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Research Article
Antioxidant, Protective Effect of Black Berry and Quercetin Against Hepatotoxicity Induced by Aluminum Chloride in Male Rats

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Abstract

Background and Objective: Aluminum chloride ($AlCl_3$) is widely used in many medical applications but with many side effects and hepatotoxicity. Black berry extract (BB) and quercetin (Qe) have major protective effects against a lot of tissues injury as they have antioxidant effects. This study aimed to evaluate the ameliorative effect of both BB and Qe against hepatotoxicity due to aluminum chloride toxicity. **Materials and Methods:** Seventy male rats were used in the study and split into 7 groups as follows: Control, $AlCl_3$, BB, Qe and the combination of either BB or Qe antioxidants with $AlCl_3$ and a combination of both antioxidants with $AlCl_3$. **Results:** Hepatic enzymes activities aminotransferases (AST, ALT) and marker of tumor necrosis factor-alpha (TNF-α) with some biomarkers of antioxidant enzymes and lipid peroxidation marker in liver hemogenates were examined. Histological examination was performed on the liver tissues as indicators of hepatic damage following $AlCl_3$ administration with/without BB and/or Qe. This study showed that BB and Qe decreased the AST and ALT levels and the oxidative stress level to a normal level after the administration of $AlCl_3$. The results clarified that treatment with $AlCl_3$ with a combination of BB with Qe has potent synergistic effect and enhanced significantly the antioxidant enzymes and declined the level of lipid peroxidation as compared with the $AlCl_3$-treated group. **Conclusion:** It is obvious from the results that both BB and Qe administration inhibited the liver damage and improved the antioxidant status of male rats induced by $AlCl_3$-toxicity.

Key words: Hepatic damage, aluminium chloride particles, black berry extract, hepatotoxicity, quercetin

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aluminium (Al) different forms are types of environmental xenobiotics that have the ability to induce cytotoxicity by generation of free radicals[1].

Aluminium metal has many medical applications such as, anti-acids, anti-perspirants and anti-allergy[2]. Aluminium can be accumulated in humans via the ingestion with juices, the diets and drinking water and this caused a significant elevation in gastrointestinal absorption and the aluminium appearance in the urinary excretion[3]. Aluminium chloride ($AlCl_3$) was proved to be teratogenic especially to the embryos when administrated excessively[4]. Polyphenols have been found excessively in berries and their leaves and they act as agents acting therapeutically on the human health and good source of antioxidant compounds[5]. Black berries are considered as the good source for polyphenols as especially flavonols, phenolic acid derivatives as well as anthocyanins[6]. Scientific reports have concluded that nutrients rich in antioxidants are very important and healthy to human bodies. Antioxidants can stabilize the effect of free radicals and prevent cellular injury that is induced by these free radicals[7]. Quercetin (Qe) is a flavonoid which is a plant-derived that is widely found in vegetables and fruits. It is a form of glycosidic flavonoids, like quercetin[8]. It has antioxidant properties and protect against a lot of diseases as heart disease, liver fibrosis, atherosclerosis and renal damage[9]. Quercetin is considered as one of the most plenteous flavonoids. Quercetin have a lot of pharmacological aspects[10] as anti-tumor effects[11], anti-oxidant[12] and antiviral activities[13]. Recently, the natural products have very lower side effects as compared with the chemicals and drugs and this has protruded as a new therapeutic strategy in medicine. Quercetin is considered as one of the plenteous natural flavonoids and it is present in several fruits and vegetables[14].

Thus, this study was conducted to assess the ameliorative roles of BB or/and Qe in alleviating $AlCl_3$ toxicity and to investigate their enhancing effect on antioxidant capacities in male rats.

MATERIALS AND METHODS

Chemicals: Quercetin (Qe) was taken from Sigma Co. It was administrated at a dose (25 mg kg⁻¹)[10]. Black berry extract was prepared by taking (10.0 g) of blackberries and then they were extracted by 95% ethanol (100 mL) for only 1 h. The solvent was evaporated by a rotatovapour under vacuum. The extract which is dried, it was stored in dark glass bottles and it was kept in deep freezer to diminish oxidative damage[5]. The dose of blackberry equal to 1.6 g kg⁻¹ that containing bout 5 mg active constituent of anthocyanin)[15]. Aluminum chloride ($AlCl_3$) (Purity 99%) was taken from Sigma Co. The dose of $AlCl_3$ was 34 mg kg⁻¹ 1/25 LD_{50}. Other chemicals were high analytically grade.

Experimental animals: At July, 2018, the male rats were taken from the animal unit in King Abdul Aziz University, Saudi Arabia. Seventy adult male rats weighing 150-180 g were housed in metal cages. This experimental study was approved by the research ethics committee of Taif University under approval number (39-31-0043). Animals were adopted before the beginning of the experiment for 2 weeks.

Experimental design: The male rats were separated into 7 groups as follows: 1st group control group, it was received 1 mg kg⁻¹ of saline as vehicle, 2nd group was treated with Aluminum chloride ($AlCl_3$) (34 mg kg⁻¹), 3rd group was treated with BB (1.6 g kg⁻¹), 4th group was received Qe in a dose of (25 mg kg⁻¹), 5th and 6th groups were treated with $AlCl_3$+BB and $AlCl_3$+Qe, respectively. The 7th group was treated with BB and Qe combination with $AlCl_3$. All groups were treated orally for successive 30 days.

Preparation of liver tissues homogenates: Liver tissues (about 0.30 g) were used for determination of oxidative stress. Liver tissues were put in cold saline sodium phosphate buffer (pH 7.4), The tissues were homogenized in cold buffer. The resultant supernatant was preserved until used.

Liver functions biomarkers: The protein level was estimated by commercial kits. Serum ALT, AST and ALP activities were measured with kits from SENTINEL CH. Serum (LDH) activity was estimated by commercial kits. The levels of proteins were evaluated[16].

Assay of serum cytokines: TNF-α levels were specified by ELISA kits spectrophotometrically at 450 nm (Immuno-Biological Laboratories Co., Ltd., (IBL) the USA.

Determination of hepatic antioxidant enzyme biomarkers: Catalase was determined according to a method of Aebi[17]. The CAT activity was U g⁻¹ tissue, Superoxide dismutase was

estimated by the method of Marklund and Marklund[18]. The SOD activity was calculated by 50% of auto-oxidation inhibition of pyrogallol.

The malondialdehyde (MDA) level was estimated as by Ohkawa *et al.*[19], MDA concentration was specified as $\mu mol\ g^{-1}$ tissue. Glutathione-S-transferase (GST) was determined by Couri and Abdel-Rahman[20] method. The GPx was determined by Hafeman *et al.*[21].

Myeloperoxidase (MPO) and Xanthine oxidase (XO) determination: Myeloperoxidase (MPO) is a peroxidase enzyme[22]. Xanthine oxidase (XO) was assayed, according to Litwack *et al.*[23] method.

Histological evaluation: A portion of the liver was kept in 10% formalin and then it was processed for histological examination as described by Gabe[24].

Statistical analysis: Data were expressed as Mean±SD (n = 10). Using analysis of variance one way ANOVA[25]. The data were considered as a significant when $p \leq 0.05$.

RESULTS

Liver functions: $AlCl_3$ administration in male rats for a period of 30 days induced a decrement significantly in total protein levels compared to the control group. It is clear that the combination of $AlCl_3$ and BB or Qe afforded slight significant decreases in comparison with the control group but with significant increase as compared with $AlCl_3$ (Table 1).

Transaminases in serum (AST and ALT) levels were increased significantly in male rats treated with $AlCl_3$ by 162.75, 97 $U\ mL^{-1}$ as compared with those in the control group which were (20.48,16.58 $U\ mL^{-1}$) (Table 1).

$AlCl_3$ treatment induced in a significant increment in serum ALP levels as compared to control group. The groups treated with $AlCl_3$ in combination with BB or Qe showed a marked increment ($p < 0.05$) in serum ALP levels compared with those in the control group.

$AlCl_3$ administration afforded a significant increase in serum LDH levels compared to in the control group. The other treated groups with $AlCl_3$ combined with BB or Qe increment significantly ($p < 0.05$) in serum LDH levels compared with those in the control group, which appeared to exhibit a significant decreases compared with those in the $AlCl_3$-treated group (Table 1).

Serum tumor necrosis factor α (TNF-α) activity: $AlCl_3$ administration elicited a increment significantly in serum TNF-α, while $AlCl_3$ in combination with BB or with Qe or both resulted in significant increases in TNF-α level compared to the control group. However, the BB or Qe-treated groups afforded non-significant decrease in TNF-α level. On the contrary, significant decline ($p < 0.05$) in TNF-α levels were noticed in all $AlCl_3$ treated groups in combination with compounds in treated groups (Table 2).

Oxidative stress biomarkers: It was apparent that treatment with $AlCl_3$ elicited a significant decrease ($p < 0.05$) in liver CAT levels as compared with those in the control group (Table 3). Whereas, treatment with combination of BB or Qe induced non-significant changes in liver CAT levels by 1.73 $U\ g^{-1}$ as compared with those in the control group.

$AlCl_3$ treatment resulted in a significant decrement ($p < 0.05$) in SOD levels compared with those in the control group (Table 3). However, the combination of $AlCl_3$ with BB and/or Qe resulted in significant decreases ($p < 0.05$) in liver SOD levels compared with those in the $AlCl_3$ treated group.

Liver MDA levels were elevated significantly in response to $AlCl_3$ treatment by 57.24 $U\ g^{-1}$ as compared with those in the control group. The ameliorating effect of BB and Q against $AlCl_3$ toxicity was highly clear.

Table 1: Liver functions of male rats treated with $AlCl_3$ or/and black berry and/or quercetin

Groups	Parameters				
	Total protein (g dL⁻¹)	AST (U mL⁻¹)	ALT (U mL⁻¹)	ALP (U L⁻¹)	LDH (µLU mL⁻¹)
Control group	7.27±0.54ᵇ	20.48±0.48ᵉ	16.50±0.20ᶠ	23.05±2.84ᶠ	139.25±5.02ᵉᶠ
Aluminum chloride (AlCl₃)	2.27±0.30ᵉ	162.75±2.39ᵃ	97.00±2.18ᵃ	69.49±1.75ᵃ	591.25±6.54ᵃ
Black berry (BB)	8.48±0.24ᵃ	13.22±1.02ᶠ	17.57±1.52ᵉᶠ	24.58±1.48ᵉᶠ	135.36±2.36ᶠ
Quercetin (Qe)	7.77±0.53ᵇ	13.60±1.24ᶠ	17.86±1.10ᵉᶠ	23.08±1.36ᶠ	137.25±7.25ᶠ
AlCl₃+BB	5.78±0.25ᵈ	68.75±1.67ᵇ	40.26±0.76ᵇ	33.27±1.25ᵇᶜ	235.25±6.35ᵇ
AlCl₃+Q	5.88±0.41ᵈ	53.55±1.12ᶜ	30.25±1.20ᶜ	31.65±1.02ᶜ	224.36±5.25ᶜ
AlCl₃+BB+Q	6.31±0.78ᶜ	39.32±1.52ᵈ	26.28±1.36ᵈ	28.45±1.68ᵈ	184.74±4.36ᵈ

Means within the same column (Mean±SE) carrying different letters and are considered as significant, the highest mean value has the symbol (a) and decreasing alphabetically, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, ALP: Alkaline phosphatase
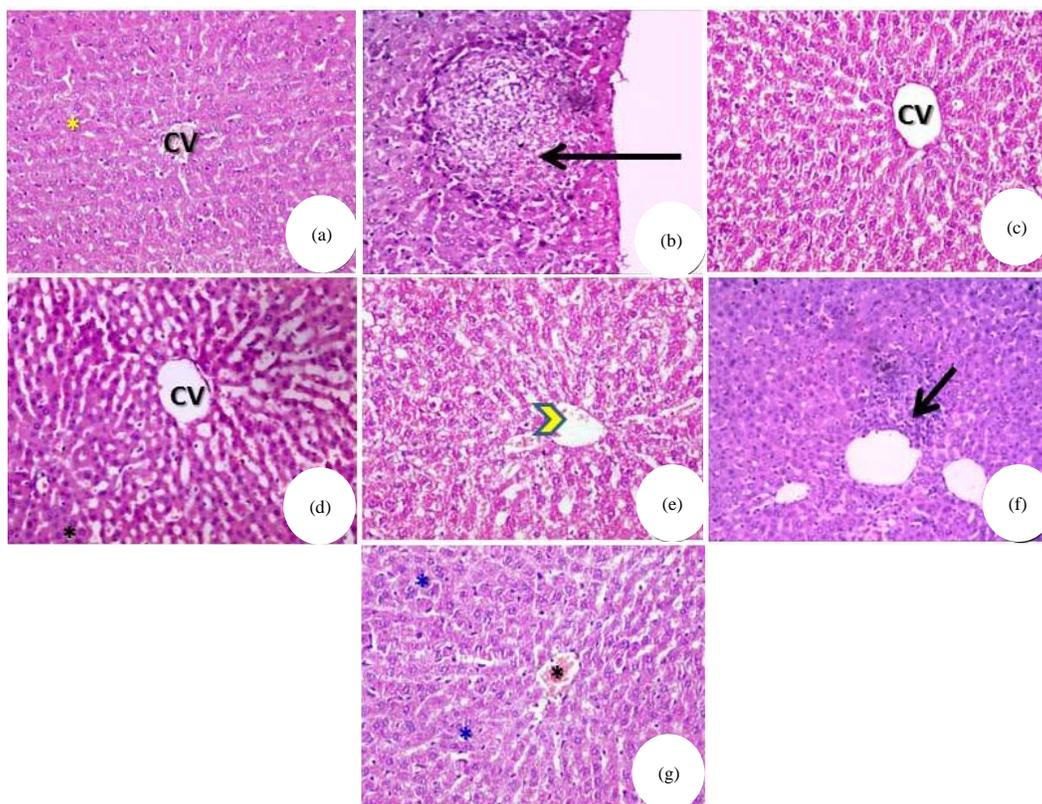
Fig. 1(a-g): Photomicrographs of liver sections in male mice of different groups by hematoxylin-eosin staining under light microscopy (X400), (a) Control group with normal hepatocytes with central normal sized nuclei (Black asterisk), (b) AlCl₃-treated group with part of granuloma with mild lobular inflammation (Black arrow), (c) BB-treated group, (d) Qe treated group with normal cords of polyhedral hepatocytes with central nuclei (yellow asterisks), (e) AlCl₃ and BB treated groups normal sized with very mild congested CV (Black asterisk) and normal cords of hepatocytes with mild fatty changes (Blue asterisk), (f) AlCl₃ and Qe treated group with mild enlarged sized of CV with peri-central lymphocytic aggregates with moderated fatty changes (Black arrow) and (g) AlCl₃, BB and Qe treated group with normal liver cords with very mild congested CV and very mild fatty change (Yellow head arrow)

Table 2: Tumour necrosis factor alpha (TNF-α) of male rats treated with AlCl₃ or/and black berry and/or quercetin

Groups	TNF-α (Pg mL^{-1})
Control group	1.51$\pm$0.51^{ef}
Aluminum chloride (AlCl₃)	78.42$\pm$1.78^a
Black berry (BB)	1.07$\pm$2.82^f
Quercetin (Qe)	1.05$\pm$1.21^f
AlCl₃+BB	25.47$\pm$1.40^b
AlCl₃+Q	21.34$\pm$1.10^c
AlCl₃+BB+Q	10.68$\pm$2.32^d

Means within the same column (Mean$\pm$SE) carrying different letters and are considered as significant, the highest mean value has the symbol (a) and decreasing alphabetically, TNF-α: Tumour necrosis factor alpha

Liver GRx activity was markedly declined in the group treated with AlCl₃ alone or in combination with BB, Qe or both compared with that in the control group (Table 3).

AlCl₃ treatment resulted in a significant decrease ($p<0.05$) in GR activity as compared to the control group. Meanwhile, a combination of AlCl₃ with either BB or Qe-induced significant decreases in hepatic glutathione reductase as compared with the control group (Table 3).

Liver GST activity was significantly decreased in groups treated with AlCl₃ alone or in a combination of BB, Qe or both as compared to the control group (Table 3).

AlCl₃ afforded a significant increase in liver MPO activity when compared with the control group. AlCl₃ in combinations with either BB or Qe-induced significant increases in MPO activity (Table 4).

AlCl₃ treatment afforded a significant increment in the hepatic XO activity when compared with control group. AlCl₃ in combination with BB or Qe-induced significant increases in liver XO activity (Table 4).

Histopathology observations: It is clear from Fig. 1a that the cross section in control group showing normal sized Central

Table 3: Oxidative/antioxidant parameters of antioxidant enzymes in hepatic tissues of male rats treated with AlCl₃ or/and Black berry and/or Quercetin

Groups	Parameters				
	Liver CAT (U g⁻¹)	Liver SOD (U g⁻¹)	Liver MDA (U g⁻¹)	Liver GR (U g⁻¹)	Liver GST (U g⁻¹)
Control group	1.78±0.41[b]	23.15±1.35[b]	4.23±0.58[e]	31.15±1.15[b]	21.65±0.72[b]
Aluminum chloride (AlCl₃)	0.56±0.12[e]	7.58±1.85[f]	57.24±1.16[a]	9.16±1.38[f]	5.65±0.98[f]
Black berry (BB)	1.72±0.36[b]	23.08±1.98[b]	4.21±0.78[e]	32.23±1.15[ab]	22.17±0.68[ab]
Quercetin (Qe)	2.00±0.65[a]	23.74±1.58[ab]	4.22±0.68[e]	32.35±1.65[ab]	22.02±1.35[ab]
AlCl₃+BB	1.02±0.63[d]	16.25±1.85[e]	21.26±2.36[b]	20.39±2.01[e]	15.32±0.57[e]
AlCl₃+Q	1.41±0.68[c]	19.85±2.26[d]	14.28±1.65[c]	25.31±1.25[d]	17.90±0.68[d]
AlCl₃+BB+Q	1.73±0.32[b]	21.28±1.65[c]	8.34±1.15[d]	29.98±2.68[c]	19.98±0.48[c]

Means within the same column (Mean±SE) carrying different letters and are considered as significant, the highest mean value has the symbol (a) and decreasing alphabetically, CAT: Catalase, SOD: Superoxide dismutase, MDA: Malondialdehyde, GR: Glutathione reductase, GST : Glutathione-S-transferase

Table 4: Antioxidant enzymes (Myloperoxidase and xanthine oxidase) in hepatic tissues of male rats treated with AlCl₃ or/and Black berry and/or Quercetin

Groups	Parameters	
	MPO (nmol min⁻¹ mL⁻¹)	XO (U g⁻¹)
Control group	17.36±1.36[e]	16.35±1.65[e]
Aluminum chloride (AlCl₃)	38.36±2.25[a]	32.77±1.36[a]
Black berry (BB)	18.54±1.65[d]	16.25±2.36[e]
Quercetin (Qe)	17.48±1.36[e]	16.36±1.25[e]
AlCl₃+BB	26.36±2.36[b]	25.39±3.52[b]
AlCl₃+Q	24.39±3.36[c]	22.85±2.36[c]
AlCl₃+BB+Q	19.65±1.69[d]	19.16±2.39[d]

Means within the same column (Mean±SE) carrying different letters and are considered as significant, the highest mean value has the symbol (a) and decreasing alphabetically, MPO: Myloperoxidase, XO: Xanthine oxidase

vein (CV) with normal hepatocytes with central normal sized nuclei (Black asterisk). Cross section in AlCl₃-treated group, showing markedly epithelioid granuloma and part of granuloma with mild lobular inflammation (Black arrow) (Fig. 1b). Cross section in BB-treated group showing normal sized CV with cords of polyhedral hepatocytes and eosinophilic cytoplasm (Fig. 1c). Cross section in Qe treated group showing normal sized CV with normal cords of polyhedral hepatocytes (yellow asterisks) with central nuclei (Fig. 1d). Cross section in AlCl3 and BB treated groups. There are normal sized with very mild congested CV (Black asterisk) with normal hepatocyte cords with mild fatty changes (Blue asterisk) (Fig. 1e). Cross section in AlCl3 and Qe treated group showing mild enlarged sized of CV with peri-central lymphocytic aggregates (Black arrow) with moderated fatty changes (Fig. 1f). Cross section in AlCl3, BB and Qe treated group, showing normal liver cords with very mild congested CV (Yellow head arrow) and very mild fatty change (Fig. 1g).

DISCUSSION

The relation between aluminium toxicity and enhancing of the oxidative stress has been reported widely in humans and in experimental animals[26]. In this study, aluminium treatment resulted in increment of the level of lipid peroxidation, while the evaluated antioxidant enzymes were declined in hepatic tissues of aluminium intoxicated rats[26].

The obtained results suggested that the aluminum exposure caused imbalance between the oxidative system and antioxidant system in the tested rats. So the oxidative stress reports free radicals imbalance in production and imbalance in cellular antioxidant system[27].

The present results indicated that there is a decline in total protein level as well as increases in liver function enzymes (AST, ALT, ALP and LDH) due to AlCl₃ administration to male rats and these results were reinforced previously by Salem *et al.*[28], as they showed that liver enzymes activity were increased due to AlCl₃ treatment and this may be due to induction of free radicals production due to AlCl₃ administration, which offensive the fatty acids of the membranes of the hepatic cells and thus eliciting lipid peroxidation, which causes membrane disorganization and then leads to the membrane fluidity decrement.

The elevation of serum triglycerides, cholesterol, LDL-C and V-LDLC levels were followed by a decrement in HDL-C level in AlCl₃ administration and this could be assigned to increasing the mobilization of fats from adipose tissues due to treatment with AlCl₃ which induce a cellular membrane impairment[29].

Similarly, the current results reported presence of increment of lipid profile markers as cholesterol and triglycerides levels with subsequent declining of HDL-C levels in AlCl₃-treated rats[26]. Accordingly, it has been reported that AlCl₃-afforded lipid peroxidation and this could be due to the lipid metabolism alteration and it is related to the alterations in lipoprotein metabolism rather than an effect directly on the oxidation of the cholesterol[30].

Similarly to the current results, Park *et al.*[31] revealed that administration of ellagic acid alleviated AlCl₃-induced dyslipidemia and this is occurred by reducing lipid profile

picture, while increasing HDL-C levels. These findings proved that both quercetin and/or black berry have the ability for free radicals scavenging activities which indirect proportionally helps declining total cholesterol, triglycerides, LDL-C and VLDL-C levels[32].

Additionally, AlCl₃ was found to induce increment in MDA level. The MDA is considered as an oxidative stress biomarker[33]. Joshi *et al.*[34] supported these observations and they showing that AlCl₃ treatment had significantly raised MDA levels of the hepatic tissues. It has been previously reported that radiation could induce the lipid peroxidation, which leading to structural and alterations to the cellular membranes. Moreover, it has been demonstrated that the AlCl₃-toxic effects may be due to the reactive oxygen species generation, which induces oxidative impairment of layers of cellular lipid. The aluminium oxidative deleterious effect may be occurring due to interaction with the cellular membranes and delicate alterations that occur in the lipids rearrangement[35].

AlCl₃ can afford oxidative damage by a lot of mechanisms. The main AlCl₃ toxic effect includes the homeostasis disturbance of some metals in the body, like Mg and Fe[36]. The aluminium chemical properties allows to mimic Mg and Fe effectively in their biological functions and generate biochemical alterations. Aluminium can affect on iron homeostasis and thus makes this metal toxic and this is very dangerous for the vitality of the body.

Likewise, AlCl₃ has the ability to replace Mg metal and can bind to phosphate molecules on the cellular membrane and DNA[37]. The lineament of the oxidative damage is namely as increment of the lipid and the protein peroxidation, decreasing the membrane fluidity and altered the antioxidant states which all are related to the aluminium toxic effects[38]. Therefore, AlCl₃ strike its toxic effect by creating a cellular oxidative stress[39].

The cause of SOD decreasing activity could be assumed to inhibition feedback mechanism or SOD inhibition as a result of excessive reactive oxygen species generation and thus oxidative injury[7].

Additionally, the results clarified that exposure to AlCl₃ caused a significant decline in glutathione levels. Glutathione level was decreased dramatically in the cytosolic fraction after radiation exposure[40]. Similarly, it has been reported that glutathione content was significantly declined in the hepatic tissues after exposure to radiation[41]. Similarly, it has been reported that glutathione content was significantly declined in the hepatic tissues after exposure to radiation[42]. So, it may be concluded that glutathione level can be declined by the effect of free radicals that are produced by any substance that could induce damage effects like aluminium.

It is possible that the decrement in the antioxidant enzyme activities after AlCl₃ treatment could be as a result of the oxidative alterations of genes that organize these antioxidant enzymes[43], thereby reducing the assembly of the antioxidant enzymes[43]. Also, a no other finding explained that aluminium can suppress the antioxidant enzymes by aluminium interaction with enzymes responsible for free radical scavenging activities[44].

This might be as a result of inhibition of NADPH generating enzymes by aluminium compounds and this resulting in retarding of glutathione regeneration. Previously, it was reported that, aluminium compounds at high doses could affect the synthesis of glutathione which could result in reduced glutathione levels[45].

Histological examination of the hepatic tissues highly support the Qe and/or BB hepatoprotective effect against AlCl₃ toxicity, where the AlCl₃ induced hepatic structure alterations were attenuated after administration of Qe and/or BB. Formerly, it has been proved that aluminium induced hepatotoxicity as reported by Salem *et al.*[28] and this toxicity was alleviated by ellagic acid treatment.

It is eligibility observation that many a lot of consumed polyphenols as Qe and/or BB have numerous potent health-enhancing activities when estimated by *in vitro* assays and when given to the experimental animals[46].

The simultaneous ingestion of antioxidant micronutrients, minimize the gastrointestinal disintegration of polyphenols. Meanwhile the addition of polyphenols may enhance efflux transporters[47] and this reflects the healthy effect on liver tissues and thus, these previous finding strengthens the obtained results in the study and confirmed the role of both BB and Qe in enhancement of health.

Regarding this study, this study was a trial to elucidate AlCl₃ side effects alone and in combination with either BB, Qe or their combination. This study included the evaluation of the effect of the materials under study on some liver function markers as well as some antioxidant enzymes (SOD, MDA, GR, GST, CAT, MPO and XO). Liver tissues were used to study the histopathological changes.

It is best known that BB and Qe have been recognized as an effective antioxidant agents, therefore, the present study aimed to elucidate the possible ameliorative role of BB and Qe[48] against the toxicity of AlCl₃ when given to normal rats.

In this study, the hepatic tissues enzymes as CAT, GPx and SOD activities were declined in the AlCl₃ treated group. The depletion of catalase activity could be assigned to increasing utilization of catalase enzyme to oppose the lipid peroxidation triggering. The depletion of glutathione content has been shown to cause an inhibition of glutathione peroxidase activity[49].

Liver GST activity was significantly in decreased in AlCl$_3$ treated groups alone or combined with BB, Qe or both as compared to the control group (Table 3). Otherwise, the noticed decline of GSH level in AlCl$_3$ group might be due to aluminium-intermediated suppression of the enzyme that is responsible for the formation of glutathione which is known as γ glutamylcysteine synthetase[50].

CONCLUSION

The current experimental study aimed to elucidate the possible protective role of either BB and/or Qe against the AlCl$_3$ hepatotoxicity. Regarding, the toxicity of AlCl$_3$ was inspected by assessment of the liver function parameters as ALT, AST, total protein and antioxidant status, thus determining the enzymes' activities like MPO, XO, SOD, CAT and GR as well as MDA and GST levels. Furthermore, histological examination was performed at the hepatic level. The BB combined with Qe were shown to converse the AlCl$_3$-induced decrease of SOD, CAT and GR activities to diminish the MDA levels and the XO activity (increased by ALCl$_3$) and to enhance the levels of GR that declined by AlCl$_3$. The results manifested that AlCl$_3$ induced the free radical's formation, which are probably cause oxidative damage and structural alterations in the liver tissues and damage liver functions. Using of BB as combined with Qe allow to protect the liver from both biological and structural damages.

SIGNIFICANCE STATEMENT

This study discovered the hepatoprotective effects of BB and/or Qe against oxidative stress induced by AlCl$_3$. A novel mechanism of ameliorative effect of BB and/or Qe against hepatotoxicity induced by AlCl$_3$ was approved as many peoples exposed to AlCl$_3$ and thus, these peoples may be exposed to hepatocellular damage with oxidative stress without any apparent cause and thus giving these peoples supplementation of BB and/or Qe will protect them against these alterations and also enhance their hepatic function biomarkers. Therefore, these results clearly demonstrate a protective role for BB and/or Qe against hepatotoxicity induced by AlCl$_3$.

ACKNOWLEDGMENTS

REFERENCES

1. Yousef, M.I. and A.F. Salama, 2009. Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. Food Chem. Toxicol., 47: 1168-1175.

2. Exley, C., 1998. Does antiperspirant use increase the risk of aluminium-related disease, including Alzheimer's disease? Mol. Med. Today, 4: 107-109.

3. Venturini-Soriano, M. and G. Berthon, 2001. Aluminum speciation studies in biological fluids: Part 7. A quantitative investigation of aluminum (III)-malate complex equilibria and their potential implications for aluminum metabolism and toxicity. J. Inorg. Biochem., 85: 143-154.

4. Cranmer, J.M., J.D. Wilkins, D.J. Cannon and L. Smith, 1986. Fetal-placental-maternal uptake of aluminum in mice following gestational exposure: Effect of dose and route of administration. Neurotoxicology, 7: 601-608.

5. Paunovic, S.M., P. Maskovic, M. Nikolic and R. Miletic, 2017. Bioactive compounds and antimicrobial activity of black currant (*Ribes nigrum* L.) berries and leaves extract obtained by different soil management system. Scientia Horticult., 222: 69-75.

6. Mattila, M.L., M. Kielinen, S.L. Linna, K. Jussila and H. Ebeling *et al.*, 2011. Autism spectrum disorders according to *DSM-IV-TR* and comparison with DSM-5 draft criteria: An epidemiological study. J. Am. Acad. Child Adolescent Psychiat., 50: 583-592.

7. Al-Eisa, R.A., F.A. Al-Salmi, R.Z. Hamza and N.S. El-Shenawy, 2018. Role of L-carnitine in protection against the cardiac oxidative stress induced by aspartame in Wistar albino rats. Plos One, Vol. 13. 10.1371/journal.pone.0204913

8. Hamza, R.Z., N.S. El-Shenawy and H.A. Ismail, 2015. Protective effects of blackberry and quercetin on sodium fluoride-induced oxidative stress and histological changes in the hepatic, renal, testis and brain tissue of male rat. J. Basic Clin. Physiol. Pharmacol., 26: 237-251.

9. Boots, A.W., G.R.M.M. Haenen and A. Bast, 2008. Health effects of quercetin: From antioxidant to nutraceutical. Eur. J. Pharmacol., 585: 325-337.

10. Naeimi, R., S. Baradaran, M. Ashrafpour, A.A. Moghadamnia and M. Ghasemi-Kasman, 2018. Querectin improves myelin repair of optic chiasm in lyolecithin-induced focal demyelination model. Biomed. Pharmacother., 101: 485-493.

11. Hirpara, K.V., P. Aggarwal, A.J. Mukherjee, N. Joshi and A.C. Burman, 2009. Quercetin and its derivatives: Synthesis, pharmacological uses with special emphasis on anti-tumor properties and prodrug with enhanced bio-availability. Anticancer Agents Med. Chem., 9: 138-161.

12. Baghel, S.S., N. Shrivastava, R.S. Baghel, P. Agrawal and S. Rajput, 2012. A review of quercetin: Antioxidant and anticancer properties. World J. Pharm. Pharm. Sci., 1: 146-160.

13. Johari, J., A. Kianmehr, M. Mustafa, S. Abubakar and K. Zandi, 2012. Antiviral activity of baicalein and quercetin against the *Japanese encephalitis* virus. Int. J. Mol. Sci., 13: 16725-16795.

14. Stavric, B., 1994. Quercetin in our diet: from potent mutagen to probable anticarcinogen. Clin. Biochem., 27: 245-248.

15. Sautebin, L., A. Rossi, I. Serraino, P. Dugo and R. Di Paola *et al.*, 2004. Effect of anthocyanins contained in a blackberry extract on the circulatory failure and multiple organ dysfunction caused by endotoxin in the rat. Planta Medica, 70: 745-752.

16. Kaplan, A. and J. Szalbo, 1984. Clinical Chemistry: Interpretation and Techniques. 2nd Edn., Lea and Febiger, Philadelphia, Page: 157.

17. Aebi, H., 1984. Catalase *in vitro*. Meth. Enzymol., 105: 121-126.

18. Marklund, S. and G. Marklund, 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem., 47: 469-474.

19. Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.

20. Couri, D. and M.S. Abdel-Rahman, 1979. Effect of chlorine dioxide and metabolites on glutathione dependent system in rat, mouse and chicken blood. J. Environ. Pathol. Toxicol., 3: 451-460.

21. Hafeman, D.G., R.A. Sunde and W.G. Hoekstra, 1974. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J. Nutr., 104: 580-587.

22. Suzuki, K., H. Ota, S. Sasagawa, T. Sakatani and T. Fujikura, 1983. Assay method for myeloperoxidase in human polymorphonuclear leukocytes. Anal. Biochem., 132: 345-352.

23. Litwack, G., J.W. Bothwell, J.N. Williams Jr. and C.A. Elvehjem, 1953. A colorimetric assay for xanthine oxidase in rat liver homogenates. J. Biol. Chem., 200: 303-310.

24. Gabe, M., 1968. Techniques Histologiques [Histological Techniques]. Masson Publisher, Paris.

25. Snedecor, G.W. and W.G. Cochran, 1982. Statistical Methods. 8th Edn., Iowa State University Press, Ames.

26. Hamza, R.Z., N.S. Al-Juaid and E.H. Althubaiti, 2018. Antioxidant effect of carnosine on aluminum oxide nanoparticles (Al$_2$O$_3$-NPs)-induced hepatotoxicity and testicular structure alterations in male rats. Int. J. Pharmacol., 14: 740-750.

27. Al-Salmi, F.A., R. Al-Eisa, R.Z. Hamza, H.E. Khaled and N.S. El-Shenawy, 2019. Protective effect of L-cysteine against sodium valproate-induced oxidant injury in testis of rats. Pharmacology, 15: 248-256.

28. Salem, A.M., T.F. Mohammaden, M.A. Ali, E.A. Mohamed and H.F. Hasan, 2016. Ellagic and ferulic acids alleviate gamma radiation and aluminium chloride-induced oxidative damage. Life Sci., 160: 2-11.

29. Onody, A., C. Csonka, Z. Giricz and P. Ferdinandy, 2003. Hyperlipidemia induced by a cholesterol-rich diet leads to enhanced peroxynitrite formation in rat hearts. Cardiovasc. Res., 58: 663-670.

30. Belaid-Nouira, Y., H. Bakhta, M. Bouaziz, I. Flehi-Slim, Z. Haouas and H.B. Cheikh, 2012. Study of lipid profile and parieto-temporal lipid peroxidation in AlCl$_3$ mediated neurotoxicity. Lipids Health Dis., Vol. 11. 10.1186/1476-511X-11-16.

31. Park, S.H., J.L. Kim, E.S. Lee, S.Y. Han, J.H. Gong, M.K. Kang and Y.H. Kang, 2011. Dietary ellagic acid attenuates oxidized LDL uptake and stimulates cholesterol efflux in murine macrophages. J. Nutr., 141: 1931-1937.

32. Makena, P.S. and K.T. Chung, 2007. Effects of various plant polyphenols on bladder carcinogen benzidine-induced mutagenicity. Food Chem. Toxicol., 45: 1899-1909.

33. Abu-El-Zahab, H.S., R.Z. Hamza, M.M. Montaser, M.M. El-Mahdi and W.A. Al-Harthi, 2019. Antioxidant, antiapoptotic, antigenotoxic and hepatic ameliorative effects of L-carnitine and selenium on cadmium-induced hepatotoxicity and alterations in liver cell structure in male mice. Ecotoxicol. Environ. Safety, 173: 419-428.

34. Joshi, R., J.P. Kamar and T. Mukherjee, 2007. Free radical scavenging reactions and antioxidant activity of embelin: Biochemical and pulse radiolytic studies. Chemico-Biol. Interact., 167: 125-134.

35. Anane, R. and E.E. Creppy, 2001. Lipid peroxidation as pathway of aluminium cytotoxicity in human skin fibroblast cultures: Prevention by superoxide dismutase+catalase and vitamins E and C. Hum. Exp. Toxicol., 20: 477-481.

36. Nordberg, J. and E.S.J. Arner, 2001. Reactive oxygen species, antioxidants and the mammalian thioredoxin system. Free Radic. Biol. Med., 31: 1287-1312.

37. Tomljenovic, L., 2011. Aluminum and Alzheimer's disease: After a century of controversy, is there a plausible link? J. Alzheimer's Dis., 23: 567-598.

38. Ferretti, G., C. Marchionni, T. Bacchetti, T. Galeazzi and N. Dousset, 2003. Effect of aluminium on lipid peroxidation of human high density lipoproteins. Free Radic. Res., 37: 515-521.

39. Bhasin, P., N. Singla and D.K. Dhawan, 2014. Protective role of zinc during aluminum induced hepatotoxicity. Environ. Toxicol., 29: 320-327.

40. Gehlot, P. and P.K. Goyal, 2009. Radioprotective effects of *Aloe vera* leaf extract on Swiss albino mice against whole-body gamma irradiation. J. Environ. Pathol. Toxicol. Oncol., 28: 53-61.

41. Srinivasana, M., A.R. Sudheera, K.R. Pillaib, P.R. Kumarc, P.R. Sudhakarand and V.P. Menon, 2007. Lycopene as a natural protector against γ-radiation induced DNA damage, lipid peroxidation and antioxidant status in primary culture of isolated rat hepatocytes *in vitro*. Biochim. Biophys. Acta-Gen. Subjects, 1770: 659-665.

42. El-Megharbel, S.M., A.S. Almalki, R.Z. Hamza, A.A. Gobouri, A.A. Alhadhrami, J.Y. Al-Humaidi and M.S. Refat, 2018. Synthesis and suggestion of a new nanometric gold (III) melatonin drug complex: An interesting model for testicular protection. Future Med. Chem., 10: 1693-1704.

43. Nehru, B. and P. Anand, 2005. Oxidative damage following chronic aluminium exposure in adult and pup rat brains. J. Trace Elem. Med. Biol., 19: 203-208.

44. Becaria, A., A. Campbell and S.C. Bondy, 2002. Aluminum as a toxicant. Toxicol. Ind. Health, 18: 309-320.

45. Orihuela, D., V. Meichtry, N. Pregi and M. Pizarro, 2005. Short-term oral exposure to aluminium decreases glutathione intestinal levels and changes enzyme activities involved in its metabolism. J. Inorganic Biochem., 99: 1871-1878.

46. Scheepens, A., K. Tan and J.W. Paxton, 2010. Improving the oral bioavailability of beneficial polyphenols through designed synergies. Genes Nutr., Vol. 5. 10.1007/s12263-009-0148-z.

47. Bohn, T., 2014. Dietary factors affecting polyphenol bioavailability. Nutr. Rev., 72: 429-452.

48. Ismail, H.A., R.Z. Hamza and N.S. El-Shenawy, 2014. Potential protective effects of blackberry and quercetin on sodium fluoride-induced impaired hepato-renal biomarkers, sex hormones and hematotoxicity in male rats. J. Appl. Life Sci. Int., 1: 1-16.

49. Hamza, R.Z., R.A. Al-Eisa, A.E. Mehana and N.S. El-Shenawy, 2019. Effect of L-carnitine on aspartame-induced oxidative stress, histopathological changes and genotoxicity in liver of male rats. J. Basic Clin. Physiol. Pharmacol., Vol. 30. 10.1515/jbcpp-2018-0064.

50. Hamza, R.Z., F.A. Al-Salmi and N.S. El-Shenawy, 2018. Nanoparticles effects on zinc oxide/green tea complex on the lipid profile and liver functions of rats after monosodium glutamate treatment. J. Applied Sci., 18: 65-70.