Biological Effects of Blackberry Juice Intake on Mice Treated with Aluminum Chloride

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ABSTRACT

Black berries, a potential source of natural anthocyanin antioxidants, have demonstrated a broad spectrum of biomedical functions including but not limited to cardiovascular disorders, advancing age-induced oxidative stress, also improves neuronal and cognitive brain functions. The aim of the present study was to determine biological effects of daily intake of blackberry juice (BBJ) in the presence of aluminum chloride ($AlCl_3$). Male Albino mice (*n:* 32) averaging 18 to 23 g body weights were classified into four equal groups of eight mice each. Dietary treatments groups were; 1) a control, 2) given $AlCl_3$ (75 mg/kg BW) intraperitoneally (ip), 3) given BBJ (1.6 g/kg BW) orally and 4) treated with $AlCl_3$ and BBJ at the same mentioned doses. Mice were daily administered their respective doses for six weeks. BBJ increased RBCs than the control group and also reduced glucose, creatinine, uric acid and bilirubin than $AlCl_3$ group. Treatment with BBJ alone reduced cholesterol, triglyceride, LDL and VLDL levels than $AlCl_3$ group or $AlCl_3$ + BBJ group, respectively. Histological examinations showed that $AlCl_3$ group showed a moderate to marked neurodegeneration change in the form of necrosis and vacuolation with an increase in neurofibrillary tangle, while $AlCl_3$ and BBJ showed improvement in brain architecture with mild necrosis and neurofibrillary tangle .On the other hand, BBJ reversed normal brain tissue contains glial and pyramidal cells showing no necrosis or vacuolation. The granular cell showed no neurodegeneration change as the control group. These results revealed that a daily intake of BBJ was able to improve the lipid profile, reduce the high levels of total cholesterol, LDL and triglycerides, resulting in playing an important role in the prevention of cardiac damage. BBJ intake during exposure to aluminum could be recommended for reverting back brain oxidative stress and neurological disorders.

Key words: Blackberry, Aluminum chloride, Mice, Lipid profile, Histological effect and blood biochemical.

INTRODUCTION

Aluminum (Al) is one of the most abundant elements in the biosphere and causes adverse effect on various organs, Reinke *et al.,* (2003). Human population is constantly exposed to Al through various sources, such as, corn, yellow cheese, salt, herbs, spices, tea, cosmetics, aluminum cooking utensils, certain beverages, drinking water, food additives and through environmental pollution Gupta *et al.,* (2005). During the last 3 decades, there have been extensive evidences that Al poisoning has demonstrated adverse effects of Al including, inducing memory impairment, personality changes and dementia in humans. Additionally, bioavailable Al seems to be associated with neurological deterioration during aging Exley (1999). High levels of Al have been linked with an increased risk of number of pathogenic disorders such as microcytic anemia, osteomalacia as well as neurodegenerative disorders Santiba *et al.,* (2007). Organs such as bone, liver, testes, kidney, and brain showed significant Al levels, and dysfunction and toxicity of these tissues and organs were noted as a result of the Al accumulation ATSDR (1999). Further extensive experimental evidences demonstrated both, *in vitro* and *in vivo*, that high Al concentrations caused oxidative stress that is described as an imbalance between free radical generation and the antioxidant defense system. Disruption of metal ion homeostasis may lead to oxidative stress, a state where increased formation of reactive oxygen species (ROS) overwhelms body antioxidant protection and subsequently induces DNA damage, lipid peroxidation, protein modification and other effects Jomova and Valko (2011). Consumers all over the world are becoming more conscious of the nutrition value, health benefits and safety of their food and its ingredients. Additionally, there is a preference for natural functional food ingredients that are believed to be safer, healthier and less subject to hazards than their artificial counterparts with increased interest in evaluation of the functional properties of naturally occurring substances, especially those that are present naturally in human diets Jiao and Wang (2005).

Fruits and vegetables contain several different types of antioxidants that may directly react with reactive oxygen or nitrogen species forming products with much lower reactivity. Alternatively, compounds in plant-based diets may increase the capacity of the endogenous antioxidant defenses Wilkinson and Clapper (1997). Many epidemiological studies have linked eating more fruits and vegetables with prevention of chronic

diseases or lower incidence of several chronic pathologies, including obesity, diabetes, cardiovascular, neurologic diseases, infections and certain cancers. Researchers are beginning to think this may be due to the wide variety of phytonutrients in plant foods, of which many can act as antioxidants, protecting our cells' DNA from oxidation, which can promote mutations and the development of cancer Vauzour *et al.,* (2010) and Tavares *et al.,* (2012).

Many research results have demonstrated that there is more than a thousand-fold difference between total antioxidants in various dietary plants. Interestingly, berries, particularly blackberries, blue berries and elder berries, are among the plants with the highest concentrations of antioxidants, Joseph et *al.,* (1999). Black berry (*Morus nigra*), are proved as a traditional and rich source of bioactive compounds, possessing important biological activities such as flavonoids (anthocyanin), some minerals (Na, K, Ca, Se, Zn and P), vitamins (vitamin A, B complex, C and E) phenolic acids (gallic acid, p-coumaric acid, caffeic acid, ferulic acid) and phenolic polymers (ellagic acids) Facchini and St-Pierre (2004) Therefore, the present study was carried out to determine the effect of black berry juice on different biological status and in counteracting aluminum toxicity in mice.

MATERIALS AND METHODS

Chemicals:

Aluminum chloride (98%; anhydrous) was obtained from Elgomhouria company (CDH Vardaan house, Indian), while fresh BB were obtained from a local market (Alexandria, Egypt), then washed, homogenized and its juice was daily freshly prepared. The BB dose (1.6g/kg BW) was utilized according to previous studies which reported that 100 g black berry contains 317 mg anthocyanin; So1.6 g black berry contains 5 mg anthocyanin Sautebin *et al.,*(2004). ½ LD_{50} (75mg/kg BW) was the tested doses of $AlCl_3$ for treated mice.

Experimental animals:

Male albino mice (*n:* 32) averaging 18 to 23 g of BW were obtained from the animal house of the Medical Research Institute, Alexandria University, Egypt. The local committee approved the design of the experiments and the protocol follows the guidelines of the National Institutes of Health (NIH). Animals received human care, and had adequate stable diet and water *ad lipitum*. Animals were acclimatized to the laboratory conditions for two weeks before being experimented.

Experimental design:

After two weeks of acclimation, animals were classified into four equal groups' eight mice each. Group 1: served as untreated control, group 2: mice were given $AlCl_3$ dissolved in distilled water at dose of 75mg/kg BW intraperitoneally (ip), group 3: mice were given BBJ at a dose of 1.6 g/kg bw orally throw oral gavages, and group 4: mice were treated with $AlCl_3$ and BBJ at the same mentioned doses. Mice were administered their respective doses on daily basis for six weeks.

Body weight and organs weight:

Body weight of mice was recorded in the beginning and at the end of the experimental period. Animals were sacrificed by decapitation, then liver, kidney and brain were immediately removed and weighed. Relative organ weights of were calculated as g/100 g body weight.

Blood sample:

Blood samples were collected from the sacrificed animals in two separatetubes, one of them containing heparin. Plasma samples were obtained by centrifugation at 4000 rpm for 20 minutes, then samples were stored at –20 °C until used for further analyses.

Biochemical parameters and enzyme activities:

Plasma samples were analyzed for glucose according to Kunst *et al.*, (1984) while uric acid and creatinine concentrations were measured according to the method of Lamb *et al.*, (2006). Total bilirubin was measured using the method of Wahlefeld and Bergmeyer (1972). Cholesterol and triglycerides (TG) were determined according to the methods of Tietz (1995) .High-density lipoprotein-cholesterol (HDL-c) was determined according to the methods of Sugiuchi *et al.*, (1995). Low-density lipoprotein-cholesterol (LDL-c) was determined by the method of Pisani *et al.*, (1995). Very low-density lipoprotein-cholesterol (VLDL-c) was calculated automatically by Roche /Hitachi Cobas C systems.

The activities of plasma aspartate transaminase (AST) and alanine transaminase (ALT) were assayed by the method of Bergmeyer and Herder (1986). Acid Phosphatase (ACP_2) activity was determined according to Hillmann (1971). Also acetyl cholinesterase (ACE) was measured by the method of Ellman *et al.*, (1961). All the aforementioned parameters were measured using commercial kits, [Bio systems S.A. (Spain), Diamond (Germany) and Randox (United Kingdom)].

Histological study:

Brain Specimen used for histological study was fixed in neutral formalin for a week at room temperature, dehydrated then cleared in xylene and embedded in paraffin wax. The paraffin sections were cut at 20 microns thickness and stained with hematoxylin and eosin for histological examination using the light microscope according to Banchroft *et al .,* (1996).

Statistical analysis:

Data were analyzed according to Steel and Torrie (1981). Statistical significance of the

difference in values of the control and treated animals was calculated by F test with 5% significance level. Data of the present study were statistically analyzed by using Duncan's Multiple Range Test (SAS), (1986).

RESULTS AND DISCUSSION

Results in (Table 1) showed that there was a significant decrease ($P \leq 0.05$) in body weight gain in $AlCl_3$ group (- 4.4g) than the control group (2.6g) while there wasa significant increase ($P \leq 0.05$) in $AlCl_3$+BBJ group (0.2g). In BBJ group there was a significant increase ($P \leq 0.05$) in body weight gain (5.0g) than other groups. This result agreed with results reported by Khattab *et al.*, (2010) who found that $AlCl_3$ induced significant decrease in final body weight. The majority of studies that utilized chronic doses of Al reported significant reduction ($P \leq 0.05$) in weight gain, Krasovskii, *et al.*, (2004) found that during three months observation of rats receiving $AlCl_3$, decrease in water and food intake and transient diarrhea occurred, which resulted in lowering of final body mass of animals in comparison to the controls, and he reported that animals exposed to chronic doses of Al consumed less food. Data indicated that treatment with $AlCl_3$ causes enlargement for liver; kidney and brain, while treatment with BBJ reverted back all the alteration to near normal (ameliorated the bad effect of $AlCl_3$). These results were also in agreement with Thangarajan *et al.*, (2013).

Treated with $AlCl_3$ caused significant increase in WBC by (53.02%), platelets by (34%) and lymphocytes by (22.02%) than the control group, while it caused decrease in total RBC by (7.13%), Hb by (6.3%) and Ht by (7.45%) (Table2).Treatment with AlCl3 and BBJ together also caused increase in WBC (37%), and platelets (5.5%) than the control group, but less than that caused by AlCl3 alone .Also $AlCl_3$+ BBJ caused reductions (3.0%) in RBC, Hb (4.0%) and Ht (2.2 %) than the control group, but also it was less than $AlCl_3$ alone. On the other hand, it was found that treatment with BBJ alone caused slightly increase in RBC level than the control and kept normal level of Hb and Ht as the control group it also decreased level of WBC than $AlCl_3$ group (36.5%) or $AlCl_3$ with BBJ group (21.2%), respectively. The reductions in platelets and lymphocytes in BBJ group was more than the control group 409.7,55.2 vs507.3,64.5 (Table 2).These results were agree with Aziz and Zabut (2011) study, they found that $Alcl_3$ decreased the total RBC count (by18%), Hb (7%) and Ht (20%), and increased WBC count (67%), lymphocytes (29%), and platelets (33%). Geyikoglu *et al.*, (2012) also found that after exposure with Al, the level of RBC, Hb and Ht revealed significant reductions in experimental group compared to the control group.

Table (3) shows that treatment mice with $AlCl_3$ alone caused increase in glucose (71.4%) than other groups but it was not significant ($P \geq 0.05$), while it caused significant increase ($P \leq 0.05$) in creatinine (27%), uric acid (23.56%) and bilirubin (25%) than the control group, BBJ group and $AlCl_3$ with BBJ group. Aziz and Zabut (2011) also found that $AlCl_3$ lowered glucose levels by 30%.

Table 1: Changes in body weight gain (g) and relative weight of organs g/100g BW in the control and different treated mice groups.

Parameters	Experimental groups			
	Control	$AlCl_3$	BBJ	BBJ +$AlCl_3$
Initial weight (g)	20.8±2.3	25.1±1.5	23.7±3.1	24.8±2.6
Final weight (g)	23.4 ± 1.6^{bc}	20.7 ± 1.9^{c}	28.7 ± 1.8^{a}	25.0 ± 4.2^{b}
Bodyweight gain (g)	2.6 ± 1.2^{bc}	-4.4 ± 0.8^{c}	5.0 ± 1.5^{a}	0.2 ± 0.03^{b}
Liver (g/100g bw)	5.4 ± 0.6^{bc}	7.9 ± 0.3^{a}	5.0 ± 0.5^{c}	5.7 ± 0.2^{b}
Kidney (g/100g bw)	1.4 ± 0.08^{b}	2.0 ± 0.15^{a}	1.2 ± 0.08^{c}	1.4 ± 0.17^{b}
Brain (g/100g bw)	1.9 ± 0.4^{ab}	2.3 ± 0.2^{a}	1.6 ± 0.1^{b}	1.9 ± 0.3^{b}

Results are expressed as mean ± SE; Means with different letters in the same row imply significant differences at $P \leq 0.05$.

Table 2: Changes in blood biochemical parameters in the control and different treated mice groups.

Parameters	Experimental groups			
	Control	$AlCl_3$	BBJ	BBJ+ $AlCl_3$
WBC($\times10^3L$)	4.6 ± 0.8^{b}	7.1 ± 0.3^{a}	5.2 ± 0.3^{b}	$6.3\pm1.8a^{b}$
RBC($\times10^6L$)	8.97 ± 0.5^{a}	8.33 ± 0.1^{b}	9.2 ± 0.2^{a}	$8.7\pm0.5a^{b}$
Hb (gm/dL)	12.4±0.6	11.6±0.7	12.1±0.5	11.9±0.8
Ht (%)	48.6±4.0	44.98±1.95	48.0±1.7	47.55±3.6
Platelets($\times10^9L$)	507.3 ± 54.1^{ab}	681.7 ± 214.9^{a}	409.7 ± 83.9^{b}	535.5 ± 137.8^{ab}
Lymphocytes (%)	64.5 ± 5.0^{b}	78.7 ± 9.7^{a}	55.2 ± 3.4^{b}	63.0 ± 3.8^{b}

Results are expressed as mean ± SE; Means with different letters in the same row imply significant differences at $P \leq 0.05$.WBC: White blood cell, RBC: Red blood cell, Hb: Hemoglobin, Ht: Hematocrit

Table 3: Changes in blood parameters in control and different treated mice groups.

Parameters	Experimental groups			
	Control	$AlCl_3$	BBJ	BBJ+ $AlCl_3$
Glucose (mg/dL)	31.5±17.3	54.1±14.7	36.0±14.7	46.9±16.1
Creatinine (mg/dL)	0.69±0.3^{b}	0.88±0.46^{a}	0.66±0.39^{c}	0.73±0.28^{b}
Uric Acid (mg/dL)	4.33±0.4^{c}	5.35±0.17^{a}	4.7±0.4^{bc}	5.0±0.26^{c}
Bilirubin (mg/dL)	0.20±0.01^{b}	0.25±0.19^{a}	0.14±0.03^{c}	0.22±0.03^{ab}

Results are expressed as mean ± SE; Means with different letters in the same row imply significant differences at $P \leq 0.05$.

$AlCl_3$ elevated levels of urea (12%), uric acid (77%) and creatinine (25%) compared to the control. Geyikoglu *et al.*, (2012) also found that after exposure with Al, the levels of urea and uric acid significantly increased. On the other hand, it was found that treatment with BBJ alone reduced glucose (33.5%), creatinine (25%), uric acid (12.2%) and bilirubin (44.0%) than $AlCl_3$ group. Ibrahim (2013) also found that, treatment with strawberry or blackberry alone in male rats showed a reduced in urea, creatinine, bilirubin and glucose compared to the control group. DeFuria *et al.*, (2009) found that BBJ supplementation could protect against insulin resistance and lower blood glucose levels in mice.

Table (4) shows that dietary treatment with $AlCl_3$ alone increased cholesterol by (28.97%), triglycerides (71.5%) LDL (11.36%) and VLDL (31.9%) than the control group, while it reduced HDL by (7.75%). On the other hand treatment with BBJ alone decreased cholesterol, triglyceride, LDL and VLDL than $AlCl_3$ group (14.5%, 44.1%, 10.6% and 20.6% respectively) and $AlCl_3$ + BBJ group (11.7%, 7.1%, 3.5% and 6.8% respectively) and the decrease was significant for cholesterol among treatment groups, also there was a significant increase ($P \leq 0.05$) in VLDL among groups. Aziz and Zabut (2011) also found that there was an increase in triglyceride (28%) and cholesterol (20%) levels by treatment with $AlCl_3$. Blueberries had also been reported to have lipid lowering properties, and thus have been suggested to be protective against atherosclerosis and cardiovascular disease. Previously, Rimando *et al.*, (2005) demonstrated a 29% decrease in LDL levels and a 7% increase in HDL levels, with significant improvement in LDL/ HDL ratio. Hassan et *al.*, (2015) found that the exposure to AL which is able to cause alterations in lipid profile where the hypo activity of lipoprotein lipases may be because of hepatic dysfunction appearing to be one of the most important factors responsible for the increment in triglycerides and cholesterol, these enzymes may be inhibited by AL, for example unspecific esterase, triglyceride lipase and pyrophosphates. The irregularities in lipoprotein profile may be a result of the decrease in the removal of low density lipoprotein (LDL) and VLDL from the circulation accompanied by or uncontrolled production of VLDL by the liver Tsutsumi *et al.*, (1995). Naidu *et al.*, (2013) found that for each 1% increase in HDL cholesterol there is a 1% decrease in the cardiovascular event rate. Also Wallace reported that dietary flavonoids have emerged as potential candidates to protect against CVD, Wallace (2011). Epidemiological studies associate regular consumption of flavonoid-rich foods and beverages with a decreased risk of CVD mortality, owing potential of these bioactive compounds in protect against LDL oxidation and prevent CVD, He and Giusti (2010).

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Table 4: Lipid profiles in the control and different treated mice groups.

Parameters	Experimental groups			
	Control	$AlCl_3$	BBJ	BBJ +$AlCl_3$
CH (mg/dL)	80.3±11.0^{c}	103.5±8.5^{a}	88.5±6.76^{bc}	100.2±8.2^{ab}
TG (mg/dL)	91.5±5.1^{b}	157.0±40.7^{a}	87. 7±18.0^{b}	94.4±9.0^{b}
HDL(mg/dL)	96.8±13.3	89.3±6.9	97.5±12.8	90.6±4.3
LDL (mg/dL)	11.0±3.4	12.3±3.3	11.0±1.8	11.4±2.1
VLDL(mg/dL)	11.8±1.3^{b}	15.5±2.7^{a}	12.3±0.5^{b}	13.2±2.2^{ab}

Results are expressed as mean ± SE; Means with different letters in the same row imply significant differences at $P \leq 0.05$. CH = cholesterol TG = triglycerides, LDL = low density lipoprotein, HDL = high density lipoprotein, VLDL = very low density lipoprotein.

difference in values of the control and treated animals was calculated by F test with 5% significance level. Data of the present study were statistically analyzed by using Duncan's Multiple Range Test (SAS), (1986).

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Liver (g/100g bw)	5.4 ± 0.6^{bc}	7.9 ± 0.3^{a}	5.0 ± 0.5^{c}	5.7 ± 0.2^{b}
Kidney (g/100g bw)	1.4 ± 0.08^{b}	2.0 ± 0.15^{a}	1.2 ± 0.08^{c}	1.4 ± 0.17^{b}
Brain (g/100g bw)	1.9 ± 0.4^{ab}	2.3 ± 0.2^{a}	1.6 ± 0.1^{b}	1.9 ± 0.3^{b}

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RBC($\times10^6L$)	8.97 ± 0.5^{a}	8.33 ± 0.1^{b}	9.2 ± 0.2^{a}	$8.7\pm0.5a^{b}$
Hb (gm/dL)	12.4±0.6	11.6±0.7	12.1±0.5	11.9±0.8
Ht (%)	48.6±4.0	44.98±1.95	48.0±1.7	47.55±3.6
Platelets($\times10^9L$)	507.3 ± 54.1^{ab}	681.7 ± 214.9^{a}	409.7 ± 83.9^{b}	535.5 ± 137.8^{ab}
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Uric Acid (mg/dL)	4.33±0.4^{c}	5.35±0.17^{a}	4.7±0.4^{bc}	5.0±0.26^{c}
Bilirubin (mg/dL)	0.20±0.01^{b}	0.25±0.19^{a}	0.14±0.03^{c}	0.22±0.03^{ab}

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Table 4: Lipid profiles in the control and different treated mice groups.

Parameters	Experimental groups			
	Control	$AlCl_3$	BBJ	BBJ +$AlCl_3$
CH (mg/dL)	80.3±11.0^{c}	103.5±8.5^{a}	88.5±6.76^{bc}	100.2±8.2^{ab}
TG (mg/dL)	91.5±5.1^{b}	157.0±40.7^{a}	87. 7±18.0^{b}	94.4±9.0^{b}
HDL(mg/dL)	96.8±13.3	89.3±6.9	97.5±12.8	90.6±4.3
LDL (mg/dL)	11.0±3.4	12.3±3.3	11.0±1.8	11.4±2.1
VLDL(mg/dL)	11.8±1.3^{b}	15.5±2.7^{a}	12.3±0.5^{b}	13.2±2.2^{ab}

Results are expressed as mean ± SE; Means with different letters in the same row imply significant differences at $P \leq 0.05$. CH = cholesterol TG = triglycerides, LDL = low density lipoprotein, HDL = high density lipoprotein, VLDL = very low density lipoprotein.

Table 5: Enzymes activity in the control and different treated mice group.

Parameters	Experimental groups			
	Control	$AlCl_3$	BBJ	BBJ+ $AlCl_3$
ALT (U/l)	118.5±2.65	156.0±42.53	121.7±14.08	132.4±25.15
AST (U/l)	449.75±45.7^{b}	543.2±36.3^{a}	387.5±51.4^{b}	415.6±48.57^{b}
ACE (U/l)	27.0±3.34^{ab}	32.2±0.96^{a}	26.0±4.97^{b}	29.4±3.05^{ab}
ACP_2 (U/l)	7.55±0.33^{b}	13.1±0.55^{a}	7.57±2.37^{b}	9.54±0.12^{b}

Results are expressed as mean ± SE; Means with different letters in the same row imply significant differences at $P \leq 0.05$.

function and increased enzymes activities while BBJ may have enhanced liver functions but there was no significant difference between the control group and different treated groups in level of ALT enzyme. Ibrahim (2013) found that treatment with strawberry or blackberry alone showed a decrease in AST and ALT levels. The presence of BBJ with $AlCl_3$ in combination groups minimized its toxic effects on AST and ALT levels, but these did not reach to the values of the control group. Geyikoglu *et al.*, (2012) also found that after exposure with Al, the enzymatic activities of ALP, AST, ALT and LDH increased. The author stated that sub chronic exposure to low doses of Al can produce serious dysfunctions in rat blood, liver and kidney, and exposure to this metal can result in greater damages. Akila *et al.*, (1998) also reported that a rise in blood transaminase activities is a sensitive indicator of damage to cytoplasmic and/or mitochondrial membranes. Enzyme activities rise when the membranes of only very few cells are damaged.

In regard to acetylcholinesterase (ACE) enzyme in blood, it was found that $AlCl_3$ significantly increased ($P \leq 0.05$) the level of the enzyme activity as compared to the control group (32.2 vs 27.0), while adding BBJ with $AlCl_3$ reduced level of ACE enzyme and treated with BBJ alone nearly kept the level of ACE as normal as the control. The observed inhibition in brain ACE activity may result from the slow accumulation of Al in the brain and formation of Al complex with high affinity for binding with the active site of this enzyme, leading to induction of oxidative stress; consequently it diminishes the activity of ACE in all parts of the brain. So, acetylcholine (AC) does not hydrolyze and accumulates in cholinergic sites leading to disturbance in the nervous system Kumar *et al.*, (2009), inhibition in ACE resulted in the accumulation ofAC, stimulation of lymphocytes and increased lymphocyte motility and cytotoxicity. Since ACE is a membrane bound enzyme, it may be removed from the binding with AC and may result in decreased activity of ACE, so the level of enzyme activity will increase in blood Mohamed *et al.*, (2011). Naidu *et al.*, (2009) found that ACE activities were significantly increased. It can be concluded that Alinduced neuronal oxidative stress and inhibition of the antioxidant system and enzyme activities could be the mechanisms of $AlCl_3$ neurotoxicity.

Table (6) and Figure (1) show histological changes in brain with $AlCl_3$ and BBJ, it was found that in the control group (Fig1-A) normal brain tissues contains glial and pyramidal cells indicating no necrosis or vacuolation. The granular cell showed no neuodegeration changes, there is no neurofillary tangle. BBJ group (Fig.1-B) revealed reversed normal brain tissue contains glial and pyramidal cells showing no necrosis or vacuolation. The granular cell shows no neurodegeneration change. There is no nerofillary tangle. $AlCl_3$ group (Fig.1-C) shows moderate to marked neurodegeneration change in the form of necrosis and vacuolation with increase in neurofibrillary tangle. $AlCl_3$ and BBJ (Fig.1-D) shows improvement in brain architecture with mild necrosis and neurofibrillary tangle.

Naidu *et al.*, (2013) found that histological examinations showed clumpy of cell neurons, or reduced pyramidal cells and scanty neurofibrillary tangle which was an indication of neurodegeneration in the treated groups when compared to the control. It was however, concluded that the oral administration of AlCl3 could induce brain damage which may impair memory and learning as seen in Alzheimer disease. These results suggest that $AlCl_3$ enhances oxidative stress in the brain, thereby disturbing the antioxidant defense of rats. Increased oxidative stress could be one of the mediating factors in the pathogenesis of $AlCl_3$, toxicity in the brain. Hamza, *et al.*, (2015) found that treatment with BBJ showed normal brain tissue formed of round and pyramidal shaped neurons surrounded by eosinophilic glial fibers as found in normal group.

From these results we suggested that 100g or 1/2cup of BB may be taking daily to avoid many health problems.

Table 6: Histological examination for brain in different treated mice group

	Experimental groups			
	Control	$AlCl_3$	BBJ	BBJ + $AlCl_3$
Necrosis	-	+++	-	+
Vaculation	-	+++	-	+
Neurofillarytengle	-	+++	-	+

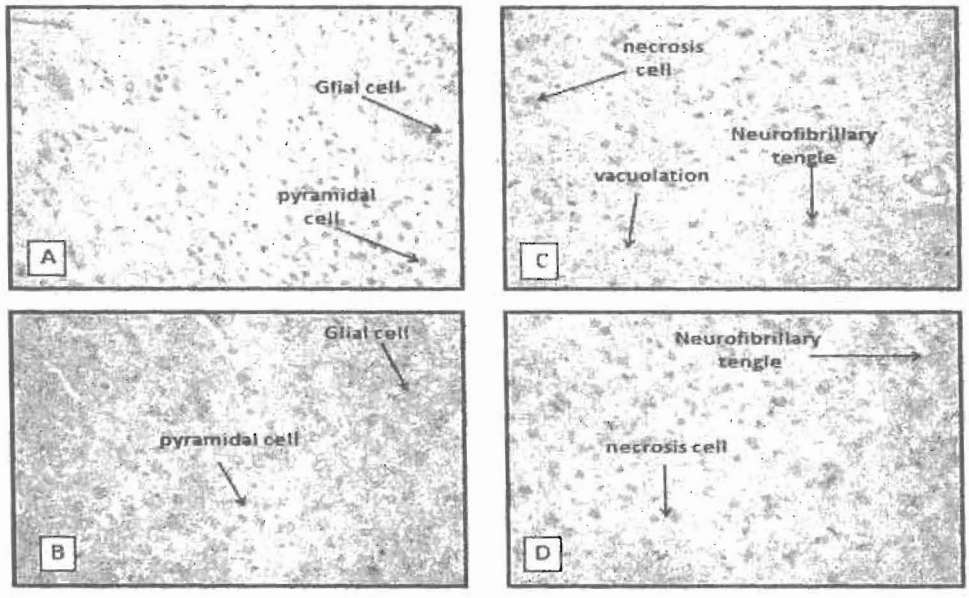

Fig.1: Photomicrographs of brain sections of control (A) and different treated mice (B): BBJ group, (C): $AlCl_3$ group and (D): BBJ+$AlCl_3$ under light microscopy.

CONCLUSION

Consumption of Blackberry juice (BBJ) was able to improve the lipid profile, reduce the high levels of total cholesterol, LDL and triglycerides. So, it can play an important role in the prevention of cardiac damage. BBJ administration during exposure to aluminum could be recommended for reverting back brain oxidative stress and neurological disorders as well as modulation of antioxidant defense mechanism. The observed beneficial effect of BBJ is probably through the synergistic anti-oxidant capacity of its various nutritional constituents. Since ever more people prefer natural therapies besides being provide further support for the investigation thereof as functional human foods.

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الملخص العربى

التأثيرات البيولوجية الناتجة من تناول عصير التوت الأسودعلي الفئران المعاملة بكلوريد الألومنيوم

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تتمتع أصناف التوت الأسود بأنها مصدر طبيعي غني بمضادات الأكسدة متمثلة في أرتفاع محتواها من الأنثوسيانين، والتي لها مدي واسع من الوظائف الطبية الحيوية بداية من أمراض القلب والأوعية الدموية الي تأثيره علي تقليل الجهد التأكسدي الذي يؤثر علي التقدم في العمر، كما يحسن من الوظائف العصبية والمعرفية للمخ. تهدف هذه الدراسة الي تحديد الأثار البيولوجية الناتجة من الأستهلاك اليومي لعصير التوت الأسود للفئران المعاملة بكلوريد الألومنيوم. تم أستخدام ٣٢ فأر من ذكور الفئران الألبينو بمتوسط وزن يتراوح من ١٨ إلى ٢٣ جرام تم تقسيمهم إلى أربع مجموعات متساوية يحتوي كلا منهاعلي ثمانية فئران، وكانت المعاملات كما يلي:١- المجموعه الضابطة ٢- تم أعطائها كلوريد الألومنيوم(٧٥ ملجم /كجم من وزن الجسم) عن طريق الحقن تحت الجلد ٣- تم أعطائها عصير التوت الأسود(١,٦ جم/كجم من وزن الجسم) عن طريق الفم ٤- تم معاملتها بكلوريد الألومنيوم+عصير التوت الأسود بنفس الجرعات السابقة وذلك لمدة ستة أسابيع. أوضحت النتائج أن تناول عصير التوت الأسود بمفرده أدى الى أرتفاع عدد كرات الدم الحمراء عن المجموعة الضابطة، وأيضا قلل من مستوى الجلوكوز والكرياتنين وحمض اليوريك والصفراء عن المجموعة المعاملة بكلوريد الألومنيوم. كما أنه أدى الى خفض مستويات الكوليسترول والجليسريدات الثلاثية، والليبوبروتينات منخفضة الكثافة، والليبوبروتينات منخفضة الكثافة جدا عن مجموعة كلوريد الألومنيوم او كلوريد الألومنيوم+عصير التوت الأسود على الترتيب. وأوضحت الفحوصات النسيجيه(الهستولوجيه) للمخ ان مجموعة كلوريد الألومنيوم ظهر لديها علامات تغير متوسطة في بنية المخ ظهرت في صورة فجوات وتشابك ليفي عصبي في حين أظهرت مجموعة كلوريد الألومنيوم+عصير التوت الأسود تحسن في بنية المخ مع وجود عدد أقل من الفجوات والتشابك العصبي. ومن ناحية أخرى وجد أن عصير التوت الأسود أظهر أنسجه مخية طبيعية تحتوي على خلايا هرمية ولا يوجد بها أي فجوات أو تشابك عصبي كما في المجموعة الضابطة. نستخلص من ذلك أن الأستهلاك اليومي لعصير التوت الأسود كان له القدرة على تحسين مستوي الدهون في الدم وخفض مستويات الكوليسترول والجليسريدات الثلاثية والتي تلعب دورا هاما في الوقاية من أمراض القلب، ويوصي أيضا بتناول عصير التوت الأسود لتقليل الجهد التأكسدي على المخ والأضطرابات العصبية الناتجة عن التعرض للألومنيوم.