Research Article

Bromelain Modulates Liver Injury, Hematological, Molecular, and Biochemical Perturbations Induced by Aluminum via Oxidative Stress Inhibition

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Aluminum (Al) is an important metal widely disseminated in various environmental compartments and largely used in daily life leading to hazardous effects via oxidative stress. Bromelain is a cheap extract from the byproduct waste of Ananas comosus stem. It has been used in several biological and therapeutic applications. So, this study was undertaken to assess the hepatoprotective potential of bromelain versus oxidative stress induced by aluminum chloride in rats. Results revealed that administration of AlCl₃ reduced the body and liver weights and increased Al concentration in the blood and liver tissue. Also, AlCl₃ caused valuable changes in hematological parameters and increased TBARS and H₂O₂ concentrations in rat liver. Enzymatic (SOD, CAT, GPx, GR, and GST) and nonenzymatic (GSH) antioxidants and protein content were significantly decreased. Furthermore, alterations in liver biomarkers such as bilirubin level and enzyme activities in both serum and liver homogenate (LDH, ALP, AST, and ALT) were detected. AlCl₃ also caused inflammation as indicated by upregulation of the inflammation-related genes [interleukin 1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), as well as matrix metalloproteinase (MMP9)], and downregulation of nuclear factor erythroid 2 (Nrf2) expression. In addition, histopathological examination showed significant variations in the liver that confirms the biochemical results. Otherwise, bromelain intake alone slumped lipid peroxidation and gotten better antioxidant status significantly. Moreover, supplementation with bromelain before AlCl₃ intoxication restores enzymatic and nonenzymatic antioxidants as well as biochemical indices and tissue architecture with respect to the AlCl₃ group. In conclusion, bromelain proved its remarkable protective power to abolish AlCl₃ toxicity. So, it might represent a new strategy in the therapy of metal toxicity by its antioxidant capacity.

1. Introduction

Aluminum (Al) is an important metal widely disseminated in various environmental compartments and largely used in daily life leading to many health problems in humans and animals [1]. The major origin of Al is due to its ingestion in different food (corn, yellow and processed cheese, baking powder, and flour) [2, 3]. Aluminum and its different compounds are used in the food industry (processing, packaging, and storage) leading to an increase in their levels in foods [4]. Also, they are used broadly in water purification [5], in medicines such as antacids, and in food additives authorizing their entrance into the body [6] and causing serious health problems in human and animal [7]. As an environmental contaminant, Al exposure generated hurtful effects on different biological systems including blood...
constituents, nervous, respiratory, skeletal, and immune systems [7]. Additionally, it is of great importance in various free radical-mediated diseases, osteomalacia, nephrotoxicity, and hepatotoxicity [8, 9]. Aluminum toxicity occurred through different mechanisms that encompass increasing blood-brain barrier permeability, interference with phosphorylation-dephosphorylation processes, and interchange of ions metabolism with successive free radicals’ production and perturbation of the second messenger system [10]. Al salts may affect enzyme activity like hexokinase, phosphatases, phosphodiesterase, and phosphooxidase [5, 11]. Moreover, Al generates reactive oxygen species (ROS) [12], resulting in oxidative deterioration of lipids, proteins, and DNA.

Great interest is directed to many plants because of their antioxidant potential. Ananas comosus (Pineapple), which belongs to the Bromeliaceae family, is one of them. Waste usage is a promising strategy to get rid of the huge waste from processing. Ananas comosus is largely cultivated in the equatorial regions worldwide and has wide beneficial known effects as antioxidant, anticancer, anti-inflammatory, and antiplatelet impact. A. comosus stem extract is a waste product rich in complex enzymes identified by bromelain which are so important in some clinical applications, especially tumor growth modulation, wound healing, anti-inflammatory effect, anti-diarrhea, and digestive help [13-15]. Bromelain has many commercial uses including food industry, pharmaceutical products such as cosmetics, for health benefits, and supplements as well as protein hydrolysates production [16, 17]. Prolonged oral use of bromelain is safe and it can be absorbed easily in the human intestinal tract without any decomposition or activity loss [18, 19]. The utilization of pineapple wastes as a source of bioactive compounds, especially proteolytic enzymes, is an alternative means. Therefore, the present study was designed to assess the potential antioxidant role of bromelain in modulating the harmful impacts induced by aluminum in male rats.

2. Materials and Methods

2.1. Chemicals. Bromelain from A. comosus (Pineapple) stem extract (600 GDU’s/g) was purchased from Holland and Barrett, England. Aluminum chloride (AlCl₃) was bought from Aldrich Chemical Company (Milwaukee, USA).

2.2. Experimental Design. The experimental design was performed following the US National Institute of Health Guidelines for the Care and Use of Laboratory Animals and Helsinki’s declaration of animal ethics as approved by the Research Ethics Committee of Alexandria University (AU14-200204-1-3). Twenty-eight male Wister rats (150-170 g) were bought from the Faculty of Medicine, Alexandria University, Alexandria, Egypt. Rats were distributed randomly in cages seven per each and kept on a commercial diet and provided with tap water ad libitum and acclimated for two weeks (temperature, 21°C; photoperiod, 7 a.m. to 7 p.m.). Animals were classified into four groups: control, bromelain (250 mg/kg), AlCl₃ (34 mg/Kg, 1/25 LD₅₀), and bromelain plus AlCl₃, respectively. Bromelain was administered one hour before AlCl₃ intoxication daily while AlCl₃ was given day after day orally for 30 days according to Saxena and Panjwani and El-Demerdash [20, 21], respectively. None of the AlCl₃-intoxicated rats showed signs of morbidity or mortality during the study. At the experiment termination, rats were anesthetized using isoflurane and then killed via cervical dislocation, and livers were immediately removed. The liver was divided into two portions: the first portion was fixed in 10% formalin for histopathology examination, and the second portion was stored at -80°C for biochemical analyses.

2.3. Measurement of Aluminum Concentration. The level of AlCl₃ was measured in the blood and liver tissue according to the method of Van Ginkel et al. [22] using the atomic absorption spectrometer (Shimadzu, AA6200).

2.4. Blood Samples. Complete blood counts (CBC) were performed in the collected blood samples by automatic methods (Sysmex kx-21n automated hematology analyzer; JAPAN CARE CO., LTD) including hemoglobin (Hb), white blood cells (WBCs), red blood cells (RBCs), platelets and hematocrit, or packed cell volume (PCV). Other blood samples were assembled for serum preparation and were left in a stand position for 30 min for blood clotting at 25°C then centrifuged at 3000 g for 15 min. The serum of each sample was taken and stored at -80°C till utilized in the determination of biochemical parameters.

2.5. Tissue Preparation. Livers were taken away and homogenized in ice-cold 0.01 mol/l sodium-potassium phosphate with 1.15% KCl buffer (pH 7.4). The homogenate was centrifuged at 10,000 g (4°C) for 20 min then the supernatants were taken and utilized for the determination of different assays.

2.6. Determination of TBARS, H₂O₂, and Glutathione Content. Thiobarbituric acid-reactive substances (TBARS), hydrogen peroxide (H₂O₂), and reduced glutathione (GSH) content were determined using the methods of Ohkawa et al., Velikova et al., and Ellman, respectively [23–25].

2.7. Determination of Antioxidant Enzyme Activities. The activities of superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), and glutathione S-transferase (GST; EC 2.5.1.18) were assessed by the methods of Misra and Fridovich, Aebi, and Habig et al. [26–28], respectively. While the activities of glutathione peroxidase (GPx; EC 1.11.1.9) and glutathione reductase (GR; EC 1.6.4.2) were evaluated according to Hafeman et al. [29].

2.8. Determination of Liver Function Biomarkers. Lactate dehydrogenase (LDH; EC 1.1.1.27) and alkaline phosphatase (ALP; EC 3.1.3.1) activities, protein content, and total bilirubin were estimated according to the methods used in the previous research [30–33]. Alanine aminotransferase (ALT; EC 2.6.1.2) and aspartate aminotransferase (AST; EC 2.6.1.1) activities were assayed using kits from Biodiagnostic, Egypt.
2.9. Molecular Analysis by Real-Time PCR. Using the RNeasy mini kit (Qiagen), total RNA was extracted from liver tissue in accordance with the manufacturer’s recommendations. 10 μg of RNA were reversely transcribed to produce first-strand cDNA. Real-time PCR was used to evaluate the relative expressions of matrix metallopeptidase 9 (MMP9), nuclear factor erythroid 2 (Nrf2), interleukin 1β (IL-1β), and tumor necrosis factor-α (TNF-α). Gene-specific primers are displayed in Table 1. A Real-Time PCR System (Applied Biosystems, USA) was used for the procedure, which consisted of 40 cycles of denaturation at 95°C for 30 s, annealing for both genes at 59°C for 30 s, and extension at 72°C for 30 s. The results were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA expression that acts as the internal control and was amplified in the same process [34]. The relative gene expression was calculated using the 2^−ΔΔCt method.

2.10. Histopathological Examinations. Livers were fixed in 10% formalin and serial paraffin sections were obtained to examine the histological changes using hematoxylin and eosin stain [35] then, slides were photographed by light microscope (Olympus BX 41, Japan).

2.11. Statistical Analysis. Data from different groups were presented as means ± standard errors (SEM) and then analyzed utilizing SPSS software (version 22, IBM Co., Armonk, NY). Comparison between groups was performed by ANOVA followed by Tukey’s post-hoc test. P value ≤0.05 was approved to be significant.

### 3. Results

3.1. Body Weight. Final body weight and body weight gain in addition to the absolute liver weight of AlCl₃-treated rats were significantly decreased as compared to control. However, bromelain supplementation alleviated this reduction with respect to AlCl₃ exposed group. Bromelain alone did not cause any significant change (Figures 1 and 2).

3.2. Aluminum Concentration in Rat Liver and Blood. The Al concentration in rat liver and blood was measured after one month of oral AlCl₃ administration (Figure 3). The level of Al in the liver and blood of the AlCl₃ intoxicated group was increased by +64.21% and +81.45% when compared to the control group, respectively. However, this concentration was significantly decreased in the liver and blood of rats treated with bromelain plus AlCl₃ by +38.56% and +47.89% as compared to the AlCl₃ intoxicated group, respectively.

3.3. Hematological Parameters. Rats administered AlCl₃ exhibited a significant decline in RBCs, Hb, PCV, and
lymphocytes concentration while WBCs, platelets, and neutrophils increased significantly as compared to the control group. Other blood parameters are not significantly changed. On the other hand, the administration of bromelain alone showed a nonsignificant change in blood parameters as compared to the control group. Rats pretreated with bromelain and then received AlCl₃ showed significant restoration near the normal level as compared to AlCl₃ treated rats (Table 2).

3.4. Lipid Peroxidation and Reduced Glutathione Content. Results revealed that the levels of TBARS and H₂O₂ were significantly (P < 0.05) increased in rats treated with AlCl₃ versus control while rats pretreated with bromelain and then intoxicated by AlCl₃ presented a significant reduction in TBARS and H₂O₂ levels as compared to AlCl₃-treated rats. Otherwise, GSH content was significantly decreased in AlCl₃-treated rats. While in the rats’ group ingested with both bromelain and AlCl₃, induction in GSH content was observed as compared with AlCl₃-treated rats. Supplementation with bromelain alone reduced the concentrations of TBARS and H₂O₂ and induced GSH content in liver homogenate significantly (Table 3).

3.5. Antioxidant Enzymes. A significant reduction (P < 0.05) in SOD, CAT, GPx, GR, and GST activity was observed in liver homogenate of AlCl₃-treated rats. Furthermore, rats taken with bromelain+AlCl₃ showed significant alleviation in antioxidant enzyme activities as compared to AlCl₃-treated ones (P < 0.05). Moreover, the treatment of rats with bromelain alone improved antioxidant enzyme activities significantly versus the control group (Table 4).

3.6. Liver Function Biomarkers. Data showed that AST, ALT, and ALP activities were significantly (P < 0.05) decreased in liver homogenate and increased in rat serum while LDH activity increased in serum and liver homogenates of rats receiving AlCl₃ with respect to control. Protein content was decreased while bilirubin was increased in rat liver homogenate, significantly. Moreover, a significant modulation in enzyme activities, protein and bilirubin contents in rats that received bromelain and then intoxicated with AlCl₃ versus AlCl₃ group was observed. Bromelain supplementation alone had insignificantly affected the measured parameters (Table 5).

3.7. Genes Expression. In the hepatic tissues of rats given AlCl₃, a considerable upregulation in the mRNA expression of the MMP9, IL-1β, and TNF-α genes and a downregulation of the Nrf2 gene were found in comparison to the control group. On the other hand, the administration of bromelain prior to AlCl₃ resulted in a considerable modification in the examined genes as opposed to the AlCl₃ group. Furthermore, the intake of bromelain alone increased the Nrf2 mRNA expression in the hepatic tissues in comparison to the control group (Figure 4).

3.8. Liver Histopathology. Light microscopic examination of the liver from control (G1) and bromelain (G2) groups showed normal architecture of hepatic lobules. However, liver sections of rats given AlCl₃ leading to calcium replacement [41]. Additionally, Al compound in the blood as citrate [42]. So, it interferes with Fe homeostasis by displacing it from transferrin leading to disturbance in iron metabolism [43]. Interestingly, bromelain supplementation for 30 days improved these parameters due to its antioxidant and chelation properties.

Blood and hematopoietic tissues rank as target organs for toxic effects of environmental chemicals; hence, they offer sensitive and reliable indicators, which could be effectively used to detect the magnitude of biochemical stress [44]. In agreement with the present results, perturbations in blood parameters in rats treated with AlCl₃ were observed and this attributed to the inhibition of erythropoiesis and

![Figure 3: Aluminum concentration in liver and blood of male rats in different experimental groups. Values are expressed as means ± standard error (SEM); n = 7 for each treatment group. Values are expressed as means ± SE; n = 7 for each treatment group. A significant difference between the groups was shown with different superscript letters (a, b, c, and d), P < 0.05.](image-url)
Table 2: Effect of bromelain (Bro), aluminum chloride (AlCl₃), and their combination (Bro+AlCl₃) on the hematological parameters in rats.

| Parameters (%) | Cont.       | Bro          | AlCl₃        | Bro+AlCl₃     |
|----------------|-------------|--------------|--------------|---------------|
| RBCs (x 10⁶/µL) | 8.03±0.274  | 8.41±0.246   | 5.78±0.264   | 8.33±0.345    |
| WBCs (x 10⁶/µL) | 10.97±0.193 | 11.51±0.418  | 13.23±0.387  | 11.96±0.340   |
| Hemoglobin (g/dl) | 13.20±0.205 | 13.82±0.455  | 9.51±0.197   | 11.79±0.202   |
| Platelets (10³/µL) | 427±12.08   | 427±14.86    | 527±13.59   | 470±13.63     |
| PCV (%)          | 47.16±1.65  | 47.16±1.42   | 38.10±1.07  | 42.09±1.25    |
| MCV (fl)         | 63.58±1.90  | 66.28±1.99   | 65.80±1.99  | 65.47±2.11    |
| MCHC (%)         | 18.90±0.620 | 19.18±0.487  | 19.90±0.528 | 19.47±0.603   |
| MCH (%)          | 32.59±0.298 | 32.46±0.301  | 31.93±0.506 | 31.99±0.415   |
| Neutrophils (%)  | 19.14±0.459 | 19.7±0.522   | 23.29±0.837 | 20.71±0.680   |
| Lymphocytes (%)  | 58.13±0.527 | 56.26±2.15   | 45.36±1.20  | 52.75±1.28    |
| Eosinophils (%)  | 1.57±0.297  | 1.60±0.170   | 1.54±0.177  | 1.58±0.276    |
| Monocytes (%)    | 4.19±0.142  | 4.07±0.170   | 4.36±0.180  | 4.17±0.166    |

RBC: red blood cell; WBC: white blood cell; hb: hemoglobin; PCV: packed cell volume; MCV: mean cell volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration. Values are expressed as means ± SEM. *P<0.05. Statistically significant variations are compared as follows: Bromelain and AlCl₃ groups are compared vs control group while Bromelain+AlCl₃ group is compared vs AlCl₃ group.

Table 3: Effect of bromelain (Bro), aluminum chloride (AlCl₃), and their combination (Bro+AlCl₃) on the level of thiobarbituric acid reactive substances (TBARS), hydrogen peroxide (H₂O₂), and reduced glutathione (GSH) content in rat liver.

| Parameters (%) | Cont.       | Bro          | AlCl₃        | Bro+AlCl₃     |
|----------------|-------------|--------------|--------------|---------------|
| TBARS (nmol/g tissue) | 30.20±0.899 | 23.49±0.599  | 44.49±1.28   | 36.31±0.928   |
| % change       | (22.22%)    | (+47.33%)   | (+20.25%)    |
| H₂O₂ (µmol/g tissue) | 63.65±1.62  | 50.35±1.36   | 90.26±1.45   | 78.92±2.02    |
| % change       | (-20.90%)   | (+41.81%)   | (+23.98%)    |
| GSH (mmol/mg protein) | 1.72±0.052  | 2.05±0.061   | 0.99±0.031   | 1.39±0.041    |
| % change       | (+19.37%)   | (-42.27%)   | (-18.95%)    |

Values are expressed as means ± SEM; *P<0.05. Statistically significant variations are compared as follows: Bromelain and AlCl₃ groups are compared vs control group while Bromelain+AlCl₃ group is compared vs AlCl₃ group.

Table 4: Effect of bromelain (Bro), aluminum chloride (AlCl₃), and their combination (Bro+AlCl₃) on the activities of antioxidant enzymes in rat liver.

| Parameters (%) | Cont.       | Bro          | AlCl₃        | Bro+AlCl₃     |
|----------------|-------------|--------------|--------------|---------------|
| SOD (U/mg protein) | 75.93±2.25  | 91.38±3.19  | 38.83±1.16   | 60.46±1.64    |
| % change       | (+20.35%)   | (-48.86%)   | (-20.37%)    |
| CAT (µmol/hr/mg protein) | 46.07±1.62  | 54.85±1.82  | 25.39±0.574  | 35.72±1.08    |
| % change       | (+19.05%)   | (-44.89%)   | (-22.47%)    |
| GPx (U/mg protein) | 1.07±0.038  | 1.26±0.041  | 0.60±0.021   | 0.85±0.025    |
| % change       | (+18.08%)   | (-43.66%)   | (-20.66%)    |
| GR (U/mg protein) | 1.23±0.042  | 1.46±0.036  | 0.70±0.023   | 0.99±0.033    |
| % change       | (+18.77%)   | (-42.53%)   | (-19.46%)    |
| GST (µmol/hr/mg protein) | 1.26±0.032  | 1.50±0.045  | 0.67±0.022   | 0.99±0.027    |
| % change       | (+19.23%)   | (-46.93%)   | (-21.22%)    |

Values are expressed as means ± SEM; *P<0.05. Statistically significant variations are compared as follows: Bromelain and AlCl₃ groups are compared vs control group while Bromelain+AlCl₃ group is compared vs AlCl₃ group.
Table 5: Effect of bromelain (Bro), aluminum chloride (AlCl₃), and their combination (Bro+AlCl₃) on enzyme activities and protein content in serum and liver of male rats.

| Parameters     | Cont.       | Bro          | AlCl₃        | Bro+AlCl₃    |
|----------------|-------------|--------------|--------------|--------------|
| Serum          |             |              |              |              |
| AST (U/l)      | 53.12 ± 1.12| 51.20 ± 1.51 | 76.88 ± 2.29 | 65.97 ± 1.75 |
| % change       | (-3.62%)    | (+44.74%)    | (+24.19%)    |              |
| ALT (U/l)      | 58.57 ± 1.28| 54.70 ± 0.84 | 82.89 ± 2.42 | 71.53 ± 2.37 |
| % change       | (-6.61%)    | (+41.54%)    | (+22.17%)    |              |
| LDH (U/l)      | 585 ± 14.32 | 563 ± 14.21  | 823 ± 18.84  | 713 ± 20.03  |
| % change       | (-3.71%)    | (+40.82%)    | (+21.95%)    |              |
| ALP (U/l)      | 59.01 ± 1.90| 58.20 ± 2.09 | 83.80 ± 2.32 | 69.47 ± 2.05 |
| % change       | (-1.38%)    | (+42.01%)    | (+17.72%)    |              |
| Bilirubin (mg/dl) | 0.730 ± 0.017| 0.732 ± 0.016| 1.02 ± 0.027 | 0.871 ± 0.028|
| % change       | (+0.25%)    | (+39.13%)    | (+19.29%)    |              |
| Liver          |             |              |              |              |
| AST (U/mg protein) | 1254 ± 3.75 | 131.32 ± 3.15| 80.43 ± 1.78 | 102 ± 2.88   |
| % change       | (+47.9%)    | (-35.78%)    | (-18.54%)    |              |
| ALT (U/mg protein) | 1624 ± 5.03 | 154 ± 3.29   | 99 ± 3.40    | 131 ± 4.21   |
| % change       | (-5%)       | (-38.73%)    | (-19.12%)    |              |
| LDH (U/mg protein) | 1001c ± 34.04| 940 ± 31.17  | 1366 ± 37.79 | 1206 ± 27.83 |
| % change       | (-6.07%)    | (+36.51%)    | (+20.58%)    |              |
| ALP (U/mg protein) | 3444 ± 9.98 | 374 ± 11.18  | 216 ± 6.21   | 291 ± 7.68   |
| % change       | (+8.87%)    | (-37.32%)    | (-15.33%)    |              |
| Protein (mg/g tissue) | 1944 ± 2.44 | 197 ± 4.29   | 128 ± 4.45   | 163 ± 4.02   |
| % change       | (+1.48%)    | (-34.15%)    | (-16.10%)    |              |

Values are expressed as means ± SEM; n = 7 for each treatment group. *Mean values within a row not sharing a common superscript letter were significantly different, P < 0.05. Statistically significant variations are compared as follows: Bromelain and AlCl₃ groups are compared vs control group while Bromelain + AlCl₃ group is compared vs AlCl₃ group.

Iron metabolism as well as alterations in erythrocyte morphology leading to anemia [45]. Furthermore, leukocytosis observed indicates the improved defense mechanism and immune system against infection induced by xenobiotics [46]. Bromelain is extensively used to improve tissue regeneration [47] and acts as an anti-inflammatory agent [19]. In this work, the administration of bromelain markedly hampered the toxic action produced by AlCl₃ on hematological parameters as it is efficient in improving blood circulation and the amendment of arterial diseases [48]. Also, bromelain is used as a natural blood thinner because it prohibits exaggerated stickiness of blood platelets [49] and affects blood coagulation by inducing fibrinolytic capability and by reducing fibrin synthesis [50].

The liver is an important organ rich in mitochondria and plays a significant role in the metabolic process. AlCl₃ inhibits the enzymes of oxidative phosphorylation leading to the cessation of energy metabolism [51]. So, oxidative stress and excess ROS production, via the Fenton reaction, have been involved in the mechanism of aluminum toxicity [21, 52] causing hepatocellular damage, apoptosis, and cellular necrosis [53, 54]. ROS Overproduction provokes injury via oxidizing cellular macromolecules such as lipids and proteins and triggering DNA injury [55]. Lipid peroxidation is a highly damaging oxidative process that occurs in phospholipid compartments of the cellular membrane following metal intoxication [56]. AlCl₃-treated rats manifested disruption in the antioxidant status where significant elevation in TBARS and H₂O₂ accompanied by a reduction in enzymatic (SOD, CAT, GPx, GR, and GST) and nonenzymatic antioxidants (GSH) in the liver homogenate were observed. The level of LPO is specified by the oxidants/antioxidants balance where the oxidants produced can be eliminated by the antioxidants [57, 58]. In agreement, previous authors showed that heavy metals work as oxidants affecting different organs leading to oxidative stress [8, 21, 54, 59–61]. On the other hand, treatment with bromelain given rise to a significant amelioration in oxidative stress markers (TBARS and H₂O₂) in AlCl₃ intoxicated rats and this reflects its immense antioxidant properties and its interaction with heavy metals [8, 62, 63] beside the presence of cysteine, an amino acid with known antioxidant properties. Also, it is an important precursor in the output of glutathione, which protects cells from toxins as free radicals incrimented in AlCl₃ toxicity [64]. Thus, bromelain supplementation could overcome AlCl₃-induced hepatotoxicity by abolishing oxidative tissue injuries.

Glutathione is a low molecular weight tripeptide with a thiol group. It plays a significant function in cell metabolism and defense versus toxicants [57]. Glutathione can directly remove free radicals or provide detoxification using GSH-dependent enzymes (GST, GR, and GPx) as substrates...
Antioxidant enzymes (SOD, CAT, GPx, GR, and GST) have a majestic role in the elimination of ROS and keeping cellular homeostasis for normal cell function as well as act as indicators of oxidative stress [66]. The significant reduction in antioxidant enzymes and GSH might be attributed to Al accumulation observed in liver cells leading to a decline in enzyme protein synthesis [59, 67]. Superoxide dismutase is implicated in the cellular defense versus oxidative injury in aerobic living organisms, where it stimulates the conversion of superoxide anion to O2 and H2O2, which is decomposed by catalase into H2O. GPx protects the membrane lipids from oxidative injury [68] and catalyzes the reaction of hydroperoxide radicals with GSH to form disulfide glutathione (GSSG) [69]. While GST is a detoxifying enzyme that acts to convert xenobiotics into water-soluble nontoxic metabolites easily excreted outside the body [70]. Aluminum may affect the synthesis of GSH through the inhibition of glutathione-synthase and glucose 6-phosphate dehydrogenase activities. Moreover, it retards the conversion of oxidized glutathione (GSSG) into its reduced form (GSH) via GR inhibition [71]. Therefore, the antioxidant defense system is so important in the protection against oxidative stress-induced by Al and disturbing antioxidant enzymes [21, 72] in rat liver via the prohibition of free radicals chain reaction. Otherwise, the observed induction in antioxidant enzyme activities might be related to the decline in radical’s generation and accumulation that are prohibited by bromelain [60]. Furthermore, it can protect against the toxic effects of ROS either by preventing their formation or interrupting their attack, via scavenging the reactive metabolites [73]. In accordance, ethanolic extract of A. comosus peel positively improved the antioxidant status by quenching and detoxifying the radicals stimulated by a carcinogenic substance and isoproterenol-caused oxidative injury in rats, respectively [20, 69]. Also, escalation in GSH content helps in the detoxification of ROS, preservation of cell integrity, and cellular components versus oxidation via the glutathione redox cycle due to its reducing features.

Toxic substances are transformed in the liver into less harmful products leading to hepatocytes damage. In the current study, rats treated with AlCl3 showed significant variations in serum and liver ALP, ALT, AST, and LDH activities as well as total bilirubin and protein. These parameters are important biomarkers for hepatocellular damage [74] and its alterations pointed out hepatocytes damage that altered the transport function and membrane permeability as well as leakage of enzymes from the cells to the bloodstream indicating hepatotoxicity [74–76]. Also, lipid peroxidation has a fundamental role in the disruption of hepatocellular membrane integrity, leading to the leakage of cytoplasmic enzymes and this confirmed the possible mechanism of oxidative stress in liver injury induced by Al [77]. Lactate dehydrogenase was significantly increased in AlCl3 intoxicated rats and this agreed with El-Demerdash [21]. This induction may be related to cellular impairment leading to disturbance in the
metabolism of carbohydrates and protein as well as energy depletion [78]. Alkaline phosphatase is a critical membrane-bound enzyme in the biological processes used as a biomarker for heavy metals toxicity. It is responsible for the detoxification, metabolism, and biosynthesis of macromolecules which are required for many biological functions. Moreover, the decline in ALP activity in liver homogenate is consistent with the findings of Ochmanski and Barabasz and Szilagyi et al. [5, 11] who referred the change in ALP to the disturbance in bone formation induced by Al in addition to the binding of Al with DNA and RNA, respectively. So, alterations in these enzymes’ activity could be expected due to cellular necrosis of the liver, kidney, and lung [79]. Protein is an essential cellular component susceptible to damage by free radicals and its depression might be linked to exaggerated leakage via nephrosis [80] or may be related to a disturbance in protein anabolic and catabolic processes. The elevation in total bilirubin may be due to diminished liver uptake, conjugation, or prolonged bilirubin output from hemolysis [21]. Moreover, results showed that bromelain (G2) rats revealed the normal histological structures of normal hepatocytes (H) with nuclei (N), central vein (CV), sinusoids (S), and Kupffer cells (K) which represented monocytes-macrophage defense system. (G3), AlCl$_3$ treated rats showed inflammation or infiltration (Ii) and enlarged bile ductular (Ebd), apoptic body (ap), branches of hepatic portal vein (Hp) surrounded by inflammatory cells (Ic). Also, leukocytic infiltration (Li) and bile ductular proliferation (1, 2, 3), branch of hepatic artery (Ha), congested portal vein (Pv) and enlarged hepatic duct (Hd) and hyperchromatic nuclei (Hn) were observed. (G4), Bromelain+AlCl$_3$ group revealed more or less normal hepatocytes (H&E X 200).
treatment attenuated hepatotoxicity induced by AlCl₃ since it could maintain hepatocytes’ integrity and minimize the liver injury caused by AlCl₃. Generally, it appears that the effectiveness of bromelain as a hepatoprotector because of its high content of active ingredients (ananasate, beta-sitosterol, campesterol, chlorogenic acid, rutin, naringenin, bromelain, vitamin A, B and C, glycosides, and flavonoids) that have potent antioxidant and anti-inflammatory activities [62, 81].

In agreement with the present results, several authors noted that Al administration significantly increased tissue TNF-α and the rise in cytokine expression suggests that the prooxidant/antioxidant balance has been upset. They also showed that during hepatocyte damage, activated Kupffer cells release growth factors and cytokines that have an encouraging effect on stellate cell activation and proliferation. Additionally, they release inflammatory mediators (TNF-α, IL-1β) that cause inflammatory leukocyte infiltration [59, 82, 83]. MMP-2 and MMP-9, two proteins associated with cell migration, were dramatically upregulated by AlCl₃. It has been discovered that human tissue inflammation increases MMP expression. MMPs have a role in the regulation of inflammatory mediators, which attract immune cells to injured tissues [84, 85]. The observed down-regulation in the Nrf2 gene’s expression level in the hepatic tissues of AlCl₃-intoxicated rats is consistent with findings made by Yu et al. [86] and Othman et al. [87], who discovered that Nrf2 deregulation is linked to Al toxicity. One of our most intriguing findings was that giving bromelain before AlCl₃ increased the Nrf2 gene’s level of expression in rat liver tissue. This would imply that one of bromelain’s primary defense mechanisms against AlCl₃-induced toxicity is the activation of Nrf2. Furthermore, the bromelain’s impact on Nrf2 expression in the liver of the treated rats coincided with the antioxidant enzymes’ functions in this investigation.

Histopathological examination of liver sections of AlCl₃ intoxicated rats showed several lesions and abnormalities in the hepatocytes as enlarged and congested veins, proliferation and leukocytic infiltration, activated Kupffer cells, and hepatocytes with pyknotic nuclei. This could be attributed to the oxidative toxicity induced by AlCl₃, which may have apparently led to severe alterations in liver architecture. Similar observations exhibited severe degenerative alterations in liver hepatocytes with hepatic cord derangement, intrahepatic hemorrhage and inflammatory cells infiltration, karyopyknosis and necrosis [51, 61], brain and testis [12, 88], and heart tissues [89] of rats treated with AlCl₃. Here, the observed degenerative alterations in liver tissues may be related to lipid peroxidation and free radicals’ accumulation along with perturbation in antioxidant status induced by AlCl₃ in rats. So, the histological examination confirmed the biochemical results and proved to be a good marker in liver failure. Based on our results, bromelain can improve most of the studied parameters in AlCl₃ intoxicated rats and could restore hepatocyte integrity and decrease liver damage. In general, it appears that the effectiveness of bromelain as a hepatoprotector in AlCl₃ toxicity may be referred to its high antioxidant constituents.

5. Conclusion

In conclusion, the current study pointed out that aluminum chloride has the potency to cause liver dysfunction via oxidative injury, alterations in the antioxidant defense system, liver function biomarkers, as well as molecular and histopathological changes. Furthermore, bromelain from Ananas comosus stem supplementation before aluminum treatment restores its toxic effects by quenching, chelating, and detoxifying the free radicals. So, our findings suggested that bromelain had a powerful antioxidant effect and could be used to develop functional healthy foods with hepatoprotective effects against complications induced by AlCl₃.

Data Availability

All data are incorporated in the manuscript.

Conflicts of Interest

The authors have declared no conflict of interest.

Authors’ Contributions

El-Demerdash F.M. was responsible for the conceptualization, methodology, manuscript preparation, and publication. Hussien D.M. was responsible for practical work, data generation, and writing the original draft. Ghanem N.F. was responsible for manuscript revision, methodology, and investigation. AL-Farga A.M. was responsible for data analysis and manuscript writing and revision.

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