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Neurodegenerative and Hepatorenal Disorders Induced Via Aluminum Chloride in Murine System: Impact of β-Secretase, MAPK, and KIM

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Abstract
Aluminum chloride ($AlCl_3$) is commonly used in daily life; meanwhile, it is the potential etiology of various neurodegenerative as well as hepatorenal diseases. Therefore, the present study was carried out to investigate the correlation between $AlCl_3$-induced biochemical alterations and the toxicity induced in various organs such as the brain, liver, and kidney. Male mice received $AlCl_3$ in an oral dose of 50 mg kg^{-1} in addition to (50 mg) in drinking water for 2 weeks. Two weeks post-$AlCl_3$ intoxication, the brain, liver, and kidney biochemical indices were assessed via molecular and western blot analysis. The results are as follows: $AlCl_3$ intoxication induced a significant elevation in serum malondialdehyde in addition to a significant reduction in serum glutathione (GSH) and superoxide dismutase (SOD) levels. Brain β-secretase (tubulin-binding protein) and tau proteins which are responsible for the synthesis of β-amyloid protein that may interfere with neuronal communication in Alzheimer's disease (AD) were also upregulated; regarding hepatic function, $AlCl_3$ elevated serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities. Moreover, it upregulated hepatic mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) protein expressions as well as renal kidney-inducible molecule-1 (KIM-1) which indicated the deleterious effect of $AlCl_3$ on these organs. These results were confirmed by histopathological investigations. So, we hypothesize that acute $AlCl_3$ administration is responsible for oxidative cell damage that interferes with brain function inducing β-amyloid accumulation, Alzheimer's disease, and neurodegenerative damage as well as hepatorenal injuries.

Keywords Aluminum chloride · β-Secretase · MAPK · JNK · KIM

Introduction

A growing body of evidence suggested that $AlCl_3$ is widely spread in the Earth's crust and is present in drinking water and in food additives. The main resources of aluminum are salt, corn, yellow cheese, tea, medicines, toothpaste, cosmetics, and cook utensils [1]. Environmental pollution with industrial waste water and cement-producing factories exposes people to aluminum [2]. Additionally, aluminum was believed to be non-toxic and quickly excreted in the urine. However, it can negatively affect human health. Aluminum is distributed mainly in the testis, liver, kidneys, and brain tissues. The toxicological effects of aluminum in humans include encephalopathy, bone disease, anemia, and Alzheimer's disease in addition to hepatorenal diseases [3].

Data accumulated for over a decade implicated that β-amyloid peptide is a central player in the pathogenesis of Alzheimer's disease (AD), and mutations in any of the three genes, amyloid precursor protein (APP) and presenilin 1 and 2 (PS1 and PS2), may lead to the early onset of AD by increasing the production of the toxic, plaque-promoting Abeta 42 peptide [4].

Beta-secretase 1 (BACE1) or beta-site amyloid precursor protein (APP) cleaving enzyme 1 is important in the formation of myelin sheaths in peripheral nerve cells [5]. β-amyloid generation requires 2 sequential cleavages of the amyloid precursor protein (APP). Extracellular cleavage of APP by BACE1 creates a soluble extracellular fragment and a cell membrane-bound fragment referred to as C99. The cleavage of C99 within its transmembrane

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domain by γ-secretase releases the intracellular domain of APP and produces amyloid-β. However, the levels of this enzyme have been shown to be elevated in the far more common late onset sporadic Alzheimer's disease [6].

Tau is a microtubule-associated protein that has a role in stabilizing neuronal microtubules and thus in promoting axonal outgrowth. Structurally, tau is a natively unfolded protein, is highly soluble, and shows little tendency for aggregation. However, tau aggregation is characteristic of several neurodegenerative diseases known as tauopathies, such as Alzheimer's disease. The middle region of tau is a Pro-rich domain that contains multiple Thr-Pro or Ser-Pro motifs that are targets of proline-directed kinases such as glycogen synthase kinase 3β (GSK3β), mitogen-activated protein kinase (MAPK), and JUN N-terminal kinase (JNK). In Alzheimer's disease and other tauopathies, these motifs become hyperphosphorylated and thereby can be recognized by several well-characterized tau phosphorylation-dependent antibodies [4].

The mitogen-activated protein kinase (MAPKs) pathway performs an intracellular signaling cascade in response to heat and stress. It can influence mitosis, cell division, metabolism, gene expression, survival, and apoptosis [7].

MAPKs and one of its member c-Jun N-terminal kinases (JNK) (stress-activated kinases) are activated by inflammatory cytokines and UV irradiation. Dysregulation of MAPK kinase pathways has been associated with cancer, neurodegeneration, and inflammation [8].

ROS-mediated prolongation of JNK activation is important for the proapoptotic function of JNK through the regulation of Bcl-2 family inactivation and promoting Bax\ and caspase-8 translocation to the mitochondria and amplifies their activation [9].

Kidney injury molecule-1 (KIM-1) is an accurate marker of kidney injury and is useful as an early diagnosis tool of kidney dysfunction [10].

The present study was conducted to investigate the toxicity of $AlCl_3$ on various organs including the brain, liver, and kidney tissues and investigated the mechanisms mediating this action.

Material and Methods

Chemicals

$AlCl_3$ was purchased from Sigma-Aldrich Co (St. Louis, MO, USA). RT-PCR kits of β-secretase, tau protein, and JNK were provided from R&D systems (MN, USA). Kits used for western blot determination of MAPK and KIM were obtained from Randox Company (UK). All other chemicals were of high analytical grade.

Animals

Male Wistar albino mice, weighing 25–30 g, obtained from the animal house of the National Research Center were used in this study. Animals were housed in cages kept at standardized conditions (22 ± 5 °C, 55 ± 5% humidity, and 12 h light/dark cycle). They were allowed free access to water and pelleted standard chow diet. Mice were fed commercial mouse chow. The diet was analyzed to contain an average amount of 60.5 mg/g Al in chow. All procedures relating to animal care and treatments strictly adhered to the ethical procedures and policies approved by the Animal Care and Use Committee of the National Research Center and complied with the Guide for Care and Use of Laboratory published by the US National Institute of Health.

Experimental Design

After 1 week of acclimatization, animals were randomly divided into two groups (20 mice each) and were divided according to the following schedule:

Group1: Animals received saline and served as control group.

Group 2: Intoxicated with $AlCl_3$ in an oral dose of 50 mg/kg for 2 weeks in addition to (50 mg) $AlCl_3$ in drinking water [11]. The dose of aluminum chloride (50 mg/kg bw) used in our experiment corresponded to 1/8 of LD50.

Blood Sampling and Tissue Preparation

Animals were followed for any sign of sickness. At the end of the experimental period, mice were weighed, slightly anesthetized by ether, and blood samples were collected from the sublingual vein. Sera were separated by centrifugation at 4000 rpm for 10 min and were kept at − 80 °C for subsequent estimation of biochemical analysis of ALT, AST, MDA, GSH, and SOD in addition to molecular analysis.

Animals were then sacrificed by cervical dislocation, and the brain, liver, and kidney tissues were carefully separated, blotted dry, weighed, and then divided into three portions. The first portion was homogenized in 4 volumes of phosphate buffer, pH 7.4, using Teflon homogenizer (Glass-Col homogenizer, Terre Haute, USA). An aliquot of this homogenate (20% *w*/*v*) was centrifuged at 4000 rpm at 4 °C for 15 min, and the supernatant was used for β-secretase, tau protein, and JNK determination. The second portion of the liver and kidney were used for western blot estimation of MAPK and KIM protein expression. The remaining portion was kept in 10% formaldehyde, and then embedded in paraffin for subsequent histopathological examination.

Measured Parameters

Electrothermal Atomic Absorption of Aluminum Chloride

Aluminum chloride was determined in the brain, liver, and kidney tissues by electrothermal atomic absorption spectrometry (GFAAS) (Perkin-Elmer Model 2100 atomic absorption spectrophotometer and HGA-700, graphite furnace with the AS-70 autosampler) according to the method of [12]. The samples from all tissues were wet-ashed in a mixture of nitric acid and perchloric acid at boiling temperature. All procedures were carried out under dust-free conditions.

Serum Alanine and Aspartate Aminotransferases (ALT and AST) Activities

ALT and AST activities were estimated spectrophotometrically using commercially available kits provided by Randox Company [13].

Serum Glutathione (GSH) Level

GSH level was estimated using the kit provided by Randox Company according to the manufacturer's instructions [14].

Serum Malondialdehyde (MDA) Level

MDA, as an index of lipid peroxidation, was measured using the kit provided by Randox Company according to the manufacturer's instructions [15].

Serum Superoxide Oxide Dismutase (SOD) Level

SOD level was estimated using the kit provided by Randox Company according to the manufacturer's instructions [11].

Real-Time PCR Analysis for β-Secretase, Tau Protein, and JNK

Target gene expression analysis was detected using real-time PCR according to specific forward and reverse primer for β-secretase, tau protein in the brain, and JNK in the liver (Table 1). Firstly, total RNA was extracted from the cerebellum brain tissue and liver samples, respectively, using SV total RNA isolation system (Promega, Madison, WI), then extracted RNA reverse transcribed into cDNA and amplified by PCR using RT-PCR kit (Stratagene, USA). Reactions were performed in a 50-μL final volume (25 μL SYBR Green Mix (2×), 0.5 μL cDNA, 2 μL primer pair mix (5 pmol/μLeach primer), 22.5 μL H2O). PCR reaction was as follows: 50 °C for 2 min (1 cycle), 95 °C for 10 min (1 cycle), 95 °C for 15 s, 45 to 60 °C for 30 s (according to optimum annealing temperature for each gene), and 72 °C for 30 s (40 cycles) then 72 °C for 10 min (1 cycle). Data from the real-time assays were calculated by Sequence Detection Software version 1.7 (PE Biosystems, Foster City, CA, USA) [16].

Table 1 Primers sequences for tau protein, β-secretase, and JNK genes

Gene	Primer sequences	
Tau	F (5′-CCCCCTAAGTCACCATCAGCTAGT-3′)	
	R (5′-CACTTTGCTCAGGTCCACCGGC-3′)	172 bp
β-Secretase	F 5'-ATGATGGAAGGCCTGGAC-3′	
	R 5′- TGATGTGTCTCCATTTAACACTGCC- 3′	92 bp
JNK	F 5′-GCCATTCTGGTAGAGGAAGTTTCTC-3′	
	R 5′-CGCCAGTCCAAAATCAAGAATC-3′	119 bp

Western Blotting for MAPK and KIM in Liver and Kidney Tissues

The liver and kidney tissues (50 mg) were homogenized using a polytron homogenizer in 1.5 ml cold lysis buffer (50 mmol/l Tris-HCL, pH 8.0, 150 mM/l NaCl, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS and 0.5 mM/l phenyl methyl sulfonyl fluoride). After boiling at 95 °C for 5 min, samples (50 μg/lane) were subjected to 12.5% SDS-PAGE gel. After gel electrophoresis, the separated proteins were transferred into nitrocellulose membranes. The membranes were blocked with 5% dry milk in PBS with 0.1% Tween 20 (PBST) for 1 h at room temperature and incubated over night at 4 °C with primary antibody against MAPK and KIM at 1:1000 (Cell Signaling Technology, Beverly, MA, USA).The membranes were washed and then incubated with a secondary horseradish peroxidase conjugated antibody (1:25000, Bio-Rad, Hercules, CA, USA) for 1 h at room temperature, followed by additional washing. Proteins were visualized by enhanced chemiluminescence (ECL plus; Amersham, Arlington Heights, IL, USA) and quantified using densitometry and the Molecular Analyst Software (Bio-Rad, Richmond, CA, USA) [17].

Tissue Histoarchitecture Study

Deparaffinized sections of 4 μm of brain cerebellum, liver, and kidney tissues were stained with hematoxylin and eosin (H&E) and examined under light microscope [18].

Statistical Analysis

Data were expressed as means ± SEM. Statistical analysis was performed using Instat-3 computer program (GraphPad software Inc., San Diego, CA, USA). One way analysis of variance (ANOVA) by SPSS 12 program followed by post hoc

Table 2 Aluminum chloride concentrations in the brain, kidney, and liver tissue expressed as mg/g wet weight

Organ Groups	Brain	Liver	Kidney
Control	1.53 ± 0.12^{a}	0.14 ± 0.02^{a}	0.19 ± 0.009^{a}
$Alcl_3$ group	3.92 ± 0.99^{b}	0.39 ± 0.03^{b}	0.46 ± 0.014^{b}

Data are expressed as mean ± S.E ($n = 15$). The significance of the differences was verified at $P \leq 0.05$. Similar letters are not significantly different from each other while different letters are significantly different from each other

test was also done. The level of significance was set at $p < 0.05$ using Tukey s test.

Results

$AlCl_3$ Concentration in Different Tissues

Table 2 showed a significant increase in aluminum concentrations and accumulation in the brain tissue greater than its concentration in the liver tissue and in the kidney tissue.

$AlCl_3$-Induced Oxidative Stress

$AlCl_3$ intoxication induced a state of oxidative stress evidenced by a significant reduction in both GSH and super oxide dismutase serum levels along with an increment of MDA serum level as compared to the control value (Fig. 1), which reflected the severe oxidative stress induced via $AlCl_3$ intoxication.

$AlCl_3$-Induced Brain Injury

$AlCl_3$ intoxication induced neurodegenerative disorders evidenced by the upregulation of brain β-secretase and tau protein gene expression by almost 6 and 14 folds respectively, as compared to the control value (Fig. 2), which reflected the initiation of β-amyloid synthesis and the incidence of Alzheimer's disease.

$AlCl_3$-Induced Liver Injury

As shown in Fig. 3, $AlCl_3$ intoxication increased significantly the serum levels of ALT and AST by 100.8% and 177.35%, respectively, as compared to the control values which reflected the severe hepatotoxicity induced via $AlCl_3$.

$AlCl_3$-Induced Liver Apoptosis

As shown in (Figs. 4 and 5), $AlCl_3$ intoxication produced a significant elevation in hepatic MAPK protein expression and JNK gene expression by almost 16 and 10 folds respectively, as compared to the control value.

It is worth noting that the $AlCl_3$ regimen exhibited a relatively high effect in this regard showing its high toxicity to the liver tissue.

$AlCl_3$-Induced Renal Injury

As demonstrated in Figs. 6 and 7, the level of KIM protein expression was significantly upregulated in the $AlCl_3$ group, reaching 10.4 folds as compared to the normal value, showing $AlCl_3$-induced renal toxicity.

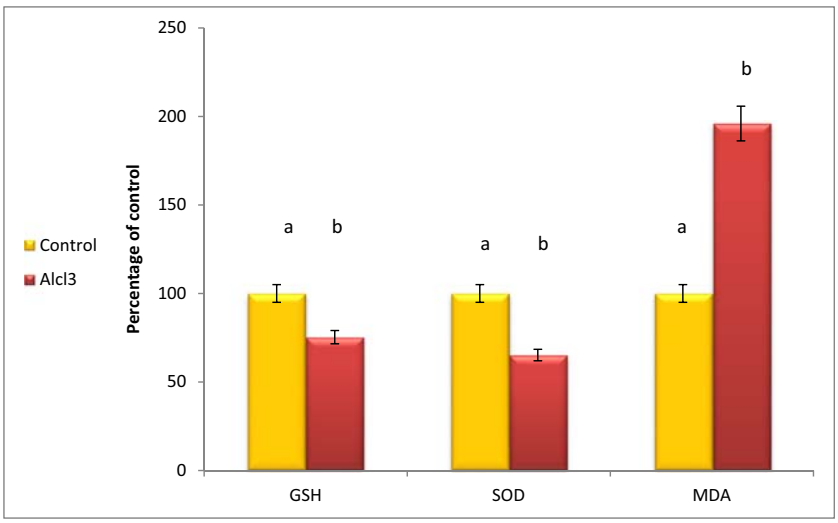

Fig. 1 Effect of $AlCl_3$ on serum GSH, SOD and MDA levels. Data are expressed as means ± SEM ($n = 10$). P value < 0.05 is considered significant. Groups having the same letter are not significantly different, while those having different letters are significantly different.

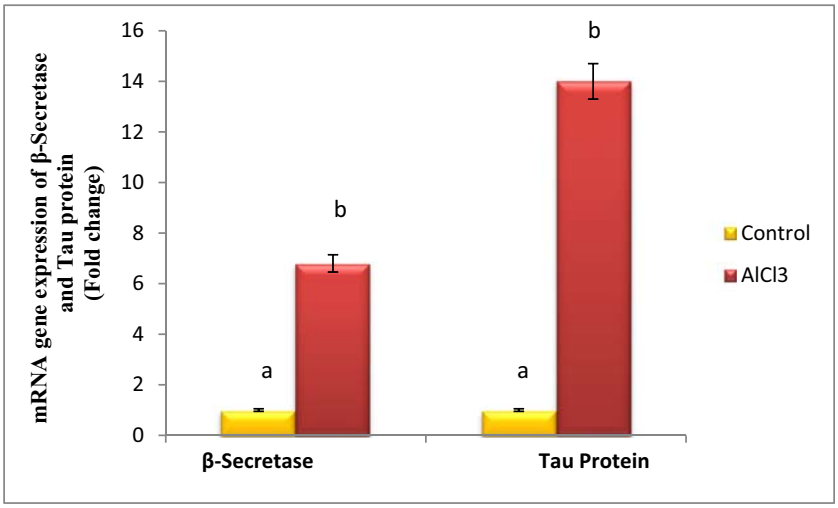

Fig. 2 Effect of $AlCl_3$ on brain β-secretase and tau protein mRNA gene expression. Data are expressed as means ± SEM (n = 10). P value < 0.05 is considered significant. Groups having the same letter are not significantly different, while those having different letters are significantly different

Brain, Liver, and Kidney Histoarchitecture

$AlCl_3$ intoxication showed remarkable changes out of normally represented in all investigated tissues. A brain lesion (Fig. 8) and ruptured liver tissues with abscess were noted. Hepatocytes showed mild dysplastic changes (enlarged nuclei and increased nucleocytoplasmic ratio) with necrotic area; multinucleated giant cells (Fig. 9) and kidney tissues showing marked degeneration of many of renal corpuscles with shrinkage of their glomeruli and degeneration of epithelial lining of many of renal tubules in addition to infiltration of inflammatory cells to the interstitium were observed in the kidney (Fig. 10).

Discussion

$AlCl_3$ is widely used in life as it was believed to be non-toxic and was quickly eliminated in urine. However, it can negatively impact human health [11]. $AlCl_3$ is highly distributed in the liver, testis, kidney, and brain tissues. The toxicological effects of aluminum in humans include encephalopathy, bone disease, anemia, Alzheimer's disease, and hepatorenal diseases [3]. Aluminum (Al) is considered part of the etiology of AD. An excess amount of Al causes amyloid neurotoxicity according to records of clinical observation and animal experiments [19].

Herein, we investigated the toxicity of $AlCl_3$ on various organs including the brain, liver, and kidney tissues and investigated some biochemical parameters that express partially the mechanisms underlying this action.

Thermal atomic absorption elucidated a significant increase in aluminum concentration in the brain tissue > liver tissue > kidney tissue; this is in harmony with the study that declared oral administration as a route of Al exposure can result in diverging accumulation of $AlCl_3$ in the liver, kidneys, stomach, and bone that was analyzed by atomic absorption

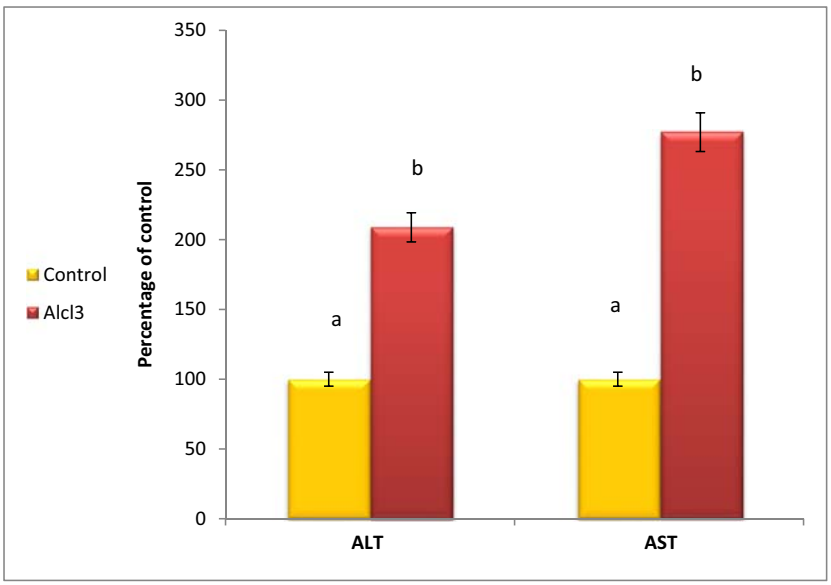

Fig. 3 Effect of $AlCl_3$ on serum ALT and AST activities. Data are expressed as means ± SEM (n = 10). P value < 0.05 is considered significant. Groups having the same letter are not significantly different, while those having different letters are significantly different.

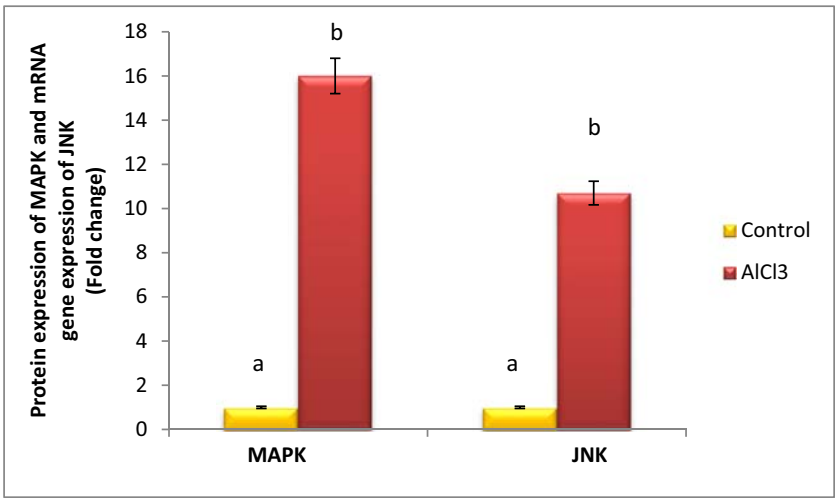

Fig. 4 Effect of $AlCl_3$ on hepatic MAPK protein expression and mRNA gene expression of JNK. Data are expressed as means ± SEM ($n = 10$). P value < 0.05 is considered significant. Groups having the same letter are not significantly different, while those having different letters are significantly different.

spectrometry; the concentration depends on the chemical form [20]. While much has been written on the variability of absorption with PH of the lumen or form of the Al administered, the variability between laboratories is still greater than the variability caused by any of these factors. For example, one laboratory recorded 27% absorption of dose of $AlCl_3$ given to rats while another recorded less than 1% for similar dose of the same compound under similar conditions of acidity to the same species which may be related to transcellular uptake or paracellular uptake of $AlCl_3$ and systemic transport [21].

Oxidative stress is recognized as one of the vital mechanisms of Al toxicity. Al induces oxidative stress through three pathways. First, Al exhibits a significant pro-oxidant activity, due to the formation of Al superoxide complex between Al^{3+} and superoxide radical anion, promoting biological oxidation both in vitro and in vivo. Second, Al induces oxidation of biomolecules by reducing ferric iron to ferrous iron, which would promote Fenton reaction in vivo systems, increasing the concentration of free radicals, such as hydroxyl radical which initiates lipid peroxidation of membrane [11]. Third, Al facilitates the production of superoxide anion through non-iron driven biological oxidations involving the photochemical, enzymatic, chemical, and biomolecular. It is well known that once free radicals generation exceeds antioxidant defense level, it can disrupt liver mitochondrial oxidative homeostasis and induce hepatocyte oxidative damages. It can also affect brain and kidney functions by binding to DNA and RNA and inhibiting hexokinases [22].

Herein, $AlCl_3$ elucidated a state of oxidative stress evidenced by the reduction of both GSH and SOD levels accompanied by increment in MDA level as compared to the control value. These toxic effects of $AlCl_3$ have been suggested to be due to the generation of ROS which results in the oxidative deterioration of cellular lipids, proteins, and DNA [23]. $AlCl_3$ was previously reported to reduce GSH contributed to its ability to either directly inhibit glutathione synthase or prevent NADPH-generating enzymes resulting in reducing NADPH and further decrease the regeneration of GSH, or both.

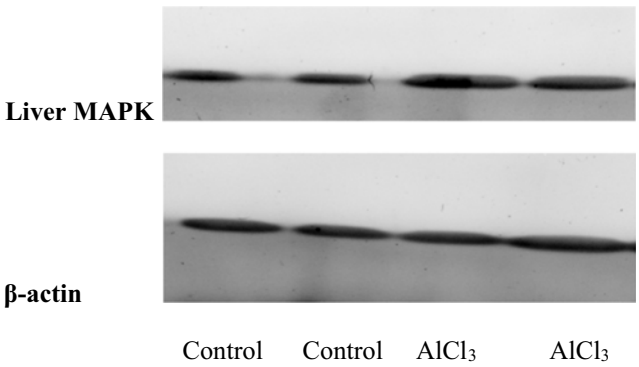

Fig. 5 Western blot analysis of hepatic MAPK protein expression in $AlCl_3$ intoxicated group

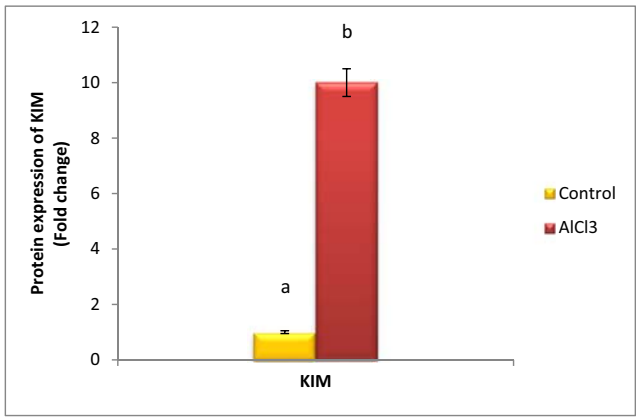

Fig. 6 Effect of $AlCl_3$ on renal KIM protein expression represented as fold change. Data are expressed as means ± SEM ($n = 10$). P value < 0.05 is considered significant. Groups having the same letter are not significantly different, while those having different letters are significantly different

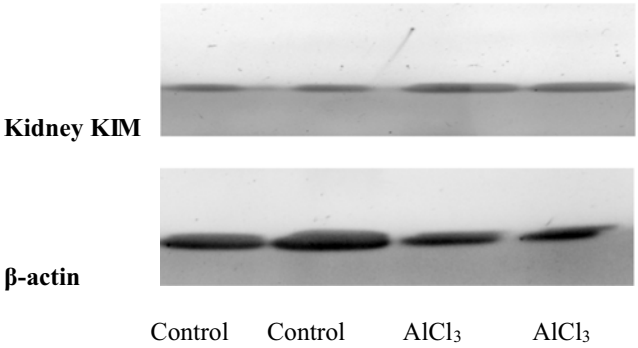

Fig. 7 Western blot analysis of renal KIM protein expression in $AlCl_3$ intoxicated group

Moreover, $AlCl_3$ can significantly reduce the antioxidant enzyme SOD. The reducing activity of this defense enzyme reflects its reduced synthesis due to higher accumulation of $AlCl_3$ and/or higher accumulation of superoxide anion (O_2^-) and H_2O_2 in renal tissue. Further, the interaction of O_2^- and NO manifested in $AlCl_3$ produce peroxynitrite ($ONOO^-$) which exaggerates renal injury. The formation of RONS (reactive oxygen and nitrogen species) triggers some of the vasoactive mediators that promote renal vasoconstriction and reduce the glomerular capillary filtration. The above events overstress the nephrooxidative insult, and this may be the possible mechanistic pathway that is responsible for the increased urea and creatinine levels [24].

Furthermore, aluminum is possibly a contributing factor in the development of Alzheimer's disease and hepatorenal diseases [3].

In this context, the present study elucidated that $AlCl_3$ caused a significant upregulation in β-secretase and tau protein gene expression, indicating that the brain of $AlCl_3$-intoxicated mice undergo severe oxidative stress and β-amyloid accumulation [25].

Previous researches have noticed that $AlCl_3$ intoxication significantly elevated β-secretase and tau protein gene expression which are considered as the main precursors of β-amyloid protein, and these concur with our findings. Alzheimer's disease is characterized by the accumulation of extracellular senile plaques and intracellular neurofibrillary tangles (NFTs) within the brain. The major component of the plaques, which was first purified and identified from AD brains, is β-amyloid (Aβ). Aβ is proteolytically processed from the amyloid precursor protein (APP) via cleavage at the β-secretase site by BACE1, followed by γ-secretase cleavage by presenilin (PS). The key component of the NFTs on the other hand,

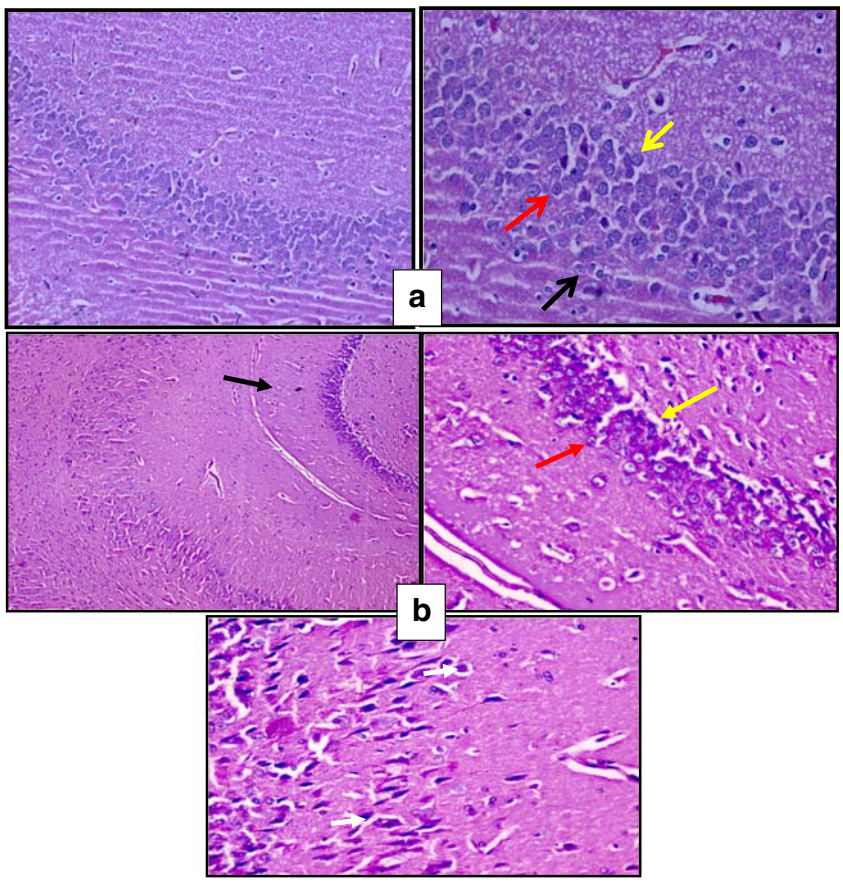

Fig. 8 **a** Brain section of normal control group, showed the cerebellum showed normal histological features, a well-defined molecular (black arrow), granular (presence of numerous closely packed small cells in the granular layer) (red arrow), and Purkinje layers (large Purkinje cells) (yellow arrow) with normal structure of neuronal cells. **b** Three brain sections of the $AlCl_3$ group, showed the cerebellum with thin, reduction in cellular size of the molecular layer (black arrow), distortion of granular cell layer (red arrow), and scattered, sparse cell distribution of Purkinje layers (yellow arrow), perineuronal edema with neuronal shrinkage (white arrow) (H&E stain,× 100, × 400,× 400)

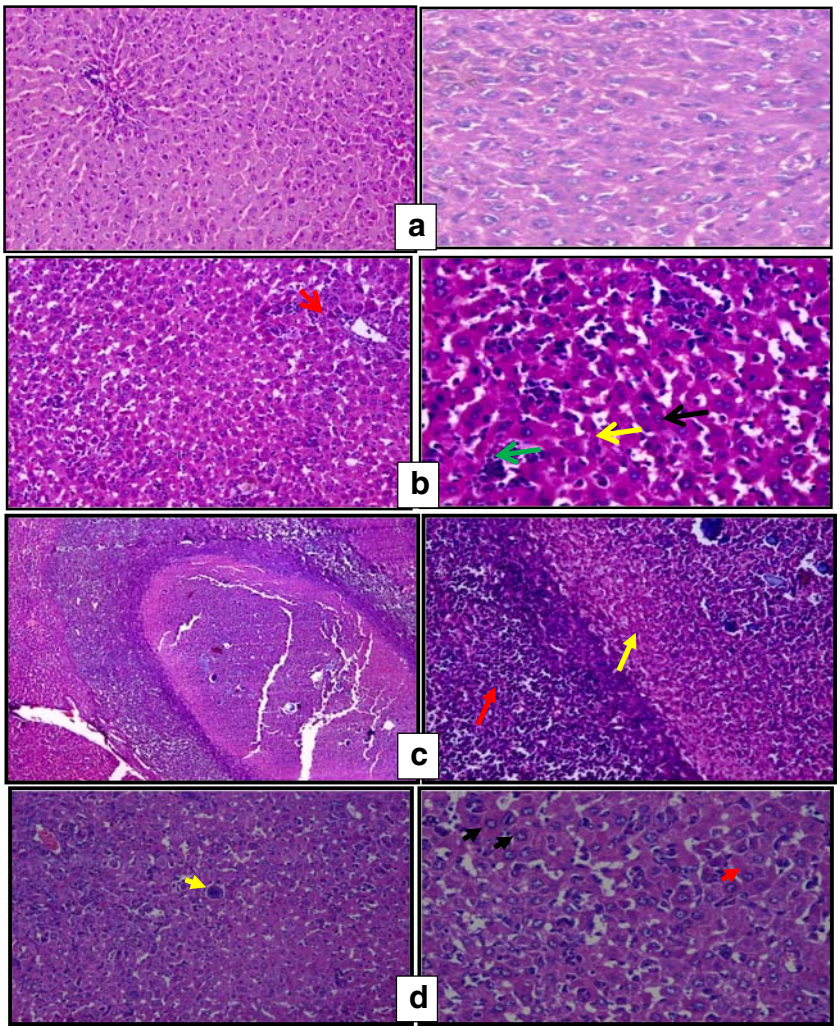

Fig. 9 **a** Hepatic tissue with normal liver architecture. **b** The $AlCl_3$ group showed hepatic tissue with disturbed architecture, hepatocytes arranged in thin and thick plates (black arrow) and dilated congested sinusoids (yellow arrow), central vein (red arrow), intralobular lymphoid aggregates (green arrow). **c** The $AlCl_3$ group showed hepatic tissue with hepatic abscess with disturbed architecture (red arrow), hepatocytes are compressed showed hydropic degeneration (yellow arrow). **d** The $AlCl_3$ group showed hepatic tissue with disturbed architecture, hepatocytes showed mild dysplastic changes (enlarged nuclei and increased nucleocytoplasmic ratio) (black arrow) and necrotic area (red arrow), multinucleated giant cells (yellow arrow) (H&E, × 200, × 400)

is the tau protein, which was originally identified as an intracellular microtubule stabilizer. Both Aβ and tau are, therefore, fundamentally involved in driving the pathogenesis of AD [26].

Beta-secretase 1 (BACE1) is important in the formation of myelin sheaths in peripheral nerve cells [5]. β-amyloid generation requires 2 sequential cleavages of the amyloid precursor protein (APP). However, BACE1 was elevated in Alzheimer's disease [6]. $AlCl_3$ intoxication exacerbated Abeta toxicity, increased ROS production, lipid peroxidation, β-secretase-1 (BACE1), and amyloid precursor protein (APP) elevation which may be related to A1 adenosine receptors agonist property [27].

Tau proteins preserve the stability and flexibility of microtubules. They are active in the distal part of axons. Hyperphosphorylation of tau proteins via γ-ATP and γ-GTP in the presence of P34 and PKC kinases causes tangling of the helical and straight filaments leading to the progression of AD [26]. Strong tau immunohistological staining was noted bilaterally in $AlCl_3$-treated rats [28, 29].

Humans and animals are exposed to various toxic agents, chemicals, and drugs in the environment on a daily basis. The liver is the site that toxic compounds get transformed into less harmful products to reduce toxicity; this will damage hepatocytes and produce hepatotoxicity. When the liver is injured, ALT and AST are spontaneously increased and released into peripheral blood; thus, serum ALT and AST are recognized as the principal indicators of liver injury [22].

In harmony, the current study revealed that $AlCl_3$ induced liver injury evidenced by the significant elevation in liver enzymes ALT and AST as compared to the control value. Increased liver enzymes pointed to cellular leakage and loss of functional integrity of liver cell membranes. Moreover, Al accumulates in the liver leading to cholestasis and necrosis noticed by the increase in ALT, AST, and ALP activities [22].

In the present study, $AlCl_3$ induced a significant upregulation in hepatic MAPK and JNK protein expressions. These data were reinforced by the study of Chan et al. [9], which

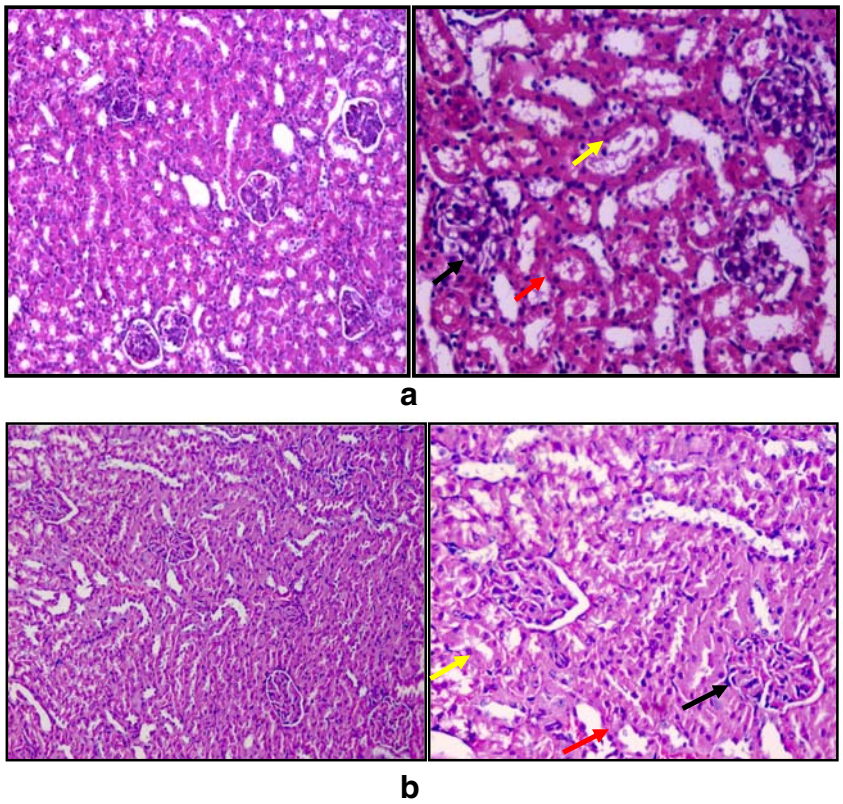

Fig. 10 **a** Kidney section from normal control group showed renal cortex showing renal corpuscle with normal glomerulus (black arrow), normal pattern of proximal convoluted (red arrow) and distal convoluted (yellow arrow) tubules (H&E, × 200, × 400). **b** Kidney section from the $AlCl_3$ group showed few of the glomeruli corpuscles are obliterated and cellular (hyperplasia of epithelial cells lining the partial layer of Bowman's capsule) (black arrow). Proximal convoluted tubules show destructed epithelial lining (red arrow), obliterated and destructed epithelial lining of distal convoluted tubules (yellow arrow), (H&E, × 200, × 400)

suggested that $AlCl_3$ infusion leads to apoptotic damage in the liver tissues.

TNF-α activates a member of the MAP3K family, which leads to JNK activation. ROS-mediated prolongation of JNK activation is important for the proapoptotic function of JNK through the regulation of Bcl-2 family inactivation and promoting Bax and caspase-8 translocation to mitochondria and amplifies their activation [9].

Previous observations pointed to the potential inflammatory and renotoxic properties of $AlCl_3$ via promoting renal tubular degeneration. In obstructive kidney disease, kidney injury molecule 1 (KIM-1) is an accurate marker of kidney injury and is useful as an early diagnosis tool of kidney dysfunction [10].

This was also highlighted by the study which declared that KIM the kidney injury molecule 1 was significantly upregulated post $AlCl_3$ intoxication [10].

Finally, histopathological investigations declared that $AlCl_3$ intoxication showed remarkable brain lesion and ruptured liver tissues with abscess, and hepatocytes showed mild dysplastic changes and necrotic area. Moreover, the kidney tissues showed marked degeneration of many of renal corpuscles with shrinkage of their glomeruli and degeneration of epithelial lining of many of renal tubules in addition to infiltration of inflammatory cells to the interstitium, and this confirms our biochemical and molecular findings.

Conclusion

$AlCl_3$ is a toxic metal that can affect various organs such as the brain, liver, and kidney via various signaling pathways in the brain; it affects β-secretase and tau protein pathway while in the liver, it affects MAPK and JNK signaling pathway. Meanwhile, in the kidney, it may affect KIM pathway.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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