	0.36±0.003^{ab}	0.33±0.001^{ab}
IV	0.63±0.02^{b}	0.61±0.02b	0.82 ± 0.02b	0.80 ± 0.02b	0.34±0.01^{b}	0.31±0.02^{b}

-Mean value ± SE

-The mean difference is significant at p <0.05-The values in the same raw carrying different letters were significantly different.



Histogram graph for table 1

3.2. Effect of $AlCl_3$ or/and *Ginkgo biloba* on DNA damage observed by comet assay in the brain of adult male albino rats

- The effects of AlCl3 or/and *Ginkgo biloba* on DNA damage observed by comet assay assessed as (Tail length (TL), %DNA in tail and Tail moment (TM)) in the brain cells of adult male albino rats are presented in **Table (2)**. Administration of $AlCl_3$ to rats of the 2nd group significantly increased DNA damage index observed by different comet assay parameters as compared with control group (1st group) after 6th and 12th week. Oral administration of *Ginkgo biloba* alone (3rd group) or with $AlCl_3$ (4th group) significantly reduced DNA damage induced by $AlCl_3$ (2nd group) as indicated by reduction in some comet assay parameters after 6th and 12th weeks.

Table (2): Effect of $AlCl_3$ and *Ginkgo biloba* on DNA damage observed by comet assay in the brain of four groups of adult male rats (n=10rats/group)

Treatment groups	Tail length		%DNA in tail		Tail moment	
	6w	12w	6w	12w	6w	12w
I	0.57±0.07[b]	0.51±0.07[b]	1.40±0.33[c]	1.12±0.29[b]	0.01±0.002[b]	0.01±0.001[b]
II	4.37±0.99[a]	5.14±0.98[a]	14.99±1.28[a]	16.81±1.99[a]	0.64±0.15[a]	0.89±0.25[a]
III	0.72±0.08[b]	0.67±0.09[b]	1.50±0.31[b]	1.64±0.37[c]	0.01±0.001[b]	0.01±0.003[b]
IV	1.65±0.41[b]	1.39±0.43[b]	5.15±0.86[b]	3.69±0.87[b]	0.11±0.004[b]	0.06±0.002[b]

-Mean value ± SE

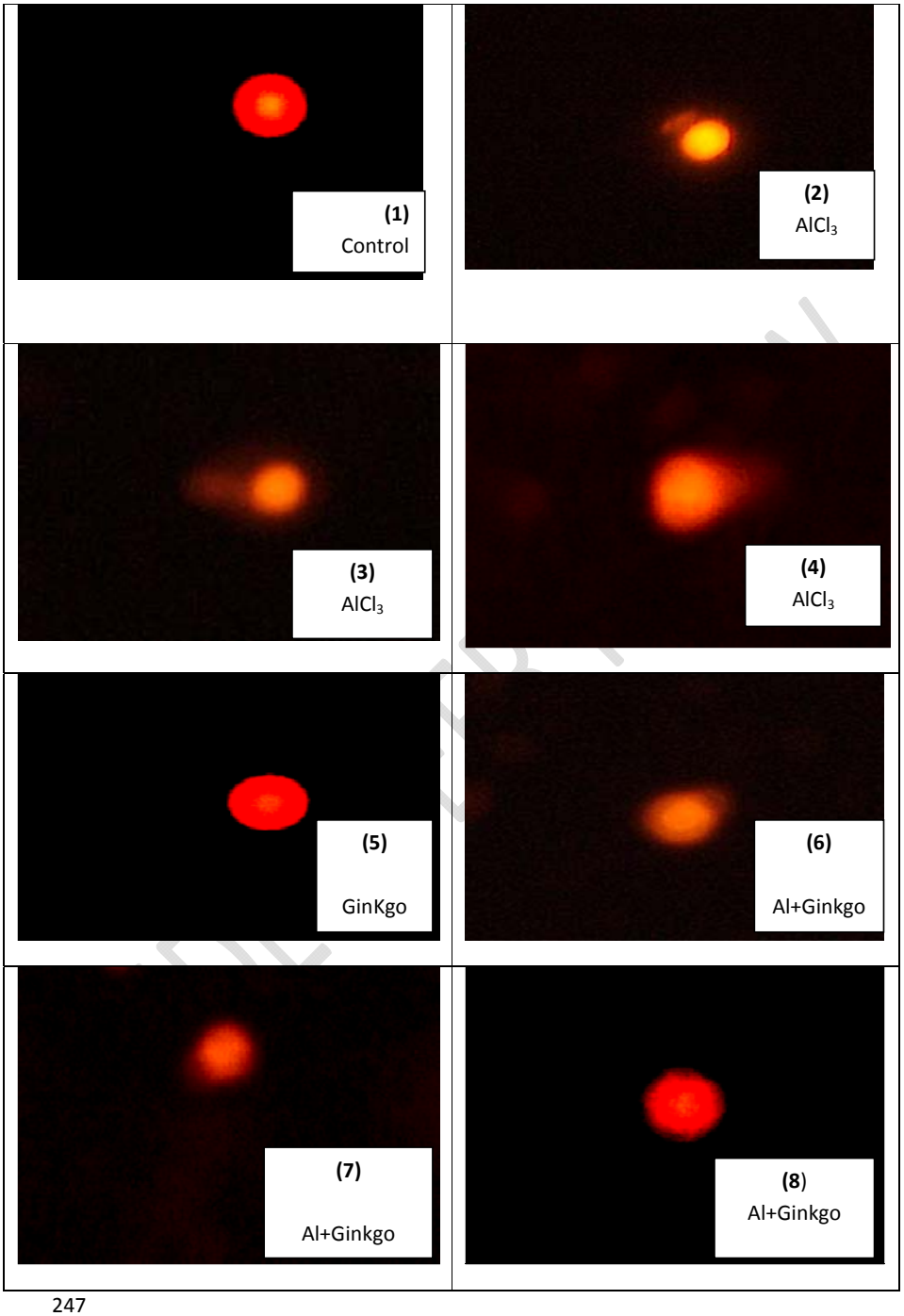
-The mean difference is significant at $p < 0.05$

-The values in the same raw carrying different letters were significantly different.

3.3. Effect of $AlCl_3$ or/and *Ginkgo biloba* on DNA damage observed by photomicrographs of comets in the brain cells of adult male albino rats:

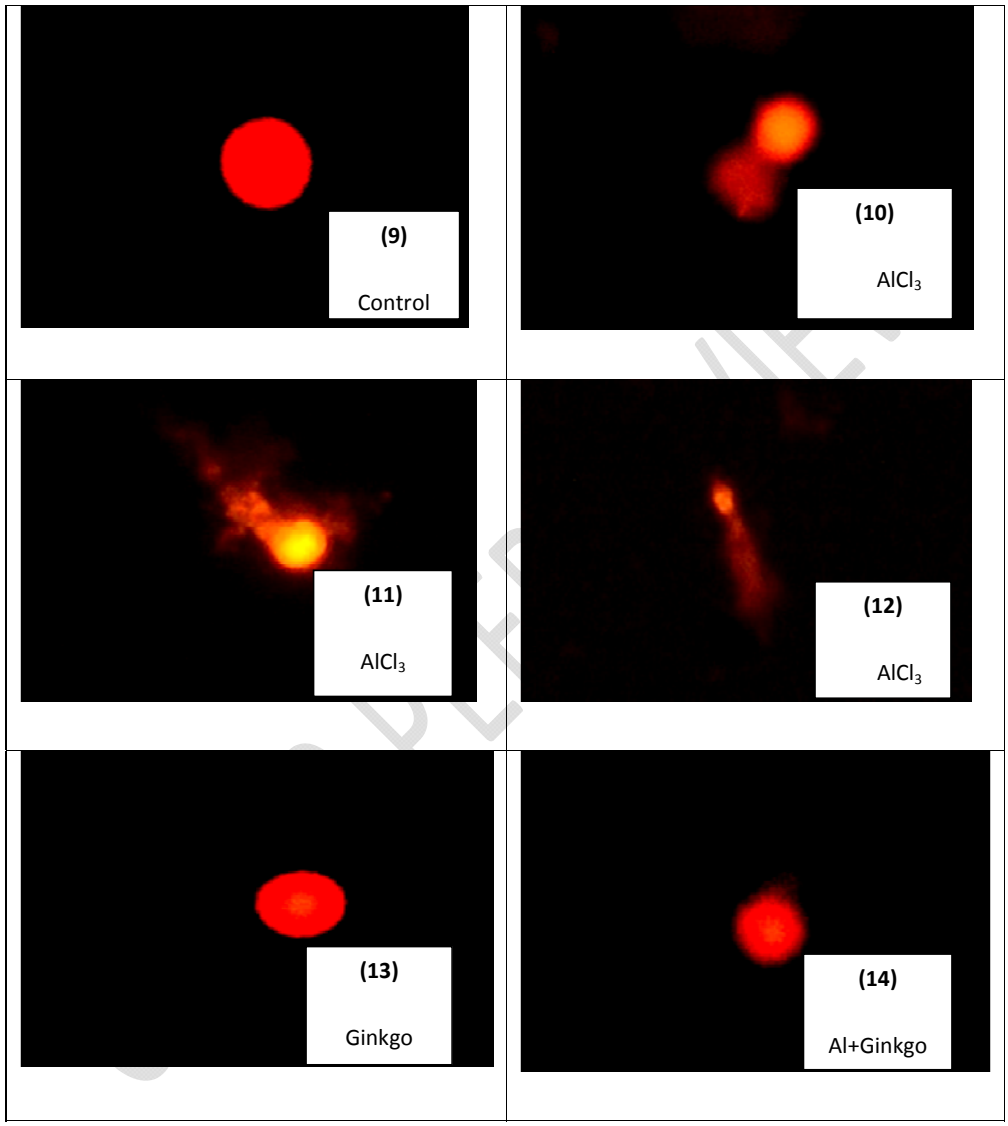
The Comet assay results of $AlCl_3$ and or *Ginkgo biloba* observed by photomicrographs in different experimental groups are shown in Figures 1 to 16. Undamaged DNA is recognized as a fluorescent core while the presence of strand breaks in the chain (damaged DNA) causes DNA to migrate and form a tail comet during the electrophoresis. There was no DNA damage in brain of control (**Fig. 1 & 9**). Rats in 2nd group intoxicated with $AlCl_3$ showed severe DNA damage in the brain cells after 6th and 12th week **(Figures 2, 3, 4, 10, 11 and 12).** No DNA damage was resulted in ***Ginkgo*-treated rats** after 6th and 12th week by microscopic examination **(Figure 5 and 13).** Oral administration of Ginkgo biloba along with exposure to $AlCl_3$ (4th group) showed slight DNA damage in the brain after 6th and 12th week **(Figure 6, 7, 8, 14, 15 and 16).**

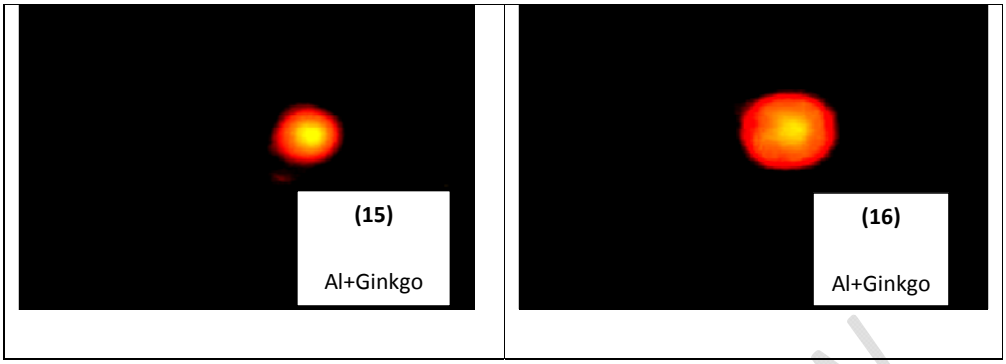
Fig. 1-8. Photomicrographs of comets in the brain cells stained with ethidium bromide in different experimental groups after 6th week (x400)


(1)
Control
(2)
AlCl3
(3)
AlCl3
(4)
AlCl3
(5)
GinKgo
(6)
Al+Ginkgo
(7)
Al+Ginkgo
(8)
Al+Ginkgo

247

Fig. 9-16. Photomicrographs of comets in the brain cells stained with ethidium bromide in different experimental groups after 12th week (x400)





250

3.4.2. Effect of $AlCl_3$ and *Ginkgo biloba* on the brain histoarchitecture

252 Within 6 weeks, Aluminum induced alteration in brain histoarchitecture.
253 Neurons with cork screw shaped neurofibrillary tangles were
254 characteristically demonstrated in cerebral cortex **(Fig. 17).** It caused
255 neurodegenerative lesions consisting of deposition of abundant amyloid
256 plaques particularly in the cerebrocortical **(Fig. 18)** and hippocampal regions
257 **(Fig. 19)** associated with neuronal degeneration and proliferation of glia cells
258 **(Fig. 20).** Brain of aluminum chloride treated rat for 6 weeks showing
259 cork strew shaped neurofibrillary tangles **(Fig. 21).** Other frequently
260 demonstrated lesions were degeneration of pyramidal nerve cells **(Fig. 22)**
261 and intense inflammatory reactions associated with focal gliosis **(Fig. 23)** as
262 well as cerebral hemorrhage **(Fig. 24)**.

263 Brain showed normal neuronal cells with large round nuclei after 6 weeks
264 **(Fig. 25).** After 12 weeks of aluminum treatment, the brain had more
265 deposition of amyloid plaques associated with congestion of cerebral blood
266 vessels, perivascular cuffing, glia cells and neuronal degeneration **(Fig. 26).**
267 After 6 and 12 weeks with less frequent cerebral hemorrhage and decreased
268 frequency of amyloid plaque deposition after 12 weeks **(Fig. 27).** Cerebral
269 blood vessels in most examined sections revealed intravascular aggregation

of leukocytes with perivascular edema and cuffing with glia cells **(Fig. 28).** Focal cerebral tissue necrosis associated with reactive gliosis was also demonstrated **(Fig. 29)**.

Histopathological examination of brain aluminum and *Ginkgo* treated rats showed improvement of the brain histoarchitecture.

The brain of *Ginkgo* treated rats alone showed normal cerebral cortex and hippocampus similar to those demonstrated in the control ones. Brain showed normal neuronal cells with large round nuclei after 12 weeks **(Fig. 30**). Brain of aluminum chloride and Ginkgo biloba treated rat for 12 weeks showing less deposition of amyloid plaques **(Fig. 31**). Brain of Ginkgo treated rats together with Al intoxication revealed marked reduction of the histopathological lesions compared to aluminum treated one. Brain showed lowered number of degenerated neurons **(fig. 32)**.

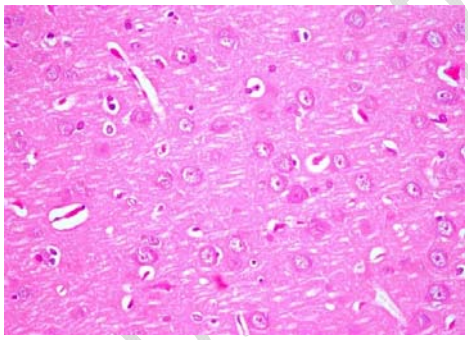


Figure 17: Brain of control rat showing normal cerebral cortex. The neuronal cells appeared normal with large round nuclei and prominent nucleoli (H&E X400).

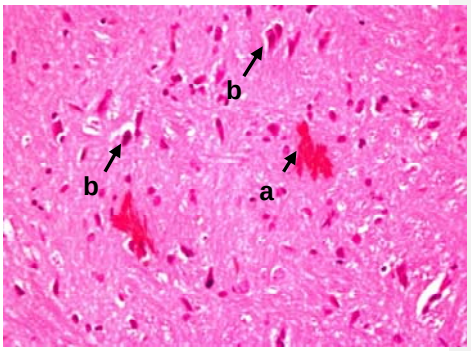
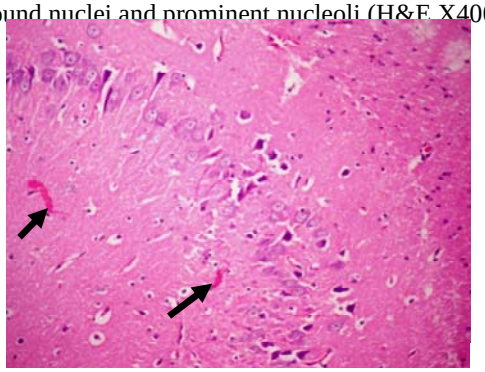
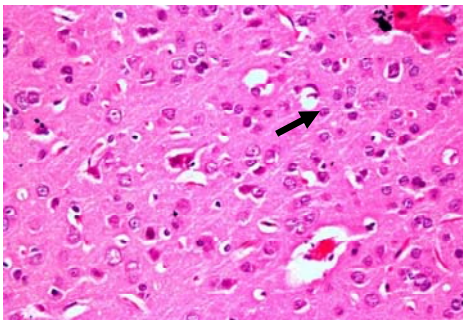
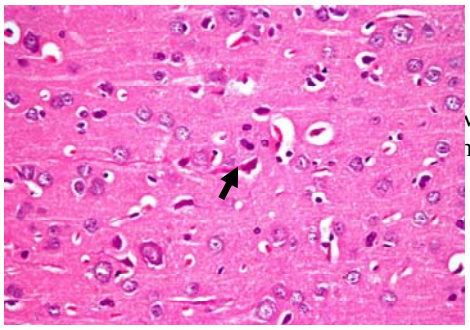


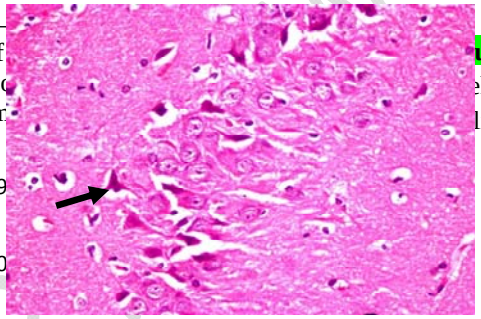
Figure 18: Brain of aluminum chloride treated rat for 6 weeks showing deposition of abundant amyloid plaques **(a)** in cerebral cortex associated with neuronal degeneration **(b)** (H&E X400).







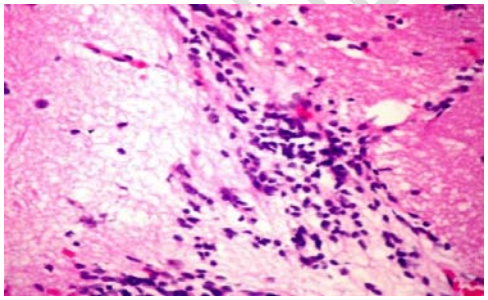
Brain of
ving depo
ippocam



ure 20: Brain of aluminum chloride treated ra
eks showing neuronal degeneration associated
liferation of glia cells (arrow) (H&E X400).

Figure 21: Brain of aluminum chloride treated rat for 6 weeks showing cork strew shaped neurofibrillary tangles (arrow) (H&E X400).

Figure 22: Brain of aluminum chloride treated rat for 6 weeks showing degeneration of pyramidal nerve cells (arrow) (H&E X400).



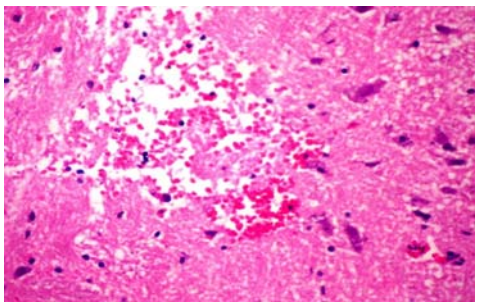
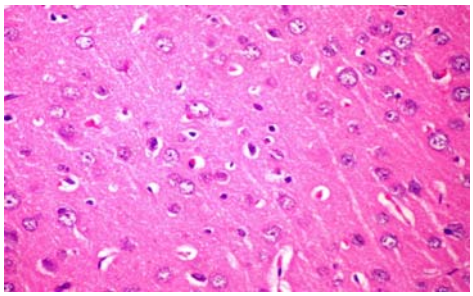


Figure 23: Brain of aluminum chloride treated rat for 6 weeks showing intense inflammatory reactions associated with focal gliosis particularly microglia cells (H&E X400).

Figure 24: Brain of aluminum chloride treated rat for 6 weeks showing cerebral hemorrhage (H&E X400).



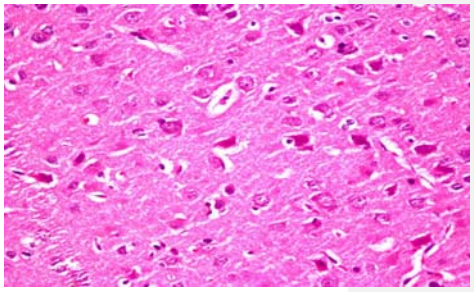
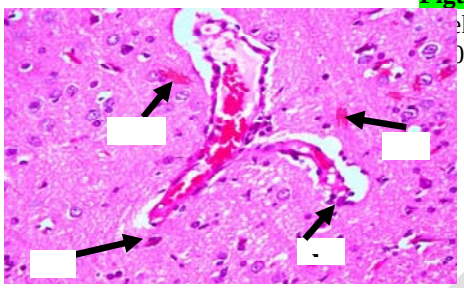


Figure 25: Brain
ks showing n
00).

Figure 26: Brain of aluminum chloride and *Ginkgo biloba* treated rat 6 weeks showing



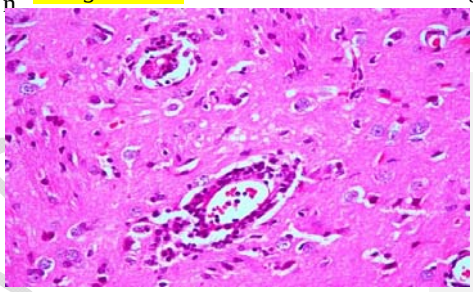
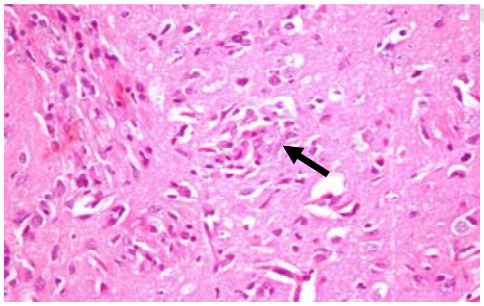


Figure 27: Brain of aluminum chloride treated rat for 12 weeks showing deposition of amyloid plaques **(a)** associated with congestion of cerebral blood vessels with perivascular cuffing with glia cells **(b)** and neuronal degeneration **(c)** (H&E X400).

Figure 28: Brain of aluminum chloride treated rat for 12 weeks showing intravascular aggregation of leukocytes with perivascular edema and cuffing with glia cells (H&E X400).



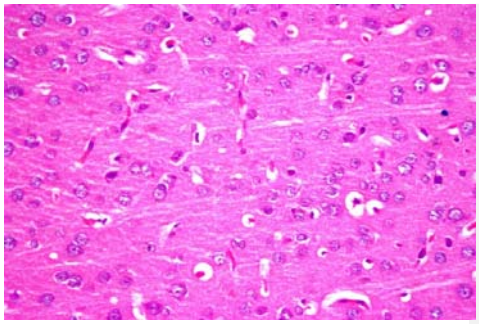
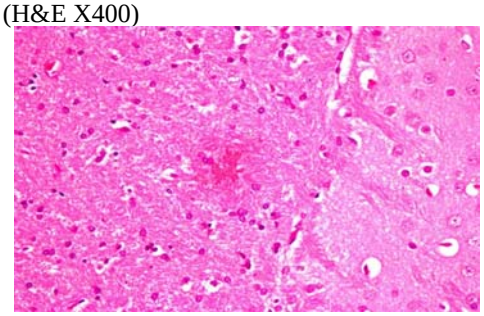


Figure 29: Brain of aluminum chloride treated rat for 12 weeks showing Focal cerebral tissue necrosis associated with reactive gliosis (arrow) (H&E X400)

Figure 30: Brain of Ginkgo biloba treated rat for 12 weeks showing normal neuronal cells with large round nuclei (H&E X400).



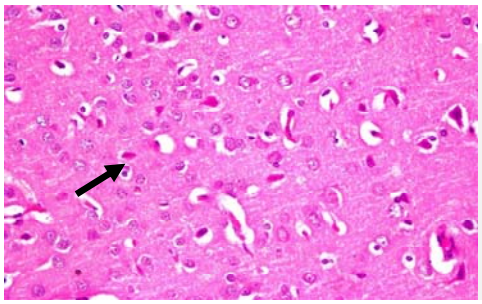


Figure 31: Brain of aluminum chloride and Ginkgo biloba treated rat for 12 weeks showing less deposition of amyloid plaques (H&E X400).

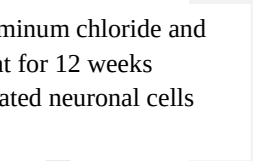


Figure 32: Brain of aluminum chloride and Ginkgo biloba treated rat for 12 weeks showing sparse degenerated neuronal cells (arrow) (H&E X400)

Disscussion

In the forebrain, such as the thalamus, hypothalamus and hippocampus; neurotransmitters play key roles in the regulation functions such as emotion and behavior. The level of these chemical also changes as a result of neurotoxicity [38].

The present study demonstrated that $AlCl_3$ induced a significant decrease in the brain level of neurotransmitters (Norepinephrine (NE), Serotonin (5-HT) and Dopamine (DA) than control group during 6 or 12 weeks of treatment. The changes in brain neurotransmitters contents were also associated with degenerative changes in brain of Al-treated rats (Figures 17-24, 27-29).These results are consistent with the findings of Xiu et al. [39] who showed that aluminum administration reduced norepinephrine content in the hypothalamus from rats. Eraz et al. [40] attributed that the reduction of NE content might be due to inhibition effect of aluminum on the enzymes activity related to NE synthesis, including dopamine-beta-hydroxylase and tyrosine hydroxylase (the rate-limiting enzyme of NE synthesis)

The protective effect of *Ginkgo biloba extract* was demonstrated by the significant increase in brain neurotransmitters contents of NE, 5-HT and DA of intoxicated rats (Fig. 26, 31 and 32). This might be attributed to the ability of *Ginkgo* extracts to stabilize mitochondrial function [41]. Our results are also similar to those reported

by Blecharz-Klin et al. [19] who showed that administration of high doses of *Ginkgo biloba extract* caused significant elevation of noradrenaline, dopamine and serotonin in rat brain.

We demonstrated that $AlCl_3$ induced a significant increase in different comet assay limits. These results are consistent with the findings of Rui & Yongjian [42] who reported that $AlCl_3$ induced DNA damage in mice hippocampus or cortex cells. Similarly, Sumathi et al. [43] showed that DNA of Al treated cells showed a comet tail indicating the DNA damage arising from the genotoxicity in the Al-treated brain cell as compared to DNA of control cells. Deleterious effects of aluminum might be attributed to increased levels of reactive oxygen species [44] as well as nitrogen species [45].

On the other hand, prophylactic treatment with *Ginkgo biloba* extract significantly reduced $AlCl_3$-induced DNA damage as indicated by reduction in different comet assay parameters in the brain of intoxicated rats during the entire experimental period. These results are consistent with the findings of El Mesallamy et al. [46] who found that *Ginkgo biloba* extract supplementation significantly diminished DNA damage caused by N-nitrosodiethylamine (NDEA) as indicated by a significant decrease in the comet assay parameters compared to control group. Similarly, Alam et al. [47] showed that *Ginkgo biloba* extract significantly diminished the level of DNA damage caused by the Technetium (^{99m}Tc). The protective effect of *Ginkgo biloba* extract was attributed to its cytoprotective effects such as its high free radical scavenging ability, which could be exerted in the nuclear, cytoplasmic and extracellular compartments [30, 48].

In conclusion, the neurotoxic effects of aluminum were mediated by inhibition of the synthesis of monoamine neurotransmitters, induction of DNA damage and

disruption of brain tissue and neural histoarchitecture. *Gingko biloba* exerts protective effects against the described consequences of aluminum toxicity.

References:

1. Walton R C, McCrohan C R, Livens F R, White K N. Tissue accumulation of aluminium is not a predictor of toxicity in the freshwater snail, Lymnaea stagnalis. Environ Pollut. 2009: 157(7), 2142-2146. doi: 10.1016/j.envpol.2009.02.009

2. Mishra PC, Dash AK, Khageswar P. Metals in environmental segments at Hirakud of Odisha. India Int Biological Sci. 2012; 1(1), 17.

3. Afifi A. Renal osteodystrophy in developing countries. Artif. Organs. 2002; 26(9), 767-769.

4. Newairy AS, Salama AF, Hussien HM, Yousef MI. Propolis alleviates aluminium-induced lipid peroxidation and biochemical parameters in male rats. Food Chem Toxicol. 2009; 47(6), 1093-1098.

5. Kutlubay R, Oguz E O, Guven C, Can B, Sinik Z, Tuncay O L. Histological and ultrastructural evidence for protective effects on aluminium-induced kidney damage by intraperitoneal administration of alpha-tocopherol. Int J Toxicol. 2007; 26(2), 95-101. doi: 10.1080/10915810701221173

6. Gonzalez MA, Alvarez Mdel L, Pisani GB, Bernal CA, Roma MG, Carrillo MC. Involvement of oxidative stress in the impairment in biliary secretory function induced by intraperitoneal administration of aluminum to rats. Biol Trace Elem Res. 2007; 116(3), 329-348.

7. Lima PD, Leite DS, Vasconcellos MC, Cavalcanti BC, Santos RA, Costa-Lotufo LV, Burbano RR. Genotoxic effects of aluminum chloride in cultured human lymphocytes treated in different phases of cell cycle. Food Chem Toxico. 2007; l, 45(7), 1154-1159. doi: 10.1016/j.fct.2006.12.022

8. Bihaqi SW, Sharma M, Singh AP, Tiwari M. Neuroprotective role of Convolvulus pluricaulis on aluminium induced neurotoxicity in rat brain. J Ethnopharmacol. 2009; 124(3), 409-415. doi: 10.1016/j.jep.2009.05.038

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

9. Matyja E. Aluminum enhances glutamate-mediated neurotoxicity in organotypic cultures of rat hippocampus. Folia Neuropathol. 2000; 38(2), 47-53.

10. Yokel RA. Brain uptake, retention, and efflux of aluminum and manganese. Environ Health Perspect. 2002; 110 Suppl 5, 699-704.

11. Shrivastava S. S-allyl-cysteines reduce amelioration of aluminum induced toxicity in rats. Bio.and Biotech. 2011; 7(2), 74-83.

12. Yao ZX, Han Z, Drieu K, Papadopoulos V. Ginkgo biloba extract (Egb 761) inhibits beta-amyloid production by lowering free cholesterol levels. J Nutr Biochem. 2004; 15(12), 749-756. doi: 10.1016/j.jnutbio.2004.06.008

13. Singh M, Mathur G, Jain KC, Mathur A. Phyto-pharmacological Potential of Ginkgo biloba: a Review. J Pharm Res. 2012; 5(10), 5028-5030.

14. Bastianetto S, Ramassamy C, Doré S, Christen Y, Poirier J, Quirion R. The ginkgo biloba extract (EGb 761) protects hippocampal neurons against cell death induced by β-amyloid. Eur. J. Neurosci . 2000; 12, 1882–1890.

15. DeFeudis FV, Drieu K. Ginkgo biloba extract (EGb 761) and CNS functions: basic studies and clinical applications. Curr Drug Targets. 2000; 1(1), 25-58.

16. Naik SR, Pilgaonkar VW, Panda VS. Neuropharmacological evaluation of Ginkgo biloba phytosomes in rodents. Phytother Res. 2006; 20(10), 901-905. doi: 10.1002/ptr.1973

17. Zhang Y, Ming L, Li JP, Li WP, Fang M. Protective effects of Egb on apoptosis of neurons. Chin J Pharmacol Ther. 2001; 6: 25-27.

18. Blecharz-Klin K, Piechal A, Joniec I, Pyrzanowska J, Widy-Tyszkiewicz E. Pharmacological and biochemical effects of Ginkgo biloba extract on learning, memory consolidation and motor activity in old rats. Acta Neurobiol. Exp (Wars). 2009; 69(2), 217-231.

19. Maclennan KM, Darlington CL, Smith PF. The CNS effects of Ginkgo biloba extracts and ginkgolide B. Prog Neurobiol . 2002; 67(3), 235-257.

20. Cheung F, Siow YL, O K. Inhibition by ginkgolides and bilobalide of the production of nitric oxide in macrophages (THP-1) but not in endothelial cells (HUVEC). Biochem. Pharmacol. 2001; 61(4), 503-510.

21. Xiao Q, Wang C, Li J, Hou Q, Li J, Ma J, Wang W, Wang Z. Ginkgolide B protects hippocampal neurons from apoptosis induced by beta-amyloid 25-35 partly via up-regulation of brain-derived neurotrophic factor. Eur J Pharmacol. 2010; 647(1-3), 48-54. doi: 10.1016/j.ejphar.2010.08.002

22. Massieu L, Moran J, Christen Y. Effect of Ginkgo biloba (EGb 761) on staurosporine-induced neuronal death and caspase activity in cortical cultured neurons. Brain Res. 2004; 1002(1-2), 76-85. doi: 10.1016/j.brainres.2003.12.018

23. Sloley BD, Urichuk L J, Morley P, Durkin J, Shan JJ, Pang PK, Coutts R T Identification of kaempferol as a monoamine oxidase inhibitor and potential Neuroprotectant in extracts of Ginkgo biloba leaves. J Pharm Pharmaco. 2000; l, 52(4), 451-459.

24. Rojas P, Rojas C, Ebadi M, Montes S, Monroy-Noyola A, Serrano-Garcia N. EGb761 pretreatment reduces monoamine oxidase activity in mouse corpus striatum during 1-methyl-4-phenylpyridinium neurotoxicity. Neurochem Res. 2004; 29(7), 1417-1423.

25. Wu WR, Zhu XZ. Involvement of monoamine oxidase inhibition in neuroprotective and neurorestorative effects of Ginkgo biloba extract against MPTP-induced nigrostriatal dopaminergic toxicity in C57 mice. Life Sci. 1999; 65(2), 157-164.

26. Huang Y, Johnson K R, Norris JS, Fan W. Nuclear factor-kappaB/IkappaB signaling pathway may contribute to the mediation of paclitaxel-induced apoptosis in solid tumor cells. Cancer Res. 2000; 60(16), 4426-4432.

27. Tang C Q. Evidence for the persistence of wild Ginkgo biloba (Ginkgoaceae) population in the Dalou Mountains, Southwestern China. American Journal of Botany. 2012; 99(8): 1408-1414.

28. Gong QH, Wu Q, Huang XN, Sun AS, Nie J, Shi JS. Protective effect of Ginkgo biloba leaf extract on learning and memory deficit induced by aluminum in model rats. Chin J Integr Med. 2006; 12(1): 37-41.

29. Thiagarajan G, Chandani S, Harinarayana Rao S, Samuni AM, Chandrasekaran K, Balasubramanian D. Molecular and cellular assessment of ginkgo biloba extract as a possible ophthalmic drug. Exp Eye Res. 2002; 75(4), 421-430.
30. Kaur T, Bijarnia RK, Nehru B. Effect of concurrent chronic exposure of fluoride and aluminum on rat brain. Drug Chem Toxicol. 2009; 32(3), 215-221. doi: 10.1080/01480540902862251
31. Stein C, Hopfeld J, Lau H, Klein J. Effects of Ginkgo biloba Extract EGb 761, Donepezil and their Combination on Central Cholinergic Function in Aged Rats. J Pharm Pharm Sci. 2015; 18(4), 634-646.
32. Chang CC. A Sensitive Method for Spectrophotofluorometric Assay of Catecholamines. Int. J. Neuropharmaco. 1964; .l, 3, 643-649.
33. Ciarlone A E. Further modification of a fluorometric method for analyzing brain amines. Microchemical. 1978; 23(1), 9-12.
34. Klaude M, Eriksson S, Nygren J, Ahnström G. The comet assay: mechanisms and technical considerations. Mutat Res. 1996; 363(2): 89-96.
35. Collins A R, Oscoz AA, Brunborg G, Gaivao I, Giovannelli L, Kruszewski M, Stetina R. The comet assay: topical issues. Mutagen. 2008; 23(3), 143-151. doi: 10.1093/mutage/gem051
36. Bancroft D, Stevens A, Turner R. Theory and practice of histological techniques. 4th edition, Churchill Livingstone, Edinburgh, London, Melbourne. . 1996.
https://doi.org/10.1046/j.1460-9568.2000.00069.x
37. Tsuga H, Haga T, Honma T. Effects of toluene exposure on signal transduction: toluene reduced the signaling via stimulation of human muscarinic acetylcholine receptor m2 subtypes in CHO cells. Jpn J Pharmacol. 2002; 89(3), 282-289.
38. Xiu C, Ren L, Li M, Liu S, Zhu Y, Liu J, Li Y. Aluminum chloride- and norepinephrine-induced immunotoxicity on splenic lymphocytes by activating beta2-AR/cAMP/PKA/NF-kappaB signal pathway in rats. Biol Trace Elem Res. 2014; 162(1-3), 168-174. doi: 10.1007/s12011-014-0149-7

39. Erazi H, Ahboucha S, Gamrani H. Chronic exposure to aluminum reduces tyrosine hydroxylase expression in the substantia nigra and locomotor performance in rats. Neurosci Lett. 2011; 487(1), 8-11. doi: 10.1016/j.neulet.2010.09.053

40. Yeh KY, Pu HF, Kaphle K, Lin SF, Wu L S, Lin JH, Tsai YF. Ginkgo biloba extract enhances male copulatory behavior and reduces serum prolactin levels in rats. Horm Behav. 2008; 53(1), 225-231. doi: 10.1016/j.yhbeh.2007.10.001

41. Rui D, Yongjian Y. Aluminum chloride induced oxidative damage on cells derived from hippocampus and cortex of ICR mice. Brain Res. 2010; 1324, 96-102. doi: 10.1016/j.brainres.2010.02.024

42. Sumathi T, Shobana C, Mahalakshmi V, Sureka R, Subathra M, Vishali, A. Rekha K. Oxidative stress in brains of male rats intoxicated with aluminium and neuromodulating effect of Celastrus paniculatus alcoholic seed extract. . Asian J Pharm Clin Res. 2013; 6(3), 80-90.

43. Moumen R. Ait-Oukhatar N, Bureau F, Fleury C, Bougle D, Arhan P, Viader F. Aluminium increases xanthine oxidase activity and disturbs antioxidant status in the rat. J Trace Elem Med Biol. 2001; 15(2-3), 89-93. doi: 10.1016/S0946-672X(01)80049-3

44. Bhalla P, Singla N, Dhawan DK. Potential of lithium to reduce aluminium-induced cytotoxic effects in rat brain. Biomet. 2010; 23(2), 197-206. doi: 10.1007/s10534-009-9278-4

45. El Mesallamy HO, Metwally NS, Soliman M S, Ahmed KA, Abdel Moaty M M. The chemopreventive effect of Ginkgo biloba and Silybum marianum extracts on hepatocarcinogenesis in rats. Cancer Cell Int. 2011; 11(1), 38. doi: 10.1186/1475-2867-11-38

46. Alam SS, Hassan NS , Raafat BM. Evaluation of Oxidatively Generated damage to DNA and proteins in rat liver induced by exposure to 99mtechnetium radioisotope and protective role of angelica archangelica and ginkgo biloba. W. Appl. Sci. 2013; 24(1), 7-17.

47. Min K, Ebeler SE. Quercetin inhibits hydrogen peroxide-induced DNA damage and enhances DNA repair in Caco-2 cells. Food Chem Toxicol. 2009; 47(11), 2716-2722. doi: 10.1016/j.fct.2009.07.033