Modulation the neuro-toxicity induced by aluminum chloride in rats using beetroots and Broccli extracts

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Abstract

Backgound: The generation of oxidative stress can be referred to Aluminium toxic effect in animals and humans. This study aimed to evaluate the role of broccoli (Br) and beetroot (Be) extarcts as antioxidant that prevents oxidative stress that associated with aluminum toxicity. **Materials and methods:**FiftyWister female were grouped into five groups (each 10 rats):Group1: control group, administered drinking water only. Group 2: (Neurogenerative) which were induced by oral administration of aluminum chloride (20mg/kg b.w) daily for one month. Group 3: Rats given aluminum chloride were treated with Rivastigmine (Ri) (1 mg/kg b.w) as a reference drug daily for five weeks. Group 4: Rats given aluminum chloride were treated with beet root extract (50 mg/kg b.w) daily for six weeks. Group 5: **Results:**Rats given aluminum chloride were treated with broccoli extract (50 mg/kg b.w) daily for five weeks. ($AlCl_3$) group showed increased ($P<0.05$) in Ach level and non-significant ($P<0.05$) change in DOP and NE levels compared to control. ($AlCl_3$+Be) was non-significant ($P<0.05$) change in Ach, DOP and NE levels compared to ($AlCl_3$) group and showed a significant ($P<0.05$) increase in Ach level compared to control. ($AlCl_3$+Br) showed a significant ($P<0.05$) increase in NE level and non-significant ($P<0.05$) change in Ach and DOP levels compared to ($AlCl_3$) group. ($AlCl_3$+Ri) showed a significant ($P<0.05$) increase in Ach, DOP and NE levels compared to ($AlCl_3$) group. Also, showed a significant ($P<0.05$) increase in Ach and NE compared to control.**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.

Keywords: Neurotoxicity- Brocolli- beetroots-antioxidants.

Background

Aluminium is the most abundant metal on earth and it was also known as a neurotoxicant. (Cheng *et al.*, 2014)**.**Several studies have indicated neurobehavioral, neuropathological, neurochemical and neurophysical effects following Al exposure. It can react with other metals in the environment to form various complexes. The widely spread use of products that containing or made from aluminium is ensuring its

presence within our body. (Kumar and Gill, 2014)**.**Aluminium gets access to the human body through the environment, food and drugs.However, there is no recognized physiological function for aluminium inside the body and as a result, this metal can also produce reverse physiological effects (Nayak, 2002).The generation of oxidative stress can be referred to Aluminium toxic effect in animals and humans. The oxidative stress has been involved in thepathogenesis of various neurodegenerative conditions including Alzheimer's disease and Parkinson's disease. (Kumar and Gill, 2014).According to the easy access of Aluminium to the central nervous system under normal physiological conditions and accumulates in thebrain, it has been reported to alter the blood-brain barrier (BBB) (Cheng *et al.*, 2014). The main toxic effects of Al are in the brain, the nervous system and the kidney.The brain is sensitive to oxidative stress due to decreased levels of antioxidants and increased levels of free radicals following toxicity, especially it is considered the most susceptible to the toxic effect of Aluminium (Taïr *et al.*, 2016).Oxidants and antioxidants play a significant role in keeping the balance between the antioxidant system of the body and free radicals which produce by metabolism or derive from environmental sources. (Singh, Sharad and Kapur, 2004).Different compounds of antioxidant that extracted from natural products (nutraceuticals)have shown in different experimentsin vitro and in vivo model theneuroprotective activity of neuronal cell death and neurodegeneration. (Kelsey, Wilkins and Linseman, 2010).Nutraceuticals extracted from therapeutic plants have shown an essential role in drug discovery due to the complexity and abundance of secondary metabolites composition with the unique structure of the molecular composition bearing a significant amount of stereo-centres revealing high specificity connected to biological activity.There are different techniques in the process of extraction of natural products and these techniques are aimed at separating a certain class of compounds from a very complex matrix.(Segneanu *et al.*, 2016).A variety of natural products which contain bioactive nutrients play a critical role in prevention and cure of various neurodegenerative diseases and other neuronal dysfunctions(Essa *et al.*, 2012). Because the plants are important components in the dietary food chain the nutritional health is completely dependent on plant-based food. The plants provide all the mineral, organic and almost essential nutrients to humans so they are a good source of chemicals that promote health. (Jahangir, 2010)

Recently, red beetroot (Beta vulgaris rubra) has been a attracting the attention as afunctional food due to its biological activity and its potential role in the improved health and preventing the disease(Clifford *et al.*, 2015).Its native is the Mediterranean and widely cultivated in America, Europe and throughout India. Several parts of this plant are used in traditional Indian medicine for numerous therapeutic properties(Jain and Singhai, 2012).The researchers studied free radical scavenging activities and shown they were rich in antioxidants due to the presence of compounds such as carotenoids, folic acids, phenols, flavonoids(Kapur *et al.*, 2012). Commonly known by Broccoli (Brassica oleracea L. var. Italica) is a plant that known by its benefits in health improvement, it contains a high amount of nutrients such as vitamins, phenolic compounds, and dietary essential minerals; however, the interest into the benefits of broccoli that exceeded its role as a basic nutrition has been rise over the years.(Ares, Nozal and Bernal, 2013).Antioxidant activity in broccoli it was shown that prevents oxidative stress that associated with many diseases. (Hwang and Lim, 2015)This study aimed to evaluate the role of broccoliand beetroot extarctsas antioxidant that prevents oxidative stress that associated with aluminum toxicity.

Material and methods

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

Plant material and extract preparation:

The beetroot and broccoli were procured from the supermarket in Jeddah.

Preparation of beetroot and broccoli Extracts

To prepare Broccoli extract, 100 g of fresh Broccoli or beetroots samples were homogenized and extracted with 1 l of 70% MeOH 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 were completely removed using a rotary evaporator. Then the aqueous fractions containing bioactive compound were lyophilized and used as dry broccoli extracts (Georgiev et al., 2010).

Animal and experimental Design

FiftyWister female rat weighing between 100 to 150gm obtained from (KFMRC), Jeddah, Saudi Arabia. The Animal House, King Fahd Medical Research Center, King Abdul-Aziz University, approved the current study. Rats were randomly grouped into five groups (each 10 rats) as following

- Group1: control group, administered drinking water only.
- Group2: (Neurogenerative) which were induced by oral administration of aluminum chloride (20mg/kg b.w) daily for one month.
- Group3: Rats given aluminum chloride were treated with Rivastigmine (1 mg/kg b.w) as a reference drug daily for five weeks.
- Group 4: Rats given aluminum chloride were treated with beet root extract (50 mg/kg b.w) daily for six weeks.
- Group 5: Rats 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 with be confirmed by cervical dislocation. The blood was collected from the inner canthus of the eye using the heparinized capillary tube and centrifuged for 15 min at 3000×g to separate the serum for estimation of biochemical parameters.

Estimation of serum biochemical parameters

For assessment of liver functions, kinetic method for the determination of activities of aspartate aminotransferase (AST) and alanine aspartate aminotransferase (ALT) according to the recommendation of the Expert Panel of the IFCC (International federation of clinical chemistry) and activities of alkaline phosphatase (ALP) determine according to the recommendation of the German clinical chemistry association. While the serum total protein concentration was estimated by biuret method as described by Gornall et al., (1949).

Oxidative stress biochemical assays:

The following biochemical markers were measured in the liver and kidney 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).

Estimation of serum Aluminum level:

Serum samples from the experimental 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) (Wolf 1982), 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. The complex color is reasonably stable for twenty hours.

Estimation of serum Acetylcholinesterase level:

using AChE(Acetylcholinesterase) ELISA Kit (Catalog No: E-EL-R0355-Elabscience). This ELISA kit uses Competitive-ELISA as the method.

Estimation of brain neurotransmitters:

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

Statistical Analysis

The statistical analysis was performed using the (SPSS program for Windows, version 20).

Results

Groups / Parameters	Control	AlCl3	AlCl3+Be	AlCl3+Br	AlCl3+Ri
HB (g/dL)	14.66±0.33	14.31±0.63	14.64±0.93	15.04±0.79	15.74±0.66
P1		0.397	0.960	0.371	**0.012**
P2		-	0.334	**0.048**	**0.0001**
P3		-	-	0.259	**0.002**
P4		-	-	-	0.054
RBCs (10^6 cells/ml)	7.78±0.55	7.48±0.86	6.76±1.11	7.12±1.12	8.30±0.24
P1		0.538	**0.039**	0.185	0.278
P2		-	0.072	0.374	**0.041**
P3		-	-	0.373	**0.0001**
P4		-	-	-	**0.005**
Platelets (cells/ml)	803.60±134.36	634.80±125.21	559.50±100.97	506.22±106.01	791.10±142.53
P1		**0.015**	**0.001**	**0.0001**	0.852
P2		-	0.174	**0.027**	**0.007**
P3		-	-	0.347	**0.0001**
P4		-	-	-	**0.0001**
WBCs (10^3cells/ml)	10.10±0.81	3.93±0.81	10.02±1.47	5.29±1.31	8.60±1.49
P1		**0.0001**	0.910	**0.0001**	**0.035**
P2		-	**0.0001**	**0.024**	**0.0001**
P3		-	-	**0.0001**	**0.015**
P4		-	-	-	**0.0001**

Table (1): Comparison of blood indices in different studied groups.
Data is expressed as mean+/- standard deviation (STD), P1: significance versus control; P2: significance versus ALCL3; P3: significance versus AlCl3 + Be; P4: significance versus ALCL3+ Br. Comparison between groups was made using OneWay ANOVA (LSD) test

In table(1), group of ($AlCl_3$) showed non-significant (P>0.05) change in Hb level and RBCs count compared with control. While showing a significant (p<0.05) decrease in platelets level and WBCs compared with control group. For ($AlCl_3$+Be) group was a significant (P<0.05) decrease in RBCs count and platelets level compared to control and increase in WBCs compared with group of ($AlCl_3$). While showing non-significant (P>0.05) change in Hb, RBCs count and platelets levels compared to group of ($AlCl_3$). Group of ($AlCl_3$+Br) was a significant (P<0.05) increase in Hb level and WBCs and significant (P<0.05) decrease in platelets level compared to group of ($AlCl_3$) and showing non-significant (P>0.05) change in RBCs count compared to group of ($AlCl_3$). For ($AlCl_3$+Ri) group was a significant (P<0.05) increase in Hb level, RBCs, platelets WBCs count compared to group of ($AlCl_3$).

Groups / Parameters	Control	ALCL3	AlCl3+Be	AlCl3+Br	AlCl3+Ri
Aluminum (mg/ml)	0.17±0.02	0.32±0.04	0.24±0.03	0.22±0.03	0.26±0.05
P1		**0.0001**	**0.0001**	**0.002**	**0.0001**
P2		-	**0.0001**	**0.0001**	**0.0001**
P3		-	-	0.330	0.231
P4		-	-	-	**0.039**

Table (2): Comparison of Aluminum level in different studied groups.
Data is expressed as mean+/- standard deviation (STD), P1: significance versus control; P2: significance versus AlCl3; P3: significance versus AlCl3 + Be; P4: significance versus AlCl3+ Br. Comparison between groups was made using OneWay ANOVA (LSD) test.

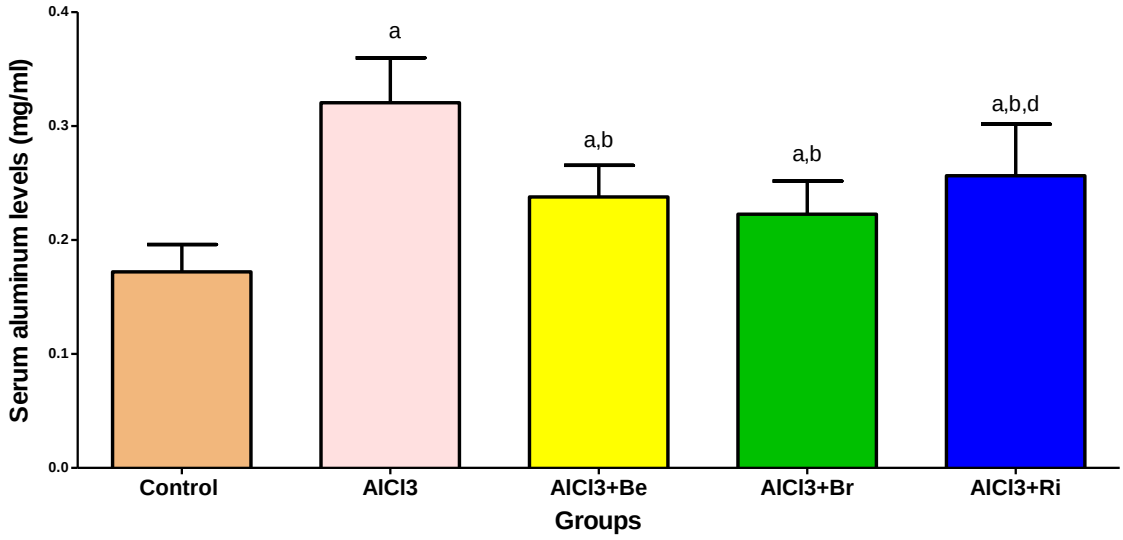

Figure (1): Comparison of serum aluminum level in different studied groups.
Control: Control group, $AlCl_3$: group exposure to Aluminium chloride, $AlCl_3$+Be: group treated with beetroot extract, $AlCl_3$+Br: group treated with broccoli extract, $AlCl_3$+Ri: group treated with Rivastigmine.

In (Table2) Figure (1)Aluminium level was significant ($P<0.05$) increase in group of ($AlCl_3$) compared with control. While treatment groups showed a significant ($P<0.05$) decrease in Aluminium level compared to ($AlCl_3$) and control group.

Groups / Parameters	Control	ALCL3	AlCl3+Be	AlCl3+Br	AlCl3+Ri
ALT (U/L)	10.10±1.81	15.77±2.29	16.03±2.44	21.45±3.93	16.57±4.91
P1		**0.0001**	**0.007**	**0.038**	**0.015**
P2		-	**0.006**	**0.001**	**0.003**
P3		-	-	0.535	0.760
P4		-	-	-	0.745
AST (U/L)	21.65±3.49	43.56±7.28	39.96±9.21	49.31±10.95	27.74±7.23
P1		**0.0001**	**0.0001**	**0.0001**	0.095
P2		-	0.331	0.133	**0.0001**
P3		-	-	**0.014**	**0.001**
P4		-	-	-	**0.0001**
ALP (U/L)	163.67±30.53	179.77±38.03	128.84±23.04	82.81±27.16	146.67±39.90
P1		0.298	**0.020**	**0.0001**	0.224
P2		-	**0.002**	**0.0001**	**0.036**
P3		-	-	**0.003**	0.222
P4		-	-	-	**0.0001**
Protein (g/dl)	9.71±1.34	8.74±1.45	9.86±0.98	9.56±0.96	6.78±0.72
P1		0.061	0.774	0.768	**0.0001**
P2		-	**0.036**	0.122	**0.0001**
P3		-	-	0.570	**0.0001**
P4		-	-	-	**0.0001**
Albumin (g/dl)	4.81±0.89	4.12±0.46	4.05±0.74	4.07±0.44	4.05±0.58
P1		**0.024**	**0.013**	**0.022**	**0.013**
P2		-	0.812	0.870	0.819
P3		-	-	0.951	0.992
P4		-	-	-	0.959

Table (3): Comparison of liver function tests in different studied groups.
Data is expressed as mean+/- standard deviation (STD), P1: significance versus control; P2: significance versus ALCL3; P3: significance versus AlCl3 + Be; P4: significance versus ALCL3+ Br. Comparison between groups was made using OneWay ANOVA (LSD) test.

In table (3), group of ($AlCl_3$) 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. Also group of ($AlCl_3$) was non-significant ($P>0.05$) change in ALP and protein level compared to control.

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

Group of ($AlCl_3$+Be) was a significant ($P>0.05$) decrease in ALP and increase in protein compared to ($AlCl_3$) group. While was a significant ($P>0.05$) increase in AST and was a significant ($P>0.05$) decrease ALP and albumin levels compared to control.

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

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

Groups / Parameters	Control	ALCL3	AlCl3+Be	AlCl3+Br	AlCl3+Ri
Uric acid (mg/dl)	4.82±1.32	6.10±1.41	6.28±1.47	4.84±1.41	6.49±1.08
P1		**0.039**	**0.019**	0.978	**0.008**
P2		**-**	0.766	**0.047**	0.518
P3		**-**	-	**0.024**	0.726
P4		**-**	-	-	**0.010**
Urea (mg/dl)	40.93±5.74	37.66±5.10	41.98±8.24	37.50±4.81	34.72±2.97
P1		0.214	0.687	0.205	**0. 021**
P2		-	0.094	0.952	0.251
P3		-	-	0.091	**0.006**
P4		-	-	-	0.289
Creatinine (mg/dl)	1.33±0.42	2.48±1.66	1.76±0.72	2.34±1.00	1.67±0.83
P1		**0.016**	0.351	0.168	0.477
P2		**-**	0.123	0.383	0.091
P3		**-**	-	0.477	0.843
P4		**-**	-	-	0.477

Table (4): Comparison of kidney function tests in different studied groups.
Data is expressed as mean+/- standard deviation (STD), P1: significance versus control; P2: significance versus ALCL3; P3: significance versus AlCl3 + Be; P4: significance versus ALCL3+ Br. Comparison between groups was made using OneWay ANOVA (LSD) test.

In table (4) group of ($AlCl_3$) was a significant ($P<0.05$) increase in uric acid and creatinine levels, and non-significant ($P>0.05$) change in urea level compared to control group.

($AlCl_3$+Be) and ($AlCl_3$+Ri) groups showed non-significant ($P>0.05$) change 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 was a significant ($P<0.05$) decrease in uric acid and non-significant ($P>0.05$) change in urea and creatinine level compared to ($AlCl_3$) group.

Groups / Parameters	Control	ALCL3	AlCl3+Be	AlCl3+Br	AlCl3+Ri
GSH-L (mmol/l)	5.08±1.99	6.70±1.17	7.34±1.75	7.69±0.79	8.55±1.31
P1		**0.018**	**0.001**	**0.0001**	**0.0001**
P2			0.330	0.151	**0.007**
P3				0.616	0.075
P4					0.211
GPX-L (mmol/l)	0.181±0.005	0.185±0.003	0.174±0.009	0.175±0.004	0.176±0.003
P1		0.191	**0.010**	**0.002**	**0. 037**
P2		-	**0.0001**	**0.0001**	**0.001**
P3		-	-	0.806	0.631
P4		-	-	-	0.819
TA-L (mg/l)	2.38±0.38	2.06±0.79	2.32±0.81	2.48±0.48	2.61±0.50
P1		0.247	0.829	0.738	0.409
P2			0.344	0.146	0.051
P3				0.587	0.299
P4					0.637

Table (5): Comparison of oxidative stress markers in liver homogenate in different studied groups.
Data is expressed as mean+/- standard deviation (STD), P1: significance versus control; P2: significance versus ALCL3; P3: significance versus AlCl3 + Be; P4: significance versus ALCL3+ Bri. Comparison between groups was made using OneWay ANOVA (LSD) test.

In table (5) For liver homogenate,group of ($AlCl_3$) was asignificant ($P<0.05$) increase in GSH level and non-significant ($P<0.05$) change in GPX and TA compared to control group.

($AlCl_3$+Ri) group showed a significant ($P<0.05$) increase in level of GSH and non-significant ($P<0.05$) change for groups of ($AlCl_3$+Be) and($AlCl_3$+Br) compared to ($AlCl_3$) group.

All treatment group showed a significant ($P<0.05$) decrease in GPX level and non-significant ($P<0.05$) change in TA level compared to ($AlCl_3$) and control groups.

Groups / Parameters	Control	ALCL3	AlCl3+Be	AlCl3+Br	AlCl3+Ri
GSH-K (mmol/l)	6.43±1.51	5.04±1.56	5.68±0.97	6.31±0.58	5.59±0.91
P1		**0.012**	0.164,	0.829	0.121
P2		-	0.232	**0.024**	0.301
P3		-	-	0.252	0.868
P4		-	-	-	0.193
GPX-K (mmol/l)	0.175±0.007	0.180±0.005	0.175±0.005	0.160±0.013	0.167±0.008
P1		0.207	0.968	**0.001**	**0.045**
P2		-	0.170	**0.0001**	**0.001**
P3		-	-	**0.0001**	**0.037**
P4		-	-	-	0.079
TA-K (mg/l)	1.04±0.22	1.78±0.47	1.85±0.41	2.07±0.66	2.60±0.23
P1		**0.0001**	**0.0001**	**0.0001**	**0.0001**
P2		-	0.737	0.144	**0.0001**
P3		-	-	0.252	**0.0001**
P4		-	-	-	**0.009**

Table (6): Comparison of oxidative stress markers in kidney homogenate in different studied groups. Data is expressed as mean+/- standard deviation (STD), P1: significance versus control; P2: significance versus ALCL3; P3: significance versus AlCl3 + Be; P4: significance versus ALCL3+ Br. Comparison between groups was made using OneWay ANOVA (LSD) test.

In Table (6) For kidney homogenate, group of ($AlCl_3$)showed a significant ($P<0.05$) decrease in GSH level and increase in TA level compared to control group and non-significant ($P<0.05$) change for GPX level.($AlCl_3$+Be) group showed a non-significant ($P<0.05$) change in GSH,GPX and TA levels compared to ($AlCl_3$) group and a significant ($P<0.05$) increase in TA level compared to control.

($AlCl_3$+Br) group showed a significant ($P<0.05$) increase in GSH level compared to group of ($AlCl_3$) and a significant ($P<0.05$) increase TA level compared to control.

($AlCl_3$+Br) and ($AlCl_3$+Ri) was a significant ($P<0.05$) decrease in GPX level compared to ($AlCl_3$) and control groups.

Group of ($AlCl_3$+Ri) was a significant ($P<0.05$) increase in TA level compared to ($AlCl_3$) and control groups and non-significant ($P<0.05$) change in GSH level.

Groups / Parameters	Control	ALCL3	AlCl3+Be	AlCl3+Br	AlCl3+Ri
AChE (ng/ml)	5.47±0.35	4.60±0.65	5.26±0.96	5.56±0.81	4.87±0.70
P1		**0.015**	0.543	0.781	0.081
P2		**-**	0.059	**0.007**	0.412
P3		**-**	**-**	0.377	0.252
P4		**-**	**-**	**-**	**0.044**

Table (7): Comparison of Acetylcholinesterase level in different studied groups.
Data is expressed as mean+/- standard deviation, a: significance versus control; b: significance versus ALCL3; d: significance versus AlCl3+ Br. Comparison between groups was made using OneWay ANOVA (LSD) test.

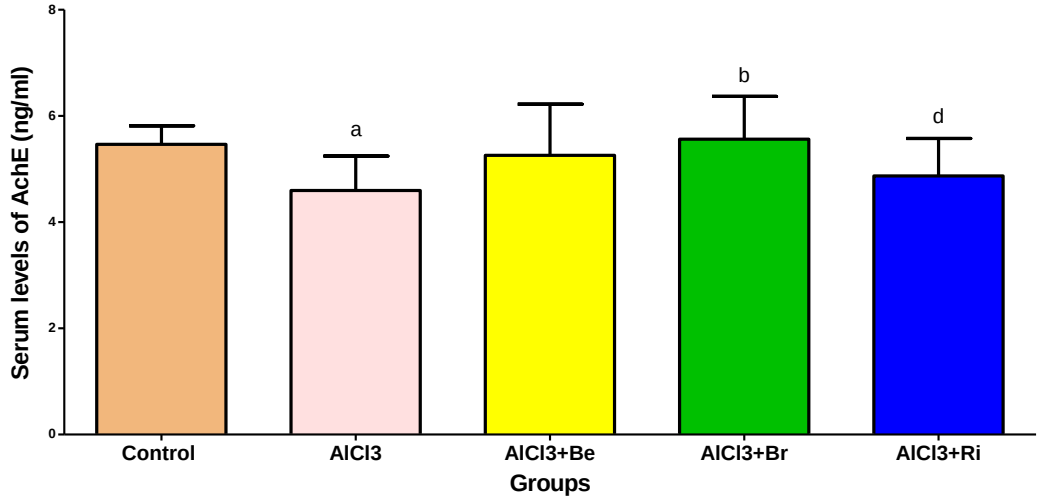

Figure (2): Comparison of serum levels of AChE in different studied groups.
Control: Control group, $AlCl_3$: group exposure to Aluminium chloride, $AlCl_3$+Be: group treated with beetroot extract, $AlCl_3$+Br: group treated with broccoli extract, $AlCl_3$+Ri: group treated with Rivastigmine.

In table (7) figure (2), group of ($AlCl_3$) was a significant ($P<0.05$) decrease compared to control group.($AlCl_3$+Br) group was a significant ($P<0.05$) increase in AChE level compared to ($AlCl_3$) group.($AlCl_3$+Be) and ($AlCl_3$+Ri) showed non-significant ($P<0.05$) change in AChE level compared to ($AlCl_3$) group

Groups / Parameters	Control	ALCL3	AlCl3+Be	AlCl3+Br	AlCl3+Ri
Ach (ng/ml)	2.35±0.08	2.44±0.10	2.48±0.10	2.47±0.08	2.53±0.07
P1		**0.029**	**0.002**	**0.004**	**0.0001**
P2		-	0.321	0.408	**0.032**
P3		-	-	0.888	0.233
P4		-	-	-	0.194
DOP (ng/ml)	0.56±0.02	0.55±0.01	0.56±0.01	0.57±0.02	0.57±0.01
P1		0.279	0.78	0.554	0.259
P2		-	0.428	0.107	**0.035**
P3		-	-	0.401	0.174
P4		-	-	-	0.596
NE (ng/ml)	0.031±0.004	0.028±0.007	0.025±0.005	2.311±0.423	1.878±0.564
P1		0.982	0.971	**0.0001**	**0.0001**
P2		-	0.988	**0.0001**	**0.0001**
P3		-	-	**0.0001**	**0.0001**
P4		-	-	-	**0.008**

Table (8): Comparison of neurotransmitters levels in different studied groups.
Data is expressed as mean+/- standard deviation (STD), P1: significance versus control; P2: significance versus ALCL3; P3: significance versus AlCl3 + Be; P4: significance versus ALCL3+ Br. Comparison between groups was made using OneWay ANOVA (LSD) test.

In table (8), ($AlCl_3$) group showed a significant ($P<0.05$) increase in Ach level and non-significant ($P<0.05$) change in DOP and NE levels compared to control.
($AlCl_3$+Be)was non-significant ($P<0.05$) change in Ach, DOP and NE levels compared to ($AlCl_3$) group and showed a significant ($P<0.05$) increase in Ach level compared to control.
($AlCl_3$+Br) showed a significant ($P<0.05$) increase in NE level and non-significant ($P<0.05$) change in Ach and DOP levels compared to ($AlCl_3$) group.
($AlCl_3$+Ri) showed a significant ($P<0.05$) increase in Ach, DOP and NE levels compared to ($AlCl_3$) group. Also, showed a significant ($P<0.05$) increase in Ach and NE compared to control.

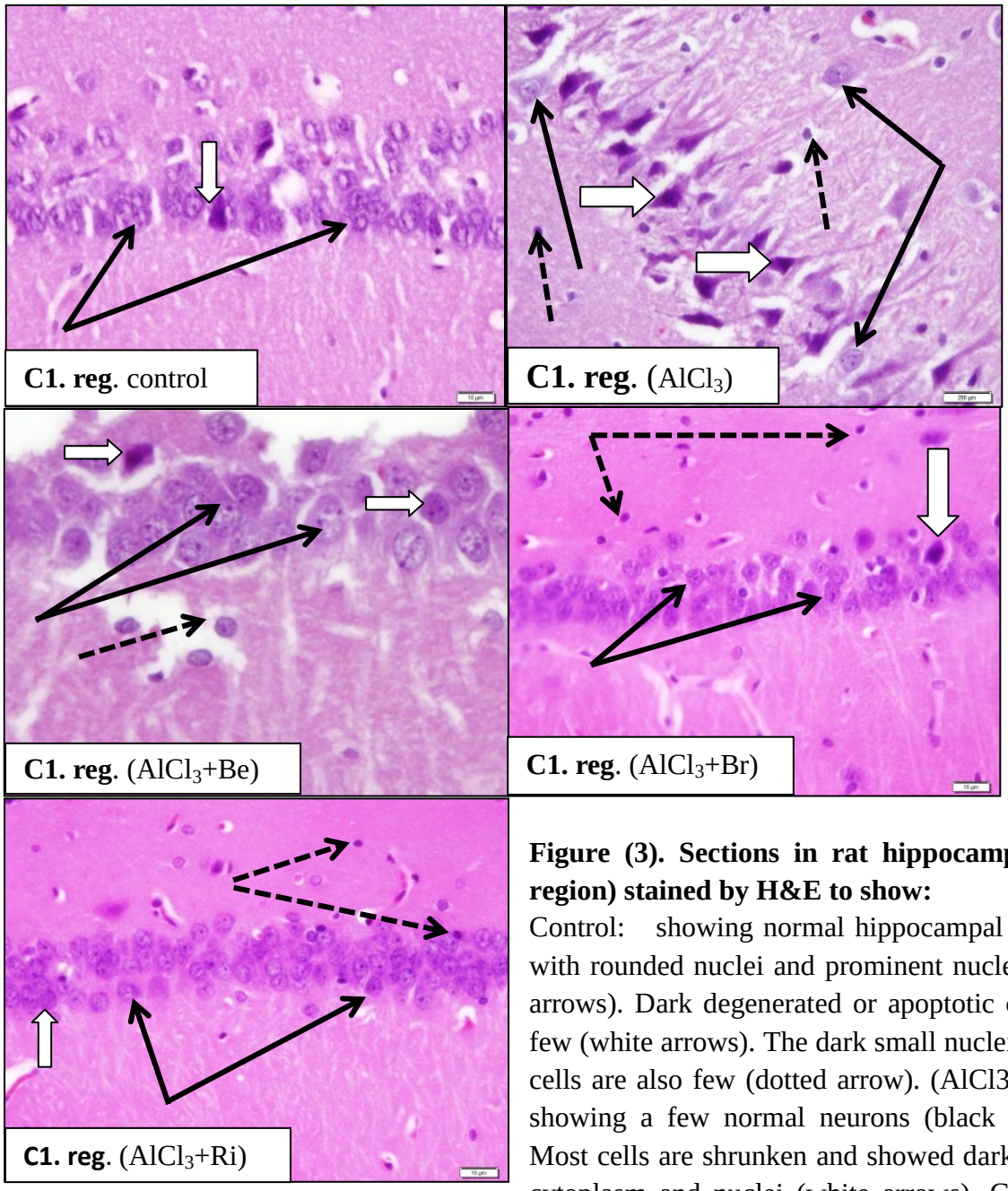

Figure (3). Sections in rat hippocampus (c1. region) stained by H&E to show:
Control: showing normal hippocampal neurons with rounded nuclei and prominent nuclei (black arrows). Dark degenerated or apoptotic cells are few (white arrows). The dark small nuclei of glial cells are also few (dotted arrow).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

Discussion:

Aluminum is 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, may prove to be useful in the reducing the progression of various pathologies associated with oxidative stress including neurodegeneration (Goschorska *et al.*, 2017). Use of plant extracts has been observed to be as effective as antioxidants in Aluminium neurotoxicity (Kumar and Gill, 2014). The decrease in level of hemoglobin and Red blood cells in Aluminium exposure group in the present study as shown in other studies (Geyikoglu & Tu 2012; Is et al. 2011), but this decrease was not statistically significant. The ages of animals at the time of exposure, different times of chronic administration and different aluminum species may the reason for this variation between the present and previous studies. One of the basic mechanisms of the toxic action of heavy metal on mammals is erythrocyte destruction (Geyikoglu and Tu, 2012). Erythrocyte is one of the major target tissues for aluminum toxicities (Zhang et al., 2016), and a hemolytic effect of Aluminium chloride is an indicator of a decline in RBC count (Harsha and Anilakumar, 2013). The aluminum can inhibition the activity of enzymes that involved in the haem biosynthetic pathway or interference with cellular iron uptake and utilization and that can affect in hemoglobin which leads to the reduction of synthesis and this can cause anemia. (Osman *et al.*, 2012). According to (Osińska, Kanoniuk and Kusiak, 2004) the defensive mechanisms which connected with white blood cells are inhibited by aluminum and this may due to an decreased level of white blood cells in group exposure to aluminum. 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 (Kovarovič *et al.*, 2017). Also, broccoli is useful to health because of its a large content of health-enhancing compounds such as glucosinolate, vitamins, phenolic compounds, and dietary essential minerals (Ares, Nozal and Bernal, 2013). Vitamin 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. Group treatment with rivastigmine showed increase significant 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. (Cheng *et al.*, 2014).

The result of this study showed that the Aluminium exposure group had a significant increase in serum level of Aluminium and these results were in accordance with those recorded by (Ekong *et al.*, 2017). Aluminium interferes with most physical and cellular processes (Jaishankar *et al.*, 2014). According to (Becaria et al. 2002) reported 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 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 (Oteiza, 2008). 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.

Increased enzyme levels of hepatic cells may cause cellular degeneration or destruction. In this study, significant increase in serum ALT, AST were reported in Aluminium-exposure group compared to control. Similar results have been reported by other studies (Oe et al. 2016; Bakour et al. 2017). The increase in enzymes activity may be due to an indicator of liver injury or dysfunction (Oe, Ac and Dawoye, 2016). Aluminum exposure can result in its accumulation in the liver and leads to liver damage because of increased enzymes levels in the serum. (Lakshmi, Sudhakar and Anisha, 2014). The present data showed that serum creatinine and uric acid levels increase 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 on a study carried out by (Krajka-Kuźniak et al. 2012) showed beetroot juice has an effect in reducing the level of creatinine which considers the marker of kidney damage. Beetroot feeding can induce metabolic alterations to protect against liver injury by preserving the integrity of the plasma membrane suppresses the leakage of enzymes and proteins.

Treated with broccoli extract showed improvement in serum biochemical parameters of liver and kidney function. Based on the study carried out by (El-baz et al. 2016) it 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, the results of the 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 (Khafaga, 2017). it can change the activity of antioxidant enzymes by inducing the production of free radical (Zahedi-amiri and Taravati, 2018). Conversely, the antioxidant enzymes are active in defense against oxidative stress because it considers as free radical scavengers (Mahgoub M. Ahmed and Nasr, 2015). In liver homogenate, Aluminium exposure group showed significant increase in the activity of GSH and this result were in accordance with those recorded by (Bhasin, Singla and Dhawan, 2012), while no significant changes were recorded in level of GPx and TA in aluminum chloride group. In kidney homogenate, 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 (Ghorbel *et al.*, 2016). Glutathione is important to counteract the damaging effects of oxidative stress and to preserve the normal reduced state of cells.(Sarhan, Shati and Elsaid, 2014). glutathione has an important role in the detoxification and metabolism of many xenobiotic compounds (Shrivastava, 2012). According to (Bhasin, Singla and Dhawan, 2012) the increased levels of reduced glutathione in Aluminium exposure animals would suggest an increased detoxification capacity of the liver. The changes in GSH may also reflect a response to aluminum-induced oxidative stress (Abubakar, Taylor and Ferns, 2003). 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 (Bhasin, Singla and Dhawan, 2012). 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 reducing glutathione synthase activity (Al-Hashem, 2009).

GPx is important antioxidant enzymes. They constitute a supportive defense mechanism against free radical (Sudjarwo, Sudjarwo and Koerniasari, 2017). it plays

an important role in protects of cells from oxidative stress by inhibiting lipid peroxidation (Ighodaro and Akinloye, 2017). the increase of GPx in group exposure to aluminum chloride may due to as a response to oxidative stress.

According to (Zahedi-amiri and Taravati, 2018) recorded increased total antioxidant capacity in rats treated with aluminum considered as a compensatory response against oxidative stress. In liver homogenates, 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 (Borowska, Ciska and Zielin, 2008; Clifford *et al.*, 2015). Free radical scavenging property of antioxidants compounds can lead to delay or inhibit of cellular damage (Lobo *et al.*, 2010). According to (Kujawska et al. 2009), Beetroot juice can prevent oxidative stress induced by xenobiotic in rats. Betalains classified as one of the highest antioxidant activity in beetroot (Aguila and Paschoalin, 2017). The betalain in beetroot has been shown it protect cellular components from oxidative injury (Clifford *et al.*, 2015).

Broccoli has been found to have stronger antioxidant. (Borowska, Ciska and Zielin, 2008). It contains numerous bioactive substances with health-promoting properties including vitamins, glucosinolate and phenolic compounds(Sarhan, Shati and Elsaid, 2014). Glucosinolate have effect in protection from oxidative stress through the elimination of ROS (Sharma, 2016). 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 (Mahgoub M. Ahmed and Nasr, 2015). Also, it has been reported to have potent neuroprotective effects (Zhang *et al.*, 2017).

AChE enzyme always receives a big interest in the study of Aluminium neurotoxicity (Taïr *et al.*, 2016). Acetylcholinesterase an enzyme that breaks down the neurotransmitter acetylcholine (Mathiyazahan and Thenmozhi, 2015). In this study Aluminium treated group was a a significant decrease in Acetylcholinesterase activity in serum. The results agree with previous studies that demonstrated a decreased activity of acetylcholinesterase (Jelenković *et al.*, 2014; Nampoothiri *et al.*, 2015). According to (Lakshmi, Sudhakar and Anisha, 2014) 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 (Chakrabarty *et al.*, 2012). Aluminum can result in the production of free radicals 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 (Bhalla, Garg and Dhawan, 2010).

The functions of Cholinergic in the central nervous system depend principally on acetylcholine. The change in the function of cholinergic neurotransmission can be due to aluminum chloride (Maya *et al.*, 2016). 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 by increasing regional cerebral blood flow in animal model (Park et al. 2017).

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 .

Ethical Approval:

As per international standard or university standard ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

Reference

Abubakar, M. G., Taylor, A. and Ferns, G. A. A. (2003) 'Aluminium administration is associated with enhanced hepatic oxidant stress that may be offset by dietary vitamin E in the rat', pp. 49–54.

Aguila, E. M. Del and Paschoalin, V. M. F. (2017) 'World ' s largest Science , Technology & Medicine Open Access book publisher Nutritional , Bioactive and Physicochemical Characteristics of Different Beetroot Formulations'.

Al-Hashem, F. (2009) 'Camel ' s Milk Protects against Aluminum Chloride-Induced Toxicity in the Liver and Kidney of White Albino Rats', 5(3), pp. 98–108.

Ares, A. M., Nozal, M. J. and Bernal, J. (2013) 'Extraction, chemical characterization and biological activity determination of broccoli health promoting compounds.', *Journal of chromatography. A*, 1313, pp. 78–95.

Bakour, M., Nawal, N. S. A., Menyiy, E., Imtara, H. and Transaminase, A. Á. C. Á. (2017) 'Antioxidant activity and protective effect of bee bread (honey and pollen) in aluminum-induced anemia , elevation of inflammatory makers and hepato-renal toxicity', *Journal of Food Science and Technology*. Springer India, 54(13), pp. 4205–4212.

Bhalla, P., Garg, M. L. and Dhawan, D. K. (2010) 'Neurochemistry International Protective role of lithium during aluminium-induced neurotoxicity', *Neurochemistry International*. Elsevier Ltd, 56(2), pp. 256–262.

Bhasin, P., Singla, N. and Dhawan, D. K. (2012) 'Protective Role of Zinc During', 2011(June 2011), pp. 320–327.

Biondo, P. B. F., Boeing, J. S., Barizão, É. O., Souza, N. E. de, Matsushita, M., Oliveira, C. C. de, Boroski, M. and Visentainer, J. V. (2014) 'Evaluation of beetroot (Beta vulgaris L.) leaves during its developmental stages: a chemical composition study', *Food Science and Technology (Campinas)*, 34(1), pp. 94–101.

Borowska, E. J., Ciska, Æ. E. and Zielin, Æ. H. (2008) 'Content of selected bioactive components and antioxidant properties of broccoli (Brassica oleracea L .)', pp. 459–465.

Chakrabarty, M., Bhat, P., Kumari, S., Souza, A. D., Bairy, K. L., Chaturvedi, A., Natarajan, A., G, M. R. K. and Kamath, S. (2012) 'Cortico-hippocampal salvage in chronic aluminium induced neurodegeneration by Celastrus paniculatus seed oil : Neurobehavioural , biochemical , histological study', 3(2), pp. 161–172.

Cheng, D., Xi, Y., Cao, J., Cao, D., Ma, Y. and Jiang, W. (2014) 'Protective

effect of apple (Ralls) polyphenol extract against aluminum-induced cognitive impairment and oxidative damage in rat', *NeuroToxicology*.

Clifford, T., Howatson, G., West, D. J. and Stevenson, E. J. (2015) 'The potential benefits of red beetroot supplementation in health and disease', *Nutrients*, 7(4), pp. 2801–2822.

Ekong, M. B., Ekpo, M. M., Akpanyung, E. O. and Nwaokonko, D. U. (2017) 'Neuroprotective effect of Moringa oleifera leaf extract on aluminium-induced temporal cortical degeneration'. Metabolic Brain Disease, pp. 1437–1447.

Essa, M. M., Vijayan, R. K., Castellano-Gonzalez, G., Memon, M. A., Braidy, N. and Guillemin, G. J. (2012) 'Neuroprotective Effect of Natural Products Against Alzheimer's Disease', *Neurochemical Research*. Springer US, 37(9), pp. 1829–1842.

Geyikoglu, F. and Tu, H. (2012) 'The genotoxic , hepatotoxic , nephrotoxic , haematotoxic and histopathological effects in rats after aluminium chronic intoxication', 29(9), pp. 780–791.

Ghorbel, I., Amara, I. Ben, Ktari, N. and Elwej, A. (2016) 'Aluminium and Acrylamide Disrupt Cerebellum Redox States , Cholinergic Function and Membrane-Bound ATPase in Adult Rats and Their Offspring', *Biological Trace Element Research*. Biological Trace Element Research, pp. 335–346.

Goschorska, M., Giewoń, K., Hasior, N., Gutowska, I., Baranowska-Bosiacka, I., Rać, M. E. and Chlubek, D. (2017) 'The protective properties of selected naturally occurring antioxidants of plant origin against fluoride-induced neurotoxicity', *Fluoride*, 50(2), pp. 203–212.

Harsha, S. N. and Anilakumar, K. R. (2013) 'Protection against aluminium neurotoxicity : A repertoire of lettuce antioxidants', *Biomedicine & Aging Pathology*. Elsevier Masson SAS, 3(4), pp. 179–184.

Hwang, J. H. and Lim, S. Bin (2015) 'Antioxidant and anticancer activities of broccoli by-products from different cultivars and maturity stages at harvest', *Preventive Nutrition and Food Science*, 20(1), pp. 8–14.

Ighodaro, O. M. and Akinloye, O. A. (2017) 'First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid', *Alexandria Journal of Medicine*. Alexandria University Faculty of Medicine, pp. 1–7.

Is, I., Aziz, A. and Zabut, B. M. (2011) 'Determination of blood indices of albino rats treated with aluminum chloride and investigation of antioxidant effects of vitamin E and C Materials & Methods', 13(8), pp. 1–7.

Jahangir, M. (2010) *Stress response and health affecting compounds in Brassicaceae*.

Jain, N. K. and Singhai, A. K. (2012) 'Protective role of Beta vulgaris L. leaves extract and fractions on ethanol-mediated hepatic toxicity', *Acta Pol Pharm*,

69(5), pp. 945–950.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B. and Beeregowda, K. N. (2014) ‘Toxicity , mechanism and health effects of some heavy metals’, 7(2), pp. 60–72.

Jelenković, A., Jovanović, M. D., Stevanović, I., Petronijević, N., Bokonjić, D., Živković, J. and Igić, R. (2014) ‘Influence of the green tea leaf extract on neurotoxicity of aluminium chloride in rats’, *Phytotherapy Research*, 28(1), pp. 82–87.

Kapur, A., Sati, S., Ranjan, A. and Gupta, P. (2012) ‘Screening methanolic extracts of Beta vulgaris roots for photoprotective activity’, *International Journal of Pharmacy and Pharmaceutical Sciences*, 4(SUPPL. 4), pp. 124–127.

Kelsey, N. A., Wilkins, H. M. and Linseman, D. A. (2010) ‘Nutraceutical antioxidants as novel neuroprotective agents.’, *Molecules (Basel, Switzerland)*, 15(11), pp. 7792–814.

Khafaga, A. F. (2017) ‘Exogenous phosphatidylcholine supplementation retrieve aluminum-induced toxicity in male albino rats’. Environmental Science and Pollution Research, pp. 15589–15598.

Kovarovič, J., Bystrická, J., Tomáš, J. and Lenková, M. (2017) ‘The influence of variety on the content of bioactive compounds in beetroot (Beta vulgaris L.)’, *Potravinarstvo Slovak Journal of Food Sciences*, 11(1), pp. 106–112.

Kumar, V. and Gill, K. D. (2014) ‘Oxidative stress and mitochondrial dysfunction in aluminium neurotoxicity and its amelioration: A review’, *NeuroToxicology*.

Lakshmi, B. V. S., Sudhakar, M. and Anisha, M. (2014) ‘NeuroToxicology Neuroprotective role of hydroalcoholic extract of Vitis vinifera against aluminium-induced oxidative stress in rat brain’, *Neurotoxicology*. Elsevier B.V., 41, pp. 73–79.

Leonoudakis, D., Rane, A., Angeli, S., Lithgow, G. J. and Andersen, J. K. (2017) ‘Anti-inflammatory and neuroprotective role of natural product securinine in activated glial cells . Implications for Parkinson ’ s disease’, 2017.

Lobo, V., Patil, A., Phatak, A. and Chandra, N. (2010) ‘Free radicals , antioxidants and functional foods : Impact on human health’.

Mahgoub M. Ahmed and Nasr, S. A. (2015) ‘Protective Effect of Broccoli and Ferulic Acid on Imidacloprid-Induced Hepatotoxicity in Rat’, *The Egyptian Journal of Biochemistry & Molecular Biology*, 32(2), pp. 1–15.

Mahieu, S., Contini, C., Gonzalez, M., Millen, N. and Elias, M. M. (2000) ‘Aluminum toxicity . Hematological effects’, 111, pp. 235–242.

Mathiyazahan, D. B. and Thenmozhi, A. J. (2015) ‘Protective effect of black tea extract against aluminium chloride-induced Alzheimer ’ s disease in rats :

A behavioural , biochemical and molecular approach', *Journal of Functional Foods*. Elsevier Ltd, 16, pp. 423–435.

Maya, S., Prakash, T., Madhu, K. Das and Goli, D. (2016) 'ScienceDirect Multifaceted effects of aluminium in neurodegenerative diseases : A review', *Biomedicine et Pharmacotherapy*. Elsevier Masson SAS, 83, pp. 746–754.

Nampoothiri, M., John, J., Kumar, N., Mudgal, J., Nampurath, G. K. and Chamallamudi, M. R. (2015) 'Modulatory Role of Simvastatin against Aluminium Chloride-Induced Behavioural and Biochemical Changes in Rats', 2015.

Nayak, P. (2002) 'Aluminum: Impacts and Disease', *Environmental Research*. Academic Press, 89(2), pp. 101–115.

Oe, Y., Ac, O. and Dawoye, Y. (2016) 'Effects of Aqueous Extract of Hymenocardia acida Leaves on Aluminium Chloride-Induced Toxicity in Male Albino Rats', 3(2), pp. 4–8.

Osińska, E., Kanoniuk, D. and Kusiak, A. (2004) 'Aluminum hemotoxicity mechanisms.', *Annales Universitatis Mariae Curie-Sklodowska. Sectio D: Medicina*, 59(1), pp. 411–6.

Osman, H. M., Shayoub, M. E., Babiker, E. M., Osman, B. and Elhassan, A. M. (2012) 'Effect of Ethanolic Leaf Extract of Moringa oleifera on Aluminum-induced Anemia in White Albino Rats', 5(4), pp. 255–260.

Oteiza, P. I. (2008) 'Aluminium and lead : molecular mechanisms of brain toxicity', pp. 789–802.

Sarhan, M. A. A., Shati, A. A. and Elsaid, F. G. (2014) 'Biochemical and molecular studies on the possible influence of the Brassica oleracea and Beta vulgaris extracts to mitigate the effect of food preservatives and food chemical colorants on albino rats', *Saudi Journal of Biological Sciences*. King Saud University, 21(4), pp. 342–354.

Segneanu, A.-E., Damian, D., Hulka, I., Grozescu, I. and Salifoglou, A. (2016) 'A simple and rapid method for calixarene-based selective extraction of bioactive molecules from natural products.', *Amino acids*, 48(3), pp. 849–58. doi: 10.1007/s00726-015-2132-9.

Sharma, D. (2016) 'iMedPub Journals Effects of Glucosinolates Rich Broccoli extract against Triazophos induced Toxicity in Wistar Rats Abstract', pp. 1–9.

Shrivastava, S. (2012) 'PROTECTIVE EVALUATION OF ZINGIBER OFFICINALE IN ALUMINIUM INDUCED TOXICITY IN RATS', 13(1), pp. 1–10.

Singh, R. P., Sharad, S. and Kapur, S. (2004) 'Free radicals and oxidative stress in neurodegenerative diseases: relevance of dietary antioxidants', *Journal Indian Academy of Clinical Medicine*, 5(3), pp. 218–25.

Sudjarwo, S. A., Sudjarwo, G. W. and Koerniasari (2017) 'Protective effect of curcumin on lead acetate-induced testicular toxicity in Wistar rats', 12(June

2016), pp. 381–390.

Taïr, K., Kharoubi, O., Taïr, O. A., Hellal, N., Benyettou, I. and Aoues, A. (2016) 'Aluminium-induced acute neurotoxicity in rats: Treatment with aqueous extract of Arthrophytum (Hammada scoparia)', *Journal of Acute Disease*, 5(6), pp. 470–482.

Zahedi-amiri, Z. and Taravati, A. (2018) 'Protective Effect of Rosa damascena Against Aluminum Chloride-Induced Oxidative Stress'. Biological Trace Element Research.

Zhang, J., Zhang, R., Zhan, Z., Li, X., Zhou, F., Xing, A., Jiang, C., Chen, Y. and An, L. (2017) 'Beneficial effects of sulforaphane treatment in Alzheimer's disease may be mediated through reduced HDAC1/3 and increased P75NTR expression', *Frontiers in Aging Neuroscience*, 9(MAY), pp. 1–12.

Georgiev, V. G., Weber, J., Kneschke, E. M., Denev, P. N., Bley, T., & Pavlov, A. I. (2010). Antioxidant activity and phenolic content of betalain extracts from intact plants and hairy root cultures of the red beetroot Beta vulgaris cv. Detroit dark red. *Plant foods for human nutrition*, *65*(2), 105-111.