

Ameliorating Effects of Fenugreek Seeds Powder against Hematotoxicity Induced by Aluminum Chloride in Male Rabbits

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Abstract

Background: Humans are exposed to aluminum from the mouth, nose and epidermal route inducing toxic effects. Accumulation of aluminum has been associated with a variety of pathologies such as anemia, osteodystrophy, joint diseases, muscular weakness, and Alzheimer's diseases. Fenugreek extracts have been shown to be neutralizing of free radicals and enhancing antioxidant status.

Objectives: The present study aimed to evaluate the ameliorative effects of fenugreek seeds against hematotoxicity induced by Aluminum chloride in male rabbits.

Materials and Methods: This study included twenty-four adult male rabbits, which were divided into 4 groups, 6 rabbits for each. Group I (control group): Animals were provided with tap water and fed with a normal diet for 30 days. Group II (Fenugreek seeds powder group): Fenugreek seeds powder was given to rabbits in food at a dose of 10 g per kilogram of diet weight/kg of body weight/day for 30 Days. Group III (Aluminum chloride (ALCl₃) group): Rabbits were treated orally with 150 mg/kg BW of AlCl₃/day for 30 consecutive days. Group IV (Aluminum chloride/fenugreek co-administered group): Fenugreek seeds flour was added at a rate of 10 g per kilogram of diet weight, and rabbits were treated orally with 150 mg/kg BW of AlCl₃/day for 30 consecutive days. At the end of the experiment and 24 hours after the last dose, all animals were anesthetized with ether and blood samples were collected by heart puncture.

Results: The results of the study showed that the treatment of male rabbits with aluminum chloride resulted in a significant decrease ($P < 0.01$) in RBCs count, hemoglobin concentration, hematocrit value, MCV, MCH, and MCHC as compared to the control group. While there was a significant increase ($P < 0.01$) in WBCs count, lymphocytes, and monocytes percentages and a significant decrease in granulocyte percentage when compared with the control group. Co-administration of fenugreek seeds powder and AlCl₃ significantly improved all haematological parameters.

Conclusion: The results showed that the administration of rabbits with aluminum chloride caused a hematotoxicity, and co-administration of fenugreek seeds powder with AlCl₃ alleviate the hematotoxicity induced by AlCl₃. The use of fenugreek seeds powder by humans can be considered beneficial in the alleviation of hematotoxicity. It is recommended that humans exposed to AlCl₃ should be advised to take fenugreek seeds powder as a rich source of antioxidants to prevent hematotoxicity induced by AlCl₃. Further studies are necessary to elucidate the exact mechanism of the anti-hematotoxic effect of Fenugreek seeds powder and the potential usefulness of fenugreek seeds powder as a protective agent against AlCl₃ induced hematotoxicity in clinical trials.

Keywords: hematotoxicity, aluminum chloride, ameliorating effects, fenugreek seeds powder, male rabbits

1. Introduction

Aluminum is a constituent of cooking utensils, medicines such as antacids, cosmetics such as deodorants, and food additives. Also, it can be found in food especially corn, yellow cheese, salt, herbs, spices, and tea. In addition, aluminum salts are widely used as flocculants in the treatment of drinking water for purification purposes [1-4]. Humans are exposed to aluminum from the mouth, nose and epidermal route inducing toxic effects to a variety of organ systems including the brain, kidney, liver, lungs as well as bone and blood [5, 6]. Aluminum accumulation in humans can occur via the diet, drinking water, vaccines, antacids,

parenteral fluids, and inhaled fumes [7, 8]. Accumulation of aluminum has been associated with a variety of pathologies such as anemia, osteodystrophy, joint diseases, muscular weakness, and Alzheimer's diseases [8, 9].

Aromatic and flavourful fenugreek seed is a popular spice and is widely used for well-recognized culinary and medicinal purposes. Fenugreek seed is used in physiological utilization for the treatment of antibacterial, anticancer, hypocholesterolemic, hypoglycemic antioxidant, and antidiabetic agent [10]. Recent researches identified antioxidant, anticarcinogenic, hepatoprotective, and other miscellaneous medicinal

effects of fenugreek [11, 12]. Fenugreek extracts have been shown to be neutralizing of free radicals and enhancing of antioxidant status [12, 13]. Fenugreek oil has many ingredients that have antioxidant activities [14, 15].

2. Objectives

The evidence reporting the amelioration by fenugreek in aluminum chloride-induced haematotoxicity in male rabbits are hardly found. So, the present study aimed to evaluate the ameliorative effects of fenugreek seeds powder against hematotoxicity induced by aluminum chloride in male rabbits.

3. Materials and Methods

3.1. Animals

24 adult male rabbits, aged between 35-37 weeks and weighing 1.5-1.8 kg, were used in the current study. The rabbits were housed in a room under standard conditions of ventilation, temperature ($25^{\circ}\text{C} \pm 2$), and humidity (60 - 70) %, rabbits were separated in a plastic cage, the animals were provided with free drinking water and standard commercial food.

3.2. Chemicals

Aluminum chloride was purchased from Sigma Chemicals Company, and rabbits were treated orally with 150 mg/kg BW of AlCl_3 /day for 30 consecutive days [6].

3.3. Fenugreek seeds

Fenugreek seeds were purchased from the Zawia market, and the fenugreek seeds were ground and added at a rate of 10 gm of fenugreek seeds powder per kilogram of diet weight that was provided to rabbits for 30 days.

3.4. Experimental Design

After one week of acclimation, the animals were randomized and divided into four groups (6 rabbits for each) as follows: Group I (control group): Animals were provided with tap water and fed with a normal diet for 30 days. Group II (Fenugreek seeds powder group): Fenugreek seeds powder was given to rabbits in food at a dose of 10 g per kilogram of diet weight/kg of body weight/day for 30 Days. Group III (Aluminum chloride group): Rabbits treated orally with 150 mg/kg BW of AlCl_3 /day for 30 consecutive days. Group IV (Aluminum chloride/fenugreek co-administered group): Fenugreek seed flour was added at a rate of 10 g per kilogram of diet weight, and rabbits were treated orally with 150 mg/kg BW of AlCl_3 /day for 30 consecutive days. At the end of the experiment

and 24 hours after the last dose, all animals were anesthetized with ether and blood samples were collected by heart puncture.

3.5. Blood Sampling

The blood samples were collected in a clean dry tube containing the anticoagulant substance EDTA (ethylene diamine tetra acetic acid) and used for the hematological studies.

3.6. Haematological Parameters

Red, white blood cells, and blood platelet counts were done by using the hemocytometer and hemoglobin content (Hb) was determined according to the method of Wong [16].

Hematocrit value (Hct) was estimated by using the heparinized capillary tubes. The mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC) were calculated according to Schalm [17] as the following equations: $\text{MCV} = \text{Hct} / \text{RBC's} \times 10$, $\text{MCH} = \text{Hb} / \text{RBC's} \times 10$ & $\text{MCHC} = \text{Hb} / \text{Hct} \times 100$.

The blood film was made after the collection of blood samples. By manual method, three blood films were made for each blood sample, stain the blood film by Giemsa stain and differential leukocyte count were done [18].

3.7. Statistical Analysis

The values were presented as means \pm SD of different groups. One-way analysis of variance (ANOVA) was carried out. For the comparison of significance between groups, Duncan's test was used as a post hoc test according to the statistical package program (SPSS version 26.0). The results were considered statistically significant when $P < 0.05$.

4. Results

The results of the study showed that the treatment of male rabbits with aluminum chloride resulted in a significant decrease ($P < 0.01$) in RBCs count, hemoglobin concentration, hematocrit value, MCV, MCH, and MCHC as compared to the control group. While there was a significant increase ($P < 0.01$) in WBCs count, lymphocytes, and monocytes percentages and a significant decrease in granulocyte percentage when compared with the control group.

The Hematological parameters in the different groups are shown in Table 1. Male rabbits that received aluminum chloride orally at a dose of 150 mg/kg BW of AlCl_3 /day for 30 consecutive days had significantly ($p < 0.01$) lower RBCs count, Hb, Ht, MCV, MCH, and MCHC than those in the control animals (Fig. 1 - 6).

Groups	Control	Fenugreek	Aluminum chloride	Aluminum chloride + Fenugreek
Parameters	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
Red blood cells count (RBCs $\times 10^6/\mu\text{L}$)	6.64 \pm 0.27	6.69 \pm 0.21	5.34 \pm 0.13**	6.48 \pm 0.31##
Haemoglobin concentration (Hb,g/dl)	12.35 \pm 0.32	12.56 \pm 0.41	9.18 \pm 0.28**	11.97 \pm 0.25##
Haematocrit value (Hct, %)	47.10 \pm 1.11	46.9 \pm 1.03	35.43 \pm 1.06**	46.02 \pm 0.86##
Mean corpuscular volume (MCV, μ^3)	71.36 \pm 0.55	71.0 \pm 0.45	66.35 \pm 0.72**	71.02 \pm 0.49##
Mean corpuscular haemoglobin (MCH, pg)	18.60 \pm 0.17	18.78 \pm 0.14	17.19 \pm 0.13**	18.47 \pm 0.27##
Mean corpuscular haemoglobin concentration (MCHC, g/dl)	26.22 \pm 0.26	26.78 \pm 0.21	25.91 \pm 0.25**	26.59 \pm 0.27##

** : Significant at ($P < 0.01$) when compared with control group, ##: Significant at ($P < 0.01$) when compared with aluminum chloride group.

Table 1. Effect of fenugreek seeds powder, aluminum chloride, and aluminum chloride plus fenugreek seeds powder on RBCs count and its indices in male rabbits.

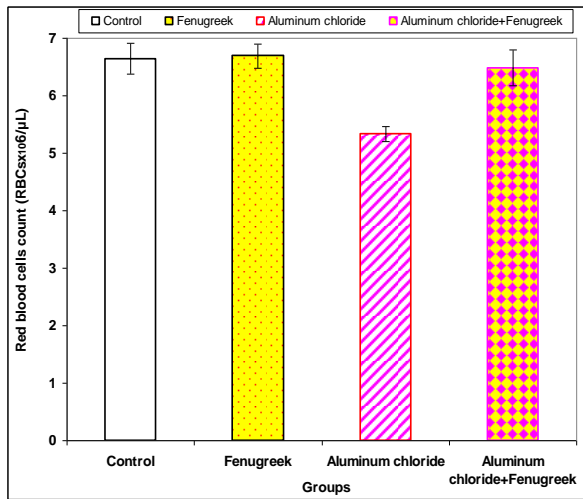


Figure 1: Effect of fenugreek seeds powder and/or aluminum chloride on RBCs count in male rabbits.

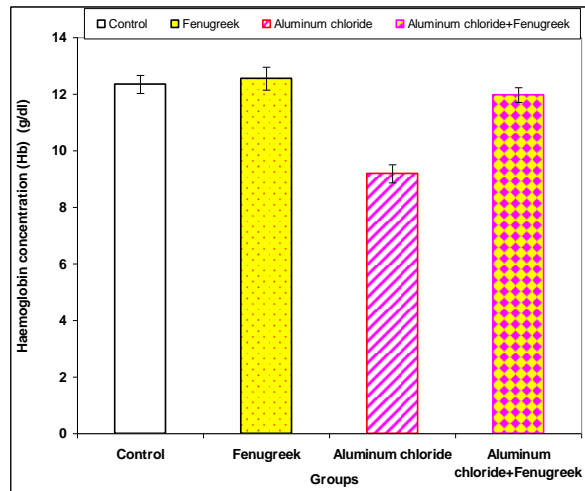


Figure 2: Effect of fenugreek seeds powder and/or aluminum chloride on hemoglobin concentration in male rabbits.

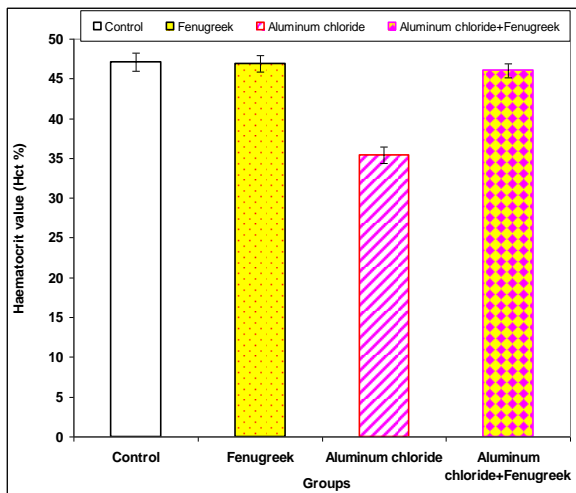


Figure 3: Effect of fenugreek seeds powder and/or aluminum chloride on haematocrit value in male rabbits.

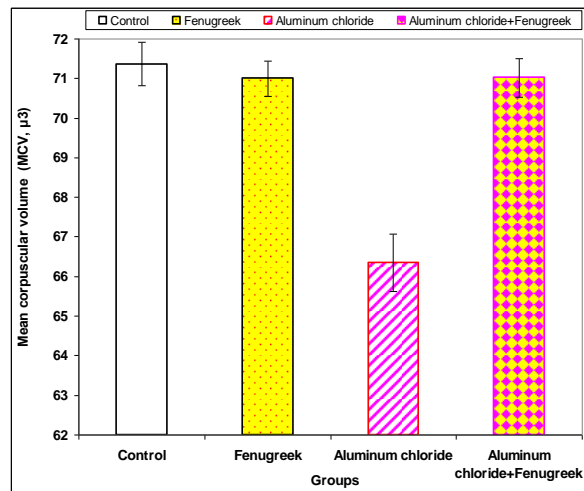


Figure 4: Effect of fenugreek seeds powder and/or aluminum chloride on Mean corpuscular volume in male rabbits.

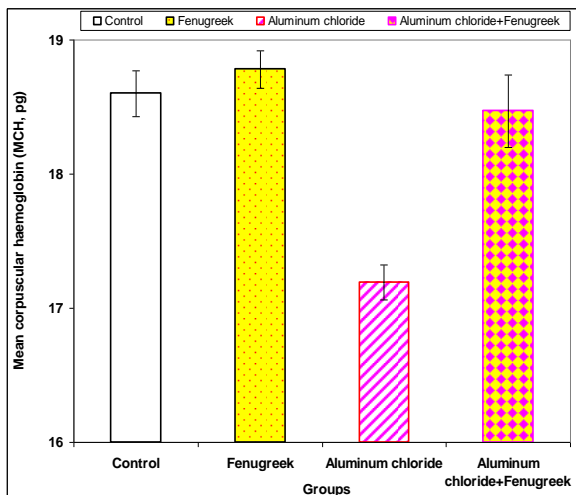


Figure 5: Effect of fenugreek seeds powder and/or aluminum chloride on Mean corpuscular hemoglobin in male rabbits.

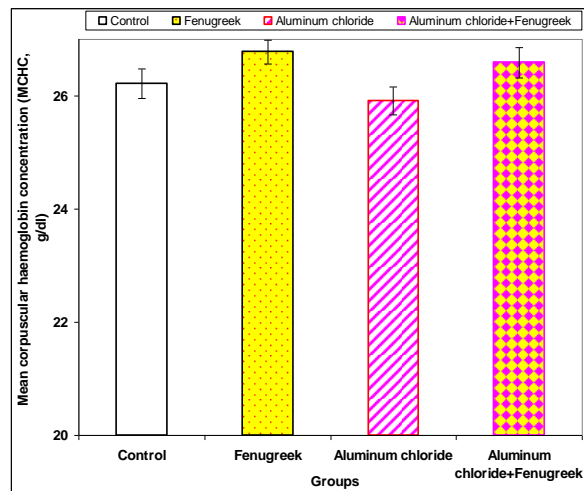


Figure 6: Effect of fenugreek seeds powder and/or aluminum chloride on Mean corpuscular hemoglobin concentration in male rabbits.

On the other hand, WBCs count, lymphocytes, and monocytes percentages, and platelet count were a significant increase and granulocyte percentage was a significant decrease when compared with

the control group (Table. 2 & Figures. 7-11). Co-administration of fenugreek seeds powder and AlCl₃ significantly improved all haematological parameters (Tabl. 1 & 2, Figures. 1-11).

Groups	Control	Fenugreek	Aluminum chloride	Aluminum chloride + Fenugreek
Parameters	Mean±SD	Mean±SD	Mean±SD	Mean±SD
White blood cells count (WBC, x10 ³ /μL)	7.79 ± 1.02	8.15 ± 1.37	12.25 ± 1.03**	9.03 ± 1.21##
Lymphocytes (%)	33.33 ± 2.51	34.04 ± 3.30	52.10 ± 2.58**	40.65 ± 2.20***
Monocytes (%)	3.65 ± 0.14	3.13 ± 0.30	5.57 ± 0.27**	4.48 ± 0.40***
Granulocytes (%)	63.02 ± 2.38	62.92 ± 3.01	42.33 ± 2.34**	54.87 ± 2.92***
Platelets count (PLT, x10 ³ /μL)	480.50 ± 45.6	483.23 ± 46.3	770.67 ± 33.3**	499.33 ± 44.0##

** : Significant at (P<0.01) when compared with control group, ## : Significant at (P<0.01) when compared with aluminum chloride group.

Table 2. Effect of aluminum chloride and/or fenugreek seeds powder on the WBCs count, differential count of leukocytes, and platelets count in male rabbits.

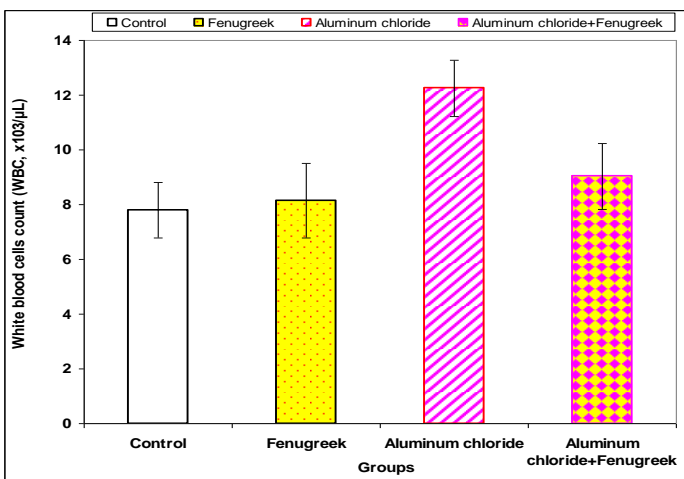


Figure 7. Effect of fenugreek seeds powder and/or aluminum chloride on WBCs count in male rabbits.

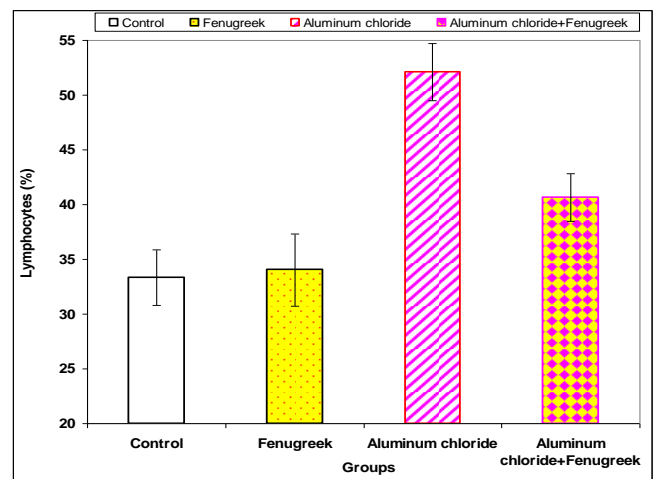


Figure 8. Effect of fenugreek seeds powder and/or aluminum chloride on lymphocytes percentage in male rabbits.

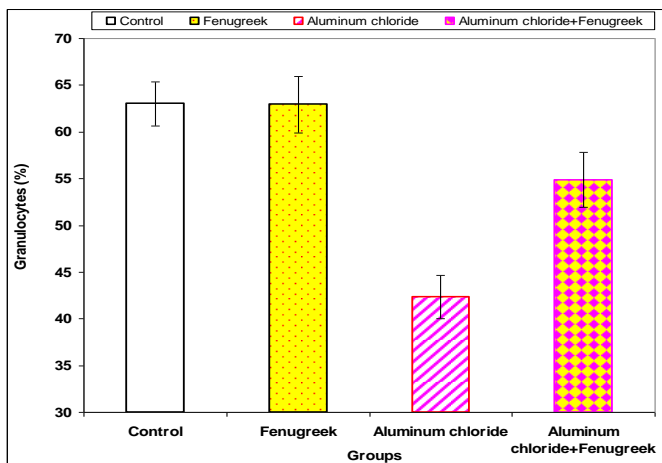


Figure 9: Effect of fenugreek seeds powder and/or aluminum chloride on granulocytes percentage in male rabbits.

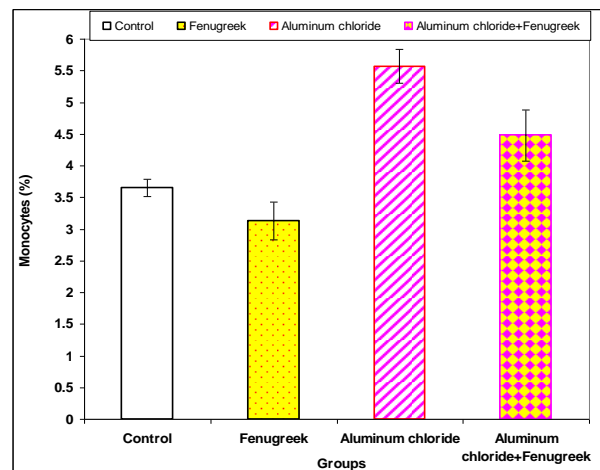


Figure 10: Effect of fenugreek seeds powder and/or aluminum chloride on monocytes percentage in male rabbits.

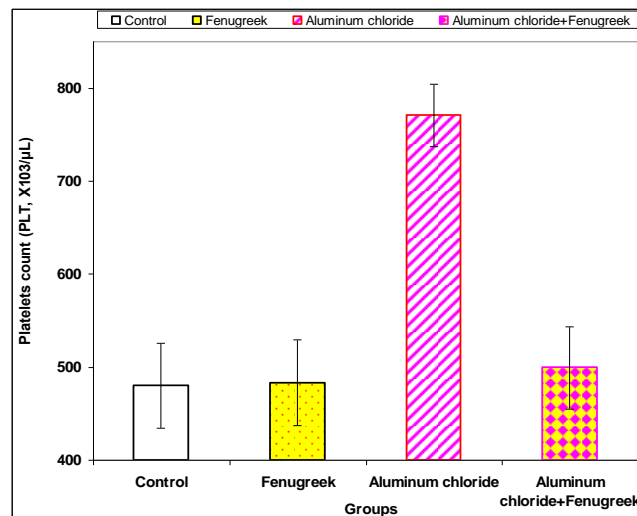


Figure. 11: Effect of fenugreek seeds powder and/or aluminum chloride on platelets count in male rabbits.

5. Discussion

Aluminum generates reactive oxygen species, resulting in oxidative deterioration of lipids, proteins, and DNA [1, 6]. The effects of aluminum chloride on hematological parameters have been studied well in experimental models [3, 4, 6, 8 and 19].

The present study showed that treatment of male rabbits with aluminum chloride were decreased red blood cell count, hemoglobin concentration, haematocrite, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and mean corpuscular volume values as compared to the control rabbits. These results run parallel to those reported by many previous studies in experimental animals [3, 4, 6, 8 and 19]. The current result agrees with Al-Hashem, [20] who recorded normocytic normochromic anemia in rats treated orally with $AlCl_3$ (0.5 mg/kg BW for 30 days). Similar observations in erythrogram values were reported by El-Sharkawy *et al.*, [8] who recorded that adult New Zealand white rabbits exposed to aluminum chloride via drinking water in a dose of 20 mg/l for 3 months showed a significant decrease in the number of red blood cells, blood hemoglobin concentration, and hematocrit value compared with the control group. The results indicated that long-term oral exposure to low doses of $AlCl_3$ promotes alterations in hematological indices. Bouasla *et al.*, [3] reported that rats consuming diets with 34 mg/kg $AlCl_3$ added showed a significant decrease in RBCs, haemoglobin content, and MCHC when compared with the controls. Osama *et al.*, [6] reported that orally treatment of rats with 150 mg/kg body weight $AlCl_3$ daily for 4 weeks caused a significant ($p < 0.05$) decrease in RBCs count, hemoglobin concentration and hematocrit value, While MCV and MCHC levels were insignificantly changed in rats with $AlCl_3$ compared to the controls. The erythrogram in this study revealed that $AlCl_3$ treatment resulted in normocytic normochromic anemia. Also, Kadhum, [4] reported that subcutaneous injection of male rats with 240, 320, and 400 ppm from aluminum chloride for 45 days caused a significant decrease ($P < 0.05$) in RBCs count, hemoglobin concentration, hematocrit value, MCV, and MCH as compared to the control group. Changes increased with an increase in the concentration of aluminum chloride injected.

These alterations induced by $AlCl_3$ may be attributed to a shortened life span of circulating erythrocytes and reduced RBCs production in bone marrow as a result of the oxidative stress as well as increase RBCs membrane fragility [6, 20]. The reduced level of hemoglobin

concentration can be associated with RBCs hemolysis which is confirmed by reduced RBCs count [6]. The induced anemia may be related to inhibition of heme synthesis, either by inhibition of enzyme activity or interference with iron incorporation or utilization [6, 21, 22], and an increase in heme oxygenase activity [6, 23, 24]. In the biological system, aluminum ions replace iron ions, reduce Fe^{+2} binding to ferritin and disturb hem synthesis [4, 25], The other cause of its inhibition of heme oxygenase, this enzyme necessary for hemoglobin formation series, stopped by the toxicity of aluminum and increase the destruction of RBCs and transformed to bilirubin [4, 26].

Once in the blood circulation, aluminum is mainly transported by plasma transferrin in its sites left vacant by iron, and to a much lesser extent by albumin [8, 27]. Aluminum has been found to induce microcytic anemia [28] or slightly hypochromic macrocytic anemia [8, 29]. The toxicity of aluminum increased free radicals in target organs such as the liver and inhibit glutathione enzyme in the liver. This important to maintain hemoglobin in red blood cell and increase removing of hydrogen peroxide (H_2O_2) and increase the lifetime of red blood cell [4, 30], reduction of some enzyme such as glutathione reductase, catalase, glucose-6-phosphate dehydrogenase lead to accumulation of toxins inside red cells [4, 26].

In fact, according to the earlier reports [3, 31-33] this anaemia could be explained by the inhibition of erythropoiesis and/or haemoglobin synthesis reduction, the same reports indicate that $AlCl_3$ could be interfering with Fe incorporation to the heme group witch induce Fe deficiency and reduce heme synthesis. Also, the observed microcytic anaemia might be due to an increase in the rate of erythrocytes destruction in haematopoietic organs. So, $AlCl_3$ might be crossing the erythrocyte membrane which is a result of its ability to initiate a lipid peroxidation [2, 3, 34, 35].

The decreases in Hct, Hb, MCV, and MCH may be due to protoporphyria and it's necessary for hemoglobin formation, the presence of aminolivolinic acid in urine refers to the failure synthesis of hem caused by toxicity of aluminum [36]. The other cause of this result caused by effects of aluminum chloride on bone marrow, especially mothers cells of red blood cells. This lead to a decrease in RBCs count and hemoglobin and reflect on the hematocrit value [37] and caused anemia, a decrease in Hb, Hct, MCV, and MCH values has often been proven to be a serious

indicator of aluminum toxicity. Aluminum induced anemia is mainly related to changes in iron metabolism [8, 19, 34, 38]. Also, aluminum exposure significantly inhibited blood activity of enzyme δ -aminolevulinic acid dehydratase (δ -ALA-D) while increased zinc protoporphyrin confirming changed heme biosynthesis, which may be responsible for heme biosynthesis inhibition [8, 31, 39].

The current study showed that exposure of male rabbits to $AlCl_3$ caused a significant increase in WBCs count, differential count of leukocytes, and platelets count when compared with the control group. These results run parallel to those reported by many previous studies [3, 6, 40-42]. Aluminum chloride treatments induce a highly significant ($P < 0.05$) increase in white blood cell and differential leukocytes count especially lymphocyte and monocyte because aluminum chloride induces infections in target organs such as the liver, brain, kidney, spleen, and smooth muscle [40]. This result is reflected in the histopathological study of the spleen, we noted increases in white pulp and occurs bleeding and degeneration in spleen tissue. The cause of this destruction because the action of free radicals and increase in lipid peroxidation this process initiate tumor in target organ [4, 43]. Also, Joshi *et al.* [42] reported that oral administration of $AlCl_3$ to male rats for 90 caused a significant increase in leukocytes count. Bouasla *et al.*, [3] recorded that rats consuming diets with 34 mg/kg $AlCl_3$ added showed a significant increase in WBCs count when compared with the controls. Also, Osama *et al.*, [6] record that the total leukocytes and neutrophil counts were increased in rats treated orally by 150 mg/kg body weight $AlCl_3$ daily for 4 weeks, which may indicate activation of the immune system [1, 6]. This increase might be indicative of the activation of defense and immune system showed that there were oedema and inflammation in the tissues [3, 35]. Kadhum, [4] reported that subcutaneous injection of male rats with 240, 320, and 400 ppm from aluminum chloride for 45 days caused a significant increase ($P < 0.05$) in WBCs count and differential leukocytes count especially in lymphocyte. Changes increased with an increase in the concentration of aluminum chloride injected. The significant increase in white blood cell levels of aluminium-treated rats might indicate activation of the immune system, a normal cell-mediated immune response [1, 41]. The increase in lymphocytes could be due to the toxic action of the aluminium ion that stimulates the hemopoietic system to release more of these cells, causing an increase in their number in the blood stream [41].

On the other hand, El-Sharkawy *et al.*, [8] recorded that a significant ($P < 0.05$) decrease in leukocytes count, and lymphocytes percentage were obtained in aluminum chloride -exposed rabbits compared with control.

Fenugreek has many active compounds, including flavonoids, steroid saponins, trigonelline, diosgenin, and 4-hydroxyisoleucine and polysaccharides, mainly galactomannans [15, 44 and 45]. These active compounds, enable fenugreek to have multiple pharmacological effects, including antioxidant, antidiabetic, antipyretic, anti-inflammatory, antineoplastic, CNS stimulant, and immunomodulatory effects [15, 46 and 47].

The results of the current study showed that the co-administration of fenugreek seeds powder to male rabbits with aluminum chloride resulted in a significant improvement in hematological parameters. These findings were identical with those of Farman *et al.*, [48] who reported that there was a significant increase in the levels of Hb, RBCs, and PCV after 7 days of fenugreek extract treatment. Also, Effraim *et al.*, [49] recorded significant differences ($P < 0.05$) in hemoglobin concentration and red blood cells count. The findings of the current study were matched with Bravo, [50] whose result indicated that in animals which received

fenugreek, RBCs count remained elevated, due to the antioxidant activity of flavonoids. Moreover, the findings of the current study were identical with Ibrahim and Hegazy, [51], who concluded that the high iron content of fenugreek seed flour stimulated hemoglobin synthesis.

Also, data obtained by Elseed *et al.*, [52] showed significant differences ($p < 0.05$) in lymphocyte, neutrophil, eosinophil, monocyte, and highly significant difference ($p < 0.05$) in basophil ratios in response to fenugreek saponin ingestion. Rosioru *et al.*, [53] reported that treatment of rats with 10% ethanol in drinking water for 30 days caused a significant increase in RBCs count, Hct value, and Hb concentration, WBCs count, and lymphocytes percentage and a decrease in neutrophils percentage as compared to the control animals. Addition of 10% fenugreek flour in the diet of ethanol-intoxicated rats for 30 days showed a tendency to restore the control values. The protective effect of fenugreek flavonoids on plasma membranes accounts for the lowering tendency of RBCs count in the ethanol+fenugreek group. However, in animals that received fenugreek RBCs count remained elevated, due to the antioxidant activity of flavonoids [50] present in fenugreek seeds, thereby elevating the antioxidant capacity of the blood. Other researchers [54], showing the influence of flavonoid compounds on RBCs count and related parameters, in Wistar rats. The high iron content of fenugreek seed flour [51] stimulated hemoglobin synthesis, even if rats were intoxicated with ethanol. Also, these findings were consistent with that of Abdel-Daim *et al.*, [15] who demonstrated that rats treated with 15 mg/ kg bw deltamethrin orally showed a significant decrease in RBCs and platelet counts, hemoglobin concentration, and hematocrit value and a significant increase in leukocytes count when compared with the control group. But, co-administration of rats with fenugreek oil contained diets (2.5% and 5%) and 15 mg/ kg bw deltamethrin orally resulted in a significant increase in RBCs and platelets counts, hemoglobin concentration and hematocrit value and a significant decrease in leukocytes count as compared with deltamethrin treated rats. Fenugreek oil kept the studied hematological parameters within normal ranges. Thus, including fenugreek oil in the diets of deltamethrin administrated rats prevented the oxidative stress induced by deltamethrin, which subsequently protects the immune and hemopoietic organs. These results indicated that fenugreek oil inhibited the toxic effects of deltamethrin on hematological parameters, and it appeared to be a promising protective agent against deltamethrin-induced hematotoxicity. Regarding the effects of fenugreek oil on deltamethrin intoxication, the current results demonstrated that administration of rats with fenugreek oil at 2.5% of diet minimized the harmful effects of deltamethrin on rats' hematological, biochemical and antioxidant status however, it did not return them to normal ranges. This may be due to the active ingredients in this dose of fenugreek oil were not sufficient to normalize the altered parameters [15]. Fenugreek oil has many ingredients that have antioxidant activities [14, 15], such ingredients enable fenugreek oil to ameliorate the altered hematological parameters in diabetic rats through its antioxidant properties [55]. The antioxidant and the protective effects of fenugreek oil are owed to their content of polyphenolic flavonoids, which have been shown to have antioxidant and free radical scavenging activities [15, 56, 57]. Fenugreek seeds powder reversed the adverse effects of diabetes induced by streptozotocin on serum levels of tissue antioxidant biomarker enzymes [58].

The corrective effect of ginger extract on alterations of erythrogram and leukogram induced by aluminum toxicity may be attributed either due to its antioxidant activity as oil of ginger contain antioxidants such as polyphenol (6-gingerol and its derivatives), flavonoids and total tannin

which reduce or scavenge free radicals, or may be due to rebuilding activities of nutrient and phytochemicals found in the extract [6, 59]. Al-Amri and Alrasheedi, [12] reported that Feeding of rats on a diet supplemented with fenugreek seeds at a concentration of 5% before and after 14 days of irradiation exposure significantly increased hemoglobin and lymphocytes percentage compared to the control group. Also, it was demonstrated the role of fenugreek seeds in protecting the spleen and increased lymphocyte, suggesting that fenugreek seeds might improve immunity. These observations are in agreement with those obtained by Sindhu *et al.*, [60] and Kandhare *et al.* [61], who reported that fenugreek seeds influenced the hemoglobin and lymphocytes count, improving hematopoietic function. Abdel-Rahman *et al.*, [62] reported that lactating female rabbits treated with fenugreek germinated and powdered seeds showed a significant increase in RBCs count, Hb concentration Hct, and MCH values. During the first lactation, period, all treated groups showed a significant reduction of WBCS count. While, animals administered germinated seeds and powdered seeds of fenugreek were showed a significant increase in WBCS count compared to the control group during the second lactation period. i.e. administration of fenugreek-germinated seeds; oil or powdered seeds to lactating female rabbits were improved RBCs count, Hct, Hb, blood indices, WBCs count and differential white blood cells count. So, the authors suggested that the administration of fenugreek-germinated seeds; oil, or powdered seeds were responsible for improvement of Immunological profile through increase phagocytic index, phagocytic capacity of macrophages, and humoral immunity. Abdel-Rahman *et al.*, [62] suggested that the administration of seed and germinated fenugreek that contain high iron content stimulated hemoglobin synthesis beside the antioxidant activity of flavonoids were responsible for the improvement of the complete blood count of lactating female rabbits. Also, fenugreek bears the potential of a powerful antioxidant activity been evaluated in human erythrocytes by the exposure to fenugreek seeds extract which showed protective effects against hydrogen peroxide-induced oxidation by protecting the erythrocytes from hemolysis and lipid peroxidation due to the presence of flavonoids and polyphenols [63]. Pradeep and Srinivasan, [64] reported that streptozotocin-induced diabetic rats caused a significant decrease in RBCs count, Hb concentration, MCV, Hct value, MCH, MCHC, and platelets count in diabetic rats. Hyperglycemia increases the production of free radicals and oxidative stress that in turn is a cause of cellular dysfunction. Dietary fenugreek seeds (100 g/kg) and onion (30 g/kg) treatment of streptozotocin-induced diabetic rats, appeared to counter the deformity of erythrocytes partially in diabetic rats by their antioxidant potential. Dietary fenugreek seeds and onion caused a decrease in glycated haemoglobin [65] and a nephro-protective [66] probably mediated by stimulating erythropoietin which enhances rapid synthesis of RBCs as indicated by the improved level of MCH and MCHC in diabetes treated groups.

Elghazaly *et al.*, [67] reported that treatment of rats with streptozotocin induced a decrease in RBC count, Ht value, Hb concentration, MCHC value, platelets count, total WBCs, monocytes, and lymphocytes and an increase in the percentage of eosinophils and neutrophils. The combination treatment of rats with Glimepiride and a fenugreek aqueous extract in streptozotocin induced diabetic in male albino rats for eight weeks caused an improvement in RBC count, Ht value, Hb concentration, MCHC value, and platelets count, total WBCs, monocytes, lymphocytes, the percentage of eosinophils and neutrophils compared to normal control. The results showed that the combination therapy induces better hematological and improves the oxidative stress biomarkers and antioxidant enzymes. Fenugreek contains iron and it can improve anemia

conditions [67]. The antioxidant property of fenugreek [68] inhibits lipid peroxidation of the erythrocytes. Improvement in platelet count may be due to the inhibitory activity of certain constituents of fenugreek on platelet aggregation [67, 69]. Fenugreek seeds may be improving immunity because they play a role in protecting the spleen and increasing the lymphocytes [60, 61 and 67].

6. Conclusion

The results showed that administration of rabbits with aluminum chloride caused a hematotoxicity, and co-administration of fenugreek seeds powder with AlCl₃ alleviate the hematotoxicity induced by AlCl₃. The use of fenugreek seeds powder by humans can be considered beneficial in the alleviation of hematotoxicity. It is recommended that humans exposed to AlCl₃ should be advised to take Fenugreek seeds powder as a rich source of antioxidant to prevent hematotoxicity induced by AlCl₃. Further studies are necessary to elucidate exact mechanism of the anti-hematotoxic effect of Fenugreek seeds powder and potential usefulness of fenugreek seeds powder as a protective agent against AlCl₃ induced hematotoxicity in clinical trials.

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