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Using Extracts from Beetroots and Broccoli to Attenuate Aluminum-Induced Neurotoxicity in Rats.

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ABSTRACT

Aluminum's harmful impact on animals and humans is often described as the production of oxidative stress. The purpose of this research was to determine whether extracts of broccoli (Br) and beetroot (Be) were effective in mitigating the oxidative stress linked to aluminum toxicity. Substances and Techniques: There were a total of 50 female Wister rats, and they were split up into 5 groups of 10 each: Group 1 received simply water for consumption as a placebo. Neurodegenerative diseases were produced in Group 2 by daily oral dose of aluminum chloride (20 mg/kg b.w.). Rivastigmine (Ri) (1 mg/kg b.w.), a reference medication, was administered daily to aluminum chloride-treated rats for five weeks (Group 3). Beet root extract (50 mg/kg b.w.) was administered once daily for six weeks to group 4 rats that had been administered aluminum chloride. Broccoli extract (50 mg/kg b.w.) was administered daily to a group 5 of rats (alluminum chloride-treated) for a period of five weeks.

The results indicated that compared to the control group, the levels of Ach were significantly higher in the (AlCl3) group (P0.05), whereas the levels of DOP and NE were not. Although the levels of Ach, DOP, and NE did not significantly alter in the (AlCl3+Be) group compared to the (AlCl3) group, they did rise significantly (P0.05) when compared to the control group. There was a statistically significant (P0.05) rise in NE levels in the (AlCl3+Br) group compared to the (AlCl3) group, but no difference in Ach or DOP levels. In comparison to the (AlCl3) group, the (AlCl3+Ri) revealed significantly higher levels of Ach, DOP, and NE (P 0.05). Also, both Ach and NE were found to be significantly (P0.05) higher than in the control group. The current research suggests that broccoli's neuroprotective properties come from its Glucosinolate, isothiocyanate, Sulforaphane, and flavonoids are only some of the bioactive components that give this vegetable its antioxidant capabilities. Therefore, broccoli's antioxidant and anti-inflammatory characteristics may have a beneficial impact on neurotoxicity.

Keywords: Neurotoxicity; Brocolli; beetroots; antioxidants.

Introduction

Al, the most common metal on the planet, has long had a bad reputation as a neurotoxin [1]. Effects on the nervous system, including behavior and pathology, as well as chemistry and physics, have been linked to Al exposure in a number of studies. In nature, it often forms compounds with other metals. Alen is present in our bodies because of the widespread usage of items containing or produced from Alen [2]. Al enters the body by contact with the environment, ingested food, or administered medications. Alinside the body, however, it has no known physiological purpose, and too much of this metal might have the opposite effect [3]. Altoxic impact is a term used to describe the creation of oxidative stress in both animals and humans. Many neurodegenerative diseases, such as Alzheimer's and Parkinson's, have linked oxidative stress to their development [4].

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Blood-brain barrier (BBB) alterations have been documented because to Al's buildup in the brain and its easy access to the central nervous system under normal physiological settings [5]. The kidneys, central nervous system, and brain are particularly vulnerable to Al's toxicity. The brain is the most vulnerable organ to Al's toxic impact [6] because of its sensitivity to oxidative stress due to poor antioxidant levels and high free radical levels after poisoning. The antioxidant system of the body and free radicals generated by human metabolism or obtained from the environment need a healthy balance of oxidants and antioxidants [7]. Antioxidant chemicals isolated from various sources in nature

Using a variety of in vitro and in vivo experimental paradigms, (nutraceuticals) have shown neuroprotective efficacy against neuronal cell death and neurodegeneration [8]. The complexity and abundance of secondary metabolites composition, as well as the unique structure of the molecular composition bearing a significant amount of stereo-centres revealing high specificity connected to biological activity, have shown that nutraceuticals extracted from therapeutic plants play an essential role in drug discovery. To isolate a specific chemical family from a large and diverse mixture, many methods of natural product extraction are used [9]. The prevention and treatment of neurodegenerative disorders and other forms of neuronal dysfunction rely heavily on a wide range of natural products containing bioactive ingredients [10]. Plants play a crucial role in the food chain that supports human nutrition, hence eating plants is essential for good health. Plants are rich in compounds that are beneficial to health since they include all the mineral, organic, and almost all vital nutrients that people need [11].

Due to its biological activity and possible involvement in enhancing health and avoiding illness, red beetroot (Beta vulgaris rubra) has recently attracted the attention as a functional food [12]. It has now been introduced to many other regions, including the Americas, Europe, and India, all from its original Mediterranean home. Different portions of this plant are employed for their medicinal benefits in traditional Indian medicine [13]. The presence of substances such carotenoids, folic acids, phenols, and flavonoids was found via the study of free radical scavenging capabilities, indicating their high antioxidant content [14].

Broccoli, or Brassica oleracea, as it is more often known,

The plant L. var. Italica) is well-known for the positive effects it has on human health due to the abundance of vitamins, phenolic compounds, and dietary important minerals it contains. However, research into broccoli's potential health advantages beyond its staple status has increased [15]. Many illnesses are linked to oxidative stress, and studies suggest that broccoli may protect against this. This research set out to determine how effective extracts of broccoli and beetroot were as antioxidants in mitigating the oxidative stress caused by Altoxicity.

MATERIALS AND METHODS

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

1.1 Plant Material and Extract Preparation

The beetroot and broccoli were procured fromthe supermarket in Jeddah.

Preparation of beetroot and broccoli Extracts was done as described in [16]. Briefly, 100 g of fresh Broccoli or beetroots samples were homogenized and extracted with 1L of 70% methanol at room temperature for 120 min with stirring to ensure the extraction, followed by centrifugation (10 min, 4°C). The supernatants were collected and methanol was completely removed using a rotary evaporator. Then, theaqueous fractions containing bioactive compounds were lyophilized and used as dry broccoli extracts.

1.2 Animals and Experimental Design

Fifty Wister female rats weighing between 100 to 150 gm were obtained from King Fahd Medical Research Center (KFMRC), King Abdul-Aziz University, Jeddah, Saudi Arabia. Ethical approval for the current study was obtained from the animal house at KFMRC. Rats were randomly grouped into five groups (each 10 rats) as following

- Group 1 (control group): Rats were administered drinking water only.
- Group 2 (Neurogenerative): Rats were orally administrated aluminum chloride (20 mg/kg b.w) daily for one month.
- Group 3 ($AlCl_3$+Bi): Rats were given aluminum chloride and treated with Rivastigmine (1 mg/kg b.w) as a reference drug daily for five weeks.
- Group 4 ($AlCl_3$+Be): Rats were given aluminum chloride and treated with beet root extract (50 mg/kg b.w) daily for five weeks.

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- Group 5 ($AlCl_3$+Br): Rats were given aluminum chloride were treated with broccoli extract (50 mg/kg b.w) daily for five weeks.

At the end of the treatment, animals were euthanized by cardiac puncture under thiopental general anesthesia and death was confirmed by cervical dislocation. Blood was collected from the inner can thus of the eye using heparinized capillary tube and centrifuged for 15 min at 3000×g to separate blood plasma for the estimation of biochemical parameters.

1.3 Estimation of Plasma Biochemical Parameters

For the assessment of liver functions, kinetic methods for the determination of activities of aspartate aminotransferase (AST) and alanine aspartate aminotransferase (ALT) were used according to the recommendation of the Expert Panel of the IFCC (International federation of clinical chemistry). Alkaline phosphatase (ALP) activity was determined according to the recommendation of the German Clinical Chemistry Association. While the plasma total protein concentration was estimated by biuret method as described in [17].

1.4 Oxidative Stress Biochemical Assays

The following biochemical markers were measured in the liver and kidney homogenates according to the manufacturers' instructions (All kits were purchased from Elabscience company): reduced glutathione (GSH) assay kit (Catalog No: E-BC-K05), glutathione peroxidase (GSH-PX) assay kit (Catalog No: E-BC-K096), total antioxidant capacity assay kit (Catalog No: E-BC-K136).

1.5 Estimation of Plasma Aluminum Level

Plasma collected from rats were mixed with an equal volume of 0.2% HNO3 to eliminate the problems of organic residue accumulation in the furnace. Aluminum was determined using calorimetric method with aluminon (triammonium salt of aurintricarboxylic acid) [18], a dye commonly used to detect the presence of aluminum ion in an aqueous solution. The compound aluminum forms a red lake color with aluminum in neutral solution.

1.6 Estimation of PlasmaAcetylcholinesterase (AChE) Level

The Competitive AChE ELISA Kit (Catalog No: E-EL-R0355- Elabscience) was used to determine AChE level according to the manufacturer's instructions.

1.7 Estimation of Brain Neurotransmitters

The following neurotransmitters were measured in the brain according to the manufacturers' instructions. All kits were purchased from Elabscience company: Acetylcholine (ACH) ELISA Kit (Catalog No: E-EL-0081- Elabscience). Dopamine(DA) ELISA Kit (Catalog No: E-EL-0046-Elabscinence).
Noradrenaline/Norepinephrine (NA/NE) ELISA Kit (Catalog No: E-EL-0047- Elabscience).

1.8 Histological Examination

Brain tissue was initially fixed with 10% neutral buffered formalin solution, embedded in paraffin, sectioned at 4 µm thickness, and stained with hematoxylin and eosin (H&E). Sections were stained with hematoxylin and eosin.

1.9 Statistical Analyses

The statistical analyses were performed using the (SPSS program for Windows, version 20). Add details here about the statistical tests that were used (t test, ANOVA etc) plus the P values.

2. RESULTS

As indicated in Table 1, $AlCl_3$ group showed non-significant ($P>0.05$) changes in Hb level and RBCs count compared with control group. On the other hand, there was a significant ($p<0.05$) decrease in platelets level and WBCs count compared with control group. For ($AlCl_3$+Be) group, there was a significant ($P<0.05$) decrease in RBCs count and platelets level compared to control and a non-significant ($P>0.05$) increase in WBCs compared with ($AlCl_3$) group. While showing a non-significant ($P>0.05$) change in Hb, RBCs count, and platelets levels compared to group of ($AlCl_3$). In ($AlCl_3$+Br) group, there was a significant ($P<0.05$) increase in Hb level and WBCs count and a significant ($P<0.05$) decrease in platelets level compared to ($AlCl_3$) group and anon-significant ($P>0.05$) change in RBCs count compared to ($AlCl_3$) group. For ($AlCl_3$+Ri) group, there was a significant ($P<0.05$) increase in Hb level, RBCs, platelets WBCs count compared to ($AlCl_3$) group.

In (Table 2) Fig. 1 showed a significant ($P<0.05$) increase in Allevel ($AlCl_3$) group compared with control. While treatment groups showed a significant ($P<0.05$) decrease and a significant ($P<0.05$) increase in Allevel compared to ($AlCl_3$) and control group, respectively.

In Table 3, ($AlCl_3$) group showed a significant ($P<0.05$) increase in ALT, AST levels and a significant ($P<0.05$) decrease in albumin level compared to control group. Additionally, ($AlCl_3$) group showed a non-significant ($P>0.05$) changes in ALP and protein levels compared to control.

All treatment groups showed a significant ($P<0.05$) increase in ALT level compared to ($AlCl_3$) and control groups.

($AlCl_3$+Be) group showed a significant ($P>0.05$) decrease in ALP and increase in protein compared to ($AlCl_3$) group. Furthermore, there was a significant ($P>0.05$) increase in AST and was a significant ($P>0.05$) decrease ALP and albumin levels compared to control group.

($AlCl_3$+Br) group showed a significant ($P>0.05$) decrease in ALP and anon-significant ($P>0.05$) change in protein compared to ($AlCl_3$) group. Also, there was a significant ($P<0.05$) increase in AST and a decrease in albumin level compared to control group.

($AlCl_3$+Ri) group showed a significant ($P<0.05$) increase in AST level and a decrease in ALP and protein levels compared to ($AlCl_3$) group. For albumin level, there was a non-significant ($P>0.05$) change compared to ($AlCl_3$) group and a significant ($P<0.05$) decrease compared to control.

Data in Table 4 revealed a significant ($P<0.05$) increase in uric acid and creatinine levels ($AlCl_3$) group and non-significant ($P>0.05$) decrease in urea level compared to control group.

($AlCl_3$+Be) and ($AlCl_3$+Ri) groups showed non-significant ($P>0.05$) changes in uric acid, urea and creatinine levels compared to ($AlCl_3$) group and showed a significant ($P<0.05$) increase in uric acid compared to control.

($AlCl_3$+Br) group showed a significant ($P<0.05$) decrease in uric acid and a non-significant ($P>0.05$) changes in urea and creatinine levels compared to ($AlCl_3$) group.

2.1 Histological Examination

Fig. 3 showed that, (AlCl3) group: showing a few normal neurons (black arrows). Most cells are shrunken and showed dark stained cytoplasm and nuclei (white arrows). Glia cells with small dark nuclei are also increased (dotted arrows). C1. reg. (AlCl3+Be): showing marked protection of hippocampal neurons. Cells looked normal with active large nuclei (black arrows). Degenerated shrunken apoptotic cells are few (white arrows). Glia cells with dark nuclei are also few (dotted arrows). C1. reg. (AlCl3+Br): showing marked protection of hippocampal neurons. Cells looked normal with active large nuclei (black arrows). Degenerated shrunken apoptotic cells are few (white arrows). Glia cells with dark and nuclei are also few (dotted arrows) C1. reg. (AlCl3+Ri): showing marked protection of hippocampal neurons. Cells looked normal with active large nuclei (black arrows). Degenerated shrunken apoptotic cells are few (white arrows). Glia cells with dark and nuclei arealso few (dotted arrows).

3. DISCUSSION

Alis recognized as a neurotoxic element in animals and humans, and it is considered as a causative agent in a range of neurodegenerative disorders. Natural antioxidants, such as those found in plants, proved to be useful in reducing the progression of various pathologies associated with oxidative stress including neurodegeneration [19]. The use of plant extracts has been observed to be as effective as antioxidants in Al-induced neurotoxicity [20].

The decrease in the level of hemoglobin and RBCs in Al-treated group in our study was similar to previously published observation [21], but this decrease was not statistically significant. The age of animals at the time of exposure, different duration of chronic administration and different animal species might explain this variation between the present and previous studies. One of the basic mechanisms of the toxic action of heavy metal on mammals is erythrocytedestruction [22]. Erythrocyte is one of the major target cells for Altoxicities [23], and a hemolytic effect of $AlCl_3$ is an indicator of a decline in RBC count [24]. Al can inhibit the activity of enzymes that are involved in the haem biosynthetic pathway or interfere with cellular iron uptake and utilization and that can affect in hemoglobin which leads to the reduction of synthesis and thiscan cause anemia [25]. Because the content of rare natural pigments (betalains), polyphenols, antioxidants, vitamins, minerals, and fiber in the beetroot, it was considered the most important vegetable in the world [26]. Also, broccoli is useful to health because of its high content of health-enhancing compounds such as glucosinolate, vitamins, phenolic compounds, and dietary essential minerals [27]. Vitamins and minerals found in beetroot and broccoli are most likely active ingredients responsible for its increase in the amount of hemoglobin in the blood and improvement of white blood cells. Rats treatment with rivastigmine showed a significant elevation in Hb and RBCs, this is due to rivastigmine may

chelate aluminum chloride and ameliorate toxic effect or stimulate enzyme involved in hemoglobin synthesis.

Aluminum (Al) is known as a toxicant agent. It linked to many neuro-disorders diseases and other serious neurodegenerative diseases [28].

The result of obtained showed that the Aluminium exposure group had a significant increase in its serum level. Aluminium interferes with most physical and cellular processes. The aluminum as a toxicant agent especially with respect to the bone, blood, and the nervous system when finding in high doses in circulation. Increased Aluminium levels in organs and

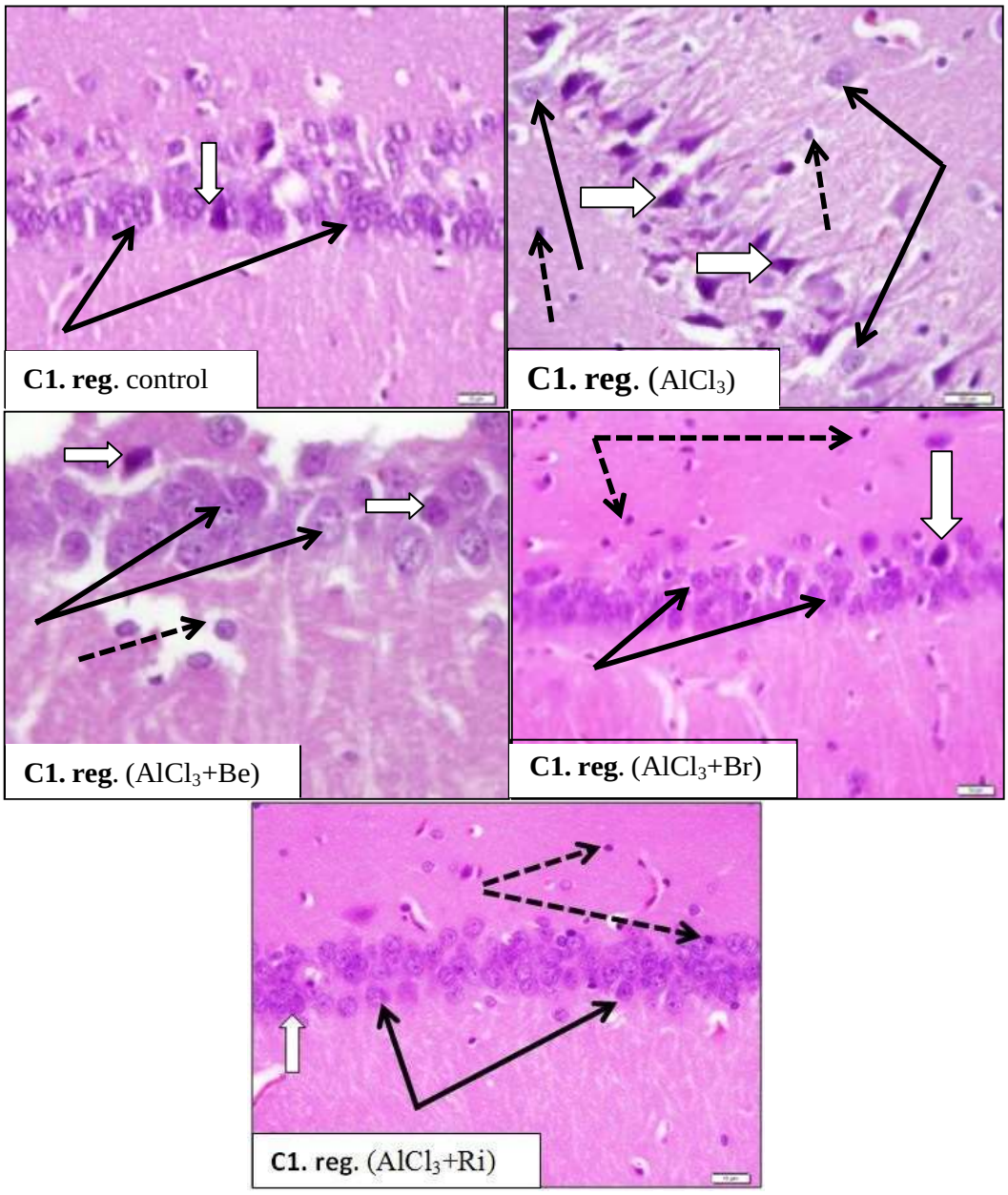

Fig. 3. Sections in rat hippocampus (c1. region) stained by H&E to show
Control: Showing normal hippocampal neurons with rounded nuclei and prominent nuclei (black arrows). Dark degenerated or apoptotic cells are few (white arrows). The dark small nuclei of glial cells are also few (dotted arrow)

tissues can lead to toxicity and dysfunction, the effects of the metal are usually linked with the local concentration. The blood-brain barrier has an active efflux through a monocarboxylate transporter in order to avoid aluminum deposition in the brain. However, this system can be affected by increasing the concentration of aluminum in the blood Reduction in serum aluminum concentration in treated group could be as a result of beetroot or broccoli extract interaction with the aluminum possibly by chelation to cause its elimination from the blood [29].

Increased hepatic enzyme levels may indicate cellular degeneration or destruction. In this study, a significant increase in serum ALT, AST were reported in Aluminium-exposure group compared to control. Similar results have been reported by other studies [30]. The increase in enzymes activity may be due to an indicator of liver injury or dysfunction [31]. Aluminum exposure can result in its accumulation in the liver and leads to liver damage because of increased enzymes levels in the serum [32]. The present data showed that serum creatinine and uric acid levels increased in Aluminium exposure group, which is accurately linked with the renal function dysfunction.

Treatment with beetroot showed improvement AST level compared with untreated group. Based previous study showed beetroot juice has an effect in reducing the level of creatinine which considers the marker of kidney damage. Beetrootfeeding can induce metabolic alterations toprotect against liver injury by preserving the integrity of the plasma membrane suppresses the leakage of enzymes and proteins [33].

Treated with broccoli extract showed improvement in serum liver enzymes and creatinine. It was concluded to that broccoli extract has the effect on liver injuries and on oxidative stress, which resulted to ameliorated serum biochemical parameters such as AST, ALP, and ALT. Also, this study indicated that the broccoli extract may be useful for the prevention of hepatotoxicity induced by oxidative stress.

Aluminum causes oxidative injury in the brain, liver and kidney. It can change the activity of antioxidant enzymes by inducing the production of free radical. Conversely, the antioxidant enzymes are active in defense against oxidative stress because it considers as free radical scavengers. In liver, Aluminium exposure group showed a significant increase in the activity of GSH while no significant changes were recorded in level of GPx and TA in aluminum chloride group. In kidney, Aluminium exposure group showed significant decrease in the activity of GSH and increase in TA level in Aluminium exposure group compared with control. Glutathione (GSH) acts as an antioxidant and a detoxifying agent [34]. Glutathione is important to counteract the damaging effects of oxidative stress and to preserve the normal reduced state of cells. Glutathione has an important role in the detoxification and metabolism of many xenobiotic compounds. The increased levels of reduced glutathione in Aluminium exposure animals would suggest an increased detoxificationcapacity of the liver. The changes in GSH may also reflect a response to aluminum-induced oxidative stress [35]. Glutathione is known to be one of the important components of an intracellular protective mechanism present in the cell and thus is an important determinant for the threshold of tissue damage caused by environmental chemicals. High doses of aluminum are able to reduce GSH levels and stimulate free radicals. Also, decreasing glutathione levels may cause by aluminum which effects in glutathione synthesis by reducingglutathione synthase activity [36].

The GPx is important antioxidant enzymes that constitute a supportive defense mechanism against free radical [37]. It plays an important role in protects of cells from oxidative stress by inhibiting lipid peroxidation. The increase of GPx in group exposure to aluminum chloride may dueto as a response to oxidative stress.

It was found that, increased total antioxidant capacity in rats treated with aluminum considered as a compensatory response against oxidative stress. Rats treatment with beetroot and broccoli extracts resulted in a partial recovery in reduced glutathione and glutathione peroxidase activity. This is due to the active components of beet roots and broccoli which prevent Free radicals production. Beetroot and broccoli consider a rich source of antioxidant compounds [38]. Free radical scavenging property of antioxidants compounds can lead to delay or inhibit of cellular damage. Beetroot juice can prevent oxidative stress induced by xenobiotic in rats. Betalains classified as one of the highest antioxidant activity in beetroot. The betalain in beetroot has been shown it protect cellular components from oxidative injury [39].

Broccoli has been found to have stronger antioxidant. It contains numerous bioactive substances with health-promoting properties including vitamins, glucosinolate and phenolic compounds [40]. Glucosinolate have effect in protection from oxidative stress through the elimination of ROS). In addition, Sulforaphane is one of broccoli component, is an antioxidant agent. In different in vivo and in vitro experiments was found that effective to reduce oxidative stress and damage of cell/tissue. Also, it has been reported to have potent neuroprotective effects [41-44].

Acetylcholinesterase an enzyme that breaks down the neurotransmitter acetylcholine. In this study Aluminium treated group showed a significant decrease in AChE activity in serum. The results agree with previous studies that demonstrated a decreased activity of AChE. It was reported a reduction in AChE activity in the brain as a response to Aluminium intoxication. Loss of cholinergic markers enzymes such as AChE is correlating with the degree of cognitive and loss of this enzymes is considered the most severe and the earliest of the biochemical changes to occur. Chronic Aluminium exposure has choline toxic effects and significant reduction of AChE activity is seen with Aluminium [45-47]. Aluminum can result in the production of freeradicals which lead to oxidative damage, and this may be responsible for the decreased AChE enzyme activity. On the other hand, Treatment with beetroot and broccoli showed improvement in AChE level compared with untreated group.

Neurotransmissions in the central nervous system are associated with learning and memory and consequently, changes in the neurotransmission would absolutely affect the behavioral responses [48-50].

The results of biochemical markers were supported wit histological examination that showed improvement in the rats treated with beet root or broccoli compred with untreated.

The functions of Cholinergic in the centralnervous system depend principally on acetylcholine. The change in the function of cholinergic neurotransmission can be due to aluminum chloride [51]. In this study, a significant increase in Acetylcholine level was reported in Aluminium-exposure group compared to the control group while the non-significant change in dopamine and norepinephrine was record in Aluminium exposure group. The high level of acetylcholine may be due to the low level of acetylcholinesterase that responsible for hydrolysis of acetylcholine to acetate and choline as shown in the results.

Rivastigmine previously showed to be beneficial in preventing neuronal degeneration byincreasing regional cerebral blood flow in animal model [52].

4. CONCLUSION

Neuroprotective role of broccoli in the present study which may result from its antioxidant properties due to its bioactive content such as glucosinolate, isothiocyanate, Sulforaphane, and flavonoids. Therefore, Broccoli can have a favorable effect on neurotoxicity due to their antioxidant and anti-inflammatory properties.

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