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Effect Wheatgrass (*Triticum aestivum)* aqueous extract albino mice exposed to aluminum chloride poisoning

Adnan M. Aldulaimi [1*], Abd-Alhadi I. Al Jumaily [2] and Sami Kh. Saeed [3]

[1,2] *Education Directorate Salah Al-deen, Ministry of Education, Iraq*

[3] *Food Science Department, College of Agriculture, Tikrit University, Iraq*

[*]Correspondence email: **Adnanmoh77@gmail.com**

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ABSTRACT

The current study was designed to observe and test the effect of the Wheatgrass aqueous extract on blood parameters and histological changes in liver and kidneys in male albino mice that were exposed to aluminum poisoning (40 mg/kg bw) for 30 days. The animals were divided into (3) groups containing five (5) animals and weighing (20-24 gm). The results of oral administration with aluminum showed a significant decrease in final body weight and the weight of the liver, spleen and testes. And concentrations of each of the RBCs, HCB, HCT, MCV, MCH, MCHC and GRAN. And a significant increase in WBC, LYM and MON. The weight of the kidneys and heart were not significantly different compared to the healthy control group. A microscopic examination of liver and kidney tissue sections of the group of animals treated with aluminum observed central vein congestion (CON), separation of the central wall vein and part of it in the liver Picture. In the kidneys Picture shows the degeneration of most urinary tubule cells (D) with clear hemorrhage within the kidney tissue. The results of the treatment with wheatgrass + aluminum had a positive effect on body weight, organs, and blood parameters. And observed the normal shape of the central vein (CV), hepatic cells (HC) and hematopoietic sinuses in the liver Picture, showing the semi-normal shape of urinary tubules (UT) and renal glomeruli (G) with hemorrhage (H) within the kidney tissue Picture, compared with the aluminum infected group. Therefore, it can be concluded that wheatgrass has a great ability to improve hematological parameters and histological changes in the liver and kidneys.

تأثير مستخلص عشبة القمح المائي في ذكور الفئران المعرضة للتسمم بكلوريد الالمنيوم

عدنان محمد احميد الدليمي [1]، عبدالهادي ابراهيم حسين الجميلي [2]، سامي خضر سعيد [3]
[1 ، 2] مديرية تربية صلاح الدين، وزارة التربية، العراق
[3]قسم علوم الاغذية، كلية الزراعة، جامعة تكريت، العراق

الخلاصة

تم تصميم الدراسة الحالية لملاحظة واختبار تأثير مستخلص عشبة القمح المائي في المعايير الدموية والتغيرات النسجية لكبد وكلى ذكور الفئران البيض المعرضة للتسمم بكلوريد الالمنيوم (40 ملغم/كغم من وزن الجسم) لمدة 30 يوماً. تم تقسيم الحيوانات الى 3 مجاميع وتحتوي كل مجموعة على 5 حيوانات واوزانها (20-24 غم). اظهرت نتائج الاعطاء الفموي بالألمنيوم انخفاض معنوي في وزن الجسم النهائي ووزن الكبد والطحال والخصى وتراكيز كل من RBCs, HCB, HCT, MCV, MCH, MCHC, GRAN. وزيادة معنوية في تراكيز كل من WBC, LYM, MON . وعدم وجود اختلاف معنوي في وزن الكلى والقلب مقارنة مع مجموعة السيطرة السليمة. كما لاحظ في انسجة الكبد والكلى للحيوانات المعاملة بالألمنيوم احتقان الوريد المركزي (CON) وانفصال جدار الوريد المركزي وتحطم جزء منها. ويوضح تنكس معظم خلايا النبيبات البولية (D) مع وجود نزف دموي واضح ضمن نسيج الكلية. اما نتائج المعاملة بمستخلص عشبة القمح المائي+ الالمنيوم كان له تأثير ايجابي على وزن الجسم النهائي ووزن الاعضاء ومعايير الدم، وملاحظة الشكل الطبيعي للوريد المركزي (CV) والخلايا الكبدية (HC) والجيبانيات الدموية (S) في نسيج الكبد. والشكل شبة الطبيعي للنبيبات البولية (UT) والكبيبات الكلوية (G) مع وجود نزف دموي (H) ضمن نسيج الكلية مقارنة مع المجموعة المصابة بالألمنيوم. لذلك يمكن الاستنتاج أن عشبة القمح لها قدرة كبيرة على تحسين المؤشرات الدموية والتغيرات النسيجية في الكبد والكلى.

الكلمات الافتتاحية: عشبة القمح ، الالمنيوم ، المعايير الدموية ، النسيجية .

INTROUCTION

Aluminum (Al) is an industrial and environmental pollutant that causes a broad spectrum of toxicity (Liu *et al*, 2016). AL is used as a food additive, in cooking pots with roughly 20% Aluminum content, in drinking water with a 0.2-mg/L concentration, as a water-purifying agent, in can bottles, in aluminum foil paper, and antiperspirant cosmetic products. Although having a low gastrointestinal absorption capacity (less than 1%), it may accumulate over time in vital organs like the kidney, liver, and brain, where it may cause apparent neurotoxicity and cytotoxicity (Othman *et al*., 2020). Although aluminum is not biologically crucial to the human body, studies have demonstrated that excessive human exposure to Al can induce oxidative damage, neuroinflammatory conditions and neurotoxic manifestations implicated in Alzheimer's disease (Rajendran *et al*., 2023). It leads to a high concentration of aluminum within the body

the serious disturbances in the functioning of the skeletal, nervous and hematopoietic systems (Kloppel *et al*., 1997). Accumulation in body tissues is associated with damage to the target organs (Becaria *et al*., 2002). the extreme exposure to aluminum due to dissimilar human daily lifestyles raised the threat of renal aluminum withholding due to the accumulation of aluminum to the renal tubules which resulted in renal dysfunction as reported by (Al Dera, 2016; Hasona & Ahmed, 2017)). Al poisoning causes oxidative stress, Apoptosis, liver and kidney toxicity, and its effect on their tissues (Ahmed *et al*., 2023).

Wheatgrass (Triticum aestivum) belonging to the Poaceae family refers to the young grass of the common wheat plant (Ozkosel *et al*., 2016; Thammana *et al*., 2016). Wheatgrass is one of the green substances that are esteemed by well-being of people as an incredible source of nutritious supplements. Wheatgrass is known as complete nourishment and is promoted to treat several conditions (Petyaev & Bashmakov, 2012; Uboh FE, *et al*.,2016). Wheatgrass juice has been known for its high nutritional and healing properties. It is a rich source of essential amino acids, enzymes, vitamins, minerals and chlorophyll. Its chlorophyll content is about 70% so it is referred to as Green blood (Shakib *et al*., 2017). Wheatgrass contains all B complex vitamins, and also calcium, phosphorus, magnesium, sodium and potassium in an adjusted proportion. It is a good source of protein and has double the measure of Vitamin A as carrots and is higher in Vitamin C than oranges (Akram, & Aftab, 2015; Amanullah, *et al*., 2015). This plant is believed to have many nutritional values; it has been shown to have anti-inflammatory, antioxidant, anti-carcinogenic, immunomodulatory, laxative, astringent, diuretic, antibacterial and anti-aging properties. Its use in acidity, colitis, kidney malfunctions, atherosclerosis and swelling is beneficial. Wheatgrass juice helps in building red blood cells and stimulates healthy tissue cell growth (Satyavati *et al*., 2011). It is used to treat many diseases such as anemia, diabetes, cancer, eczema, constipation, kidney swelling, and the common cold (Sareen *et al*., 2014).

The study aims to know the effect of Wheatgrass aqueous extract on blood parameters histological changes of the liver and kidneys in male albino mice exposed to Aluminum chloride toxic.

MATERIAL AND METHODS

Prepare of experimental samples: Wheatgrass was obtained by planting wheat seeds in the organic fertilizer in ponds dedicated to agriculture. It was sprayed with sufficient water until it was germinated and exposed to light. When the length of the herb was about 10-12 cm it was

harvested and dried in a clean place in the shade. And then it was ground by an electric grinder to get a powder. The powder was filled in plastic bags and closed for use. The current study's procedures were carried out in conformity with the guidelines of the Ethics Research Committee of the College of Agriculture, Tikrit University.

Extract preparation: The aqueous extract was obtained using a method (Harborne, 1984), 100 g of Wheatgrass powder was weighed in an analytical balance, In a flask add 200 ml of distilled water and leave for 24 hours in the refrigerator after stirring, The treatment was then mediated by the medical gauze. The washing process was then returned using 100 mL of distilled water and the filtration was returned. The washing and re-filtration process was then repeated, using 50 mL distilled water. Then put the filtrate for evaporation in the rotary evaporator at 50 C° of until a concentrated liquid is obtained. Then, the extract was placed in plastic containers by freezing at -20 °C until use.

Aluminum chloride: Aluminum chloride (AlCl3) was purchased as yellow solid anhydrous salt from the Bashir office in Baghdad/ Iraq.

Experimental animals: Male white mice were used in the experiment, weighing 24-26 gm, obtained from the Faculty of Veterinary Medicine - Tikrit University. Weights ranged between 23-25gm. It was randomly placed in stainless steel cages, The aspect of taking care of the cages' cleanliness and sterilization was observed from time to time, with the change of sawdust every three days, at a temperature of 25 + 2 ° C and a light period of 12 hours and the basal diet was given as recommended in (Mizil & Al-Zamely, 2002). In addition to research samples and free water as desired, the experiment lasted for 30 days, and measured the initial body weight using a mettler-sensitive balance. the animals were divided into 3 groups containing each group 5 animals as follows.

Group 1: (Control group).

Group 2: Administered Aluminum chloride with 40 mg/kg b.w. by gavage.

Group 3: Administered with Wheatgrass aquatic extract 400 mg/kg b.w + aluminum chloride 40 mg/kg b.w by gavage.

On the last day of the experiment, animals fasted for 12 hours, the final body weight of the animals was taken and anesthetized by chloroform and sacrificed by cutting the jugular vein, and blood samples were collected and placed in tubes containing an anticoagulant for blood analysis.

Then the organs that including the liver, kidneys, spleen and testes were taken and weighed with a sensitive scale.

Hematological methods: Complete blood counts (CBCs) (whole blood analysis). The hematological analysis of White blood cells (WBCs) count, lymphocyte (LYM), monocyte (MON), granulocyte (GRAN), Red blood cells (RBCs) count, hemoglobin concentration (HGB), hematocrit (Hct), mean cellular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined by using automated hematology analyzer (Syamex model: K-1000, Japan) (Haen, 1995). In addition to, most of the blood tests performed by using modified methods (WHO, 1989).

Histopathological methods: After taking the blood samples, livers and kidneys were dissected out and fixed in 10% formalin for 24h. The specimens were washed in tap water, dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax. Sections of about 6mm thickness were prepared and stained with hematoxylin and eosin for histopathological examination (Drury & Wallington, 1980).

Statistical Analysis

The data of results in the present study were analyzed by using the ANOVA analysis, utilizing the general linear model of the Statistical Analysis System (SAS, 2001). Also, the significant differences were evaluated by using Duncan's multiple-range test (Duncan, 1955), and significance level is based on the level of probability ($P<0.05$).

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RESULTS AND DISCUSION

Table (1) Results showed administered aluminum, it led to a significant decrease in final body weight and the weight of the liver, spleen and testes. The weight of the kidneys and heart were not significantly different compared to the healthy control group. As for the group that gave Wheatgrass extract + aluminum, it led to a significant increase in the weight of the liver, spleen and testes, while the weight of the kidneys and heart did not differ significantly compared to the group that gave the aluminum.

Table (2) shows that administered aluminum, it led to a significant increase in the concentration of WBC, LYM, MON. Significant decrease in GRAN compared to the control group. While observed administrated as for the group that gave Wheatgrass extract + aluminum,

it resulted in a significant decrease in the concentrations of LYM and MON. Significant increase in GRAN, and WBC concentration was not significantly different in comparison with the aluminum group. When administered aluminum, it led to the significant decrease in the concentrations of RBCs, HCB, HCT, MCV, MCH and MCHC compared to the control group. As for the group that gave Wheatgrass extract + aluminum, there was a significant decrease in the concentrations of RBCs, HCB, HCT, MCV, MCH and MCHC compared with the group Infected with aluminum table (3).

Table (1) Effect administered orally of Wheatgrass in body weight and organs weight of albino mice exposed to aluminum poisoning.

Type of transaction	**Measured Standards (g)**							
	Testicular	**spleen**	**liver**	**heart**	**kidneys**	**Initial body weight**	**final body weight**	**Increase in weight**
Control	0.51 c ±0.005	0.13 c ±0.005	2.06 a ±0.011	0.15 a ±0.011	0.44 a ±0.011	24.44 b ±0.271	32.73 a ±0.663	8.29 a ±0.506
Aluminum	0.87 a ±0.011	0.24 a ±0.011	1.30 c ±0.057	0.13 a ±0.005	0.56 a ±0.005	23.17 b ±0.578	17.50 c ±0.771	-5.67 b ±0.631
Wheatgrass + Aluminum	0.67 b ±0.011	0.19 b ±0.011	1.76 b ±0.005	0.20 a ±0.057	0.49 a ±0.057	25.78 a ±0.190	24.21 b ±0.577	-1.57 c ±0.399

The figures followed by vertically different letters mean that there are significant differences at the probability level ($P \leq 0.05$).

Table (2) Effect administered orally of Wheatgrass in WBC, LYM, MON and GRAN of albino mice exposed to aluminum poisoning

Type of transaction	**Measured Standards (g)**			
	WBC (10^9/L)	**GRAN(%)**	**MON(%)**	**LYM (%)**
Control	4.66 b ±0.33	11.80 a ±0.40	12.30 b ±0.17	75.90 c ±0.57
Aluminum	8.50 a ±0.17	2.90 c ±1.15	14.10 a ±0.57	83.00 a ±0.57
Wheatgrass + Aluminum	7.20 a ±0.57	8.30 b ±0.40	13.30 ab ±0.17	78.40 b ±0.57

The figures followed by vertically different letters mean that there are significant differences at the probability level ($P \leq 0.05$).

Table (3) Effect administered orally of Wheatgrass in hematological of albino mice exposed to aluminum poisoning

Type of transaction	**Measured Standards (g)**					
	MCHC (g/Dl)	**MCH (pg)**	**MCV(fl)**	**HCT(%)**	**HCB (g/l)**	**RBCs (10^{12}/L)**
Control	41.40 a ±0.11	20.50 a ±0.17	45.10 a ±0.57	40.30 a ±0.17	13.20 a ±0.11	8.60 a ±0.76
Aluminum	35.00 c ±0.57	14.20 c ±0.57	39.30 b ±0.57	33.10 b ±0.57	6.90 c ±0.57	4.53 b ±0.57
Wheatgrass + Aluminum	38.90 b ±0.57	17.30 b ±0.05	43.40 a ±0.57	39.00 a ±0.57	9.40 b ±0.11	6.92 a ±0.57

The figures followed by vertically different letters mean that there are significant differences at the probability level ($P \leq 0.05$).

A microscopic examination of liver and kidney tissue sections of the group of animals treated with aluminum for 30 days observed central vein congestion (CON), separation of the central wall vein and part of it in the liver Picture (3). In the kidneys Picture (4) shows the degeneration of most urinary tubule cells (D) with clear hemorrhage within the kidney tissue (H) compared with the control group Pictures (1 and 2). when giving the Wheatgrass extract + aluminum observed normal shape of the central vein (CV), hepatic cells (HC) and hematopoietic sinuses in the liver Picture (5), shows the semi-normal shape of urinary tubules (UT) and renal glomeruli (G) with hemorrhage (H) within the kidney tissue Picture (6) compared with the aluminum-infected group Pictures (3 and 4).

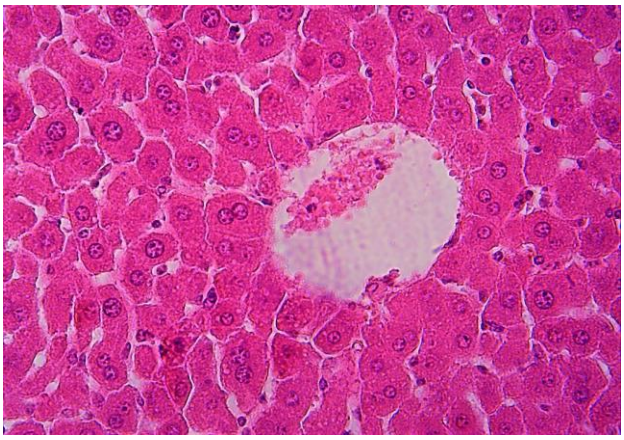
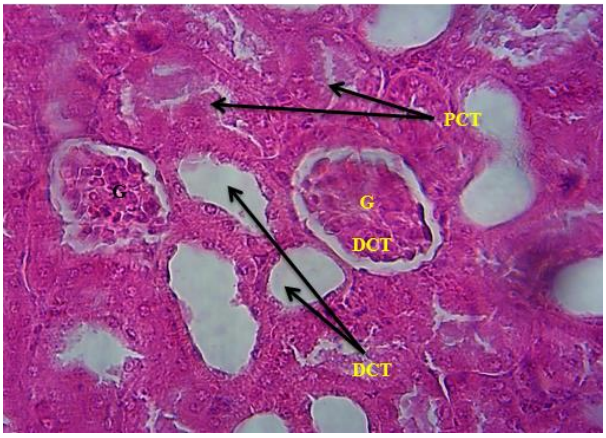
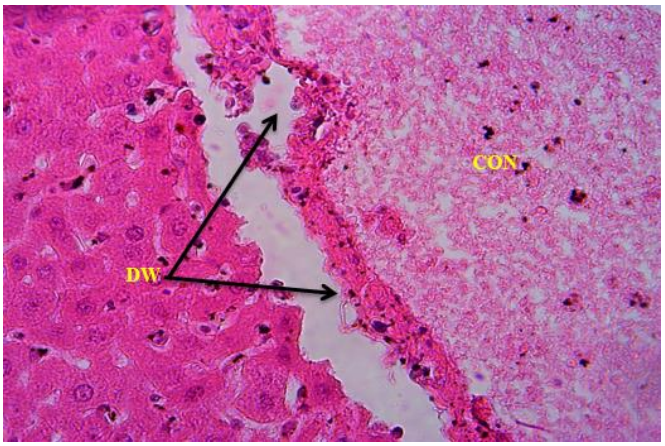


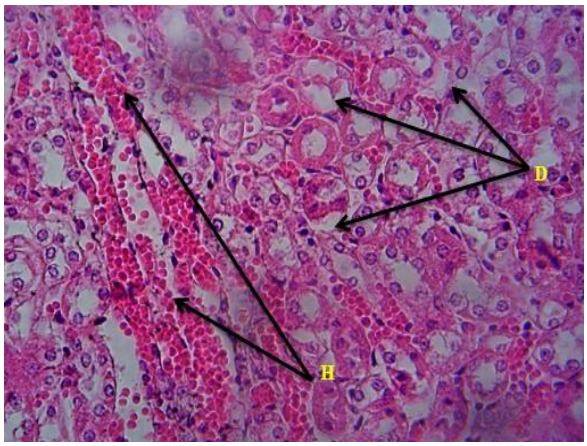
Fig (1): Histological images of the liver and kidneys of healthy male mice infected with aluminum chloride and treated with wheatgrass + aluminum chloride



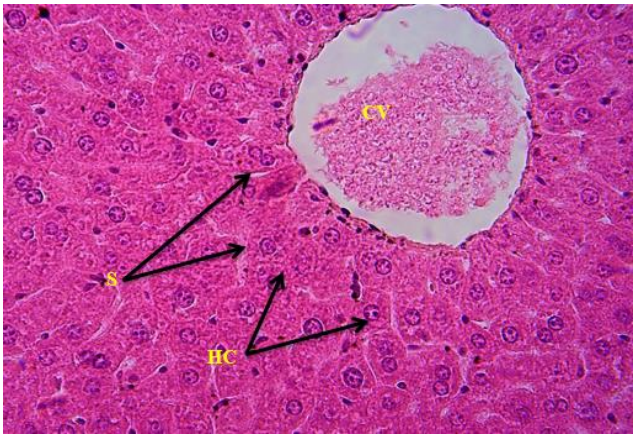
Picture (2) kidney section control group showing renal glomerulus (G) and proximal twisted tubules (PCT) and distal (DCT). H & E 400X.



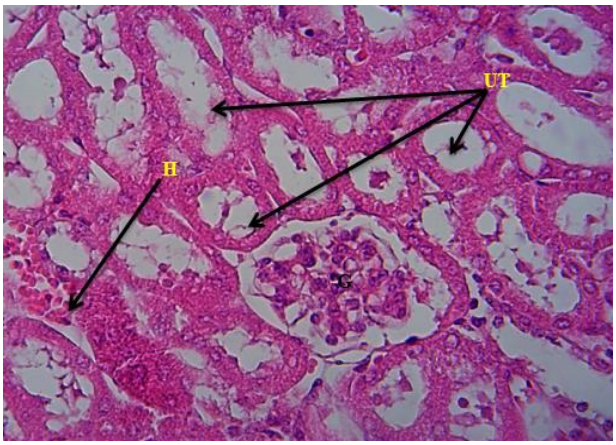
Picture (3) Liver section of aluminum treated group showing central vein congestion (CON), separation of central wall vein and Destructed of part of the wall (DW). H & E 400X.



Picture (4) kidney section of the group treated with aluminum showing the degeneration of most urinary tubule cells (D) with clear hemorrhage within the kidney tissue (H) H & E 400X.



Picture (5) liver section of the group treated with aluminum + wheatgrass, showing the normal shape of the central vein (CV), hepatic cells (HC) and hematopoietic sinuses (S). H & E 400X.



Picture (6) kidney section of the group treated with aluminum + wheatgrass, showing the semi-normal shape of urinary tubules (UT) and renal glomeruli (G) with hemorrhage (H) within the kidney tissue H & E 400X.

The dosage of mice with aluminum chloride has led to a reduction in body weight, this may be due to the effect of aluminum chloride on metabolism, enzymes and accumulation in different tissues, as well as its induced oxidation stress (Sallam *et al*., 2005). The reason may be due to damage to the gut, preventing food absorption. The reduction in RBCs, HCT and HB might be due to an inhibition of hemoglobin and erythropoiesis synthesis and to an increase in the rate of erythrocytes destruction (Maruyama *et al*., 2001). Several mechanisms have been proposed for aluminum-induced anemia, and the proposed mechanisms appear to involve inhibition of heme synthesis, either by inhibition of enzyme activity and interference with iron incorporation or utilization (Kaiser & Schwartz, 1985; Han *et al*., 2000) . These can be a marker of anemia with subsequent results of inhibition of erythropoiesis in the hemopoietic system (Lavanya *et al*., 2001). Aluminum was able to induce changes in hematological parameters. Any changes made in their structure and number can cause very large physiological changes animals (Kalaiselvi *et al*., 2015). The white blood cells are the regulators of the immune system and the increase in WBCs count may be due to generalized immune responses and a protective response to mental stress (Nussey *et al*., 2002). In general, Stimulation of lymphopoiesis and enhanced release of lymphocytes from lymph myeloid tissue under toxic stress may lead to an increase in WBCs number (El-Sayed *et al*., 2007). Aluminum chloride intoxication produced various pathological lesions in the liver and kidney cells in male mice, such as central vein congestion (CON),

separation of central wall vein and Destructed of part of the wall (DW) in liver, the degeneration of most urinary tubule cells (D) with clear hemorrhage within the kidney tissue. Aluminum chloride-induced renal damage. Reduction of oxidative stress and inflammation may be considered as the main pathways of action (Al Dera, 2016). Aluminum toxicity causes severe imbalances in the oxidant–antioxidant system and, as a consequence, generates reactive oxygen species, which are highly reactive and can cause damage to nucleic acid, lipids, and proteins (Kinawy, 2019). The researchers stated Hassan *et al*, (2023) that exposure of mice to ALCL3 causes a significant decrease in renal function, inflammation, apoptosis, and excessive renal pathological damage with interstitial fibrosis as evidenced by endothelial, glomerular, and tubular grade .Concomitant treatment of Wheatgrass with aluminum chloride showed prominent recovery and normal architecture with mild residual degeneration. The reason may be that wheatgrass contains high levels of antioxidants and inhibit DNA oxidative damage and are effective in suppressing the superoxide radical (Kulkarni *et al*., 2006; Rana *et al*., 2011). And especially high in chlorophyll along with some other constituents, The chlorophyll which has more oxygen and conveys more oxygen to the blood. Thus the number of RBC will increase and blood oxygen levels will rise rapidly with the drinking of wheatgrass squeezed juice and utilizing wheatgrass juice. This is a key indicator of recovery from various abnormalities, ailments and diseases. Oxygen acts as a crucial element to numerous body parts, especially the cerebrum uses 25% of the oxygen supply (Ahmed *et al*., 2023). it strengthens the cells, detoxifies the liver and bloodstream, and chemically neutralizes the contaminating elements. Wheatgrass contains a high level of flavonoids and phenolic, which an antioxidant properties. Keeping these in view, the plant extracts rich in phenolic and flavonoids can be used in treating diseases such as cancer, stomach ailments, anemia and other blood-related diseases that result mainly from the free radicals generated in the body (Zendehbad *et al*., 2014) . the composition of wheatgrass, which includes vitamins, minerals, amino acids, enzymes, chlorophyll, and bioactive compounds. These components contribute to its antioxidant, anti-inflammatory, antimicrobial and immunomodulatory properties **(**Singh *et al*., 2023). The researchers indicated that wheatgrass has great potential to diminish the stress-mediated complications and improve the antioxidant status (Tripthl *et al*., 2021).

CONCLUSION

The current study showed that wheatgrass extract has a clear effect in improving the results of blood parameters, liver and kidney tissue in male rats from the effects of aluminum chloride poisoning, and this may be related to the active and antioxidant components found in wheatgrass.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

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