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Research Article

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A Pioneer Study on the Anti-Inflammatory Activities of Copper (I)-Nicotinate Complex Against Alzheimer Disease.

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ABSTRACT

To find out the effect of [Cu (I)-(nicotinic acid)2]1Cl- complex (Cu- N complex) on aluminum induced Alzheimer's disease in Wistarrats, memory impairment was induced by aluminum chloride, (10 mg/kg bwi.p.) for 30 d. To study the activity of (Cu- N complex) (400 µg/kg B.W.), Wistar rats were administered for 30 d along with aluminum chloride. Biochemical parameters of oxidative stress were estimated in brain after the treatment. The major finding of this study is that aluminum enhanced oxidative stress. Cu- N complex showed a significant improvement in increasing of TAC and reduction of the oxidative stress by reduction of MPO, AchE and NO, in the histopathological investigation, it restoreshipocummal neuron to normal status and there was a decreased in DNA damage in the treatment group. The present study clearly demonstrated the beneficial effects of (Cu- N complex) that shows good antioxidant properties, and may act as a key to treat Alzheimer's disease.

***Key words** : Anti-inflammatory, Copper (I)-Nicotinate, Alzheimer Disease.*

INTRODUCTION

The first case of Alzheimer's disease (AD) was observed by Alois Alzheimer in 1901, with the Histological findings, including "plaques" and "tangles" in the upper cortical layer [1]. Alzheimer's disease begins with memory loss of recent events (short-term memory impairment) and finally robs the patients' sense of self. AD is involved in 50%–70% of dementia cases, and nearly half of people over the age of 85 suffer from it [2].

Alzheimer brains have low levels of acetylcholine (ACh), which can arise from the accumulation of beta amyloid (βA) protein fragments that form hard plaques that can in turn interfere with the ability of ACh to affect synaptic transmission and initiate inflammatory processes that produce reactive oxygen species. Research suggests that βA opens channels in cell membranes, permitting calcium ions (Ca2+) to enter the cell and triggering several processes leading to mitochondrial dysfunction, inflammation, and cell death [3]. Some research suggests that, in the early

stages of AD, βA has an antioxidant function so that efforts to reduce it might be counterproductive. Another research has found only a weak relationship between the amounts of βA and the severity of AD. βA may be the end result of a destructive chain of events and hence more symptomatic than problematic. Another possible cause of cell death in AD is a chemical change in a protein (tau) that keeps microtubules stable. This causes a neuron's microtubules to pair with other tubules producing tau (neurofibrillary) tangles that result in tubule disintegration and block neurotransmitters, leading to cell death. Reactive oxygen species (oxygen ions, peroxides, and free radicals) can result in cell death by initiating a chain reaction that leads to damage of cell membranes, mitochondria, lipids, and proteins. Damage from toxic excitatory amino acid neurotransmitters, especially glutamate, can produce excitotoxicity and cell death. Excitotoxicity can occur even with normal glutamate levels if glutamate receptor sites become overstimulated[4]. The receptor most involved in excitotoxicity is N-methyl-D-aspartic acid (NMDA). If NMDA sites are overactivated, high levels of Ca2+ can enter the cell, causing a permanent depolarization of the post-synaptic neuron and creating reactive oxygen species and other substances that cause cell death. Potential mechanisms have also linked excitotoxicity to βA and tau tangles[4]. Damage from toxins, chemicals, and trauma can produce inflammation, another factor in AD. Inflammation often results from persistent oxidative stress, but other determinants include βA, protease inhibitors, pentraxins, inflammatory cytokines, and prostaglandin-generating cyclooxy-genases. Unhealthy neurons contain low levels of N-acetyl-aspartate (NAA), which may also be an issue. Exposure to pollutants can make the blood-brain barrier permeable to toxins, thus causing oxidative stress, inflammation, and βA accumulation [5, 6].

Certain medicinal plants and their combinations mentioned in Ayurvedicmaterialmedica have been evaluated in various experimental studies for their memory enhancing property. Brāhmī (Bacopamonieri), has a positive effect on learning and both short-term and long-term memory. In a recent double-blind, placebo-controlled clinical trial[7]. Maṇḍūkaparṇī (Centellaasiatica). This extract was also found to be effective in preventing cognitive deficits in an intracerebroventricularStreptozotocin model of AD in rats[8]. Haridrā (Curcuma longa),on oral administration to alcohol-fed rats, caused a significant reversal of brain lipid peroxidation, thus indicating a neuro-protective role[9].

The current possibilities of treatment were solving just the symptoms of this disease. There exist two classes of drugs with differing mechanisms of action that are licensed for the treatment of AD. These are as follows [10, 11]

• acetyl cholinesterase inhibitors for the treatment of mild and moderate AD (donepezil, galantamine, and rivastig-mine); and

• the N-methyl-d-aspartate receptor antagonist, memantine, for the treatment of moderate-to-severe AD. These drugs do not prolong patients' survival, but they are able to delay the most serious phases of AD, increase memory, quality of life and self-sufficiency of AD patients, and lower caregivers' burden. A number of other drugs also influence behavioral disorders and mood (antidepressants or neuroleptic drugs), sleeping disorders, and other symptom.

Nicotinic acid (NA) or vitamin B3 was essential for many biological processes namely for the production of energy [12]. Copper (Cu) complexes of a number of compounds have increased anti-inflammatory efficacy over the native compounds [13]. The metalloviatamin; copper (I)-nicotinate complex [CuCl (HNA)2];stimulates the blood flow and prevents gastric congestion and capillary damage in experimental animals [14]. Also, it had superoxide dismutase (SOD) mimic activity [15]. Recent reports have demonstrated the potential usefulness of Cu N-glycinate in immunotherapy of drug resistant cancers through induction of interferon- γ and/or tumour necrosis factor-a [16]. Nicotinic acid-copper complex (CuNA) has been shown to exert diverse bioactivities. In particular, it can stimulate blood flow and prevent gastric congestion [14], reduce total lipids in sera hepatic tissue as well as regulate levels of alanine transaminase, aspartate transaminase, alkaline phosphatase, gamma glutamyltranspeptidase and oxidative markers such as nitric oxide (NO) and lipid peroxidation in rat models [17]. The Cu complex may serve as a novel chemical restoring agent in fatty degenerated liver cells and for renewal of their structure and functions.[17]. This work has been designed in order to evaluate the prophylactic effect of a bioactive copper (I) nicotinate complex (Figure 1) as a food additive against the oxidative stress status in Alzheimer's disease induced in rats.

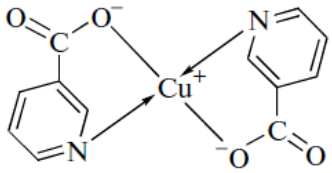


Figure 1. The proposed structures of Cu (I)–(NA) 2 complexes.

MATERIALS AND METHODS

The experimental work of the present study was conducted at Medical Biophysics Laboratory at King Fahd Medical Research Center (KFMRC), King Abdul- Aziz University, Jeddah, Kingdom of Saudi Arabia.

Animals:

Forty albino Wister male rats weighing between 200 to 250gm obtained from (KFMRC), Jeddah, Saudi Arabia. The Animal House, King Fahd Medical Research Center, King Abdul-Aziz University, approved the current study. Rats allowed acclimatizing to the laboratory environment for one week before the experiment.

Experimental Design

Forty white male Wister Lewis rats weight (190-225) grams.

G1- Healthy reference (normal.) group, 10 rats, Group G1, daily gavage withDistilled water for 4 weeks.

G2- Alzheimer group (AD group), 10 rats, daily intraperitoneal injection of AlCl3 (10 mg/kg bw) and D- galactose (60mg/ kg day) for 30 days.

G3 - Treated group (Before AlCl3 treatment), 10 rats, daily intraperitoneal Injection of AlCl3 (10 mg/kg bw) and D-galactose (60mg/ kg day) for 30 days treated with Cu - N Complex (400 µg/kg B.W.) for 4 weeks.

G4 - Treated group (After AlCl3 treatment), 10 rats, daily intraperitoneal with Cu– N Complex (400 µg/kg B.W.) for 4 weeks then treated with injection of AlCl3 (10 mg/kg bw) and D- galactose (60mg/ kg day) for 30 days.

All the groups were killed at the end of each successive week for biochemical and histopathological assessments. Blood and brain samples were collected. The brain was weighted and divided into two parts: the first part was homogenized in cold saline and then centrifuged at 3000g for 5 min at 4 1C and the supernatant was frozen at -80 C for biochemical assays. The second part was fixed in 10% phosphate-buffered formalin for light microscopic study.

Chemicals:

All chemicals, which were used in this study, were of analytical grade and supplied from different Companies for medical and commercial service.

Preparation of Cu (I)-nicotinate complex

The [Cu (I)-(nicotinic acid)2]1Cl- complex was synthesized as described by Gohar and Dratoviscky in 1975[18] .

Biochemical Determination

Biochemical parameters of oxidative stress and Antioxidant:

Sera samples were collected,Nnitric oxide (NO) levels were estimated in serum by the colorimetric methods described by [19], and total antioxidant capacity (TAC) according to the method described [20],

Hippocampus samples were divided into three portions:

The first portion was separated for determination of acetycholinesterase (AchE) according to the method described[21],Myeloperoxidase (MPO) according to the method described[22].

The second portion was used for histopathological investigation according to the method described [23].

Comet assay,the third portion for measuring DNA single strand breaks [24]

Statistical analysis:

Statistical analysis performed using Microsoft office excel and SPSS 16.0. The variability degree of the results was expressed as mean ± standard of means (mean ±SD). The significance of the difference between samples was used one way anova.

RESULTS

Effect of CU – N complex on Serum Oxidative Stress Marker

The effect of CU – N complex on NO and TAC of rats is shown in table (1). Significant increase in NO and depletion in serum TAC in AD group. In contrast the treatment of AD-induced group rats with CU – N complex produced very highly significant decreased in NO and exhibited non-significant increase in serum TAC.

Table (1) Effect of CU –N complex on NO activity and TAC in serum rat.

Groups	NO (mM/L)	TAC (mM/L)
G1	2.620±0.3820	3.506±0.463
*p	-	-
**p	0.00	0.005
G2	14.063±0.5648	1.726±0.323
*p	0.00	0.005
**p	-	-
G3	8.345±0.9564	2.641±0.033
*p	0.00	NS
**p	0.00	NS
G4	4.178±0.4477	2.861±0.087
*p	0.02	NS
**p	0.00	NS

G1: normal G2: AD group G3: Cu -N complex before AlCl3 treatment G4: Cu -N complex after AlCl3 treatment

*p:p value with respect to normal group,**p:p value with respect to Alzheimer group. P value < 0.05 is significant, NS:non-significant

Effect of treatment of CU –N complex on brain AchE activity and MPO

The activity of AchE and MPO in AD group portion of rat hippocampus tissue homogenate is given in table (2).

Treatment with CU-N complex, very highly significant decreases the concentration of AchE level and MPO as compared to the AD group.

Table (2) Effect of CU –N complex on AchE activity and MPO in rat brain.

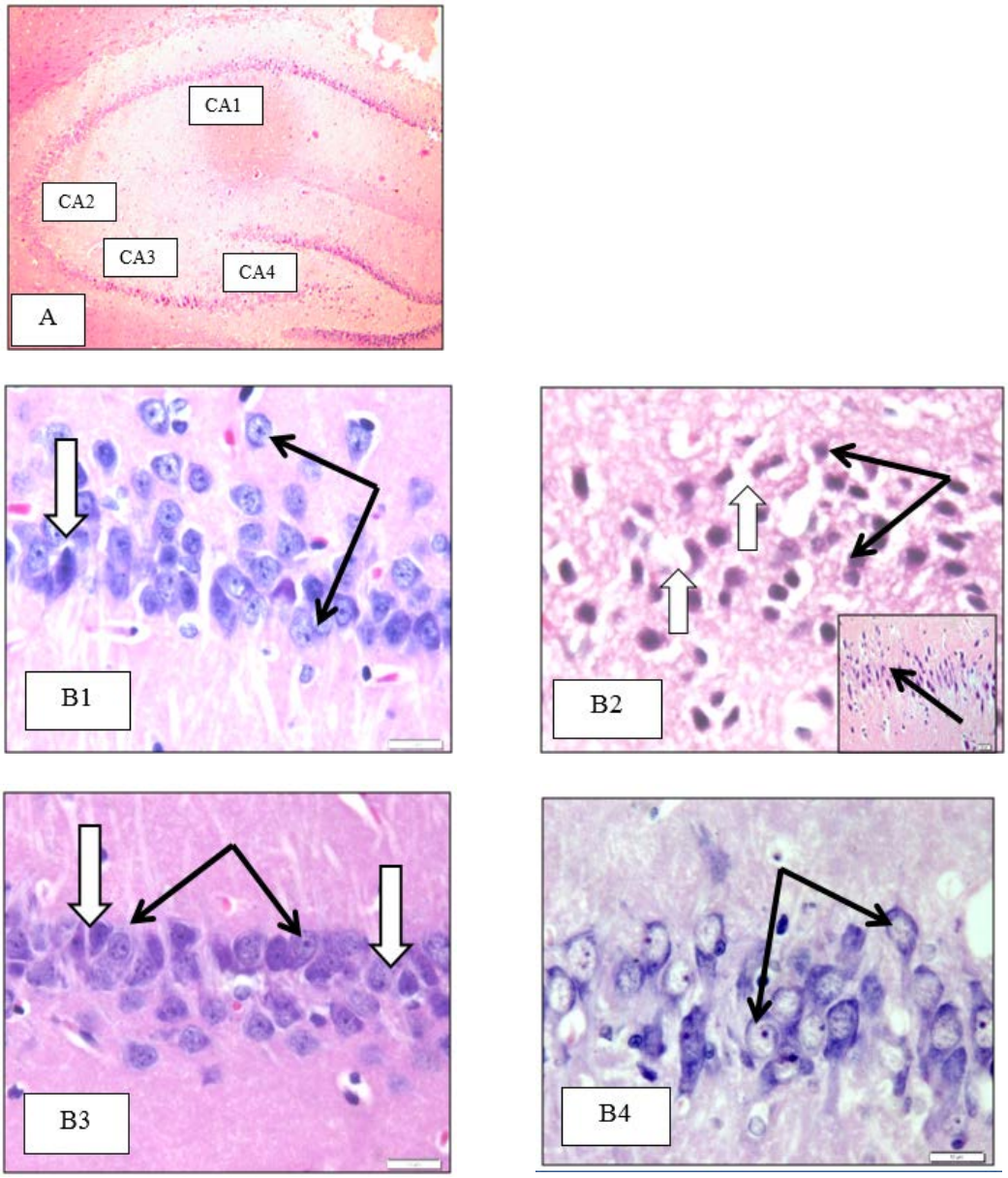
Groups	AchE (u/g)	MPO (u/g)
G1	746.62±4.459	0.382±0.180
*p	-	-
**p	0.00	0.00
G2	893.96±4.251	0.822±0.100
*p	0.00	0.00
**p	-	-
G3	766.16±4.132	0.778±0.020
*p	0.00	0.00
**p	0.00	NS
G4	743.39±5.680	0.584±0.136
*p	NS	0.0007
**p	0.00	0.001

G1: normal G2: AD group G3: Cu -N complex before AlCl3 treatment G4: Cu -N complex after AlCl3 treatment

*p:p value with respect to normal group,**p:p value with respect to Alzheimer group. Pvalue<0.05 is significant, NS: non-significant.

Histopathological investigation of brain hippocampus section in different studied groups

Microscopic examination of brain section of control rat showed normal morphological structure of the hippocampus, Cellsshowed active lightlystained nucleiwith few degenerated cells (Figure 1B). On the other hand, microscopic investigation for brain section of ALCL3intoxicated rat (AD-induced group) demonstrated various sizes of amyloid plaques in the hippocampus, shrinkage degenerated neurons. Cells looked dark stained with ill-defined degenerated nuclei. Wide spaces were observed between cells (Figure 2B). Histological investigation of brain section of AD-induced rats before treated with CU-N complex in a dose of 000000 mg /kg b.w revealed more or less normal histological structure of the hippocampus and potential protection of Hippocampal neurons from such effect (Figure 3B). Histological investigation of brain section of AD-induced rats after treated with CU-N complex, all amyloid plaques that are formed under the influence of AlCl3 administration disappeared, markedly restore neurons to its normal status (Figures 4B). Moreover, the treatment with CU-N complex showing normal histological structure of hippocampus with dislocation of some hippocampus cells in AD-induced rats treatment with CU-N complex.



A. general structure with the different hippocampal l zones: C1-C4

1B: Normal: showing normal hippocampal neuron density. Cells showed active lightly stained nuclei (black arrows). Few degenerated cells could be seen (white arrow). 2B :(AlCl3): (Alzheimer model) showing shrinkage degenerated neurons. Cells looked dark stained with ill-defined degenerated nuclei (black arrows). Wide spaces were observed between cells (white arrows). 3B: Cu -N complex before AlCl3 treatment showing moderate restoration of normal neuron stature (black arrows) still few cells looked shrunken and degenerated (white arrows) 4B: Cu -N complex after AlCl3treatment showingmarked restoration of normal neuronal structure (arrows) most cells looked active similar to control

Assessment of direct and oxidative DNA damage- comet assay

The DNA damage caused in the cell as a result of AlCL3 treatment was examined by single cell gel electrophoresis (comet assay) (Table3). The results indicated that the DNA of Al treated cell showed a comet tail indicating the DNA damage arising from the genotoxicity in the Al treated cell as compared to DNA of normal cell. The average % of DNA damage in the Al treated group was 76% as compared to normal control (20%). In the combined treatment groups, damage to DNA was before ALCL3 treatment with CU-N complex damage was 70%, and appreciably less (44%) in G4 group as compared to Al treated rats.

Table (3) Effect of CU-N Complex supplementation on DNA damage index observed by single cell

Groups	DNA damage (%)
G1	20±6
G2	76±15
G3	70±24
G4	44±12

G1: normal G2:AD group G3: Cu -N complex before AlCl3 treatment G4: Cu -N complex after AlCl3 treatment

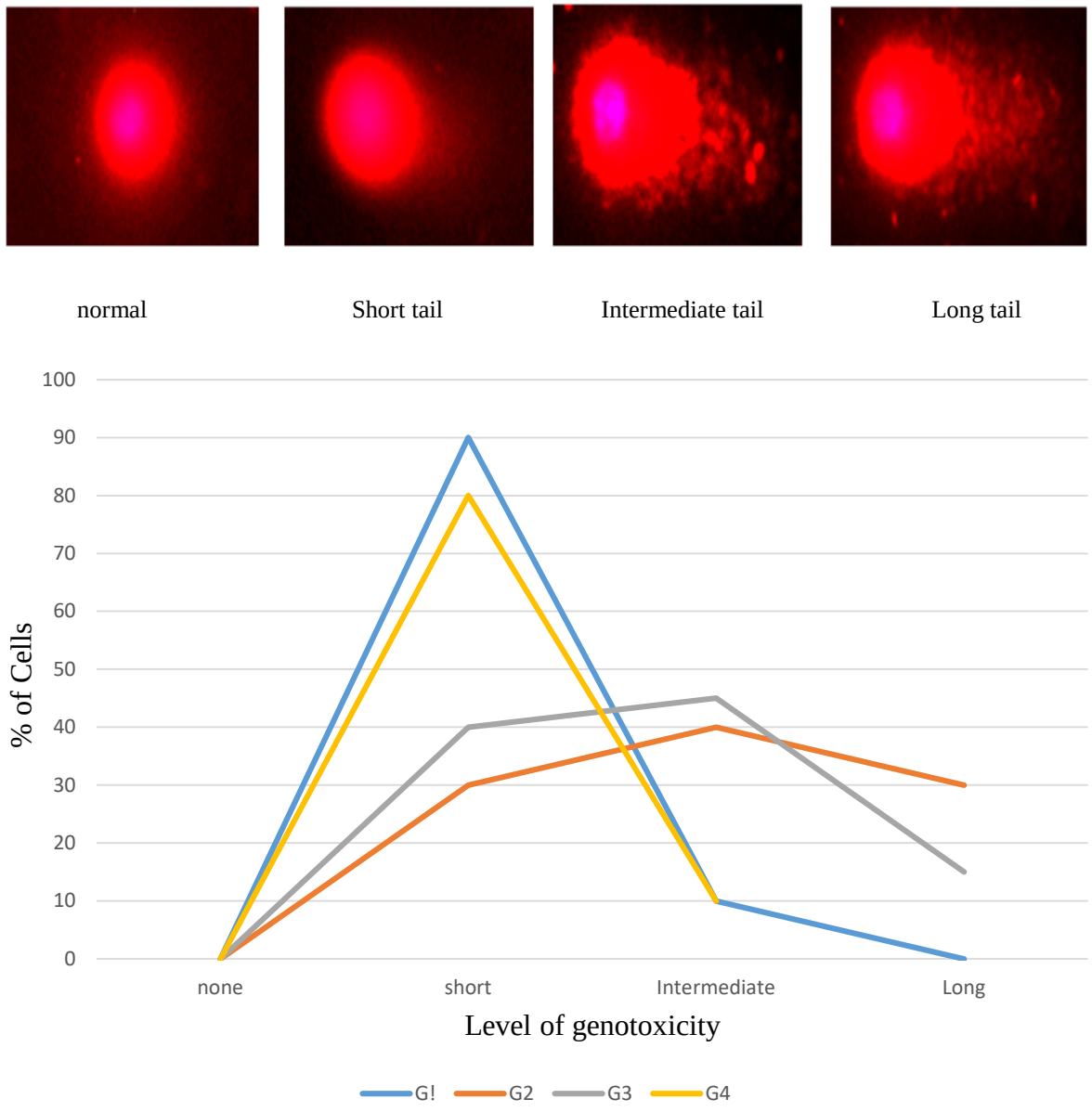


Figure (3). The effect of Cu- N Complex on DNA damge in rats' brain. The Top panel shows a typical of comet assay for each level of gentotoxicity. The values are shown as the mean ±SEM.

DISCUSSION

The number of patients suffering from Alzheimer's disease (AD) all over the world was rising continually and becomes one of the biggest challenges for most societies throughout the world[25]. It was believed that oxidative damage to critical molecules occurs early in the pathogenesis of AD and precedes pronounced neuropathological alterations. However, the onset of AD is not caused by a single factor, but by interaction of multi-factors, such as Aβ multifactors, inflammatory factors, oxidative stress, gene mutation, and neurotransmitter [7]. Moreover, there was no effective drug due to its complex pathogenesis, so seeking for drugs for treating and preventing AD and exploring its mechanism have been one of research hotspots.

Aluminum has been implicated as most important risk factor in aging related changes [26]and particularly in neurodegenerative disease [27]. The mechanism of aluminum induced neurodegeneration is not clearly known. However, it has been reported that aluminum potentiates the activity of ferrous (Fe2+) and ferric (Fe3+) ions to cause oxidative damage leading to neurodegeneration[28]. Moreover, aluminum promotes the formation of amyloid-β plaque [29] and aggregation of tau protein in Alzheimer disease [30]. In our study, as we did not observe any appreciable change in the diet consumption of the rats subjected to AlCl3 treatment so it can be anticipated

This effect could possibly be due to increased peroxidation of lipids as a consequence of oxidative stress in the animals following Al treatment, which has also been pointed out in our earlier studies [31].Complexation of nicotinic acid with various metals, e.g. manganese, cobalt, nickel, copper and zinc had been previously reported [32]. The metalloviatamin; copper (I)-nicotinate complex [CuCl (HNA)2] has been shown to exert diverse bioactivities. In particular, it can stimulate blood flow and prevent gastric congestion [14], reduce total lipids in sera hepatic tissue as well as regulate levels of alanine transaminase, aspartate transaminase, alkaline phosphatase, gamma glutamyltranspeptidase and oxidative markers such as nitric oxide (NO) and lipid peroxidation in rat models [18]. Additionally, it also exhibits SOD mimic activity in patients with hepatocellular carcinoma. The metal complexes of nicotinic acid with other small molecules such as isonicotinate and acetylacetonate were previously reported [32, 33]. The aforementioned nicotinic acid-copper complexes were formed through coordination with the carboxylate oxygen- and/or pyridine nitrogen-atoms [33, 34], which are electron donors. These electron-donating groups are commonly found in many natural compounds such as flavonoids (rutin, taxifolin, epicatechin and luteolin), phenolics (catechol and resveratrol) and drugs (aspirin, ibuprofen and oxaprozin), all of which were reported to form metal complexes with potent superoxide scavenging capacities [35, 36]. For example, flavonoid copper complexes were shown to exhibit higher SOD activity than the parent free flavonoids [37]. In the process of oxidative metabolism, oxygen radicals highly response to proteins and lipids to damage cell membrane and tissues, thus giving rise to cellular degeneration and death. The present study showed that oxidative stress was found in Alzheimer group of rats which indicated by statistically significant elevation in the mean levels of MPO, AchE and NO and significant decrease in the activities of TAC. Dickstein et al.[38] stated that growing evidence appears to implicate oxidative stress as the common factor rendering the brain vulnerable to environmental insults, and it has been shown to play an important role in the pathogenesis of AD. They reported that accumulated oxidative stress affects nitric oxide (NO) function to relax endothelial vasculature, increases vascular endothelial permeability, and further reduces CBF. These are thought to occur because of the reduced bioavailability of NO and the increase in free radicals. Nitric oxide is a well-known free radical[39] and after reacting with superoxide anion radials forms peroxynitrite[40] which then adversely acts on various macromolecules. Further, an important regulator of NOS activity is free Ca2+ which via calmodulin can activate the NOS enzyme activity and thus leads to the increased amount of NO [41]. Al toxicity has been reported to cause an increase in calcium levels. The increased amount of Ca2+ will bind to calmodulin, which further gets associated with NOS and activates the enzyme thus leading to the increased production of NO in brain regions.

The finding of significant elevation of brain NO level after AlCl3 administration in ovariectomized rats is in agreement with our and the previous studies [42, 43].The NO elevation in brain tissue may be related to Al-induced nitric oxide synthase (NOS) activity with consequent increase in NO production in rat brain tissue and microglial cells [43]. Those authors found that cerebellar levels of inducible NOS (iNOS) protein in rats were significantly elevated following both shortand long-term AlCl3administration [44].We also measured the levels of MPO activity

which might also explain the relationship between CU-N complex and neuroprotective effects. MPO and its resultant cytotoxic oxidants may potentially promote protein nitration and lipid peroxidation in AD brain contributing to neuronal dysfunction and memory loss [45, 46]. Increased MPO expression in activated microglia, astrocytes and neurons in cortex and hippocampus also worsens neuronal sprouting and synapticmaturation thus contributing to AD pathology [47, 48]. Moreover, increased plasma MPO was reported in AD patients indicating a clear link between MPO and neurodegeneration[49, 50]. The dynamic relationship between inflammation and neurodegeneration raises the possibility of a novel therapeutic approach aimed at suppression of MPO gene expression or enzyme activity. For example, statins [51] and liver X-receptor ligands [52] decrease MPO mRNA expression, possibly explaining the protection provided by these agents in human and animal models of AD. Thus MPO may serve as a valuable therapeutic target by mitigating the production of toxic inflammatory oxidants in Alzheimer's disease[53]. The present findings could support the hypothesis that decreased TAC activity could lead to an accumulation of H2O2. This could increase the stimulation of lipid peroxidation and protein oxidation, resulting in cellular damage [54]. Increasing the production of oxygen and nitrogen reactive species in mild cognitive impairment AD leads to a rapid consumption of plasma antioxidants. So, the antioxidant systems failed to protect the organism against the oxidative damage with subsequent development of the pathological alterations that characterize the neurodegenerative disorder [29, 55].Our results elucidate that Cu- N complex enhance cellular defense mechanisms against free radicals and lipid peroxidation by increasing utilization of copper in cells and tissues. The absorbed copper complex in cells and tissues could react with apoenzymes and apoproteins leading to formation of copper dependent enzymes and proteins respectively; these are mostly antioxidant. These reactions may account to suppress copper-induced lipid peroxidation[17]. Also the pretreatment with Cu-N complex had gastroprotective effect by preventing the down regulation in the antioxidants activity whereby fighting each of peroxide elevation and subsequently lipid peroxidation. The blocked in peroxide level by the antioxidant activity of Cu-N complex emphasize its role as a potent antioxidant, since Cu-N complex affect activity of several cuproenzymes controlling oxidation-reduction reactions (such as copper-zinc superoxide dismutase enzyme) as a cofactor and as a prosthetic component.Critalli et al. assessed the plasma level of TAC in AD patients (mild, intermediate and advanced disease) versus control groups. They found significantly lower TAC in AD patients (all stages) as compared to control group [56]. Also, Aldred et al. [57]showed a significant changes in TAC (decrease) in severe AD with respect to controls. In another study, a significant decrease of TAC levels in AD patients was reported [58]. Zifrilla et al. observed low levels of plasma TAC in light-moderate and severe AD groups when compared to healthy control [59].This reduction might be due to malnutrition and high-speed production of free radicals in patients [60]. In contrast to the reported studies, some studies have not found a significant difference in plasma TAC between the AD and control groups [61, 62]. According to the results obtained from our study, it was seemed the AD group have higher levels of oxidative stress and lower levels of antioxidant. The cholinergic system (i.e., acetylcholine) is imperative for making memory or encoding, the creation of long-term memory from the new memory or storage and the recall of the memory or recovery [78]. AchE was hydrolytic enzyme of Ach, which catalyzes Ach to be decomposed into choline and acetic acid, whose activities indirectly reflex their indecomposition rate in brain. Both ChAT and AchE keep the dynamic balance of Ach, which played vital important roles in learning and memory. The data of the current study showed significant increase in brain AchE activity AlCl3 -intoxicated rats. Gulya et al. [63] showed that aluminum causes cholinergic system dysfunction that may contribute to learning and memory deficits observed in Alzheimer's dementia. Zhang et al. [64] reported that increasing AchE activity in AlCl3 overloaded rats. Kaizer et al. [65]suggested that AlCl3 exposure increased AchE activity via allosteric interaction between AlCl3 and the peripheral anionic site of the enzyme molecule, leading to the etiology of AD pathological deterioration[66]. AlCl3 promotes the formation of amyloid-ß plaques (Aß 1-42) which significantly reduced brain Ach level leading to greater hippocampal Ach reduction accompanied by more memory impairment[67].The present study showed that treatment of AD-induced rats with Cu-N complex produced significant increase in brain Ach and significant decrease in brain AchE levels. Moreover, significant decreases in levels of oxidant markers MPO and NO and significant increases in levels of antioxidants TAC were reported in comparison with AD group. In addition, many researchers are trying to develop new therapies to treat AD. Acetylcholinesterase inhibitors are still largely used, since it has been hypothesized that cognition impairment may be related to a decrease in acetylcholine levels or an increase in AchE activity [35].Microscopic examination of brain sections of treatment rats showed that did not produce any histological changes in the hippocampus, this may be related to antioxidant effect. Cu-N complex was reported to function as potent superoxide scavenging capacities

[68]. Changes in the structure of synapse was also seen after Al treatment, which may affect neurotransmission and hence learning and memory processes in rats and the same is also observed in a number of studies [69-71]. AlCl3 in the present study produced degenerative changes in hipocummpal neurons similar to those described in literature of Abd El-Rahman (2003) which demonstrated that AlCl3 administration causes the appearance of neuritic plaques with a dark center in the hippocampus, typical for the Alzheimer's disease[72]. Treatment of AlCl3 -intoxicated rats with Cu-N complex revealed more or less normal structure of the hippocampus, i.e., most of β-amyloid plaques that were formed under the effect of AlCl3 administration disappeared. This result is in agreement with Cardounel et al. [73]who observed that dehydroepiandrosterone can protect against β-amyloid toxicity in hippocampal cells. On the other hand, Cu-N complex was found to be effective therapeutic substance as it restoreshipocummal neuron to normal status and even the neuron looked more numerous, larger and with active large vesicular nuclei. Cu-N complex significantly reduced the levels of ROS thereby preventing lipid peroxidation, composition and fluidity of the membranes which may account for the structural improvement as seen in the present study. The present study thus shows the protective effects of Cu-N complex by containing the cytotoxic effects inflicted by AlCl3.In in vitro studies, the Comet assay has been shown to detect genetic damage induced by different genotoxic agents such as radiation [74], herbicides [75], and heavy metals [76]. DNA fragmentation and increase in the appearance of comets have also been reported in other studies as a consequence of AlCl3 exposure [77]. Aluminum was known to increase the levels of reactive oxygen species[78, 79]as well as nitrogen species as seen in the present study also, which were known to cause damage to various macromolecules and also to DNA. An increase in the AlCl3 levels was also observed, which may result in more production of free radicals and thus contribute to the cytogenetic effects resulting from AlCl3 toxicity. Damage to DNA was one of the markers and typical characteristic of apoptosis[80] and the present study shows that Al toxicity can lead to faster apoptosis as seen in the electron micrographs which clearly revealed disruption of cells.CU-N complex, on the other hand revealed neuroprotective effects as evidenced by decreased DNA damage in the combined treatment group. A similar inhibition of DNA fragmentation was observed in G4 rats after CU-N complex treatment. Further, CU-N complex has been shown to possess antioxidative properties[81, 82] and hence may decrease the levels of free radicals and ultimately the damage to DNA.

CONCLUSION

In conclusion, this study demonstrated that Cu-N complex has the ability to improve cognitive function after treatment with CU-N complex by the regulation of the antioxidant system and exert diverse bioactivities.

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