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Research Article

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Selenium and Melatonin Attenuates Inflammation and Oxidative Stress in the Brain of Aged Rats with Aluminum Chloride -induced Alzheimer.

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ABSTRACT

Melatonin (MEL) and Seleniun (Se) are identified as potential antioxidants that can detoxify different Reactive Oxygen Species (ROS) in neurological diseases. The relationship between Se and MEL in detoxification remains unclear. The present study explores whether selenium and melatonin protects against experimental Alzheimer (AD) (aluminum chloride)-induced brain, and blood oxidative stress, and cytokine production in rats. Memory impairment was induced by aluminum chloride AlCl3 (100 mg/kg) for 42 days through oral gavages. To study the activity of MEL and Se 10 mg/kg (composed of 9.9 mg/kg melatonin and 0.1 mg/kg selenium (sodium selenite)), it was administered to Wistar rats for 49 days in addition to aluminum chloride. With the help of Morris water maze and passive avoidance test, learning and memory impairment were measured whereas the biochemical parameters of oxidative stress were measured in brain post treatment. The prominent finding of this study is that the oxidative stress is enhanced by $AlCl_3$. A significant improvement was shown by Selenium and Melatonin in terms of reduction in the oxidative stress through the reduction of AST, ALT, MDA level and further it counteracted the AChE activity. MEL-Se significantly lowered, an increase in TNF-alpha, IL-1beta and IL-6 levels and increasing SOD, GSH, CAT, GSSG-R and Na+- k+ ATPase activity. The present study clearly demonstrated the beneficial effects of selenium and melatonin through regulation of the antioxidant level and cytokine production, and this may act as a key to treat Alzheimer's disease.

Key words: *Aluminum chloride; Alzheimer's disease; Oxidative stress; Cytokines, Selenium and Melatonin*

INTRODUCTION

Millions of people die every year due to Alzheimer's disease (AD), the most common form of dementia. Unfortunately, to date, there is no effective treatment for the disease. Abnormal accumulation of extracellular amyloid-β peptides (Aβ) in amyloid plaques is one of the pathological hallmarks in the brain of AD patients. Inflammation and oxidative stress are two of the major factors resulting in neurodegeneration during AD pathogenesis. Aβ-induced astrocyte activation is involved in the production of proinflammatory cytokines and reactive oxygen species which contribute to synaptic loss and memory decline. Increased lipid peroxidation as well as protein and DNA oxidation are found in AD brains [1]. Antioxidant treatments in the early stages of pathogenesis were able to alleviate the functional impairment and to reduce brain Aβ in AD mouse models [2]. Microglia is nothing but innate immunocyte that reside in brain region and there are literary proofs that Microglia play an crucial role in AD's inflammatory response through the section of inflammatory cytokines such as interleukin (IL)-1β and Tumor Necrosis Factor α (TNF-α) [3].

Since neuroinflammation plays a role in AD, anti-neuroinflammation is considered as a potential strategy to treat AD. Human body requires dietary minerals called micronutrients in a very small quantity. Various literature provide evidence on the protective efforts of antioxidant nutrients on cells against the environmental agents' deleterious effects [4]. In such a scenario, Seleniun (Se), being one of the essential micronutrients in both animals and human beings, got greater attention now due to its functions at the active sites of glutathione peroxidase. Selenium plays a primary role in a number of physiological, biochemical processes such as biosynthesis of coenzyme Q, ion flux regulation across the membranes, stimulation of antibody synthesis and maintenance of the keratin integrity [5]. Selenium exhibits protective efforts due to the presence of seleno-enzymes that are known to protect DNA and other components in cell being damaged from oxidative stress [6]. Though the treatment is debated one [7], the elderly people are advised to intake antioxidants in order to help them prevent age-associated and free radical mediated neurodegenerative diseases. The use of Melatonin (MEL) as cytoprotective agent gained the wide exposure in this scenario.

Pineal gland is the source for circulating melatonin and various studies prove that when the plasma melatonin gets decreased, it is one of the symptoms of advancing age. The melatonin plays an important role in protecting the central nervous system which is document in many studies. Melatonin, through its antioxidative action, inactivate the oxygen reactants by donating the electron, thus results in the formation of inactive metabolites. When melatonin reacts with hydroxyl radical (.OH), it results in the formation of N1-acetyl-N2-formyl-5-methoxykynuramine [8].

Various studies have proved the melatonin's antioxidative action via stimulation of enzymatic reactions [9]. In a study conducted by Barlow-Walden et al. [10], when melatonin was administered in homogenates of rat brain, there is a significant increase in the activity of Glutathione Peroxidase (GPx) was observed. Antolin et al. [11] inferred from their study findings that melatonin protected Harder's gland cells from the damaging actions of free radical action of porphyrins and increased expression of mRNA which is the reason behind the Superoxide Dismutase (SOD) production. It is reported at many instances that melatonin (MEL) possess anti-inflammatory (and occasionally pro-inflammatory) properties in many species and in human beings as well [12]. According to the literature [13], MEL administration lessens Aβ-induced pro-inflammatory cytokine levels in rat and mouse brains which is to be noted. It is easy to understand that melatonin is representing a naive class of anti-inflammatory agents [14] whereas the research findings with regards to its role in reducing neuroinflammation via diverse mechanisms [15] is proving the same. Melatonin offers neuroprotection at the level of mitochondrial function [16]. So, it is possible to prose melatonin as a potential therapeutic agent in the reduction of AD severity. The current study suggests to use multi-targeted approaches by formulas which contains one or more antioxidant compounds since this kind of approach is promising than using single-agent approach. On the basis of this approach, the current study is designed to investigate the neuroprotective effect of MEL and Se against AlCl3-induced cognitive dysfunction and associated cerebral damage in rats.

Materials and Method

Chemicals

For the study, Aluminum chloride, melatonin and selenium were procured from standard reagents, Hyderabad. The chemicals used in this study were of high analytical grade procured from Sigma–Aldrich Company (USA).

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.

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 I (control group): Rats were administered distilled water for 49 days.
- Group II (AlCl3 -treated group): Rats were administered Aluminium chloride (100 mg/kg, p.o.) for 42 days through oral gavages.
- Group III (melatonin and selenium (MEL and Se) – treated group): Rats were treated with 10 mg/kg, p.o. (composed of 9.9 mg/kg melatonin and 0.1 mg/kg selenium (sodium selenite)) daily for 49 days.
- Group IV (MEL and Se + AlCl3 - treated group): MEL and Se was applied daily for 49 days from 7 days before AlCl3 administration and lasted until the animals received AlCl3 daily for 42 days.

All animals were sacrificed by decapitation following the day 42 after administration with AlCl3

Evaluation of Behavioral Parameters

Morris water maze

The apparatus contains a large-sized circular swimming pool (150 x 45 cm; water filled up to the depth of 30 cm at 28 ± 1°C) divided into four equal quadrants and a Perspex platform. At acquisition phase, the researcher placed a small platform about 1 cm above the water level. Each rat was subjected to four consecutive trials with 5 minutes break. Each animal was placed in the different quadrants gently for each trial such that it faces the wall of the pool and given 120 s for locating the platform. Next, it was allowed to stay up to 20 s in the platform. The animals were guided to reach the destination and if an animal failed to reach the platform within the timeframe of 120 s, then it was allowed to remain there for next 20 s. One 19^{th} and 20^{th} day of the experiment, the animals were allowed to attend two consecutive training sessions. The mean time to reach the visual platform was measured as acquisition latency. On day 21 and 42, after AlCl3 administration, mean time to locate the hidden platform was recorded as first retention latency and second retention latency respectively [17].

Passive avoidance task

In the apparatus, there are two chambers such as light and dark which are present with a metal grid floor. These chambers were separated from each other by a wall with a door. The test was done on two consecutive days. During the acquisition trial, each and every animals was placed in the light chamber independently. After entering into the dark chamber, an electric shock (40 V, 0.5 mA for 1 second) was delivered at the feet of the animal through grid. Immediately, the rat was taken out and returned to cage. After 24 hours, rats were placed again in the light chamber and the time taken to enter the dark chamber was calculated as step-through latency. The test was ceased in case if the animal did not enter the dark chamber within a 5-minute test period whereas the step-through latency was noted as 300 seconds [18].

Estimation of Biochemical Parameters

Serum Biochemical analysis

As per Tietz [19], dichromatic rate technique was used at 340 nm wave length to measure Aspartate Aminotransferase (AST) and Creatinine Alanine Aminotransferase (ALT). Based on Kakkar et al. [20], Xanthine oxidase system was used to generate superoxide radicals (O2 -) in order to assess the Superoxide Dismutase (SOD) activity.

Tissue Sample Preparation

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 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 (MDA, GSH, CAT, and glutathione reductase, GSSG-R) and acetyl cholinesterase (AChE). As per Yoshioka et al., [21], Thiobarbituric Acid Reactive Substances (TBARS) assay kit was used to assay alondialdehyde (MDA). Aebi [22] method was followed to estimate the Catalase (CAT) activity. Likewise Ellman [23] method was used to determine the reduced glutathione (GSH) whereas Hafeman et al. [24] method was employed to measure the Se-dependent glutathione reductase activity. Based on the method proposed by Tsakiris et al. [25], Na^+ K^+ - ATPase activity in brain homogenate was assayed. Lowry et al. [26] method was followed to determine the protein content in the brain tissue. Brain AChE activity was measured colorimetrically using a Quimica Clinica Aplicada SA kit (QCA, Amposta, Spain), den Blaauwen et al. [27].

Measurements of inflammatory cytokines

After the animals were sacrificed on 42^{nd} or 49^{th} day, immediately the cytokines (IL-1β, IL-6, and TNF-α cytokines) levels in 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.

Statistical Analysis

The results of the experiment is expressed here as mean ± SEM with each group containing six 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

I- Melatonin and Selenium administration attenuates AlCl3 induced learning and memory impairments

In Morris water maze, the animals took more time to reach the platform and also decreased step-down latencies when the passive avoidance test is conducted. This shows that the aluminium-chloride-treated group suffered from learning and memory impairments whereas, in the group administered with MEL and Se, the time taken to reach the platform in Morris water maze is decreased and increased step-down latencies which infers that the memory action in drug-treated animals got strengthened.

II - Effect of Melatonin and Selenium on some Biochemical parameters in AlCl3-Induced Alzheimer's Disease

AlCl3 exposure produced significant increase ($P < 0.0001$) in AST and ALT, and significant decrease $P < 0.0001$ in SOD in AlCl3 control group when compared to normal control group. Melatonin and Selenium administration along with AlCl3 exposure showed increase in SOD, and ameliorated elevated levels of serum liver enzymes (ALT and AST) significantly when compared to AlCl3-treated group (Table 1).

Table (1). Effects of selenium and melatonin on changes of serum Liver Enzymes, and Superoxide Dismutase content in rats with AlCl3-induced AD.

Parameter / Groups	**AST µ/L**	**ALT µ/L**	**SOD µ/L**
GI	56.42±6.3	37±3.5	1.98±3.2
GII	159±3.3#	71±5.8#	0.5±4.6#
GIII	46±6.5*	36±5.1*	2.04±5.7*
GIV	100±4.2*	53±6.4*	1.02±7.2*

The values of the experiment are expressed here as mean ± SEM. The Prism 6.0 software was used to conduct one-way ANOVA (One-way Analysis of Variance) followed by Dunnett's test. #$P < 0.01$ compared with normal control; *$P < 0.01$ as compared with aluminum control.

III - Effect of Melatonin and Selenium on brain Antioxidant Parameters, lipid peroxidation, Na+- k+ ATPase and ACHE activity in AlCl3-Induced Alzheimer's Disease

AlCl3 exposure produced significant decrease ($P < 0.0001$) in catalase, GSH, glutathione reductase and Na+- k+ ATPase and significant increase $P < 0.0001$ in MDA and in the enzymatic AChE activity in brain homogenate in AlCl3 control group when compared to normal control group. Melatonin and Selenium administration along with AlCl3 exposure showed increase $P < 0.0001$ in catalase, GSH, GR, Na+/K+-ATPase activity and significant decrease $P < 0.0001$ in MDA and counteracted the AChE activity in GIV group when compared to AlCl3-treated group (Table 2).

Table (2). Effects of selenium and melatonin on changes of oxidative stress markers content in brain homogenate induced by AlCl3 overload in rats.

Groups / Parameters	**GI**	**GII**	**GIII**	**GIV**
Catalase (µmole/min/mg)	19.66± 1.53	6.85±0.63#	23.58±0.89*	17.325±1.8*
Glutathione reductase (µmol/min/mg)	22.09± 1.4	9.89±0.78#	36.15±1.89*	29.54±4.9*
GSH (µmol/min/mg)	27.26±1.09	13.21±3.89#	35.15±182*	31.26±5.8*
MDA (nmol/g)	209.2±20.3	320.1±32.3#	199.8±29.5*	212.2±41.9*
Na+- k+ ATPase µmol Pi/ mg	7.79±1.82	4.29±1.5#	8.53±2.87*	6.08±2.78*

AChE (U/mg)	397.6 ± 33.2	724± 39.2#	415± 23.9*	494± 29.1*

The values of the experiment are expressed here as mean ± SEM. The Prism 6.0 software was used to conduct one-way ANOVA (One-way Analysis of Variance) followed by Dunnett's test. #P < 0.01 compared with normal control; *P < 0.01 as compared with aluminum control.

IV - Melatonin and Selenium suppressed the neuroinflammatory response in AlCl3-Induced Alzheimer's Disease

The protein expression of TNF-α, IL-1β, and IL-6 was quantified using enzyme-linked immunosorbent assay (ELISA) kits. As shown in Figure 1, a basal level of TNF-α, IL-1β, and IL-6 expression was detected in the brain homogenate of the AlCl3 group. Treatment with MEL +Se significantly blunted the AlCl3 -induced activation of TNF-α, IL-1β, and IL-6 expression. As shown in Figure 1, the values of TNF-α, IL-1β, and IL-6 protein expression in the G III group were lower than GI and better response in GIV group.

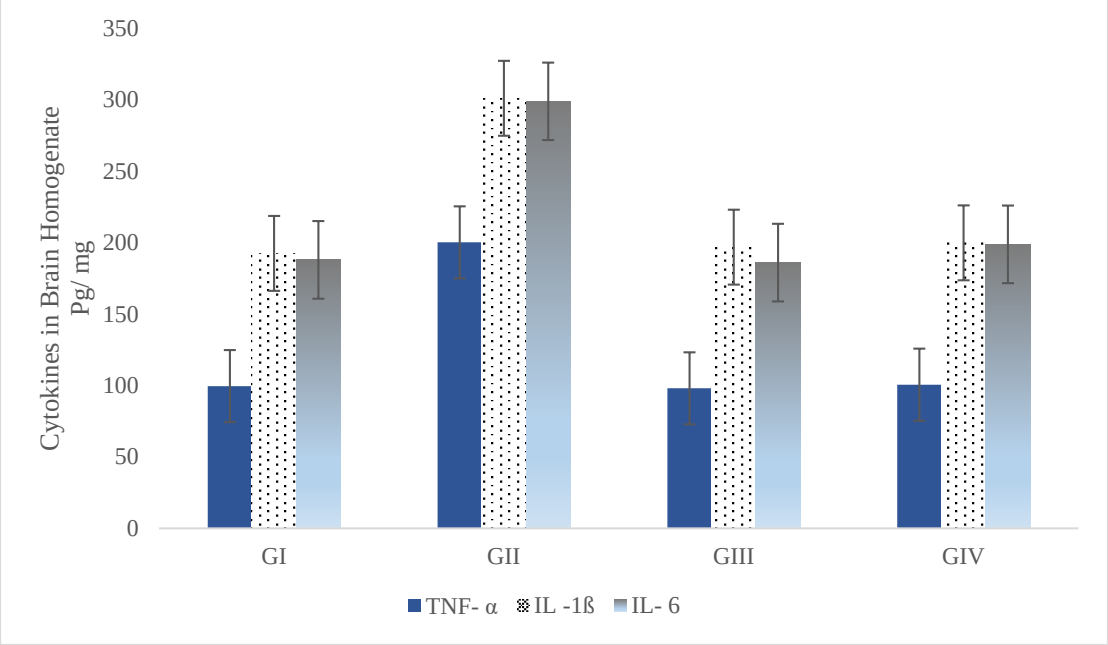

Figure 1: Cytokine levels in brain homogenate of the rats. Levels of cytokines in the homogenate of the brain were measured by ELISA. Data (means ± SD) were compared by one-way ANOVA.

DISCUSSION

One of the pathological mechanisms of AD is hyperoxidation injury induced by free radicals. Studies indicate that both sedimentation of amyloid β (Aβ) and the intertwinement of nerve fibers, the main pathogenesis of AD, are related to hyperoxidation injury induced by free radicals [28]. Our results indicated that AlCl3 significantly reduced contextual memory in passive avoidance test and spatial memory in Morris water maze test. In the passive avoidance test, AlCl3 treated rats do not remember the aversive stimulus and enter earlier into the dark chamber associated with shock as compared to the control rats that could remember. In the Morris water test, AlCl3 over loaded rats showed less capacity to retrieve and retain the location of hidden platform with the help of spatial information even after several days. MT-Se co-administration reversed AlCl3 induced memory deficits, which

indicate its memory enhancing effect. In the current study, elevated serum ALT and AST activities is indication of abnormal liver function.

The effect of AlCl3 on liver function was observed by many studies [29]. When MT-Se is supplied at the dose rates of 10 mg/kg (composed of 9.9 mg/kg melatonin and 0.1 mg/kg selenium), there is a great credibility expressed in protecting the cells against hepatocellular damage. The current study findings suggested that the hepatic protective action of MET and Se is effective, at least show partial effect with regards to 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 glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) in an effective manner so that an defensive alliance is created against ROS and the cells are protected from oxidative damage. 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 [30].

Selenium and Melatonin administration used in different experimental models that deployed animals and human beings showed increased levels of GSH and GSH-Px activities [31]. In the current study, the Aluminium chloride was administered chronically for 42 days which enhanced the learning and memory deficits of the animals significantly. This finally resulted in marked oxidative stress which can be inferred through the increase in lipid peroxidation and decrease in reduced glutathione, catalase and glutathione reductase activity. The above mentioned changes may have been brought by the reduction in axonal mitochondria turnover, Golgi disruption or reduced synaptic vesicles which is induced by AlCl3 treatment. All these above reactions finally ended up in the release of oxidative products such as malondialdehyde, carbonyls, peroxynitrites and enzymes such as superoxide dismutase within the neurons [32]. Due to the relation between oxidative stress and cognitive dysfunction, the agents that modulate the Reactive Oxygen Species can be considered as potential anti-dementia agents. The CAT activity was higher in the groups that received MEL and Se compared to control groups. When MEL and Se are administered chronically, it was found that it reduced oxidative damage induced by chronic AlCl3 administration. From this results, it can be inferred that the SOD activity in AD when subjected to treatment with MEL and Se, represents an important facet of the $O_2^{\cdot-}$ inactivation. Reduced glutathione act as the direct scavenger of free radicals since it is the abundantly found intracellular antioxidant that acts as a substrate for glutathione peroxidase enzyme that catalyzes the detoxification of H_2O_2. The current study observations inferred that the MEL and Se successfully restored reduced glutathione, increased catalase and glutathione reductase activity in GIV-treated rats [33].

In ischemic cascade, Lipid Peroxidation (LPO) is one of the primary markers denoting the oxidative damage. This is important since the major constituent of the brain is PUFA i.e., Polyunsaturated Fatty Acids (PUFA) and if it undergoes LPO, then that results in the production of lipid peroxides that can abruptly affect different cellular functions such as receptors, signal transduction mechanisms, transport systems and enzymes [34] in which proteins are involved. The current study observations are increased MDA and decreased antioxidant defense system in the AlCl3 group when compared to the control group.

There is a significant inhibition of MDA level is observed coupled with attenuated decrease in the antioxidant capacity in the brain when the ischemic rats were treated with MEL and Se. The studies conducted earlier stressed the importance of attenuating oxidative stress so as to evolve the neuroprotective strategies which can result in improved neuronal survival [35]. Likewise, the study conducted by Gupta and Sharma [36] mentioned that there was a decrease in amount of lipid peroxidation and mitochondrial enzyme complexes (I, II, and IV) in Huntington's disease-induced rats' brain striatum because of agomelatine, a dual agonist of melatonin receptors; though the treatment increased the activities of GSH antioxidant enzymes such as catalase and superoxide dismutase.

From the current study results, it is demonstrated that MEL and Se prevented the reduction in Na+/K+-ATPase activity associated with AlCl3 treatment. According to the literature [37], 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. With regards to impaired neuronal function, the Na+, K+-ATPase activity is significantly less in the AD patients' brain cells compared to the normal controls [38]. This enzymes 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+ equilibrium through neuronal membranes.

The studies that were conducted recently prove that that the role played by Na^+-K^+-ATPase is important in AD and could be a potential neuroprotective modulator against AD. Amyloid induces a series of actions such as mitochondrial dysfunction, oxidative stress and impairment of Na+, K+-ATPase activity 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 the selection reduction of Na+, K+-ATPase activity which is then followed by the calcium homeostasis loss resulting in cell degeneration. However, the current treatment fails in impairing the Mg2+-dependent ATPase or Na+/Ca2+ exchanger activity. According to the literature [39], for the Ca2+ 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+ and K+-ATPase expression or activity are induced by oxidative stress, cholinergic activity and low concentrations of Zn [40]. AD can be prevented from the effects exerted by various elements such as s-Ethyl cysteine and s-propyl cysteine [41], citicoline [42], rivastigmine [43], Vit E [44], memantine and tea polyphenol [45]. To be precise, various study results infer that there is a close relationship between the NMDA receptor in intact cells and the Na+, K+-ATPase activity [46]. According to the study [47], Aβ oligomers are said to modify the calcineurin activity which further enhances the Na+, K+-ATPase activity through dephosphorylation [48]. Besides these actions, Ca2+ influx through the NMDA receptor triggers calcineurin and protein phosphatase 1 which results in the modification of Na+, K+-ATPase activity [49]. So, the amount of endogenous antioxidants enzymes such as CAT, GR, Na+/K+-ATPase and SOD 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 MEL and Se whereas it reflected in the Na+, K+-ATPase activity too when compared with the Aluminium chloride group.

Cholinergic hypofunction is partly one of the reasons behind early memory deficit, a characteristic of AD, with hyperactivity of acetylcholinesterase (AChE). This enzyme is a postsynaptic enzyme that ends the cholinergic synaptic transmission through the acetylcholine hydrolysis [50]. This is usually a prominent one and overexpressed by neurites that are associated with β-amyloid plaques in Alzheimer patient's brain [51]. We can also assume that AChE, when overexpressed, can contribute to the amyloid pathology of AD since β-secretase activity which is mandatory to generate β-amyloid from β Amyloid Precursor Protein (βAPP) and this is under muscarinic receptor regulation [52] which further suggests that when there is a decline in cholinergic neurotransmission, then it would be favouring the formation of β-amyloid. Further, amyloid fibrils formation is highly favoured when there is a direct physical interaction between AChE and β-amyloid [53]. Progressive cognitive deterioration is coupled with overexpression of human AChE in transgenic mice [54].

The current study results shows that when aluminium chloride is chrnonically administered in rats, there is a significant increase in brain AChE activity as seen in the previous study [55]. Aluminium ions do interact with the AChE peripheral sites, make a modification in its secondary structure preceded by enhancing its activity. When MEL and Se are co-administered to AlCl3 intoxicated rats, there is a possible neuroprotection which is expressed through the reduction of AChE activity. When the AChE activities are inhibited, then Ach levels increase with positive effects on the cognitive events [56].

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 [57], neuro-inflammatory process and neurodegenerative process go in parallel with each other. As discussed in the earlier studies, when Aβ peptide gets accumulated and aggregated in the hippocampus, it leads to glial cells activation which in turn initiates a neuroinflammatory response with the involvement of inflammatory cytokines and over-production of inflammatory cytokines which actually enhances the glial cell activation. In AD pathogenesis, the glial cells activation and proinflammatory mediators release are the important players.

When the IL-1β levels are elevated, it means that the risks for AD are high. In the AD brain where the overexpressed IL-1β in activated glia is present, it can directly induce the glial activation further and increase the capability of Aβ towards glial cell activation. Additionally, the synthesis and processed of the β APP is promoted by IL-1β that further enhances other AD-relevant cytokines, such as IL-6 and TNF-α expression [58]. The current study results inferred that the IL-1β, IL-6, and TNF-α levels in the brain homogenates have elevated in a significant manner than what is measured in brains of the rats which were not treated with aluminum chloride. This study's results agree with the earlier study reported the elevated levels of IL-1β, IL-6, and TNF-α cytokines in Alzheimer Disease patients and Alzheimer Disease animals [59]. Finally, the results conclude that Alzhemeric rats could not balance the levels of IL-1β, IL-6, and TNF-α cytokines in brain. However it is possible for the MEL and Se to restore the relative balance of cytokines (through dose-dependent exposure) and thus by it can attenuate the cognitive impairment in AD rats. The future studies must look into the possible mechanisms in order to improve the cognitive function and effects in other AD models for other neurodegenerative disorders. To conclude, the Aluminium Chloride ($AlCl_3$), when administered, cause memory dysfunction through combined actions of brain lipid peroxidation, complicated inflammatory action and cholinergic neuronal dysregulation.

When calcium ion (Ca^{2+}) enters, if coupled with oxidative stress, it leads to neuropathic pain and hippocampal injury. In the sensory neurons and hippocampus, the following are expressed such as melastatin 2 (TRPM2), TRP vanilloid type 1 (TRPV1) and Transient Receptor Potential (TRP). Further the TRPM2 and TRPV1 activations during the process of oxidative stress are linked to neuronal death. To researcher's knowledge, the role played by MEL and Se in AZ rat brains are not reported. However the modulator roles of Se and MEL on TRPM2 channel in CHO cells (i.e., Chinese Hamster Ovary) and HEK-293 (Human Embryonic Kidney-293) cells were reported earlier [60].

According to recent study results [61], mitochondrial homeostasis is influenced by Selenium since it stabilizes the inner mitochondrial membrane that enhances the electron transport chain activity. Additionally, a recent findings about the role played by Selenium for modulation of Ca2+ entry and apoptosis through TRPV1 channels found in the patients' neutrophils who suffer from polycystic ovary syndrome [32]. In a study conducted by Espino et al. [62], it was reported that melatonin can delay calcium ion overload-induced leukocyte apoptosis in advanced age which may likely be due to its antioxidant properties. Melatonin possesses redox properties since it contains an electron-rich aromatic ring that allows indole to act as an electron donor. . Thus melatonin efficiently inverses the mitochondrial oxidative stress process through the effective removal of ROS formed in mitochondria [63].

CONCLUSION

Melatonin (MEL) and Seleniun (Se) are identified as potential antioxidants that can detoxify different Reactive Oxygen Species (ROS) in neurological diseases. Through a set of processes such as modulation of polyol oxidative reactions and selenium-dependent glutathione peroxidase (GSH-Px) antioxidant pathways, both MEL and Se reduce the TRPM2 and TRPV1 channel activation. Further it enhances the cytokine production, thus making it a potential therapeutic agent in treating oxidative stress-associated neurodegenerative diseases such as AD.

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