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Protective Effects of Hydroalcoholic Extract of *Cichorium intybus L.* against Aluminum Chloride-induced Toxicity in Rats

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ABSTRACT

Background: Aluminum is a potent inhibitor of numerous cation-dependent biological processes. Traditional medical practitioners have used plant extracts for the treatment of liver disorders for centuries. The present study aimed to assess the hepatoprotective activity of hydroalcoholic extract of *Cichorium intybus L.* against aluminum chloride (AlCl3)-induced toxicity in rats.

Methods: The hepatoprotective activity of extracts (hydroalcoholic extract) at 50 mg/kg body weight was compared with Alcl3-treated animals. The animals were assigned to four groups with seven animals in each group. The first group represents control, the second group received aluminum chloride, the third group received C. intybus extract, and the fourth group received C. intybus plus aluminum chloride. The duration of the injection was 15 days. The injection was administered by intraperitoneal method and the sampling was performed one week after the last injection.

Results: There were significant changes in serum biochemical parameters, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzyme in Alcl3-intoxicated mice, which were restored towards normal values in C. intybus-treated animals. Histopathological examination of liver tissues further substantiated these findings.

Conclusion: The results ascertain that the herb extracts of C. intybus possess significant hepatoprotective activity.

Keywords: Aluminum chloride, Cichorium intybus, Enzyme, Hepatoprotective effects, Poisoning

Introduction

The liver is the largest internal gland in the body that can be damaged by medications and drugs, chemicals, toxins, viruses, and parasites; nonetheless, in many cases, the mechanism of pathogenesis is unknown. There are different names for each liver disorder. The role of the liver in detoxification, the synthesis of proteins and enzymes, as well as the storage of glycogen and some of the salts and vitamins, is well known [1]. Liver diseases, such as cirrhosis, fatty liver, and chronic hepatitis are critical world health issues. The medical management of these diseases is currently inadequate despite their frequent occurrence and high morbidity and mortality. Even though there are newly developed medications used to treat chronic liver disorders, completely successful treatment is not available for the prevention of these disease. Furthermore, the currently used medicines for liver diseases have side effects [2]. Traditional medical practitioners have used plant extracts for the treatment of liver disorders for centuries [3]. Polyphenolic compounds are a large group of herbal chemical compounds with well-known therapeutic and protective effects [4].

During the metabolic process, excess free radicals are generated and may cause damage to the liver. Therefore, the liver is more likely to be injured in the body than any other organ. Recently, the strategies for the prevention and treatment of aluminum poisoning have focused on the use of chemical compounds extracted from plants [5]. Aluminum is the most abundant metal element, accounting for about 18% of the Earth's crust. In general, nature is the third element after oxygen and silicon in terms of quantity. Aluminum is not pure and is mainly found in the form of a combination of hydroxide, silicate, sulfate, and phosphate [1]. Food is one of the ways in which aluminum enters the body. Cheese, baking powder, and tea are among the foods containing the highest amount of aluminum [6].

Considering the use of aluminum in the water

purification industry, it has been demonstrated that after the purification process, the amount of aluminum in water increases by about 40%-50%. Nonetheless, the amount of aluminum entered into the body does not include more than 3% of the total amount of aluminum entering the body [7]. According to the World Health Organization (WHO/IPC), the average amount of aluminum entry through food in different countries is estimated to be less than 15 mg per day (0.11-3.5 mg) [8]. The production and storage of food in aluminum containers can also contribute to the amount of aluminum entering the body [9]. It turns out that reducing PH in the food environment and increasing its baking time will increase the amount of aluminum in the food [10]. Aluminum can also enter the body through the air. The amount of aluminum in the non-urban areas is 5 µgr/m3, while is far higher in industrial cities. On average, humans may be exposed to about 20 mg of aluminum per day [11]. The presence of aluminum in some pharmaceutical products may also increase the amount of aluminum intake. The amount of aluminum introduced into the body can be much more than the amount of aluminum entering the body through food. Significant amounts of aluminum can enter the body through analgesics and antioxidants [12]. Furthermore, the use of aluminum in cosmetics increases the amount of aluminum received [13]. There are reports that aluminum can also enter the body through calcium supplements [14]. The overall estimate of the amount of aluminum intake per day is estimated at an average of 25 mg [1].

Aluminum is a potent inhibitor for many cation-dependent biological processes. Most toxic substances are lipophilic and therefore accumulate in different tissues and cannot be easily excreted. Biotransformation converts these substances into polar metabolites and can be eliminated. This process reduces the half-life of the toxins and reduces the biological activity and harmful effects in the body. Biotransformation of toxins and medications is carried mainly in the liver [15].

Research has illustrated that hepatotoxicity induced by aluminum increases serum levels of aspartate transaminase (AST) and aka alanine aminotransferase (ALT) liver enzymes, depending on the dose [16]. In cases of human and animal poisoning with aluminum, liver tissue, kidneys, and even central nerves are damaged. Aluminum can be absorbed in three ways: oral, inhalation, and dermal; nonetheless, it is absorbed very rarely through the skin. The possibility of breathing the aluminum particles directly into the brain. Gastrointestinal absorption depends heavily on PH and the presence of complex ligands, in particular carboxylic acids that are absorbable. For instance, intestinal absorption is dependent on the presence of citrate. Citrate does not absorb itself, rather it markedly enhances the intestinal absorption of aluminum [17]. Aluminum can cause cell injury in the liver. The hypothesis states that aluminum binds to the skeletal proteins of the membrane of the plasmid affecting the permeability of the cell membrane and ultimately leading to the death of hepatocytes [18].

In order to eliminate the problems caused by poisonings, numerous natural herbs have been used to treat and protect the liver in traditional medicine of different nations [19]. One of these natural herbs used for liver treatment and protection is chicory. The scientific name of the chicory plant is *Cichorium Intybus L*. This plant reduces the destructive effects of poisoning and improves liver function [20]. In terms of traditional medicine, all parts of the chicory, especially the root and leaves, improve the function of the stomach and gallbladder and serve as an appetizer for improving digestive function [21]. Other properties of the chicory plant are urinary stimulation, analgesic effect, sweat production, stomach uplift, blood purifier, laxative effect, and strengthening of liver function. Enhancing the elimination of germs and microbes, it also is used to relieve inflammation and skin tumors. All parts of this plant, especially the root and leaves, can be used [22].

Considering that the use of aluminum can damage the human body, the present study aimed to assess the chemical and hepatoprotective activity of chicory extract against Alcl3-induced liver damage in rats.

Materials and Methods

Chicory leaves and stalk: Dry chicory leaves and stalk (500 g) were exhaustively extracted with ethanol 70% at room temperature. The mixture was filtrated and evaporated. After filtering the solution with a Whatman-filter-paper (No. 1 filter), was withdrawn the extract using a rotary machine under vacuum conditions, and the extract was prepared [23]. This research was conducted in 2018.

Animals: A total of 28 adult male mice (4-6 weeks old) weighing 24-28 gr. were obtained from the animal house of Avicenna Research Institute, Mashhad, Iran. The animals were housed in plastic (polypropylene) cages at room temperature (25ºC±1ºC) and 50±5 humidity and exposed to a 12 h light: 12 h dark cycle. The mice were allowed free access to water and standard laboratory diet until the start of the experiment. All animal procedures were approved by the Animal Welfare Committee of the School of the Veterinary Medicine, Ferdowsi University of Mashhad, Iran, and the national laws for experiments on animals.

Animal treatment schedule: The first, each mouse was weighted. Their weight was between 24 and 28 grams. Thereafter, the dose of each injection was calculated based on the weight and each rat received intraperitoneal injection. The injectable solution of aluminum chloride was prepared by dissolving aluminum chloride powder in distilled water at a specified concentration. Mice were assigned to four

groups: seven animals in each group with similar conditions and nutrition. It is noteworthy that the duration of injection was 15 days and the sampling was performed one week after the last injection.

Groups were categorized as follows:

1) Control group: In this group, the maintenance conditions were similar to those of other groups, with the exception that no treatment was performed for them.

2) Group 1: Intraperitoneal injection (IP) of chicory extract (50 mg/kg).

3) Group 2: Intraperitoneal injection (IP) of Alcl3 (20 mg/kg).

4) Treatment group: Intraperitoneal injection (IP) of aluminum chloride (Alcl3) at 20 mg/kg with intraperitoneal injection (IP) of chicory extract (50 mg/kg).

Daily injections were taken for 15 days. One week after the last infusion, animals were anesthetized with ether and they were dissected. Blood was taken from the heart of the mice by syringe before the autopsy. After the autopsy, the liver of each rat was sampled for histological examination. The body weight of the animals was measured by their liver weight. These weights were compared (Table 1).

After the examination of macroscopic lesions, the liver samples were fixed in 10% buffered formalin (Merck, Darmstadt, Germany) for 96 h. Tissue samples were then dehydrated and cleared by a series of graded alcohols and xylene before being embedded in paraffin. The sections (5 µm) were stained with hematoxylin and eosin for observation under a CX21 light microscope (Olympus, Tokyo, Japan). An Olympus (U-TVO 63XC) camera mounted on the microscope was used to take microphotographs. The histological structure of the liver, including capsule, blade, hepatocyte, and bile ducts were studied. Serum isolated from blood were sent to the lab. The results of each group were compared with those of the control group.

A sample (size approx. 1 mm3) was taken from each group with electron microscopy (EM). The tissue samples were placed in 2% glutaraldehyde in 1 M cacodylate buffer for embedding in resin. The glutaraldehyde-fixed samples were re-fixed in 1% osmium tetroxide in 1 M cacodylate buffer. These samples were dehydrated by the progressive lowering temperature method, embedded in epoxy resin (TAAB Laboratories Equipment Ltd, UK), and cut in 1-µm-thick sections. The sections were stained with toluidine blue and then examined for pathological lesions.

Statistical analysis: The statistical analysis was carried out using SPSS software (version 11.5). Differences between groups were analyzed using statistical testing (Mann Whitney u test). All data are presented as mean±standard deviation (SD). A p-value of less than 0.017 was regarded as statistically significant.

Results

Macroscopic finding

The compassion between the body weight of the mice and their liver weight revealed that during Alcl3 poisoning, the weight of the liver increased. Nevertheless, when the chicory extract is used to treat poisoning, liver weight is approaching normal weight. Moreover, the mice which were only injected with chicory extract exhibited no significant difference with those in the control group (Diagram 1).

Table 1. Weight of mice and their livers

		Weight based on Gram						
Body Weight (gr.)		27	26	27	28	24	26	25
Liver Weight(gr.)	Control group	1.91	1.79	1.95	2.14	1.68	1.81	1.70
	Group 1 (chicory extract)	1.90	1.77	1.91	2.15	1.60	1.83	1.71
	Group 2 (injection of Alcl3)	2.25	2.37	2.61	2.41	2.20	2.00	2.25
	Treatment Group (Alcl3+ chicory extract)	1.95	1.93	2.05	2.30	1.99	1.97	2.02

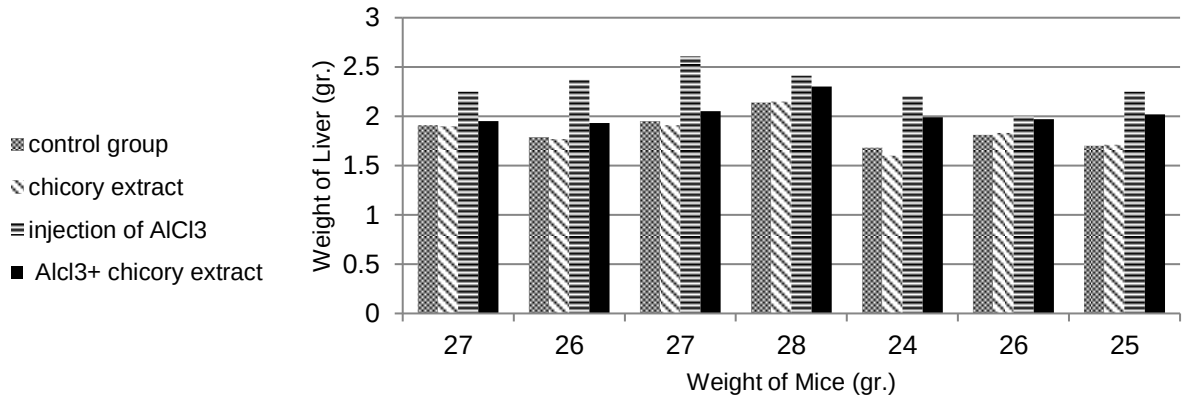

Diagram 1. Comparison between the weight of mice and their livers

Histopathological finding

In this study, the lesions, including inflammation, hepatocellular necrosis, hemorrhage, vacuolar degeneration of hepatocytes, and other lesions in the liver, were investigated by light microscopy.

Group 1. There was no definite lesions. There was only a small amount of hemorrhage and inflammation (Figure 2).

Group 2. The lesions in this group included hepatocyte necrosis, inflammation in the liver, and vacuolar degeneration in hepatocytes (Figure 3 and 4).

Treatment group: The lesions in this group included hyperemia and mild degeneration (Figures 5).

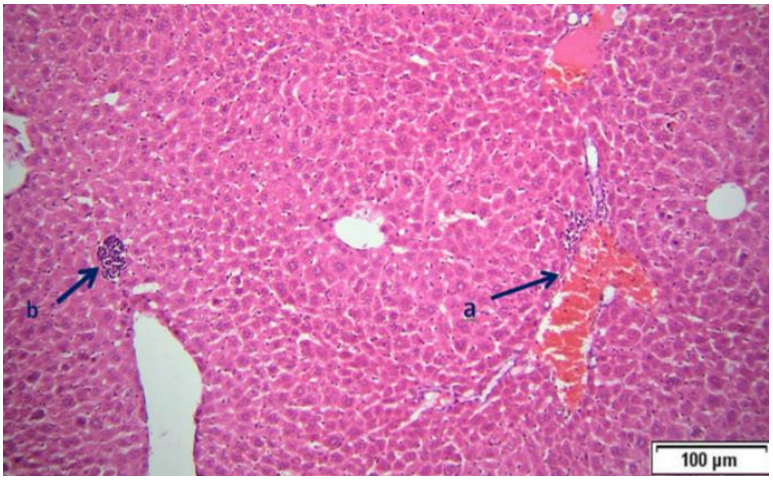

Figure 1. Mild hemorrhage (a) and mild inflammation (b). Hematoxylin and Eosin staining (100×)

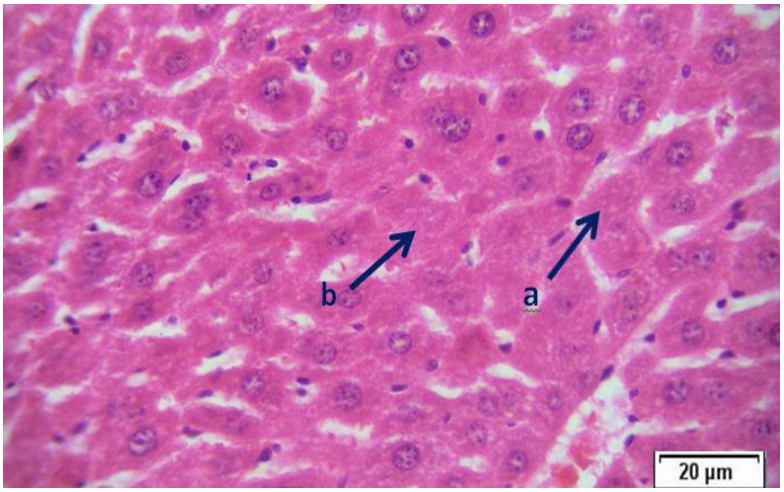

Figure 2. Hepatocyte necrosis with Alcl3 poisoning (a & b). Hematoxylin and Eosin staining (400×)

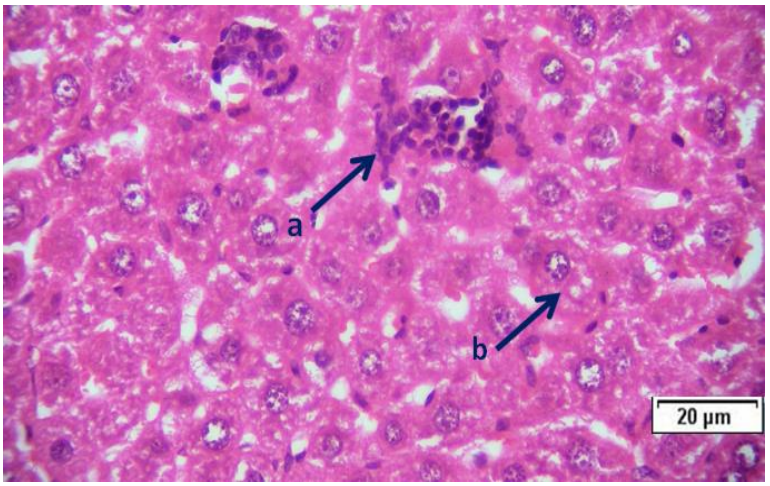

Figure 3. Inflammation in the liver tissue (a) and vacuolar degeneration in hepatocytes (b) with Alcl3 poisoning Hematoxylin and Eosin staining (400×)

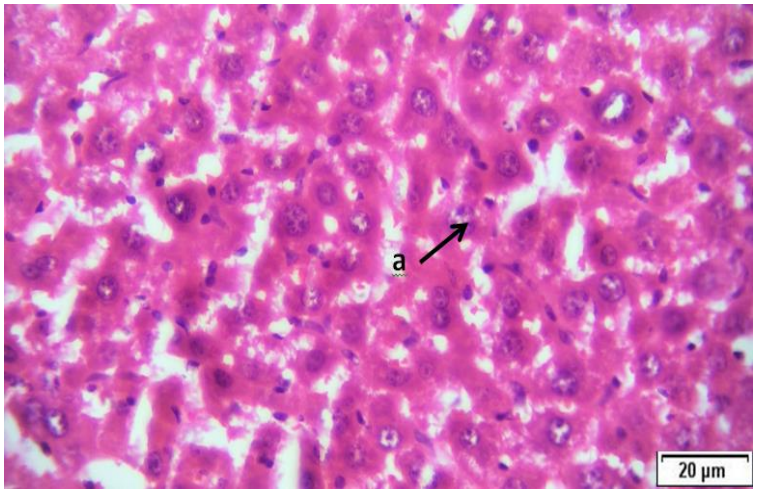

Figure 4. Mild degeneration in the simultaneous injection of chicory extract and Alcl3 (a). Hematoxylin and Eosin staining (400×).

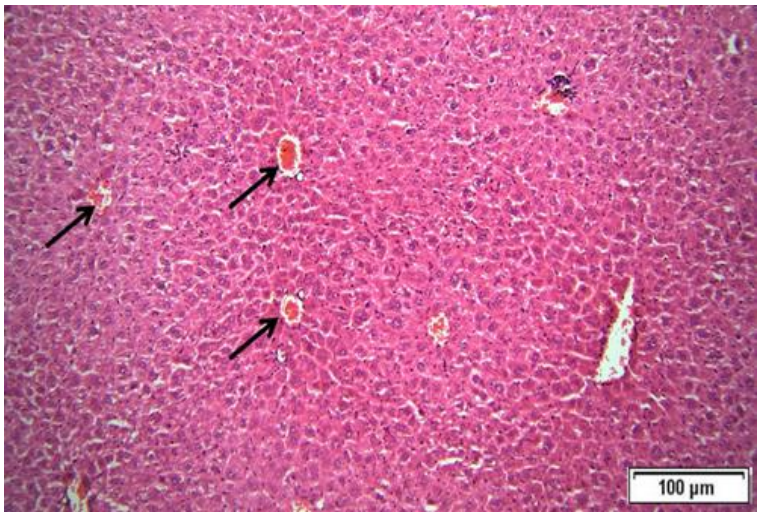

Figure 5. Hyperemia in the simultaneous injection of chicory extract and Alcl3 (arrows). Hematoxylin and Eosin staining (100×).

Serological finding

Isolated serum was sent to the Avicenna Laboratory. In the serological study, both ALT (SGPT) and AST (SGOT) enzymes were measured, and the results were evaluated in different groups. The results of each group were compared with the control group. The serological results are presented in Table 2 and compared in diagrams 2 and 3.

The mean of enzymes measured in groups that had been injected with chicory extract, significantly decreased, demonstrating the positive effect of chicory on liver hepatocyte cells.

Table 2. Serological results

	Control group	Group 1	Group 2	Treatment group
AST (U/L)	125	64	369	191
ALT (U/L)	22	15	238	47

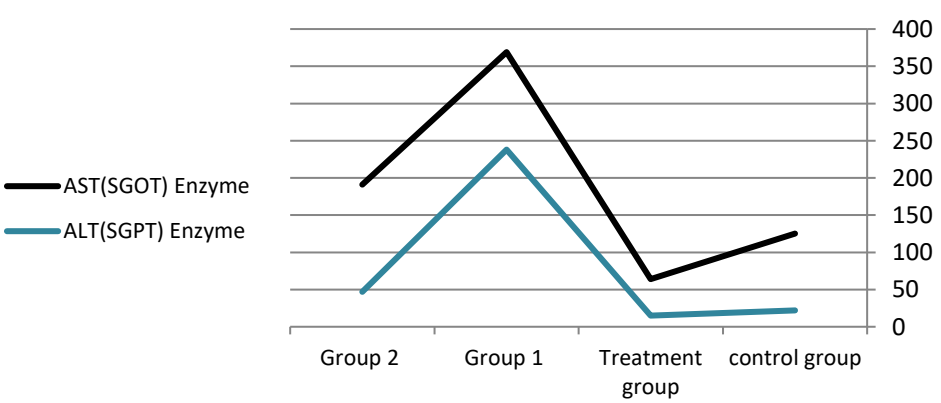

Diagram 2

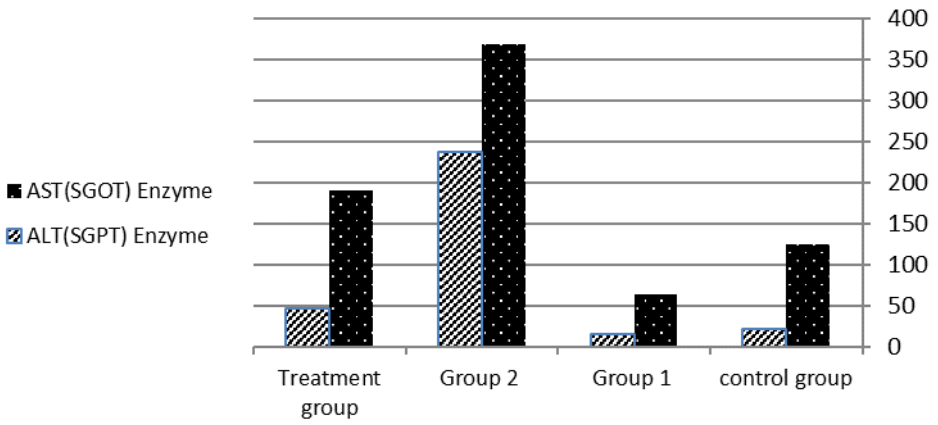

Diagram 3.

Discussion

As evidenced by the results of this research, the induction of aluminum poisoning did not create macroscopic lesions in the liver. Of course, longer infusions may have a damaging effect on the liver. According to the comparative results displayed in Table 1, the negative effect of aluminum poisoning is quite obvious. This negative effect can be fully confirmed by increasing liver weight in group 2. On the other hand, weight loss of liver in the treated group and the closer of this weight to the control group is a reason for the positive effects of chicory extract on liver function. These results are well presented in Diagram 1.

The serological results in Table 2 display that the average level of enzymes- ALT (SGPT) and AST (SGOT)- increased in group two that was poisoned with Alcl3. These results also confirm the increase in liver weight. On the other hand, the results in the treatment group highlight the positive effects of chicory extract on the treatment of aluminum-induced poisoning. These results are associated with a decrease in the average level of enzymes. Furthermore, histological studies pointed out that in group one, the lesion was not descriptive in comparison with group two (poisoned with Alcl3) in which liver lesions, including necrosis in hepatocytes, inflammation, and vacuolar degeneration were clearly evident. These histological changes reflect the destructive effects of Alcl3 on the liver tissue, as emphasized in various studies.

In the treated group, that received a combination of aluminum and chicory injections, damage significantly reduced and only hemorrhage and mild degeneration were observed. This is a reason for the positive effects of chicory extract on the treatment of Alcl3 poisoning. The presented results were completely consistent with the findings of the studies by transmission electron microscopy (TEM) in which there were similar lesions as observed in light microscope studies.

Investigations in this field have demonstrated that the chicory plant has a high protective effect on carbon tetrachloride toxicity in the animal model [20]. In 2012, researchers isolated the phenolic compound from the root of chicory and confirmed the role of hepatic protection in mice against liver damage from Carbon tetrachloride [12]. Androli et al. (2007) reported that liver toxicity induced by dose-dependent aluminum increases the serum levels of ALT, AST, and ALP liver enzymes [1]. In the same vein, in their study, Jamshidzadeh et al. (2006) revealed that chicory extract with low concentrations of carbon tetrachloride poisoning has a protective effect on Liver cells; however, the chicory extract with high modulus has toxic properties [24].

One of the oxidative substances that generates free radicals causing liver damage and liver toxicity is Iodine Thiostamate. Madani et al. (2005) reported that the use of the polyphenol extract of Chicory plant protects the liver cells against the damage caused by the free radicals produced by the Iodin Thiostamate. The results illustrated that the polyphenolic extract of chicory plant has a protective effect on liver cells [25]. Aluminum is transmitted in the circulation as binding to β-globulin, called transferrin, which is essentially the main carrier of iron in vertebrates [26]. It seems that the albumin is also partially attached to the aluminum. Currently, the toxic effects of aluminum have been considered in recent years [12]. The recent studies have pointed out that the mechanism of the effect of aluminum compounds on body tissues is different. In the liver tissue, lysosome, base membrane, and cell membrane are affected by aluminum compositions. The long-term administration of aluminum, and changes in mitochondria, rough endoplasmic reticulum (RER), glycogen, and hepatocyte nucleus have been confirmed [18].

Conclusions

Given the data of the present study, it can be deduced that chicory can be considered an effective remedy for liver diseases, as confirmed by its common uses in different polyherbal mixtures or formulation across the globe. Both histological and serological studies indicate that lesions were markedly educed in chicory treated groups, and liver enzymes were improved.

Therefore, it can be concluded that chicory plant as a herbal medicine plays a major role in mitigating the effects of poisoning caused by Alcl3. This role can be described by reducing the amount of lesions in the liver cells and improving liver enzymes.

Conflict of Interests

The authors declare that they have no conflict of interest.

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Compliance with Ethical Guidelines

This study was approved by the Ethics Committee, Ferdowsi University of Mashhad, Mashhad, Iran (Code: IR.UM. REC.1400.301).

Authors' Contributions

AN, ZM and NDM conception, designed the study and performed the experiments. All authors were involved in data analyses and interpretation and writing the manuscript. All authors read and approved the final manuscript.

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