

**Immuno-enhancement of barely β -glucan or barley extracts
on arsenic trioxide-induced immunosuppressed rat model**

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Abstract

The present investigation aimed to study the biological effects of barely β -glucan or barley extracts on arsenic trioxide-induced immunosuppression. Fifty-four male albino rats (weighing 100 ± 25 g) were divided into eight groups, each group including six rats. Group 1: PBS was injected intraperitoneally into the rats, while saline was given orally (negative control). Groups 2–8 received daily intraperitoneal injections of As_2O_3 (5 mg/kg) for 4 weeks. Group 2 serves as a positive control. The rats in groups 3, 4, and 5 were received daily doses of β -glucan extracts of 40, 80, and 160 mg/kg bw/day, respectively, with ATO (arsenic trioxide). Groups 6, 7, and 8 were given barley extract at doses of 0.7, 1.4, and 2.8 g/kg bw/day, respectively, with ATO. At the end of the experiment, all of the rats were slaughtered for biochemical, hematological, and histopathological analysis. The obtained results revealed that barley β -glucan and barley extracts increased CAT and GPx activity while lowering MDA levels in the spleen tissue of rats. It

also improved haematological and histopathological changes caused by arsenic trioxide. The levels of IgG and IgM in the groups treated with barley β -glucan extract were considerably greater than those in the groups treated with barley extract. The cytokine levels, hepatic and renal biomarker markers showed affection results due to treatment with arsenic trioxide. Our findings show that barley extracts and barely β -glucan extracts could be employed as a component of functional foods to boost immunity.

Keywords: barely β -glucan, barley, immunity, Arsenic trioxide, inflammation.

1- Introduction

Patients with hematological malignancies, notably acute promyelocytic leukaemia, are treated with arsenic trioxide (As_2O_3). Arsenic is widely distributed throughout nature, and it can be found as a component of both organic and inorganic compounds. Arsenic inorganic (arsenite, As^{+3} or arsenate, As^{+5}) compounds include arsenite, which is the most deadly form, and arsenate, which is less poisonous than organic arsenic-containing molecules (Ojo *et al.*, 2018). Acute arsenic poisoning damages organs such the kidney, liver, gut, and brain (Zhao *et al.*, 2017 & Guo *et al.*, 2016). Arsenic's harmful effects are caused by an increase in oxidative stress (Turk *et al.* 2019). Zarei *et al.*, (2019) found that ROS are involved in arsenic-induced oxidative damage to macromolecules in cells, which leads to apoptosis. It's thought that arsenic contributes to spleen poisoning (Hosseinzadeh *et al.*, 2019).

Several critical components of immune systems to protect the body from infection have been identified in studies during the last decade (Iwasaki & Medzhitov 2015). The focus was on barley (*Hordeum vulgare L.*), which, while being the world's fourth-largest grain crop, is used in the food industry. Due to its high abundance of soluble dietary fibre, particularly β -glucans, pectin, and arabinoxylans, barley has recently become the subject of research (Bader *et al.*, 2018). Barley whole grains and their outer bran layer are high in functional compounds like fibre, phenolic acids, flavonoids, phytosterols, alkyl resorcinols, benzoxazinoids, lignans, alpha-tocopherol (vitamin E), and folate (Zeng *et al.*, 2020). β -glucans, lignans, and vanillic acid are three anti-inflammatory compounds found in barley (Andersson *et al.*, 2008).

The effects of whole-grain barley and extracted barley β -glucans on glycaemic control, but their effects on the T2D (type 2 diabetes) management are less well documented (Li *et al.*, 2020 & Higa *et al.*, 2019). The β -glucans are a type of polysaccharide made up of linked D-glucose monomers joined together by β -glycosidic linkages. β -glucans from cereals are mostly linear, unbranched molecules made up of glucose units linked by $\beta(1\rightarrow3)$, and $\beta(1\rightarrow4)$ glycosidic bonds (Johansson *et al.*, 2004). Barley β -glucans are plentiful, easy to extract, and have a high biological activity. In terms of metabolic advantages on satiety, energy intake, weight loss, and insulin sensitivity, barley or oat β -glucan consumption by human participants has yielded mixed findings (Li *et al.*, 2020; Zhang *et al.*, 2021; Andersson *et al.*, 2008).

Furthermore, β -glucan is a potent activator of humoral and cell-mediated immunity, and frequent consumption of β -dglucan boosts immunological activity considerably (Novak & Vetvicka 2009). The bioactivity of beta-glucans has been discovered to have antioxidative, antiviral, boosts immunological, and anticancerogenic properties, which are predominantly seen in fungal beta-glucans. The immune boosting properties of cereal beta-glucans, as well as their function in avoiding infections, chronic inflammation, and cancer (Suchecka *et al.*, 2017& Zhang *et al.*, 2021).

As a result, the current study looked at the effects of barely β -glucan extracts and barley flour extracts on sodium trioxide-induced immunosuppression, oxidative stress, haematological, biochemical, and histopathological markers.

2- Materials and methods

2-1- Materials

Arsenic trioxide (ATO, As_2O_3 , MW: 197.84) with trace metal basis purity of 99.99% was purchased from Sigma-Aldrich (St Louis, MO, USA). Methanol and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Merck, Germany. All biochemical assay kits were purchased from Randox Laboratories Ltd, Diamond Road Crumlin, Co, Antrim, UK, BT294QY.

2-2- Barley flour preparation

The naked barley (*Hordeum vulgare* variety, Giza 135) used in this study obtained from the Barley Research Department, Field Crop Research Institute,

Agriculture Research Center, Giza, Egypt. A high-speed electric mill (mesh size 20 mm) was used to grind the barely grains for making barley flour.

2-3- Barley extract preparation

In 1000 ml of distilled water, 100 g of barley whole meal were dispersed. After that, the mixture was heated for two hours at 30-40 ° C, and the water soluble extract was filtered, then concentrated in a dark container using a rotary evaporator. The concentrate was dried at 50 °C in an oven for 2 hr. (Hosseini *et al.*, 2013).

2-4- Extraction of β -glucan

With a few modifications, β -glucan was isolated from barley flour following the method described by Wood *et al.*, (1978). Fifty grams of barley flour were added to 500 ml of distilled water, the pH was adjusted to 10 with Na₂CO₃ (20% v/w), and the mixture was rapidly agitated for 30 minutes at 45°C. The mixture was centrifuged for 15 minutes at 1006 g (3000 rpm) at 4°C (Model 3K30, Sigma, Germany). The supernatant was adjusted to pH 4.5 with 2M HCl before centrifugation (20 minutes at 1789 g (4000 rpm) at 4°C) to separate precipitated protein, which was discarded. By slowly swirling an equal volume of ethanol (97%) was added to the supernatant, to precipitate the β -glucan. The solution was centrifuged at 1006 g (3000 rpm)/10 min after 12 hours at 4 °C. The precipitate was dried for 1.5 hours at 50 °C with forced air circulation in an oven for 2 hr (model 400/D 200 C, Nova Ética).

2-5- Chemical composition of extracts

The proximate composition and total dietary fiber of two extracts (barley β -glucan and barley extracts) were determined by AACC (2000).

2-5-1- Characterisation of β -glucan concentrate

The method for determined the barley β -glucan and barely were disclosed by Wood *et al.*, (1991). Extractable were milled to pass a 1.0 mm screen and hydrolysis with sulfuric acid according to McCleary & Draga (2016). The content of glucose in the solutions was analyzed by HPLC, [Hewlett Packard HP1047 (pump)]. This was carried out in order to identify the recovery percentage of β -glucan content.

2-5-2-Antioxidant activity

The antioxidant activity of barely β -glucan and barely extracts was measured using the DPPH (1,1-diphenyl-2-picrylhydrazyl) experiment (Hatano *et al.*, 1988). Each extract (0.1 ml) at concentrations ranging from 0.1 to 0.5 mg/ml were combined with 3.9 ml of DPPH (0.025 g/L methanol). Ascorbic acid (10–100 μ g/ml) was employed as a positive control. After that, the samples were mixed and kept in a dark room for 15–20 minutes. At 515 nm, the absorbance was measured. The following equation was used to calculate the ability to scavenge:

$$\text{Scavenging activity (\%)} = [(\text{Absorbance of control} - \text{Absorbance of sample}) / \text{Absorbance of control}] \times 100$$

2-6- Biological assay

2-6-1- Animals

This experiment is designed in the same way as the previously (Reeves *et al.*, 1993). In this study, fifty-four male albino rats in a healthy state with a starting weight of 100 ± 25 g were chosen. Rats were kept in conventional health laboratory settings for 1 week. Temperature was adjusted at $25^{\circ}\text{C} \pm 2$ and 12 h light–dark and the rats were fed a basic diet and water ad libitum before the initiation of the experiment. All experimental methods were carried out at the Food Technology Research Institute, Agriculture Research Center, Giza, Egypt, in compliance with the guide to the care and use of laboratory animals-NRC (2011) (NIH Publication No. 85-23, Revised 2011).

2-6-2- Arsenic trioxide preparation

Phosphate-buffered saline (PBS) was used to dissolve arsenic trioxide (5 mg/kg/day) and maintained at 4°C (Hemmati *et al.*, 2018). The rats in the groups that got treatments (barely β -glucan or barley extracts) with ATO (Arsenic trioxide) received treatments orally by gavage every day, 1 hour before receiving ATO intraperitoneally.

2-6-3- Experimental design

After a week of acclimation, the rats were randomly separated into 8 groups of six rats each. Group 1: PBS was injected intraperitoneally into the rats, while saline was given orally (negative control). Groups 2–8 received daily intraperitoneal injections of As_2O_3 (5 mg/kg) for 4 weeks. The second group serves as a positive control. The rats in groups 3, 4, and 5 were given daily doses of

barely β -glucan extract of 40, 80, and 160 mg/kg bw/day, respectively, plus ATO. Barley extract was given to groups 6, 7, and 8 at doses of 0.7, 1.4, and 2.8 g/kg bw/day, respectively, with ATO. For four weeks, all rats were gavaged with their respective medications on a daily basis. At regular intervals, the body weight was recorded (once a week).

2-6-4-Collection of blood and biochemical assays

The animals were fasted for 12 hours, anaesthetized, and euthanized at the end of the treatment period; blood samples were obtained from the animal's ocular plexus under diethyl ether anaesthesia. The samples were allowed to clot for 20 minutes at room temperature before being centrifuged for 10 minutes at 1006 g (3000 rpm) to separate the serum and utilized it for biochemical analysis. Following the collection of blood samples, all the rats were slaughtered, and their spleens were dissected.

Spleens were completely cleaned and weighed in ice-cold normal saline (10% w/v) before homogenates were prepared in phosphate buffer saline (50 mmol/l, pH 7). To assess antioxidant activity and lipid peroxidation, homogenates were centrifuged for 10 minutes at 11180 g (10000 rpm) (4°C). The Aebi method (1983), which requires measuring the breakdown of hydrogen peroxide at 240 nm, was used to assess catalase activity (CAT). Glutathione peroxidase activity (GPx) was measured by measuring the decrease in GSH concentration after incubation in the presence of H₂O₂ and sodium azide (NaN₃) (Hafeman *et al.*, 1974). Lipid peroxide assayed by measuring the thiobarbituric acid reactive substances (TBARS) as described by Ohkawa *et al.*, (1979) and expressed as nmoles of

malondialdehyde (MDA)/mg protein. Lowry *et al.*, method (1951) was used to determine protein concentrations. The activities of enzymes were expressed as U/mg protein. According to Larsen (1972) & Orsonneau *et al.*, (1992) respectively, determined serum creatinine and urea. Sheehan *et al.*, (1988) and Durum *et al.*, (1985) used enzyme-linked immunosorbent assays (ELISA) to evaluate serum levels of inflammatory factors (tumor necrosis factor- α) TNF- α , and IL-1 β (interleukin-1 β) levels. The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also measured by the method of Bergmeyer & Harder (1986).

In whole blood, haematological parameters such as total leucocyte count, total red blood cells (RBC), total white blood cells (WBC), and haemoglobin were measured (Armour *et al.*, 1994). Using enzyme-linked immunosorbent assays, serum levels of immunoglobulin G (IgG) and immunoglobulin M (IgM) were measured (Temple *et al.*, 1993). The spleen or thymus index = [spleen or thymus weight (g)/body weight (g)]/100.

2-6-5- Histopathology analysis

Tissues from the spleen were fixed in 10% formalin, embedded in paraffin, sectioned at 4 μ thicknesses, and stained with hematoxyline-Eosin (yoon *et al.*, 2001). The histopathology laboratory of Cairo University's Faculty of Veterinary Medicine performed the examination.

3- Statistical analysis

With three replicates, the data were given as mean \pm SE. The results were analysed using SPSS version 16.0 and a one-way ANOVA. At $p < 0.05$, the results were considered statistically significant (Snedecor & Cochran, 1980).

4- Results

In present investigation, barley flour was employed to extract β -glucan. It is representing 5.8% of the total extract. Furthermore, HPLC was used to identify the barley β -glucan, which was found to be 73.18% of the yield (5.8%). The chemical composition of barley and barely β -glucan extract was determined (Table 1). According to the computed data, barley β -glucan extract resulted in the most abundant component was total dietary fiber compared with barley extract. Regarding total dietary fiber (Soluble dietary fiber and insoluble dietary fiber), beta glucan consisted of major compositions soluble dietary fiber. On the other hand, protein content of barely extract was higher than that of barely β -glucan extract. The results showed that barely β -glucan extract had a higher percentage of antioxidant activity than barley extract.

Table (1): Chemical composition of barley and barley β -glucan extracts

Chemical components (%)	Barely β -glucan extract	barley extract
Moisture	4.78 \pm 0.252	7.387 \pm 0.223
Protein	3.73 \pm 0.120	14.53 \pm 0.145
Fat	1.8 \pm 0.026	2.327 \pm 0.137
Ash	2.726 \pm 0.12	1.75 \pm 0.061
Total dietary fiber	85.247 \pm 0.219	14.83 \pm 1.56
Crude fiber	7.839 \pm 0.145	4.589 \pm 0.546
Antioxidant activity	48.46 \pm 0.61	29.41 \pm 2.743
β -glucan	73.18	4.76

Data are expressed as the mean \pm SE (n= 3 replicates)

Fig. (1) Illustrates the levels of MDA and antioxidant enzyme activity (GPx and CAT) of rat spleen tissue. The results showed that ATO treatment (G2) significantly enhanced MDA (Figure 1c) level and decreased the activity of antioxidant enzymes (GP_x and CAT) compared with the control negative G1 and other groups. Whilst, barley β -glucan and barley extracts plus As₂O₃ treatments significantly ameliorated elevation of MDA levels and deterioration CAT and GPx activity compared to rats received As₂O₃ alone (G2). The rats given 160 mg/kg barley β -glucan (G5) had significantly higher CAT activity than the other groups, according to the findings (Fig. 1a). GPx activity was increased in both the control group (G1) and the rats given 160 mg/kg β -glucan (G5) and 2.8 g/kg barley extract (G8), respectively (Fig.1b).

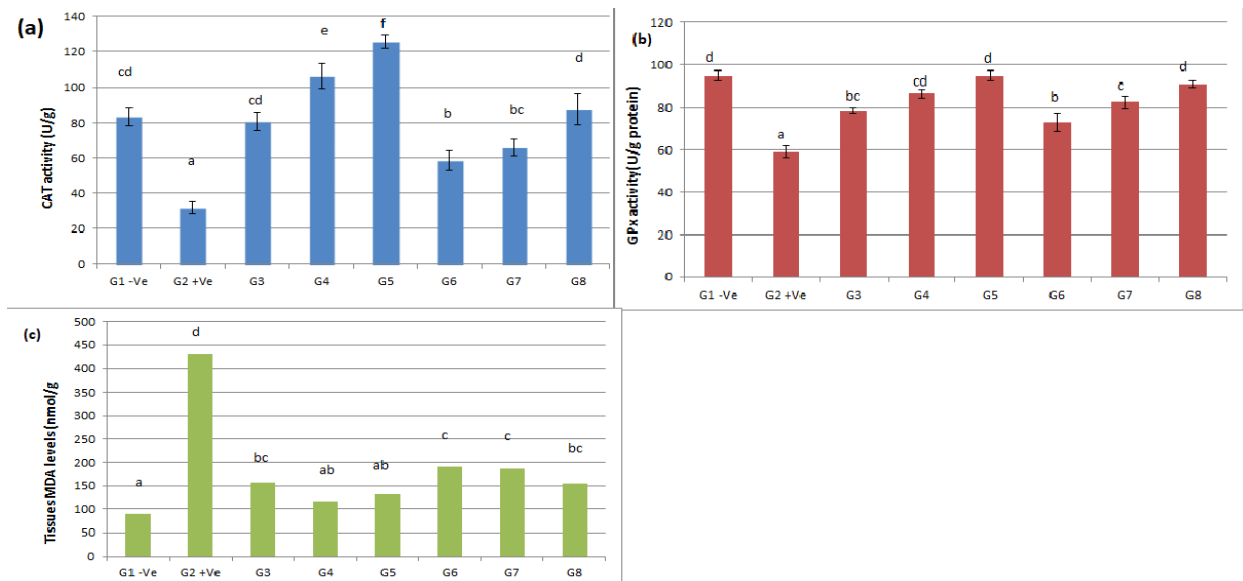


Figure (1): Effects of β -glucan and barley extracts on antioxidant enzyme activity and MDA levels in rats' spleen tissue. Values are expressed as mean \pm SE, $P \leq 0.05$ (n= 3 replicates), a, b, c and d denoted CAT, GPx, MDA, respectively. G1: Control Negative (-V);

G2: control positive (+V) rats injections As_2O_3 (5 mg/kg); G3, G4, G5 The rats with barely β -glucan extract, plus ATO daily (40, 80, and 160 mg/kg bw/day, respectively); G6, G7, G8, Barley extract was given to groups with ATO daily (0.7, 1.4, and 2.8 g/kg bw/day, respectively). Data columns of the groups with different superscripts (a, b, ...) for the same parameter are significantly different compared with G2.

Fig. 2 (a,b) shows the results of immunoglobulin (IgG and IgM) responses of rats to various dosages of barely β -glucan and barley extract. When compared to the other groups, serum IgG and IgM levels in group 2 (rats only given ATO) reduced dramatically. At doses dependent, IgG and IgM levels were significantly higher in the groups treated with barley β -glucan extract than in the groups treated with barley extract. The treatment with 160 mg/kg barely β -glucan (G5) resulted in a significant increase in blood IgG levels when compared to control positive (G2). The doses of 80 mg/kg barely β -glucan and 1.4 g/kg barley extract resulted in a non-significant increase in blood IgG levels in the G4 and G7 groups as compared to the control positive group (G2). Furthermore, when compared to control positive rats (G2), rats given dosage of 80 mg/kg barely β -glucan extract (G4) had considerably higher serum levels of IgM. In the groups G5, and G8, rats given dosages of 160 mg/kg barley β -glucan and 2.8 g/kg barley extract showed a non-significant increase in serum IgM when compared to the control positive group (G2).

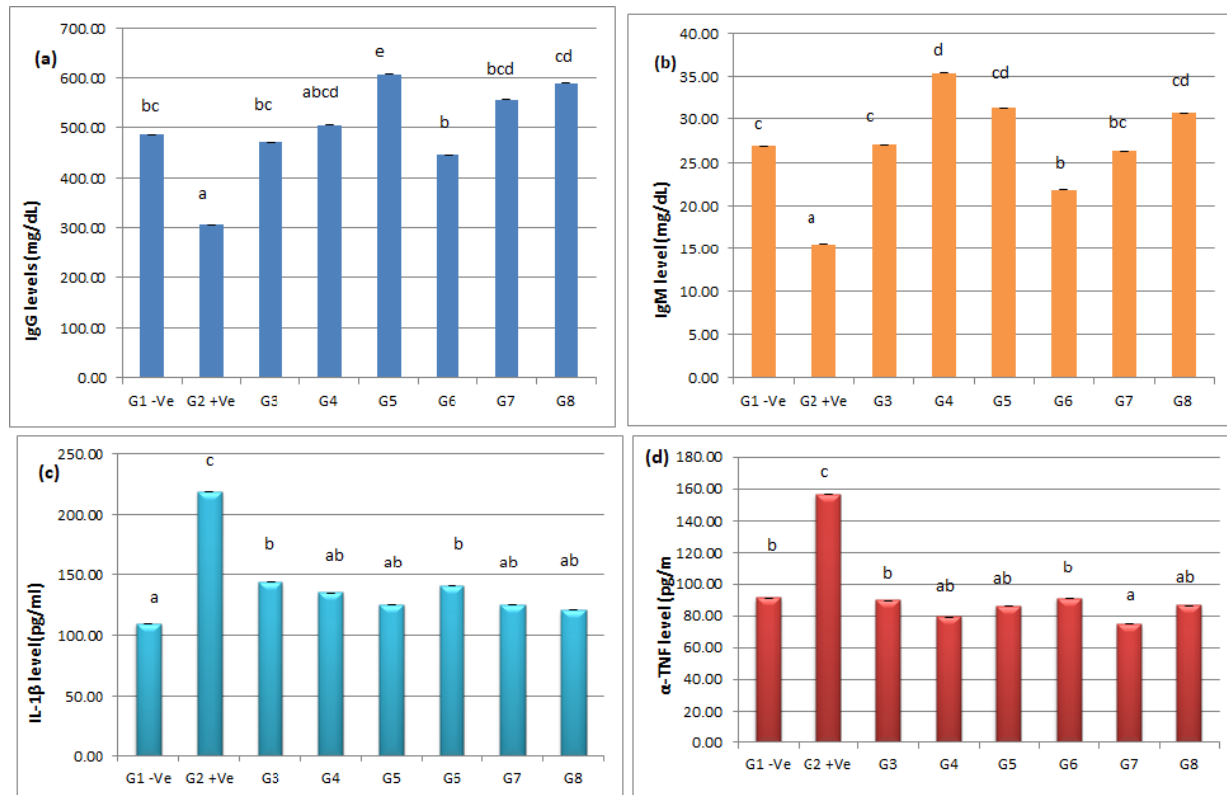


Figure (2): The effects of various doses of barely β -glucan and barley extract on IgG, IgM, IL-1 β , and α -TNF levels in rats induced Ar₂O₃. Values are expressed as mean \pm SE, $P \leq 0.05$ ($n = 3$ replicates), a, b, c, and d denoted IgG, IgM, IL-1 β , and α -TNF levels, respectively. G1: Control Negative (-V); G2: control positive (+V) rats injectionsAs₂O₃ (5 mg/kg); G3, G4, G5 The rats with barely β -glucan extract, plus ATO daily (40, 80, and 160 mg/kg bw/day, respectively); G6, G7, G8, Barley extract was given to groups with ATO daily (0.7,1.4, and 2.8 g/kg bw/day, respectively). Data columns of the groups with different superscripts (a, b,...) for the same parameter are significantly different compared with G2.

The serum IL-1 β and α -TNF levels are provided in Fig. 2 (c,d). According to the findings, the control positive group (G2) had higher levels of serum IL-1 β and α -TNF levels than the control negative and other test groups. On the other hand, when barley β -glucan and barley extracts were used, the elevation was attenuated. However, there was non-significant difference in serum IL-1 β levels between rats treated with 80, 160 mg/kg barely β -glucan (G4 and G5), 1.4, and 2.8 g/kg barley

extract (G7, G8), respectively. Moreover, no variations in serum α -TNF levels were detected between G4 (rats given 80 mg/kg barley β -glucan), G5 (rats received 160 mg/kg barley β -glucan), and G8 (rats treated with 2.8 g/kg barley extract), respectively.

Furthermore, when As_2O_3 -treated rats (G2) were compared to control negative rats (G1), serum renal function (urea and creatinine in Fig. 3 a,b), liver enzyme (AST and ALT in Fig. 3 c,d), and MDA levels all increased significantly (Fig. 3e). However, rats given varied doses of barley β -glucan or barley extracts showed the better liver enzyme and renal function protection than rats given ATO only (Fig. 3a,b,c, and d). In the other words, treatments with barely β -glucan or barley extracts appeared to reverse this parameter significantly towards normal Group (Control Negative G1).

Additional biochemical characteristics of the serum are shown in Fig. 3. Arsenic trioxide has been found to be a potent inducer of lipid peroxidation in serum. However, after administration rats with barley β -glucan or barley extracts at different doses are the better protection for liver enzymes and kidney function was found more in rats treated with ATO only. In the other words, treatments with barely β -glucan or barley extracts appeared to reverse this parameter significantly towards normal in Group (G1).

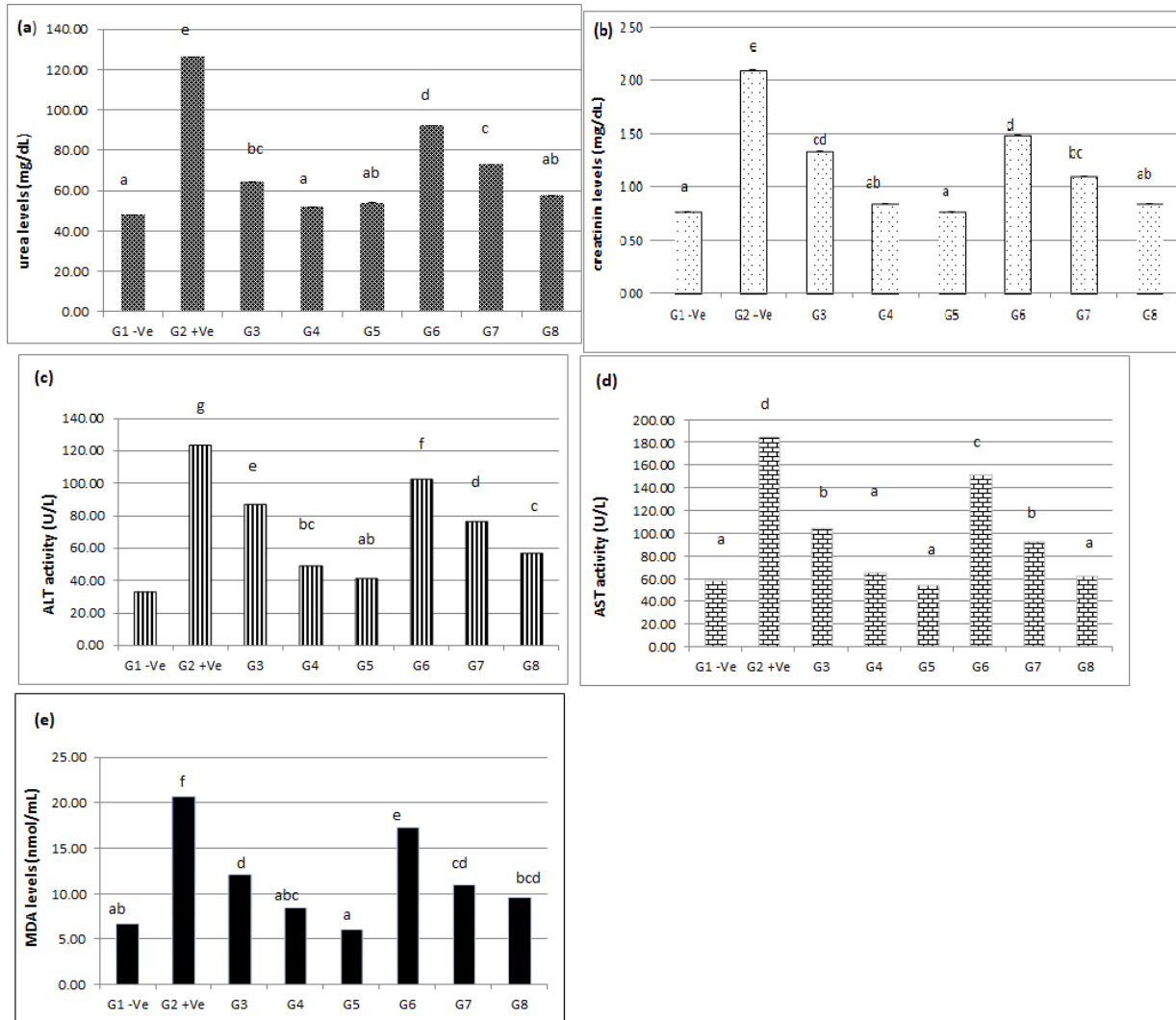


Figure (3): Biochemical serum parameters of arsenic trioxide induced rats treated with barely β -glucan and barley extracts. Values are expressed as mean \pm SE, $P \leq 0.05$ ($n= 3$ replicates), a, b, c, d and e denoted Urea, creatinine, ALT, AST and MDA levels, respectively. G1: Control Negative (-V); G2: control positive (+V) rats injections As_2O_3 (5 mg/kg); G3, G4, G5 The rats with barely β -glucan extract, plus ATO daily (40, 80, and 160 mg/kg bw/day, respectively); G6, G7, G8, Barley extract was given to groups with ATO daily (0.7, 1.4, and 2.8 g/kg bw/day, respectively). Data columns of the groups with different superscripts (a, b,...) for the same parameter are significantly different compared with G2.

Values of hematological parameters are showed in Table 2. In erythrocytes, significant variations in haemoglobin, red cell count, white cell count, Haematocrit, and platelet count were noted. When compared to control values (G1). Arsenic trioxide deteriorates haemoglobin, red cell count, white cell count, and haematocrit, while increasing plate count (G2 rats given ATO alone). After receiving barley β -glucan or barley extracts, arsenic trioxide-treated rats significantly improved. These extracts were significantly reduced in platelet count, and increasing Hemoglobin, red cell account, white cell count, and Haematocrit values compared to control values (G1). There were no significant differences in hemoglobin levels between G1, G4, G5, G7, and G8 rats fed basal diet, 80 mg/kg β -glucan, 160 mg/kg β -glucan, 1.4 g/kg barley extract, and 2.8 g/kg barley extract, respectively. Rats which were orally administrated with β -glucan at a dose 160 mg/kg (G5) showed a potential protective role against arsenic trioxide induced alterations by elevation of red cell count values near to the control negative.

Table 2: Effects of β -glucan and barley flour extracts on Haemoglobin, Red cell count, white cell count, Haematocrit, and Platelet count Erythrocytes counts among the experimental rat groups.

Groups	Haemoglobin (g/dL)	Red cell count (10^6 /uL)	white cell count (10^3 /cmm)	Haematocrit (%)	Platelet count (10^3 /cmm)
Control Negative(G1)	15.20 \pm 0.608 ^a	5.77 \pm 0.296 ^a	12.63 \pm 0.65 ^a	46.13 \pm 1.65 ^{ab}	261.67 \pm 6.36 ^a
Control Positive (G2)	8.37 \pm 0.467 ^c	3.07 \pm 0.297 ^e	7.40 \pm 0.47 ^e	35.57 \pm 1.91 ^c	396.67 \pm 9.94 ^d
40 mg/kg β -glucan extract (G3)	11.33 \pm 0.639 ^b	4.63 \pm 0.22 ^{cd}	10.63 \pm 0.35 ^{bcd}	43.73 \pm 2.77 ^{ab}	311.00 \pm 16.26 ^{bc}
80 mg/kg β -glucan extract (G4)	16.13 \pm 0.612 ^a	5.57 \pm 0.15 ^{ab}	9.20 \pm 0.38 ^{cd}	45.30 \pm 2.72 ^{ab}	318.67 \pm 10.17 ^c
160 mg/kg β -glucan extract (G5)	15.70 \pm 0.656 ^a	5.87 \pm 0.26 ^a	8.90 \pm 0.92 ^{de}	44.77 \pm 1.70 ^{ab}	283.33 \pm 6.01 ^{ab}
0.7 g/kg barley extract (G6)	11.27 \pm 0.504 ^b	4.17 \pm 0.203 ^d	11.93 \pm 0.26 ^{ab}	40.73 \pm 1.60 ^{bc}	319.00 \pm 10.12 ^c
1.4 g/kg barley extract (G7)	14.60 \pm 0.67 ^a	4.97 \pm 0.18 ^{bc}	10.73 \pm 0.49 ^{bc}	48.07 \pm 3.36 ^{ab}	292.33 \pm 5.21 ^{abc}
2.8 g/kg barley extract (G8)	16.43 \pm 0.26 ^a	5.23 \pm 0.12 ^{abc}	9.40 \pm 0.61 ^{cd}	50.20 \pm 2.83 ^a	295.00 \pm 11.79 ^{bc}

Data are expressed as the mean \pm SE, (n= 3 replicates). Means within the same column carrying different superscripts are significant different at $P \leq 0.05$

The neutrophils, lymphocytes, monocytes, and eosinophils in rats treated with ATO were tested to confirm the effects of barely β -glucan or barley extracts on immune related blood leukocyte count in this study (Table 3). Significant alterations were identified in neutrophils, lymphocytes, monocytes, and eosinophils when the control negative (G1) group was compared to the other groups. In control positive (G2) rats injected with ATO (5 mg/kg/day), the percentage of neutrophils, lymphocytes, and monocytes significantly decreased. Eosinophils, on the other hand, increased significantly in the same group. At different doses, barely β -glucan or barley extracts ameliorated the percentage of neutrophils, lymphocytes, and

monocytes, causing these values to rise. In addition, these doses resulted in a decrease in the percentage of eosinophils.

Table (3): Effects of different doses of β -glucan and barley flour extract on leukocyte subset count in rats

Groups	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
Control Negative(G1)	52.00 \pm 4.93 ^a	43.33 \pm 2.03 ^a	8.17 \pm 1.30 ^a	2.83 \pm 0.44 ^{ab}
Control Positive (G2)	27.33 \pm 2.73 ^d	25.33 \pm 2.73 ^b	2.17 \pm 0.44 ^b	5.67 \pm 0.44 ^d
40 mg/kg β -glucan extract (G3)	40.00 \pm 2.65 ^{bc}	46.00 \pm 2.00 ^a	8.00 \pm 0.866 ^a	2.83 \pm 0.44 ^{ab}
80 mg/kg β -glucan extract (G4)	47.67 \pm 3.71 ^{ab}	47.67 \pm 1.45 ^a	8.67 \pm 1.20 ^a	2.70 \pm 0.36 ^{ab}
160 mg/kg β -glucan extract (G5)	47.33 \pm 3.844 ^{ab}	45.67 \pm 2.33 ^a	9.63 \pm 0.59 ^a	1.57 \pm 0.35 ^a
0.7 g/kg barley extract (G6)	34.67 \pm 2.60 ^{cd}	44.00 \pm 2.65 ^a	8.13 \pm 0.52 ^a	2.87 \pm 0.19 ^{ab}
1.4 g/kg barley extract (G7)	44.00 \pm 2.89 ^{abc}	47.33 \pm 2.33 ^a	7.87 \pm 0.98 ^a	3.20 \pm 0.44 ^b
2.8 g/kg barley extract (G8)	39.67 \pm 4.06 ^{bc}	42.33 \pm 1.20 ^a	9.17 \pm 0.44 ^a	2.83 \pm 0.44 ^{ab}

Data are expressed as the mean \pm SE, (n= 3 replicates). Means within the same column carrying different superscripts are significant different at $P \leq 0.05$

In rats treated with ATO, the effects of barley β -glucan or barley extracts on initial body weight, final body weight, body weight change, spleen index, and thymus index were assessed (Table 4). At different doses, rat body weight change while spleen index and thymus index were similar. The groups administered barley β -glucan or barley extracts resulted in a slight increase in the thymus index.

Table (4): Initial body weight, final body weight, body weight change, spleen index, and thymus index of rats in different experimental groups.

Groups	Initial body weight (g)	Final body weight (g)	Body weight change (%)	Spleen index (%)	Thymus index (%)
Control Negative(G1)	101.67±1.67 ^a	128.33±1.67 ^a	20.72±2.36 ^b	0.48±0.03 ^a	0.53±0.011 ^{ab}
Control Positive (G2)	125.00±0.00 ^b	135.33±0.33 ^a	7.63±0.23 ^c	0.16±0.022 ^b	0.34±0.03 ^c
40 mg/kg β-glucan (G3)	123.33±1.67 ^b	153.33±4.41 ^b	19.42±2.82 ^b	0.54±0.103 ^a	0.46±0.04 ^b
80 mg/kg β-glucan (G4)	123.33±1.67 ^b	150.00±5.77 ^b	17.53±3.45 ^b	0.59±0.12 ^a	0.50±0.045 ^{ab}
160 mg/kg β-glucan (G5)	121.67±3.33 ^b	155.00±2.89 ^b	21.38±3.46 ^b	0.57±0.103 ^a	0.46±0.014 ^b
0.7 g/kg barley extract (G6)	125.00±0.00 ^b	155.00±2.89 ^b	19.299±1.50 ^b	0.53±0.082 ^a	0.50±0.023 ^{ab}
1.4 g/kg barley extract (G7)	121.67±1.67 ^b	150.00±2.89 ^b	18.79±2.61 ^b	0.41±0.011 ^a	0.51±0.05 ^{ab}
2.8 g/kg barley extract (G8)	100.00±0.00 ^a	151.67±4.41 ^b	33.96±1.89 ^a	0.45±0.08 ^a	0.61±0.03 ^a

Data are expressed as the mean ± SE (n= 6 replicates). Means within the same column carrying different superscripts are significant different at P ≤ 0.05.

Fig. (4) Shows the results of histopathological examinations of spleen tissues. Spleen tissue from the group that received the basal diet (G1) and those injected with ATO and treated with 80 mg/kg barley β-glucan (G4), 160 mg/kg barley β-glucan (G5), 1.4 g/kg barley extract (G7), and 2.8 g/kg barley extract (G8) showed the normal histological structure of lymphoid follicle. In comparison to the control group, lymphocytic necrosis and depletion with the appearance of tangible body macrophages were observed in the spleen tissue of the As₂O₃-treated rats (G2).

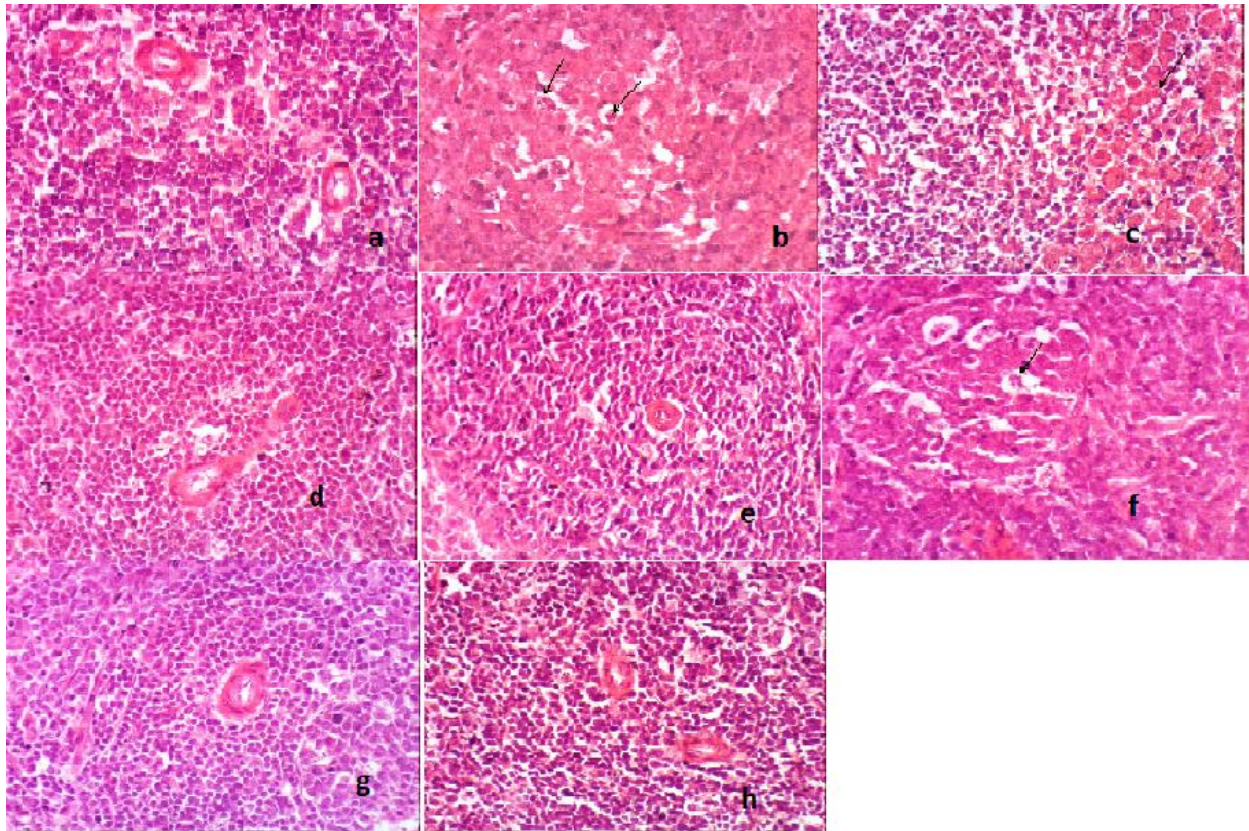


Fig. (4): (a) Spleen of rat from group 1 showing the normal histological structure of lymphoid follicle, (b) Spleen of rat from group 2 showing lymphocytic necrosis and depletion with appearance of tangible body macrophages, (c) Spleen of rat from group 3 showing congestion of blood sinuses, (d) Spleen of rat from group 4 showing no histopathological alterations, (e) Spleen of rat from group 5 showing no histopathological alterations, (f) Spleen of rat from group 6 showing slight lymphocytic necrosis and depletion with appearance of tangible body macrophages, (g) Spleen of rat from group 7 showing no histopathological alterations, (h) Spleen of rat from group 8 showing no histopathological alterations

5- Discussion

The goal of the present study was to evaluate biologically barley or barely β -Glucan extracts, on immunomodulation due to its containing antioxidant activity and anti-inflammatory properties, Barley has traditionally been used as a folk remedy for a range of ailments, including immune system support and antioxidant

protection (Din *et al.*, 2018). Mikulajová *et al.*, (2007) discovered that it contains the flavonoid catechin, which has a strong antioxidant effect and contributes to antioxidant capacity.

According to the data, the protein content of barely β -glucan extract was found to be lower than that of barley extract. Because the single-step extraction method effectively separated them from the barley flour to obtain barely β -glucan, furthermore, the results of this study show that barley is the best source for β -glucan which was similar as that reported by Limberger-Bayer *et al.*, (2014). Our findings show that barely β -glucan extract has a higher level of antioxidant activity than barley extract, which has anti-inflammatory properties. In addition, the findings of the current study consistent with those of previous studies (kofuji *et al.*, 2012). β -Glucans are a major component of the soluble dietary fibre β -(1,3-1,4)- D-glucan, which is isolated from barley. Furthermore, because of their wide range of biological activities, including lowering blood cholesterol levels, they are widely employed as supplements and food additives (kofuji *et al.*, 2012).

The effects of inorganic arsenic (As_2O_3) on inflammation and immunity in experimental rats were studied in the second part of this study. In previous research, arsenic-induced oxidative stress, inflammation, and a heat shock response have been described in the brain, liver, and immunological organs (Zhao *et al.*, 2017& Guo *et al.*, 2016). Furthermore, arsenic-produced reactive oxygen species (ROS) react with polyunsaturated fatty acids, increasing lipid peroxidation (Zhao *et al.*, 2017). Lymphocytic necrosis and depletion with the appearance of tangible body macrophages, the present study looked at the negative effects of 5 mg/kg

arsenic trioxide intraperitoneal injection in rats, which causes necrosis in spleen lymphocytic cells by increasing oxidative stress in the spleen and serum, and the subsequent amelioration by barley extract and barley β -glucan extract in adult male rats. In most cells, electron leakage from the mitochondrial electron transport chain is the main source of ROS. It has been suggested that an increase in reactive oxygen species (ROS) as a biochemical mediator of apoptosis can cause cell death (Zarei *et al.*, 2019).

Natural extracts with immunosuppressive and anti-inflammatory properties are being investigated in depth. The goal of this study was to see if barley β -glucan and barley extract could protect against the harmful effects of arsenic trioxide as an antioxidant agent. Furthermore, the current study found that arsenic may produce significant alterations in antioxidant activity markers and histological spleen tissues in experimental rats, suggesting that arsenic trioxide could increase oxidative stress. In arsenic-treated rats, the same results have been described (Hemmati *et al.*, 2018 & Kumar *et al.*, 2021). According to research (Hemmati *et al.*, 2018), MDA is a good indicator of endogenous lipid peroxidation. As for oxidative stress biomarkers, we assessed antioxidant enzyme activity detoxifying after ATO treatment. According to the findings, arsenic trioxide administration resulted in a significant increase in MDA (G2), indicating oxidative damage to the spleen, whereas anti-oxidative enzyme activities (GPx and CAT) were reduced in comparison to other groups. The antioxidant enzymes were strongly regulated by the administration of barley extracts, particularly barley β -glucan extracts. As a result, barley β -glucan and barley extracts increased antioxidant activity (CAT and GPx) and decreased lipid peroxidation (MDA levels) in the spleen tissue of rats,

which is a key organ in the immunological response. This suggests that barley β -glucan has antioxidant properties and can restore the activity of ATO-affected enzymes. These findings support previous research (Ciftci *et al.*, 2018) that found barely β -glucan to have significant antioxidant activity and potentially protective benefits against cell damage.

Arsenic toxicity increases the expression and release of the pro inflammatory cytokines interleukin (IL-6, IL-8, and IL-1 β), the production of which is linked to the generation of reactive oxygen species (Gao *et al.*, 2015). The administration of arsenic trioxide resulted in alterations in cytokine levels in the current investigation. TNF- α and IL-1 β concentrations increased significantly in rats treated alone with ATO (G2). This conclusion is consistent with previous findings that ATO-induced oxidative stress likely promotes inflammation, and ROS overproduction causes increased expression of pro-inflammatory molecules such as tumour necrosis factor- α (TNF- α) (Li *et al.*, 2017). A high concentration of arsenic produced TNF- α release from mononuclear cells and induced apoptosis in T cells, according to Yu *et al.*, (2002) & Liu *et al.*, (2020).

From the above mentioned results, arsenic trioxide-induced oxidative stress damages the cellular membrane, causing inflammation and the production of pro-inflammatory cytokines. Furthermore, the findings indicate that barley extracts or barely β -glucan extracts have anti-inflammatory properties and decrease ATO-induced inflammation. The levels of cytokines (IL-1 β , and α -TNF) were also higher in rats given only ATO (G2) than in the control group (G1). These parameters were lowered after treatment with barley or barely β -glucan extracts.

The results might be attributed to the antioxidant and neutrophil inhibitory activities of β -glucan. In addition, β -Glucan's antioxidant affects and reduction of cytokines have also been demonstrated in other studies (Chaichian *et al.*, 2020 & Mikulajová *et al.*, 2007). α -TNF can also be employed as a biomarker for the toxicity of test substances (Kumar *et al.*, 2014). According to results, Barley β -glucan can increase endotoxin clearance via scavenger receptors by lowering α -TNF generation. These findings are consistent with the information provided by Vetvicka & Oliveira, (2014) & Arican *et al.*, (2005). Therefore, β -glucans, lignans, vanillic acid, and arabinoxylan are the major anti-inflammatory components in barley (Zeng *et al.*, 2020).

A weak immune system can cause infections, tumor growth, and immunodeficiency-related disorders. Immunoglobulins are a group of proteins that function as antibodies. To eliminate pathogens and neutralize toxins, they combine with antigens (Cannon *et al.*, 2010). The immune system is made up of a variety of interdependent cell types that work together to protect the body from bacterial, parasite, fungal, and viral infections, as well as tumor cell growth. Many of these cell types have specific functions. B cell lymphocytes are responsible for antibody-mediated immunity (humoral immunity). They produce immunoglobulins (antibodies), which are proteins that bind to specific antigens (Ojeka *et al.*, 2016). The present research supports this theory, demonstrating that when ATO is used, the immune state deteriorates. Immunoglobulin levels (IgG, IgM) were shown to be significantly higher in rats given either barely β -glucan extracts or barley flour extracts, although the 80, 160 mg/kg β -glucan (G4, G5) and 2.8 g/kg barley extract (G8) were shown to be more beneficial in terms of all immunoglobulin

concentrations. According to Chan *et al.*, (2009), β -glucans immediately enter the proximal small intestine and are captured by macrophages. They are then internalized and fragmented into smaller sized β -glucans. The small β -glucan fragments are then released by macrophages and taken up in the bloodstream by circulating granulocytes, monocytes, and dendritic cells. After that, the immunological response is triggered (Chan *et al.*, 2009).

Several experimental studies have reported antibacterial, antifungal, antiviral, and antioxidant activities of β -glucans (Erkol *et al.*, 2012; & Iraz *et al.*, 2015). Barley β -glucans can regulate the immune responses and combine innate and adaptive immunity (Zeng *et al.*, 2020). In several investigations (Erkol *et al.*, 2012 & Iraz *et al.*, 2015), β -Glucans are immune stimulants that boost both natural and adaptive immunity.

In the current investigation, arsenic trioxide-induced rats had higher serum AST and ALT activity, which could indicate liver tissue injury. Increased levels of these liver biomarkers in the blood suggest morphological and functional damage to the liver (Hosseini *et al.*, 2013). Furthermore, the increased serum transaminases (AST and ALT) after ATO treatment disrupted not only the synthesis of energetic macromolecules, which are required for several vital and metabolic functions, but also the detoxification processes (Turk *et al.*, 2019), because barely β -glucan and barely extracts were effective in lowering these parameters. These findings corroborate the findings of Liu *et al.*, (2020), who found that ATO caused hepatotoxic and haematological alterations in rats. Previous studies have shown that barely β -glucan lowers oxidative damage and protects liver and kidney

function (Zeng *et al.*, 2020), as well as that polyphenols present in barley, such as (+)-catechin, protocatechuate, and quercetin, have hepatoprotective characteristics (Quan *et al.*, 2018). Similarly, serum creatinine and urea, both renal function markers, increased significantly in rats following ATO treatment, possibly indicating purine and pyrimidine breakdown and decreased kidney function. Many research looked at the effects of ATO on serum creatinine and urea levels in rats (Arcidiacono *et al.*, 2019). As a result, the health benefits of dietary β -glucans may reach the poorest populations, such as patients with chronic renal disease and healthy people with systemic pro-aging or inflammatory disorders, raising the risk for kidney and vascular damage (Arcidiacono *et al.*, 2019). Furthermore, uremic toxin produced by bacteria is reduced by barely β -glucan (Zeng *et al.*, 2020).

By improving the ability of macrophages, neutrophils, and natural killer cells to respond to and fight a variety of threats such as bacteria, viruses, fungi, and parasites, beta-1,3-glucans help the body's immune system defend against foreign invaders (Sofi *et al.*, 2017).

According to obtained data, ATO reduced haemoglobin, red cell count, white cell count, neutrophils, lymphocytes, monocytes, and haematocrit while increasing plate counts and eosinophils. Arsenic trioxide toxification and increased oxidative stress in blood cells could be the causes of these changes in the hematopoietic system. As a result, ATO affected bone marrow function (Hosseinzadeh *et al.*, 2019). By attenuating the effects of the above variables, the administration of barely β -glucan and barley extracts decreased hepatotoxicity in arsenic-poisoned

rats. The scavenging of free radicals may be due to barley β -glucan and barley flour extracts.

Previous studies have suggested that arsenic exposure changes haematological parameters, and these data support that hypothesis (Ojo *et al.*, 2018 & Hosseinzadeh *et al.*, 2019). The absolute values of RBC count and haemoglobin serve as crucial indications of anaemia. ATO therapy can also cause immunological suppression in rats, due to alterations in lymphocyte and neutrophil populations (Taheri *et al.*, 2016). Monocytes are an important part of the immune system because it can also destroy invading pathogens through phagocytosis and antibody production. Monocyte levels were also reduced after treatment with ATO, presumably because of an association with inflammation (Taheri *et al.*, 2016).

The spleen's failure is the root cause of most illnesses. Modern medicine's immune function corresponds to the defence capacity (Noh *et al.*, 2019). The immunological response in this investigation was compatible with the BW response, which is a clinical marker of illness. Furthermore, ATO has been demonstrated to damage the spleen and thymus glands, both of which are essential organs in the immune system. Rats' spleens and thymus glands lose weight.

In the case of oral administration treatment, the current study found that barely β -glucan extracts or barley flour extracts improved immunity in immune compromised rats. ATO administration reduced the weight of the spleen and thymus, indicating direct toxicity (Aljeboori & Gzar 2020). These extracts, on the other hand, mitigated this decrease. Previous studies found that the barley and barely β -glucan groups had lower liver weights, kidney weights, and spleen

weights (Zeng *et al.*, 2020). These findings provide indirect evidence that barely β -glucan extracts or barley extracts boost immune responses in the body.

Histological alterations in the spleen are significantly linked to oxidative stress indicators. Histological investigations following arsenic trioxide treatment revealed lymphocytic necrosis and depletion with the appearance of tangible body macrophages, which were consistent with prior reports (Aljeboori & Gzar, 2020). Histopathological findings support the ability of barley β -glucan (80 and 160 mg/kg) and barley extracts (1.4 and 2.8 g/kg) to protect against spleen damage (with a corresponding decrease in MDA levels in the tissue). As a result, the findings show that β -glucan and barley extracts can be used as dietary supplements to protect humans and animals from arsenic toxicity. Barley can protect spleen tissue against steroid-induced structural alterations, according to Shehata *et al.*, (2019).

Barley β -glucan has been demonstrated to improve human health, particularly by lowering postprandial blood glucose and LDL cholesterol levels (Bell *et al.*, 1999). Barley-glucans are widely available, easy to extract, and have high biological activity (He *et al.*, 2021).

6- Conclusion

Based on the findings, it can be concluded that barely β -glucan extracts and barley extracts were beneficial in treating the immunosuppression caused by As_2O_3 . Our findings showed that barely β -glucan extracts and barley extracts enhanced biochemical, hematologic, immunological, and histopathological

markers, suggesting that they could be useful in immune therapy. These findings imply that barley extracts and barely β -glucan extracts could be useful in the development of functional foods and pharmaceuticals for immunological boosting.

7- Abbreviations

PBS: Phosphate-buffered saline; As₂O₃: Arsenic trioxide (ATO); MDA: Malondialdehyde; TBARS: Thiobarbituric acid reactive substances; GPx: glutathione peroxidase; CAT: catalase; ELISA: enzyme-linked immunosorbent assays : TNF- α : tumor necrosis factor- α); IL-1 β : interleukin-1 β ; ALT: alanine aminotransferase; AST: aspartate aminotransferase RBC: total red blood cells; WBC: total white blood cells; IgG:immunoglobulin G; IgM: immunoglobulin M; ROS: reactive oxygen species; BW: body weight. T2D: type 2 diabetes; HPLC: High-Performance Liquid Chromatography

8- Declaration of Conflicting Interests

The authors declare that there are no conflicts of interest.

9- Ethical Approval and Consent to participate

This experiment was carried out in the animal house of the Food Technology Research institute, Agriculture Research Center, Giza, Egypt. An animal housing and experiments were conducted in strict accordance with the Guide for the Care and Use of Laboratory Animals 8th Edition 2011.

10- Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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