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ROLE OF COMBINED ADMINISTRATION OF COPPER - NICOTINIC ACID COMPLEX AND COENZYME Q10 AGAINST ALUMINIUM CHLORIDE – INDUCED OXIDATIVE STRESS IN RAT BRAIN

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ABSTRACT

Alzheimer's disease (AD) is the most common chronic neurodegenerative disorder associated with aging. This study aimed to explore the modulatory effects of Coenzyme Q10 (CoQ10) (10 mg mg/kg., b.w.) and [Cu (I) – (nicotinic acid) 2] 1 Cl – complex (Cu –N complex) (400 µg/kg., b.w.) on aluminium chloride (AlCl3) -100 mg/kg b.w.) induced rat model of AD. Our results revealed that oral administration of AlCl3 for 42 days significantly elevated the levels of brain myeloperoxidase(MPO) activity and IL-1β levels, while Catalase and Na+/ K+ ATPase activity were markedly decreased. Plasma ferric reducing ability of plasma (ferric reducing/antioxidant power (FRAP) and superoxide dismutase (SOD)levels were noticeably decreased but Aspartate Transaminase (AST) concentrations were significantly increased, confirming that abnormal inflammatory response is associated with AD. Treatment by CoQ10 and Cu–Ncomplex restored the above mentioned parameters to about normal levels comparable to those of AD, indicating that IL-1β and Na+ / K+ ATPase activity may be considered as new biomarkers for AD. Histopathological and comet assay examinations confirmed the neuroprotective effect of CoQ10and Cu – N complex. The present data advocate the possible beneficial effect of CoQ10 and Cu – N complex protecting the cells against hepatocellular damage and as therapeutic modality for Alzheimer's disease via its anti-inflammatory/antioxidant mechanism.

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Introduction

Alzheimer's disease (AD), is a series of chronic progressive mental deteriorations occurring during or before the senile period characterized by progressive dementia as a result of brain dysfunction syndrome. The incidence of AD increases rapidly with age and, at the same time, AD is associated with high disability and mortality which have contributed to it becoming the fourth most frequent cause of death after heart diseases, tumor and stroke. AD is therefore likely to become a serious social and economic problem. Therefore, how to prevent and treat AD effectively has become an important subject for research. According to the AD mitochondrial hypothesis, defects in mitochondrial metabolism and particularly in the electron transport chain (ETC) may play a role during the early stage of AD pathogenesis [1]. β-amyloid peptide (Aβ peptide) has been associated with mitochondria from AD patients, showing mitochondrial fragmentation in various brain regions [2]. As extensively reviewed, Aβ accumulation in the mitochondria leads to different mitotoxic events, such as

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permeabilization of membranes, reduction of respiratory function, and disturbance of the mitochondrial calcium homeostasis [3]. Among these mechanisms, impaired Oxidative phosphrylationfunctions have been frequently observed in AD patients and in different AD transgenic mice (tg) mouse and cellular models [4]. Although the source of the oxidative damage remains uncertain, several lines of evidence have pointed to mitochondrial dysfunction as the cause [5]. "Mitochondrial antioxidants" are consequently candidate therapies for AD [6]. Coenzyme Q_{10} (CoQ_{10}) is especially attractive in light of favorable animal studies in a murine model of one neurodegenerative disease (Huntington's disease) [7] and favorable clinical trials in another (Parkinson's disease) [8]. Coenzyme Q_{10} is a potent antioxidant in both mitochondria and lipid membranes [9]. It is the only lipid-soluble antioxidant synthesized endogenously. Coenzyme Q_{10} acts directly by protecting cellular components from free radicals and indirectly via regenerating other antioxidants such as α-tocopherol and ascorbate [10]. It may induce some genes, and some of the effects of exogenously administered CoQ_{10} may be due to this property. The fundamental role of CoQ_{10} in mitochondrial bioenergetics and its well-acknowledged antioxidant properties constitute the basis for its clinical applications, although some of its effects may be related to gene induction mechanisms [11]. Despite the development of Alzheimeric drugs that display efficacy in patients with Alzheimer, new drugs with more potent anti neurodegenerative activity and fewer side effects are still required. With regard to many of the biologically active copper chelating complexes [12] the copper (I) – Nicotinate complex that exhibiting antioxidant activity as well as therapeutic pharmaceutical activity against Newcastle diseases [13], anti-inflammatory effect on gastric ulcer [14], reduction of adverse effects of 5-Fluorouracil in patients with hepatocelluler carcinoma [15], curative effect against induction of fatty liver in experimental animals [16], curable clinical signs of rheumatoid arthritis in rats[17], improved the skin burns in the experimental animals [18], efficiently prevented induced nephrotoxicity by Aflatoxin B1 (AFB1) specifically by promoting phase II detoxificating glutathione S-transferase activity [19], protected neurons and glial cells injured by 4-dimethylaminoazobenzene exposure [20]. Our observations [21] suggested that $AlCl_3$ induced AD associated pathologies, which might be due to its multiple pharmacological actions. Increasing evidence indicates that aluminum exposure and oxidative stress play crucial roles in the initiation and development of Alzheimer's disease (AD). Aluminum chloride ($AlCl_3$) and d-galactose (d-gal) combined treatment of mice is considered as an easy and cheap way to obtain an animal model of AD. So the present investigation was designed to examine the combination effects of Cu-nicotinate complex {Cu(I)-(nicotinic acid)$_2$Cl, [CuCl (HNA)$_2$]} (Cu –N complex) Figure (1),and CoQ_{10}on various neuropathological changes induced by $AlCl_3$ in in hippocampus of the male brain rats.

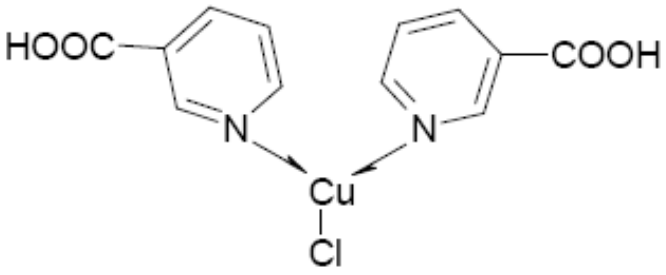


Figure 1. Copper (I)-Nicotinate complex

Materials and Methods

Animals

The study used young male wistar rats (Central Animal House, KAU, SA) weighing 200–280 g at the beginning of the study. Prior to experiments, the animals were acclimatized to laboratory conditions at room temperature. Animals were kept under standard conditions of a 12-hr light/dark cycle with food and water ad libitum in plastic cages with soft bedding. The above said manipulations were carried out during the light phase i.e., 09.00 am and 05.00 pm.

Materials

Copper nicotinic acid complex was prepared according to Goher [22].

Coenzyme Q10 and Aluminum chloride ($AlCl_3$, MW = 133.34) was purchased from Sigma-Aldrich Co. (Munich, Germany). All chemicals which were used in this study, were of analytical grade and supplied from different companies for medical and commercial service.

Induction of Alzheimer's disease (AD) in rats

Induction of AD in rats was carried out by administering $AlCl_3$ orally at a dose of 100 mg/kg body weight and D-galactose(60mg/Kg) daily for 42 days, according to the procedure described by Karam et al. [23].

Experimental Design

For this study, a total of Forty Wistar albino male rats of weight 200–280 g were selected. These animals were divided into four groups, each consisting ten animals respectively.

Group 1 (control group): Rats were administered distilled water for 49 days

Group2($AlCl_3$ -treated group): Rats were administered $AlCl_3$(100 mg/kg, B.W) and D-galactose (60mg/Kg) for 42 days through oral gavages.

Group 3 (co-treated group): Rats were treated with CoQ_{10}(10 mg/kg) and of Cu Nicotinate complex (400 µg/kg B.W.) daily for 49 days.

Group 4 (treated group): CoQ_{10} and Cu-N complex were applied daily for 49 days from 7 days before $AlCl_3$ administration and lasted until the animals received $AlCl_3$ daily for 42 days.

Serum Biochemical analysis

Ferric reducing/antioxidant power (FRAP) was assessed according to the Benzie and Strain [24]**.** Aspartate Transaminase (AST) activity was measured using a dichromatic rate technique at 340 nm wave length according to Tietz [25]. Superoxide dismutase (SOD) activity was assessed using a Xanthine oxidase system to generate superoxide radicals (O2 -) as described by McCord and Fridovich [26].

Oxidative Stress Markers of Brain hippocampus Homogenate

Hippocampus samples were divided into three portions:

i- The first portion was separated for determination of catalase, MPO, N^+/ K^+ ATPase and IL-1β cytokine. Using ether anesthesia, the animals were sacrificed and the brain of every rat was removed, washed with ice-cold saline in order to remove blood. This is then stored at -80ºC. Later, the stored brain hippocampus was taken, minced into small pieces and then 10% homogenate was prepared using phosphate buffer (0.1 M, pH 7.4) containing 1 mmol Ethylene Diamine-Tetra-Acetic acid (EDTA), 0.25 M sucrose, 10 mM potassium chloride (KCL), and 1 mM Phenyl Methyl Sulfonyl Fluoride (PMSF) with a homogenizer (REMI) fitted with a Teflon plunger, which was centrifuged at 800 rpm for 30 min at 4°C to yield the supernatant. This supernatant was used at later times to estimate the antioxidant parameters CAT, and MPO. Aebi [27] method was followed to estimate the Catalase (CAT) activity. MPO activity can be calculated through the reduced amount of H_2O_2 by colorimetric analysis at 460 nm [28]. Based on the method proposed by Tsakiris et al. [29], Na^+/ k^+ ATPase activity in hippocampus homogenate was assayed. IL-1β cytokine level in hippocampus homogenates were measured using Enzyme-Linked Immunosorbent Assay (ELISA) kits based on the instructions provided by the manufacturer (CUSABIO, Wuhan, China). The samples were analyzed in duplicate and the mean values of the concentrations were used for statistical analysis. Lowry et al. [30] method was followed to determine the protein content in the hippocampus tissue.

ii- The second portion was used for histological study; the hippocampus specimens were fixed in Bouin's solution. After fixation, specimens were dehydrated in an ascending series of alcohol, cleared in two changes of xylene and embedded in molten paraffin. Sections of 5 microns' thickness were cut using rotary microtome and mounted on clean slides. For histological examination, sections were stained with hematoxylin and eosin [31].

iii- The third portion was used for measuring DNA single strand breaks (Comet assay) [32].

Statistical Analysis

The results of the experiment are expressed here as mean ± SEM with each group containing ten rats. The statistical analysis was performed for intergroup variation between different groups using ANOVA (One-way Analysis of Variance) with the help of GraphPad Prism version 5.0 followed by Dunnett's Multiple Comparison Test (DMCT). The results were considered as statistically significant when $P < 0.05$.

Results

Effect of CoQ_{10} and Cu-N complex on some main members of anti-oxidative defense enzyme system in $AlCl_3$ -Induced Alzheimer's Disease

$AlCl_3$ exposure produced significant increase ($P < 0.0001$) in AST and significant decrease $P < 0.0001$ in SOD and FRAP in $AlCl_3$ control group when compared to normal control group. CoQ_{10} and Cu-N complex administration along with $AlCl_3$ exposure showed increase in SOD and FRAP, and ameliorated decreased level of serum liver enzyme AST significantly when compared to $AlCl_3$-treated group (Table 1).

Table 1. Effect CoQ_{10} and Cu – N complex on Serum Oxidative Stress Marker

Groups	FRAP (µM)	SOD (U/L)	AST (U/L)
G1	154.37+5.530	2.127+0.1206	55.292+5.7059

*p	-	-	-
**p	0.00	NS	0.00
G2	101.33+6.416	1.518+0.0816	146.141+5.5010
*p	0.00	NS	0.00
**p	-	-	-
G3	170.72+3.748	2.006+0.0667	49.019+3.6180
*p	0.00	NS	0.002
**p	0.00	NS	0.00
G4	119.03+1.077	2.787+0.5339	111.690+2.6777
*p	0.00	NS	0.00
**p	0.00	.04	0.00

G1: normal **G2**: AD group **G3**: CoQ_{10}+Cu – N complex **G4**: $AlCl_3$ + $CoQ_{10}$and Cu -N complex

*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 CoQ_{10} and Cu-N complex on brain hippocampus Antioxidant Parameters, Na^+/ k^+ ATPase and IL-1β cytokine activity in AlCl3-Induced Alzheimer's Disease

$AlCl_3$ exposure produced significant decrease ($P < 0.0001$) in catalase and Na^+/ k^+ ATPase and significant increase $P < 0.0001$ in MPO and in the IL-1β cytokine activity in hippocampus homogenate in $AlCl_3$ control group when compared to normal control group. CoQ_{10} and Cu-N administration along with $AlCl_3$ exposure showed increase $P < 0.0001$ in catalase, Na^+/ k^+ ATPase activity and significant decrease $P < 0.0001$ in MPO and counteracted the IL-1β cytokine activity in G4 group when compared to $AlCl_3$-treated group (Table 2).

Table 2. Effect of CoQ_{10}+Cu –N complex on, MPO, Na^+/ k^+ ATPase, Catalase and 1L-1β in rat hippocampus

Groups	**Catalase µmol/min/mg**	**MPO (u/g)**	**Na^+/ K^+ ATPase µmol Pi/mg**	**1L-1ß pg/ mg**
G1	22.290+0.53	0.047+0.005	8.938+0.8082	11.100+0.4497
*p	-	-	-	-
**p	0.00	NS	0.00	0.00
G2	6.907+0.673	0.681+0.041	5.099+0.5882	31.160+1.2186
*p	0.00	NS	0.00	0.00
**p	-	-	-	-
G3	20.28+0.302	0.048+.0.004	8.938+0.3383	9.600+0.8287
*p	.0031	NS	NS	.035
**p	0.00	NS	0.00	0.00
G4	16.73+0.580	0.226+0.023	7.942+1.3062	27.769+0.5877
*p	0.00	NS	NS	0.00
**p	0.00	NS	.0002	0.00

G1: normal **G2**: AD group **G3**: CoQ_{10}+Cu – N complex **G4**: $AlCl_3$ + $CoQ_{10}$and Cu -N complex

*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

Histopathological investigation of brain hippocampus section in different studied groups

Microscopic examination of brain hippocampus section of control rat showed normal morphological structure of the hippocampus, cells showed active lightly stained nuclei with few degenerated cells (Figure 2.B). On the other hand, microscopic investigation for brain section of $AlCl_3$intoxicated 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 2.C). The histopathological investigation of the brain hippocampus sections of the rats supplemented with the combined therapy (CoQ_{10}, Cu-N complex) after$AlCl_3$ intoxication showed necrosis of few neurons and more or less normal neurons in male rats except the appearance of pale supporting cells in rats (Figure 2.D). The neurons revealed a healthy appearance with small condensed surrounding cells.

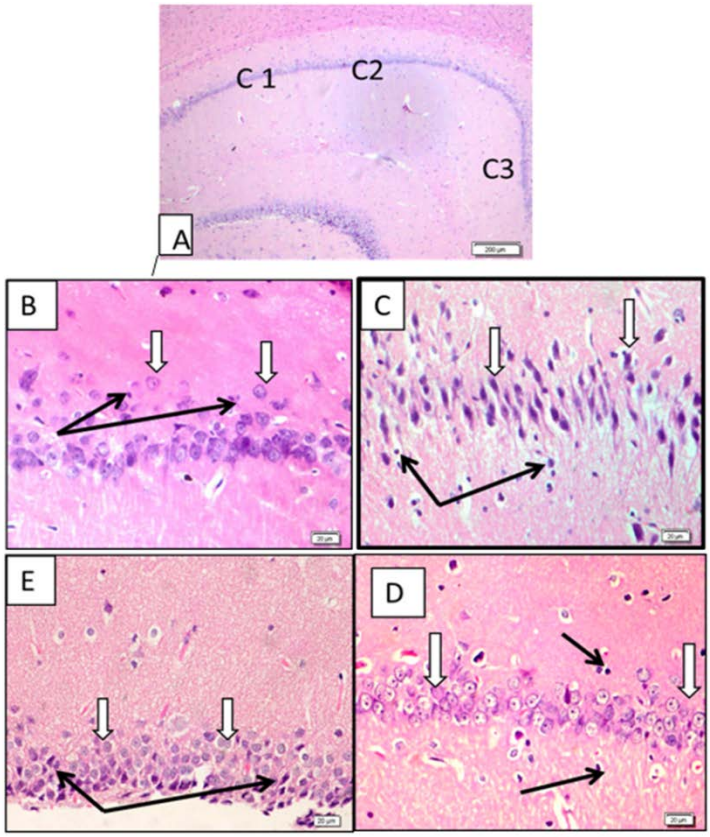


Figure 2. A: sections in rat hipocummpus to show: low power of control hippocampus different parts (C1.C2 and C3).

B: G1 (control) higher magnification of hipocumpucal neurons (C1 region) showing large lightly stained nuclei with well-defined nucleoli (white arrows). Glia cells are small with dark nuclei (thin black arrows).

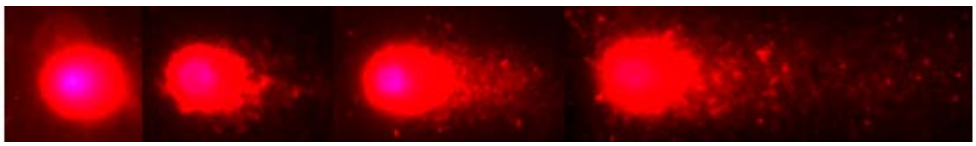
C: G2 (Alzheimer model) showing marked shrinkage of neurons which become dark stained (degenerated) or under went apoptosis. Glial cells were increased (thin black arrows)

D: G3 (Co treated group) hippocampal neurons appeared normal. All or most have large vesicular active nuclei and normal arrangement (white arrows). Glia cells are few (black arrows)**.**

E: G4 **(**treated group) Potential neuronal protection was observed (white arrows) still many neurons looked shrunken and degenerated (thin black arrows) (H&E x400).

Assessment of direct and oxidative DNA damage- comet assay

The DNA damage caused in the cell as a result of $AlCL_3$ treatment was examined by single cell gel electrophoresis (comet assay) (Figure 3). The results indicated that the DNA of $AlCL_3$ treated cell showed a comet tail indicating the DNA damage arising from the genotoxicity in the $AlCL_3$treated cell as compared to DNA of normal cell. The average % of DNA damage in the $AlCL_3$ treated group was 76% as compared to normal control (15%). In the combined treatment group damage was 9%, and appreciably less (35%) in G4 group as compared to $AlCL_3$ treated rats.



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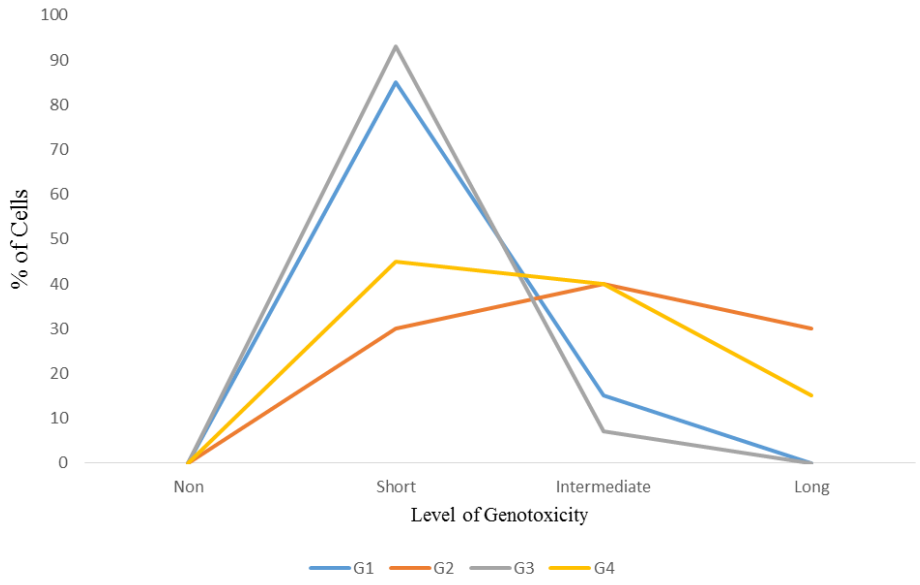

Figure 3. The effect of CoQ_{10} and Cu – N complex on DNA damage in rats hippocampus. The top panel shows a typical of comet assay for each level of genotoxicty. The values are shown as the mean ±SEM

Discussion

Alzheimer (AD) patients suffer from cognitive obstacles and reduced learning and memory. Senile plaques are one of the main pathological characteristics of AD, and are generally formed by the deposition of Aβ peptide [33]. The precise mechanistic basis leading to the development of both neuroinflammation and OS changes in AD is not clear, although the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway has been reported to become activated in both settings. In our previous study, intraperitoneal injection of D-galactose in combination with $AlCL_3$ injection was used to establish rat models of AD [21]. Antioxidants have been investigated as a strategy to attenuate the impact of aging and to extend lifespan. However, untargeted antioxidants often fail to modulate lifespan, possibly because only a small fraction of these antioxidant molecules is taken up by the mitochondria. Mitochondrial impairment and oxidative stress are thought to be important in aging, and the principal risk factor for AD is aging [34]. Coenzyme Q_{10} is a key component of the mitochondrial respiratory chain, and, for a number of years, it was mainly known for its role in oxidative phosphorylation; its presence was then demonstrated in other subcellular fractions and in plasma lipoproteins, where it is endowed with antioxidant properties. Coenzyme Q is synthesized endogenously, and an approximate amount of 3–5 mg per day is consumed within the diet [35]. Coenzyme Q_{10} could potentially play a therapeutic role in Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, Friedreich's ataxia, and other neurodegenerative disorders [36]. The observation that Mitochondrial Coenzyme Q (MitoQ) is able to delay Aβ peptide-induced paralysis in the transgenic nematodes is consistent with work performed using Aβ-induced toxicity in transgenic mice, by preventing the onset of AD-associated cognitive decline. The other studies also found that MitoQ is able to protect or elevate complex IV activity in AD model. This is consistent with a study in azide-treated rats, in which the authors demonstrate that a reduction in complex IV activity is associated with learning impairment [37]. The Coenzyme Q_{10} concentrations used in vitro are within the range seen in plasma from patients with AD (0.86 μM) and Parkinson's disease (0.5 μg/ml at baseline and 2 – 4 μg/ml with CoQ_{10} supplementation at 300–1200 mg/day), and therefore appear to be feasible target levels in the brain [38]. Although brain tissue and MitoQ levels were not increased with oral CoQ_{10} supplementation, oxidative damage to brain proteins was attenuated by CoQ_{10} supplementation, so oral CoQ_{10} may be a viable antioxidant strategy for neurodegenerative disease, including AD [39]. Deficiency of certain vitamins as nicotinic acid has deleterious effects on brain. Complexation of copper with nicotinic acid was shown to have potentials in medical practices, as decreasing inflammation, anti-oxidant properties and controlling lipid peroxidation [40], Metal coordination complexes have been rapidly developing in medical practices [41]. Together with other findings results demonstrating the considerable uptake of CoQ_{10} suggests that CoQ_{10} exerts its effects by acting on the mitochondria by preventing the production of mitochondrial radicals, improving mitochondrial efficiency and/or stability, or by some unknown mechanism. In this study, we investigated the effect of CoQ_{10} and Cu – N complex on a $AlCL_3$ model of Alzheimer's disease. Nedecky et al. [42] showed that copper complexes showed a diverse of in vitro biological activities, by neutralizing the charge of copper ion, intercalating DNA, interacting with proteins or increasing the lipophilicity of the complexes which facilitates their transport through all membranes. In this aspect, El-Saadani et al. [43] concluded that in rats Cu –N complex was absorbed by different organs intact without ligand exchange and the uptake by brain and heart was relatively slow and they referred this to be due to that homeostatic mechanisms and

utilization of copper [44]. Our study shows that rats with $AlCl_3$-induced AD exhibit reduces total antioxidant status, catalase, Na^+/K^+ ATPase and SOD activity and increased MPO and IL-1β content in the supernatant of the hippocampus compared with control rats. A previous study [21] showed that increases acetylcholinesterase activity, reactive oxygen species, and lipid peroxidation, and reduces total antioxidant status and SOD activity in the brain, possibly because the accompanying Aβ peptide plaque formation activates local as well as microglial and astrocytic inflammatory responses, thereby producing oxidative stress. In addition, $AlCL_3$ is extremely reactive with carbon and oxygen, and compromises the integrity of the blood brain barrier (BBB) and increases its permeability. Chronic exposure to $AlCl_3$ increase inflammatory processes in the central nervous system. The effect of $AlCl_3$ on liver function was observed by many studies [45]. Our study shows that rats with $AlCl_3$-induced AD exhibit increases in AST activity, when CoQ_{10} and Cu-N is supplied at the dose rates of Cu – Nicotinic acid complex (400 µg/kg., b.w.) and CoQ_{10} (10 mg mg/kg., b.w.) there is a great credibility expressed in protecting the cells against hepatocellular damage. The current study findings suggested that the hepatic protective action of $CoQ_{10}$and Cu-N is effective, at least show partial effect with regards to AST and antioxidant property. Since the oxidative damage is mediated by free radicals, it becomes mandatory to inquire the status of endogenous antioxidant enzymes such as reduced serum FRAP, catalase (CAT), superoxide dismutase (SOD) and in an effective manner so that a defensive alliance is created against ROS and the cells are protected from oxidative damage. The brain level of myeloperoxidase (MPO) was measured as a marker of inflammation. MPO expression in astrocytes leads to a specific pattern of phospholipid peroxidation and neuronal dysfunction contributing to AD. From the reports, it can be inferred that there is difference found in the activities of antioxidant enzymes in alzheimeric rats. Though the enzyme activities are reported as reduced, some are contrary and reported as high activity and some, no change in the enzyme activity at all. These contradictory reports may be due to various reasons such as tissue specificity, duration, disease severity or other experimental conditions. From the current study results, it is demonstrated that CoQ_{10} and Cu-N complex prevented the reduction in Na^+ / K^+ ATPase activity associated with $AlCl_3$ treatment. In this aspect and according to the literature [4], amyloid impairs glucose transport in hippocampal and cortical neurons is an effect that involves the process of membrane lipid peroxidation. This peroxidation process generally explains well about the reduced Na^+ / K^+ ATPase activity. The inhibition of Na^+ / K^+ ATPase during its long-term incubation with amyloid beta ($A\beta_{42}$), is caused by the change in the thiol redox status of cells leading to induction of glutathionylation α-subunit of Na^+ / K^+ ATPase [46]. This enzyme plays an important role in regulating the entry of Potassium and exit of sodium from the cells and take the responsibility to ensure the Na^+ / K^+ ATPase equilibrium through neuronal membranes. With regards to impaired neuronal function, the membrane-bound enzymes Na^+ / K^+ ATPase activity is significantly less in the AD patients' brain cells compared to the normal controls. The studies recently proved that that the role played by Na^+ / K^+ ATPase is important in AD patients and could be a potential neuroprotective modulator against AD. Amyloid peptide induces a series of actions such as oxidative stress, mitochondrial dysfunction, and membrane-bound enzymes Na^+ / K^+ ATPase impairment in hippocampal neurons which are further attenuated by basic Fibroblast Growth Factor (bFGF). When the cultured rat hippocampal neurons are exposed to Aβ peptide, it results in reduction of Na^+ / K^+ ATPase activity which is then followed by the calcium homeostasis loss resulting in cell degeneration. According to the literature [46], for the Ca^{2+} to be elevated and occurrence of neuronal injury, it is sufficient when Na^+ / K^+ ATPase activity is inhibited. Further, there are number of studies have inferred that the substances which actually restore or enhance the Na^+ / K^+ ATPase activity are induced by oxidative stress, low concentrations of Zn and cholinergic activity [47]. To restore the activity of Na^+ / K^+ ATPase, it is proposed to use reducing agents, which promote normalization of the redox status of cells and deglutathionylation of the protein. Alzheimer can be prevented from the effects exerted by various elements such as s-Ethyl cysteine and s-propyl cysteine, rivastigmine, Vit E, citicoline, memantine and tea polyphenol [48]. To be precise, various study results infer that there is a close relationship between the NMDA receptor in intact cells and the the membrane-bound enzymes Na^+ / K^+ ATPase activity [49]. According to the study [49], Aβ peptide are said to modify the calcineurin activity which further enhances the Na^+ / K^+ ATPase activity through dephosphorylation [50]. Besides these actions, the modification of the membrane-bound enzymes Na^+ / K^+ ATPase activity results from Ca2+ influx through the NMDA receptor triggers calcineurin and protein phosphatase 1 [51]. So, the amount of endogenous antioxidants enzymes such as CAT, and SOD as well as Na+/K+-ATPase were measured in order to evaluate the oxidative stress. The table 2 shows the outcome of the study i.e., various enzyme levels are restored when treated with CoQ_{10} and Cu-N whereas it reflected in the Na^+ / K^+ ATPase activity too when compared with the $AlCl_3$ group. In AD pathogenesis, the cytokines such as IL-1β, IL-6, and TNF-α play an important role which is document well in the previous past. According to the study [52], neuro-inflammatory process and neurodegenerative process go in parallel with each other. In AD pathogenesis, the glial cells activation and proinflammatory mediators release are the important players. An increase in expression of inflammatory cytokines, interleukin-1β (IL-1β), IL-6 and tumor necrosis factor-α (TNF-α) has been detected in brain of AD model animal and IL-1-positive microglia present with Aβ peptide -containing plaques, a similar event occurs in activated astrocytes. The role of astrocytes in neuroinflammation has been highlighted in the past years with many observations both in vivo and in vitro depicting the importance of these glial cells in this process Increased levels of INF-γ, IL-1β, IL-6, and TNFα induce astrocytes to adopt a classical activation state (increased activation of NF-κB pathway, production of ROS and NO, and release of IL-1β, IL-6, and TNFα), while increased levels of IL-4 and IL-13 induce an alternative activation (increased secretion of IL-4 and decreased production of ROS and NO); oppositely, high levels of IL-10 and TGF-β induce astrocytic

deactivation (reduced immune surveillance and proinflammatory signaling). In the present study, we found that $AlCl_3$ significantly impaired the learning and memory capability of rats 42 days. The cognitive decline was accompanied by an abnormal hippocampal structure and neuronal degeneration. Moreover, increased rates of hippocampal neuron apoptosis and secretion of pro-inflammatory cytokine IL-1β suggesting the presence of neuroinflammation induced by $AlCl_3$. These results are consistent with those of previous reports [53] in that the accumulation of Aβ peptide may activate astrocytes and trigger the generation of proinflammatory cytokines, which further accelerate the production of Aβ [54]. This study revealed that CoQ_{10} and Cu – N complex significantly ameliorates the neuroinflammation characterizing Alzheimer's disease in $AlCl_3$ rats due to its proteolytic activity as well as its anti-inflammatory effect. These findings were well confirmed by the histological examination and the comet assay of the brain hippocampus tissue of the treated rats. In the current study, histological examination of the hippocampus of rats in AD model group revealed that the pyramidal layers in CA1 region of the hippocampus showed marked shrinkage in size of small and large pyramidal cells respectively with some cell loss. These indicated that $AlCl_3$ and D-galactose treatment impaired the memory and spatial learning abilities of mice. Choosing hippocampus for studying the pathological findings of AD comes from the fact that the hippocampus remains one of the most vulnerable brain regions to AD, and its degeneration may directly underlie memory deficit, the earliest symptom of AD [55]. These histological results are in agreement with Paduraiuet al. [56] and Nirmala et al. [57]. They demonstrated in their study that the cytoplasm of neurons was shrunken, the nuclei were side-moved and dark-stained, neurofibrillary degeneration and neuron loss were observed in hippocampus of rat received $AlCl_3$. Moreover, Yassin et al. [58] observed that sections of rat brains receiving only $AlCl_3$ (17 mg/kg) for 4 weeks showed brain necrosis, plaques with loss of normal structure, outlines, and nuclei of cells. There was marked shrinkage in size of granule cells with some cell loss and marked vacuolation. This indicates a clear evidence of chronic inflammation and oxidative damage [59] and the same result was confirmed later by Abo El-Khair et al. [60] and Lynch [61]. Inflammatory changes are features of AD. Several studies have reported this observation and have shown that activated cells cluster around Aβ peptide -containing plaques [62]. Our finding coincides with finding of Nobakht et al. [63] as they reported that there was reduction in neuronal population in hippocampus of rat model with AD. Serrano-Pozo et al. [34] mentioned that neuronal loss is the main pathological substrate of the cortex and hippocampus which is evident in sections stained with hematoxylin and eosin, it can be more readily shown with a Nissl staining or a NeuN immunohistochemistry. It is reported that the neuronal loss is a common pathway for a large number of degenerative processes in AD and can be triggered by various factors, such as Aβ peptide, perturbed calcium regulation, glutamate, ischemia, inflammatory processes or oxidative stress [64]. Moreover, neuronal loss in the hippocampus due to Aβ peptide deposition might induce glucose dysregulation leading to hepatic insulin resistance which is one of the mechanisms of cognitive dysfunction in AD [65]. Molecular & polymorphic layers revealed enlarged and excess astrocytes and widened blood capillaries. This confirmed a result detected previously by Hashem et al. [66] as they found numerous astrocytes and microglial cells in the dentate gyrus granule cell layer of the hippocampus of their AD model. Numerous dilated blood capillaries were also, observed by Abo El-Khairet al. [60]. While, AD group treated with CoQ_{10} and Cu – N complex showed great improvement in the brain morphological structure. In the current work, studying the protective effect of CoQ_{10} and Cu – N complex in group 4 showed obvious improvement of the histological picture. The histopathological changes in this study are presented by preservation of most cells and markedly decreased apoptosis of cells were observed in C1 region. It is reported that treatment of D-galactose can cause degeneration changes in brain, such as increased AChE activity and ROS level, and depression of antioxidant defense enzyme system [21]. High levels of ROS can induce a progressive increase in the percentage of DNA fragmented brain [67]. Our comet assay results highlight reduction in the percentage of cells showing the highest amount of oxidized bases, indicating a potential role of CoQ_{10} and Cu – N complex in modulating DNA repair mechanisms. For instance, the increased level of DNA fragmentation, that is used to identify apoptotic cells, is a stringent morphological criterion for the evidence of apoptosis. Taken together with our results, all of these events allow us to suggest that apoptosis is the major cell death mechanism at least in part, in present model. The comet assay results showed that the fragmented DNA was significantly reduced after treatment with CoQ_{10} and Cu – N complex. As hypothesized, CoQ_{10} dramatically reduced apoptotic cell death, attenuated ATP decrease, and hindered DNA fragmentation elicited by all apoptotic stimuli. This was accompanied by inhibition of mitochondrial depolarization, cytochrome c release, and caspase 9 activation. Because these events are consequent to mitochondrial. The permeability transition pore (PTP) opening, we suggest that the antiapoptotic activity of CoQ_{10} and Cu – N complex [21] could be related to its ability to prevent this phenomenon. The blocked in peroxide level by the antioxidant activity of copper nicotinate highlighted its role as a potent antioxidant. Since copper nicotinate affect enzymes activity both as a cofactor and as a prosthetic component of several cuproenzymes controlling oxidation-reduction reactions including particularly copper-zinc SOD enzyme (Cu–Zn SOD). In the present paper, CoQ_{10} and Cu – N complex exerts a direct protective effect on brain cells, kinetics, lipid peroxidation and DNA fragmentation. The present data advocate the possible beneficial effect of CoQ10 and Cu – N complex protecting the cells against hepatocellular damage and as therapeutic modality for Alzheimer's disease via its antiinflammatory/antioxidant mechanis

Conclusion

Results of the present study demonstrated that CoQ_{10} and Cu – N complex could be as anti-inflammatory agent in the treatment of oxidative stress and apoptosis associated neurodegenerative diseases including AD.

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