

Barley Grass (*Hordeum vulgare* L.) Protects against Dieldrin-Induced Hyperlipidemia and Hepatorenal Toxicity in Rat

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Abstract The objective in this article was to evaluate the total phenol and proanthocyanidin contents of Fresh *Hordeum vulgare* L. grass aqueous extract (HGAE) and to determine its antioxidant capacity, using standard phytochemical methods. It aimed also to determine the protective capacity of this extract against dieldrin-induced hepatotoxicity and nephrotoxicity in rat. The animals were separated *into* six groups: control (C), dieldrin (6 mg/kg i.p) (D), HGAE (10 and 20 ml/kg, orally) and D plus HGAE (HGAEII+D). The experiment lasted 10 days. Our results indicated that total phenol and proanthocyanidin contents, were higher in aqueous barley grass extract. Dieldrin-induced oxidative stress in liver and kidney was indicated by marked increase in lipid peroxidation, reduction in the catalase activity and thiol group levels (-SH). Pretreatment with HGAE significantly reduces these alterations. HGAE was also found subsequently to provide a protection against dieldrin-induced hepatic and renal injury respectively by averting the hepatocyte deep necrotic lesions and improving renal tubular and glomerular architecture. It also restored serum lipid profile and liver and kidney relative weights. We concluded that aqueous extract of barley grass has the potential to ameliorate liver and kidney injury induced by dieldrin mainly through its potent antioxidant properties due to its richness in phenolic compounds.

Keywords: barley, dieldrin, liver, kidney, oxidative stress

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1. Introduction

Previous investigations have been focused on natural molecules to identify consumer concerns about safety and toxicity [1]. Polyphenols are some examples of these natural molecules with strong antioxidant properties found in foods. Phenolic acids, flavonoids and proanthocyanidins are polyphenols that may have higher beneficial effects on human health offering protection against chronic diseases. These biological effects are mainly attributed to their antioxidant activities in scavenging free radicals, inhibition of peroxidation and chelating transition metals [2].

Among important food sources of polyphenols are the cereals. World literature, especially, Greek, Roman and Egyptian literatures point to the dietary and medicinal properties of barley (*Hordeum vulgare* L.). It has also been used as a component of numerous health foods [3]. Preceding research has revealed the potential of *Hordeum vulgare* L. as source of various antioxidants and naturally healthy compounds in seeds, bran, leaves and sprout

[3,4,5]. Consumption of barley which contains many medicinally active phytochemicals is usually associated with improvement in health [6,7]. It contains a wide spectrum of vitamins, minerals, as well as eight essential amino acids [7].

Barley grass, which are the young leaves of barley harvested approximately 10 days after sowing the seeds, have recently received much attention as a functional food. The highest concentrations of nutrients are present in the green mass during short period of the vegetation and that the nutrient profiles of green cereal plants change quickly as they grow [8]. Young plant parts are characterized by increased contents of phytonutrients, vitamins, provitamins, antioxidants, and others bioactive substances with protective functions in the humans compared to seeds [8]. It has also been reported that barley young leaves possess beneficial properties such as hypolipidemic, antidepressant, and antidiabetic actions [9,10] and likewise protect against health disorders including excessive cholesterol levels, blood pressure, immune response and cancer prevention, and reduces inflammation and pain by acting as a free radical scavenger [11,12].

Previous studies have suggested that pesticides exert their toxic effects in part by stimulating the production of reactive oxygen species (ROS) [13,14]. Dieldrin is a member of the organochlorine pesticides family causing a wide variety of health problems and multiplying the risk of carcinogenic process [15,16,17,18].

There is paucity of available in literature regarding the biological activity of aqueous barley grass extract *in vivo*. Based on these reports, this present study was designed to evaluate the phenolic contents of fresh *Hordeum vulgare* L. aqueous grass extract and to assess the effect of this extract against dieldrin-induced hepatorenal injury as well as its lipid-lowering potential.

2. Materials and Methods

2.1. Plant Material and Preparation of Extract

2.1.1. Samples

In order to prepare the extract, we used barley seeds with Manel variety, provided by the National Institute of Agronomic Research of Tunisia.

2.1.2. Growing of the Grass

The grass of *Hordeum vulgare* L. was grown indoors until required for experiments. Earthen pot was filled with 20 cm of growing medium composed of 3 parts of soil and one part of compost. Overnight soaked barley seeds were then evenly spread over and further covered with 1 cm of soil. Small quantities of water were sprinkled evenly over soil and 3–4 h indirect sunlight was allowed daily for growth of grass. On the tenth day, when grass is about 15 cm tall, it is cut 0.5 cm above the surface of soil. To harvest continuous supply of fresh grass, pots were similarly planted at one-day interval [19].

2.1.3. Preparation of Fresh Grass Juice

Forty grams of above harvested fresh grass was grounded in a laboratory mortar and the juice was squeezed out through four layers of wet muslin cloth. The residue was twice suspended in 3 ml of sterile water and similarly squeezed. The filtrate was made up to 20 ml and administered as *Hordeum vulgare* L. grass aqueous extract (HGAE). Each day, the fresh extract was prepared prior to administration [19].

2.2. Animals and Reagent

Male Wistar rats weighing 120–150 g were used from the Tunisian Company of Pharmaceutical Industries (SIPHAT, Rades, Tunis, Tunisia). The rats were housed under controlled conditions of temperature ($22 \pm 2^\circ\text{C}$) and light (12:12 light/dark). They were maintained on a standard diet and water was available *ad libitum*. Dieldrin and all other chemicals were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Animals were cared for in compliance with the Tunisian code of practice for the Care and Use of Animals for Scientific Purposes in accordance with the Principles of Laboratory Animal Care (NIH publication #85–23, revised in 1985). The

experimental protocols were approved by the Faculty Ethics Committee (Faculty of Sciences, Bizerta, Tunisia).

2.3. Experimental Design

Rats were randomized *into* six experimental groups ($n = 10$) as follows: (1) control group (C) received equal daily volumes of vehicle (corn oil) during 10 days, (2) animals received daily intraperitoneal injections (i.p.) of dieldrin (D) diluted with corn oil at doses of 6 mg/kg body weight (b.wt) [15] during 10 days, (3) and (4) rats administered fresh HGAE respectively at the oral doses of 10 ml/kg (HGAEI) and 20 ml/kg (HGAEII) [19] during the first three days, subsequently they received both HGAE and equal volumes of vehicle in the seven days that remain, (5) and (6) animals received respectively HGAEI and HGAEII pre-treatment in the first three days, then they received HGAEI or HGAEII plus dieldrin (HGAEI+D or HGAEII+D) in the seven days that remain.

All the above treatments were carried out each day in the morning under similar constant conditions, as far as possible. Rats were fed and observed daily. Body weights were recorded daily throughout the study.

2.4. Phytochemical Studies of HGAE

2.4.1. Total Polyphenol Contents

Total phenolic contents were determined by the Folin-Ciocalteu method [20]. 200 μl of HGAE diluted sample were added to 1 ml of 1:10 diluted Folin-Ciocalteu reagent and 800 μl of saturated sodium carbonate 75 g/l. The reaction mixture was incubated at 45°C for 30 min, and the absorbance was measured at 765 nm. Tannic acid ($0\text{--}80 \mu\text{g}\cdot\text{ml}^{-1}$) was used for the calibration curve. The results were expressed as tannic acid equivalent in mg total polyphenols per gram of fresh weight plant (mgTAE/g FW). Each sample was measured in triplicates.

2.4.2. Total Proanthocyanidin Contents

The total proanthocyanidin contents of HGAE sample were determined according to the method of Scalbert et al. [21] with slight modification. 2.5 ml of 1% vanillin solution in methanol (MeOH), and 2.5 ml of 3.6 N H_2SO_4 in MeOH were added to the MeOH-diluted sample. Then the mixture was allowed to stand for 20 min at 30°C . The absorbance was measured at 500 nm. Total proanthocyanidin content of samples was expressed as mg tannic acid equivalent (TAE) per gram of fresh weight plant.

2.5. Protective Effects of HGAE

2.5.1. Lipid Profile Measurement

One day after the end of the experiment, the animals were fasted for 12 hours and were then sacrificed by exsanguination *via* cardiac puncture followed by rapid decapitation. Serum was collected by centrifugation (3000 g for 15 min), aliquoted and frozen at -80°C until biochemical analysis of total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C) and triglycerides (TG). Low density lipoprotein-cholesterol (LDL-C) was calculated using Friedewald's formula [22].

2.5.2. Total Bilirubin Measurement

Total bilirubin was estimated by using commercially available kits (Biomagreb, Tunisia).

2.5.3. Lipid Peroxidation Measurement

Lipid peroxidation was determined by the quantification of malondialdehyde (MDA), according Buege and Aust [23]. Liver and kidney homogenates were centrifuged at 1000 g for 10 min at 4°C to sediment cell debris and mitochondrial samples. Supernatants were suspended in PBS, pH=7.4, mixed with BHT-TCA (Trichloroacetic acid, Butylhydroxytoluen) solution (1% w/v BHT dissolved in 20% TCA), centrifuged at 1000 g for 35 min and finally mixed with 0.5 N HCl and 120 mM TBA (Thiobarbituric acid) in 26 mM Tris and heated in water bath at 80°C for 10 min. After cooling, the absorbance of the resulting chromophore was measured at 532 nm. MDA levels were determined by using an extinction coefficient for MDA-TBA complex of $1.56 \times 10^5 \text{ xM}^{-1} \text{ xcm}^{-1}$.

2.5.4. Non-protein Sulfhydryl Groups Measurement

We used Hu and Dillard's method [24] to determine tissue non-protein sulfhydryl (-SH) compound levels.

2.5.5. Catalase Activity

Catalase (CAT) activity was assayed by measuring the initial rate of H_2O_2 disappearance at 240 nm [25]. There action mixture contained 33mM H_2O_2 in 50Mm phosphate buffer pH 7.0 and CAT activity calculated using the extinction coefficient of $40 \text{ mM}^{-1} \text{ cm}^{-1}$ for H_2O_2 .

2.5.6. Protein Determination

The protein content of the analysis sample was determined by the method of Lowry et al. [26] using bovine serum albumin (Sigma, St. Louis, MO) as a standard.

2.5.7. Histological Analysis and Organ Weight

The organs were removed and weighed immediately after the rodent's dissection and fixed overnight at room temperature in paraformaldehyde 4% in 0.1M phosphate buffer, pH 7.4. The samples were dehydrated with ethanol and toluene series and embedded in paraffin. For histopathological analysis, serial sections (5 μm) were mounted on gelatin-coated glass slides, cut and stained with hematoxylin and eosin. Tissue preparations were observed and micro-photographed under a light BH₂ Olympus microscope.

2.6. Statistical Analysis

Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison as the *post hoc* test and significance of difference between treatments was accepted at $p < 0.05$. Data are expressed as mean \pm standard deviation of the mean.

3. Results

3.1. Phytochemical Studies

The aqueous extract of *Hordeum vulgare* L. grass (HGAE) was centrifuged at 4500xg for 10 min and

lyophilized. The extract yielded 11.82 g of residue which expressed 13% of initial weight used (40 g). The phytochemical studies of HGAE revealed the presence of total phenolic content and total proanthocyanidin content at a rate of $168.33 \pm 0.34 \text{ mg TAE.g}^{-1}\text{FW}$ and $6.66 \pm 0.2 \text{ mg TAE.g}^{-1}\text{FW}$, respectively (Table 1).

Table 1. Extraction Yield, Contents of Total Phenols and Total Proanthocyanidins in HGAE

Sample	Extraction Yield (%)	Total Phenols Content (mg TAE.g ⁻¹ FW)	Total Proanthocyanidins Content (mg TAE.g ⁻¹ FW)
HGAE	13	168.33 ± 0.34	6.66 ± 0.02

Values are expressed as mean \pm S.D of triplicate measurement. *Hordeum vulgare* L. grass aqueous extract (HGAE). Extraction yield (%) = (sample extract weight/sample weight) \times 100%. TAE.g⁻¹FW, tannic acid equivalent (TAE) per gram of fresh weight plant.

3.2. Effect of HGAE on Body Weight

There were no differences between control and treated rats body weights throughout the course of the study (data not shown).

3.3. Effect of HGAE on Liver and Kidney Relative Weight

Figure 1A shows a significant increase of the relative liver weights after i.p. injection of dieldrin. Organ relative weights were comparable to that of control subsequent to barley grass extract treatment. Pretreatment with the higher dose of HGAE significantly ($p < 0.05$) decreases relative liver weight, almost to the levels seen in control. In contrast, dieldrin treatment caused a significant decrease in kidney relative weight (Figure 1B). HGAE extract alone at the two doses did not affect this parameter, while pretreatment with the two doses of HGAE increased ($p < 0.05$) the kidney relative weight compared to D groups, around to the level seen in control.

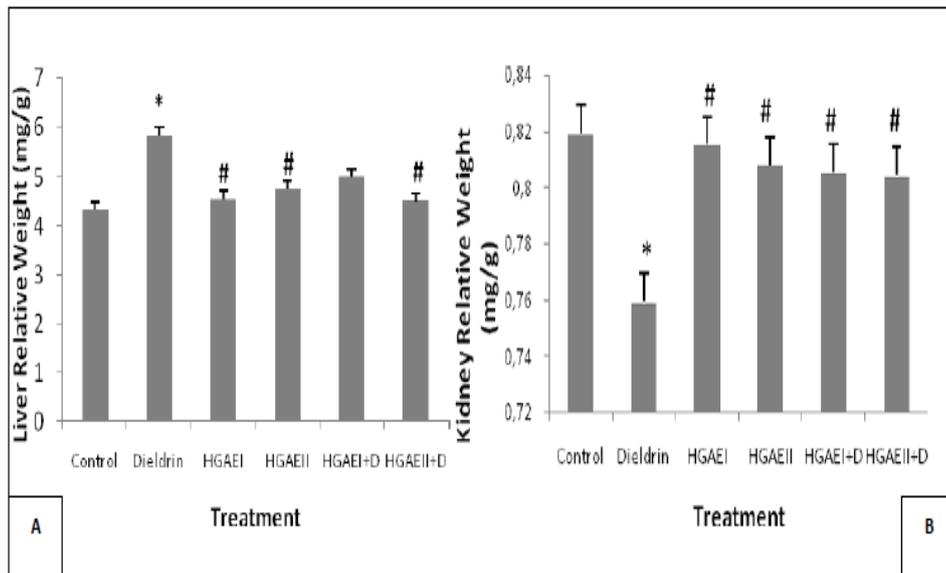
3.4. Effect of HGAE on Lipid Profile

Table 2 showed that subacute dieldrin exposure of rats induced considerable lipid metabolic disorders. In fact, dieldrin treatment significantly ($p < 0.05$) increased the TG, TC and LDL-C plasma contents and decreased the HDL-C level. Pretreatment with HGAE significantly ($p < 0.05$) protected against the disturbance of lipid metabolic parameters induced by the toxic. Importantly, HDL-C level was increased in a dose-dependent manner respectively by 15 and 20% in HGAEI+D and HGAEII+D groups compared to dieldrin group and becomes again comparable with control group. Lipid profile remained unchanged at the two doses of extract alone (Table 2).

3.5. Effect of HGAE on Total Bilirubin Level

Figure 2 shows the serum level of total bilirubin in control and various experimental groups of rats. An elevation in serum bilirubin level in dieldrin group was highly significant ($p < 0.05$) when compared with the control rats. Pretreatment with both doses of HGAE caused marked reduction ($p < 0.05$) in the elevated level of

total bilirubin induced by dieldrin administration, while HGAE alone had no significant effect.



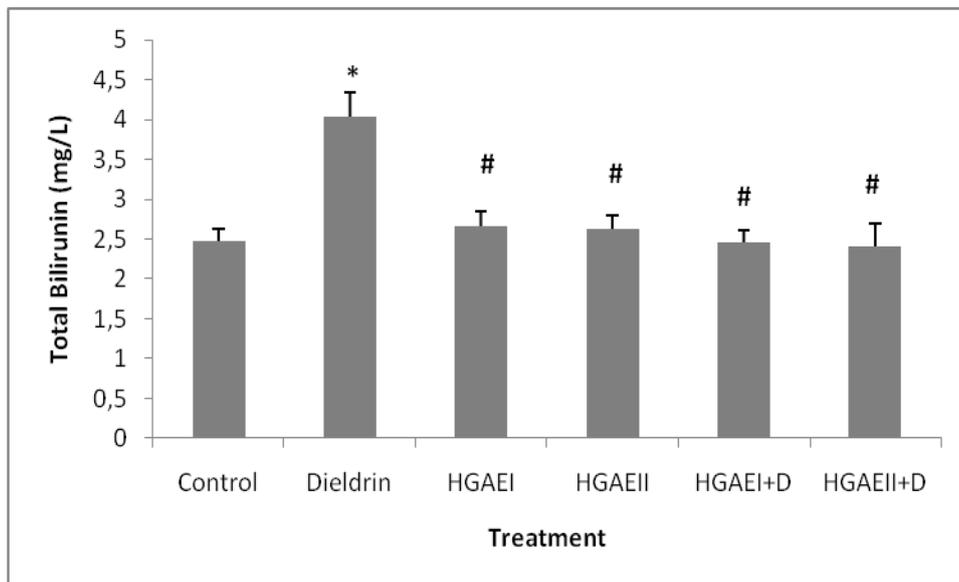
Values (means ± standard errors of the mean (SEM), n=10) are significantly different (p<0.05) in Tukey’s multiple comparison post hoc test. *: p<0.05 compared to control group and #: p<0.05 compared to dieldrin group. HGAE, *Hordeum vulgare L.* grass aqueous extract; D, dieldrin.

Figure 1. Effect of Barley Grass Extract on Liver and Kidney Relative Weight

Table 2. Effect of Barley Grass on Serum Lipid Profile of Rats

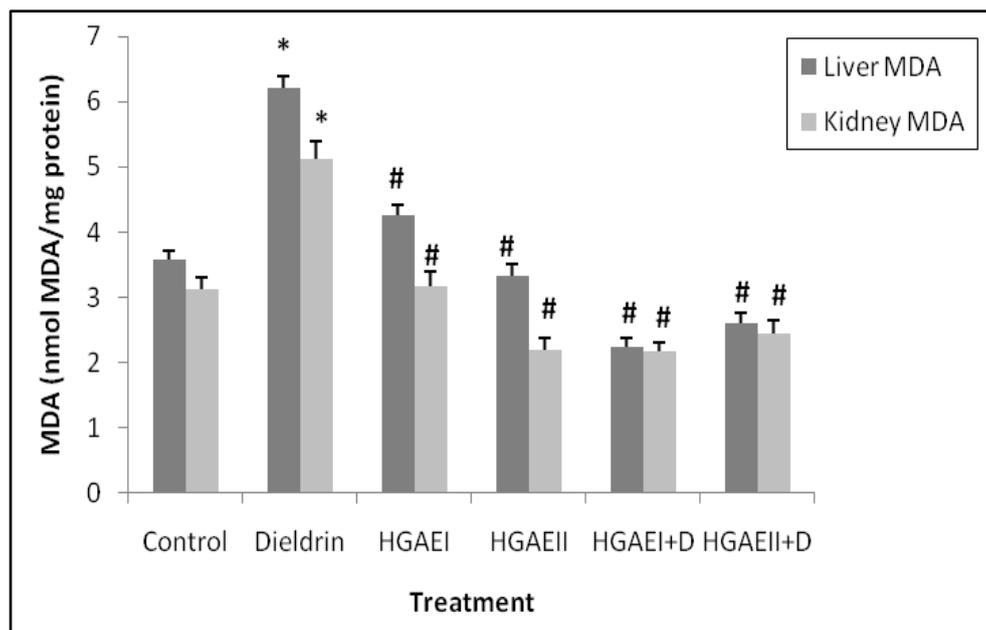
	TG (g/l)	TC (g/l)	HDL-C (g/l)	LDL-C (g/l)
Control	0.96 ± 0.094	0.56 ± 0.025	0.25 ± 0.013	0.18 ± 0.039
Dieldrin	1.56 ± 0.218*	0.69 ± 0.031*	0.20 ± 0.008*	0.21 ± 0.045*
HGAEI	1.01 ± 0.082#	0.54 ± 0.008#	0.21 ± 0.006	0.13 ± 0.021#
HGAEII	1.03 ± 0.105#	0.57 ± 0.012#	0.22 ± 0.005	0.13 ± 0.023#
HGAEI+D	1.09 ± 0.059#	0.56 ± 0.043#	0.23 ± 0.021#	0.14 ± 0.046#
HGAEII+D	0.87 ± 0.041#	0.57 ± 0.025#	0.24 ± 0.025#	0.15 ± 0.029#

Dieldrin (6 mg/kg b.wt/day) is administered intraperitoneally (i.p.) in corn oil during 10 days. Control animals received the vehicle. Treated animals were administered fresh *Hordeum vulgare L.* grass aqueous extract (HGAE) respectively at the oral doses of 10 ml/kg “HGAEI” and 20 ml/kg “HGAEII” during the first three days, subsequently they received both HGAE and equal volumes of vehicle in the seven days that remain. Pre-treated rats received respectively HGAEI and HGAEII pre-treatment in the first three days, and then they received both respectively HGAEI or HGAEII and dieldrin (HGAEI+D/HGAEII+D) in the seven days that remain. Values (means ± standard errors of the mean (SEM), n=10) are significantly different (p<0.05) in Tukey’s multiple comparison post hoc test. *: p<0.05 compared to control group and #: p<0.05 compared to dieldrin group.



Values (means ± standard errors of the mean (SEM), n=10) are significantly different (p<0.05) in Tukey’s multiple comparison post hoc test. *: p<0.05 compared to control group and #: p<0.05 compared to dieldrin group. HGAE, *Hordeum vulgare L.* grass aqueous extract; D, dieldrin.

Figure 2. Effect of Barley Grass Extract on Total Bilirubin Level



Values (means \pm standard errors of the mean (SEM), n=10) are significantly different ($p < 0.05$) in Tukey's multiple comparison post hoc test. *: $p < 0.05$ compared to control group and #: $p < 0.05$ compared to dieldrin group. HGAE, *Hordeum vulgare L.* grass aqueous extract; D, dieldrin.

Figure 3. Effect of Barley Grass Extract on Hepatic and Renal Lipoperoxidation

3.6. Effect of HGAE on Hepatic and Renal Lipid Peroxidation

As depicted in [Figure 3](#), the levels of hepatic and renal MDA, an end product of lipid peroxidation, was significantly ($p < 0.05$) increased in dieldrin-intoxicated rat compared with that in normal controls. Conversely, pretreatment with HGAE significantly ($p < 0.05$) ameliorated the abnormal levels of MDA in a dose-dependent manner.

3.7. Effect of HGAE on Hepatic and Renal Antioxidant System

Treatment with dieldrin notably ($p < 0.05$) decreased the non enzymatic antioxidant activity of non-protein sulfhydryl groups (NPSH) and the enzymatic antioxidant activity of catalase (CAT) in rat's hepatic and renal tissues when compared with control group ([Table 3](#)). In contrast HGAE pretreatment greatly ($p < 0.05$) restituted the activity of antioxidant system (NPSH and CAT) of

the dieldrin-intoxicated rats, approximately to the levels seen in normal control ([Table 3](#)).

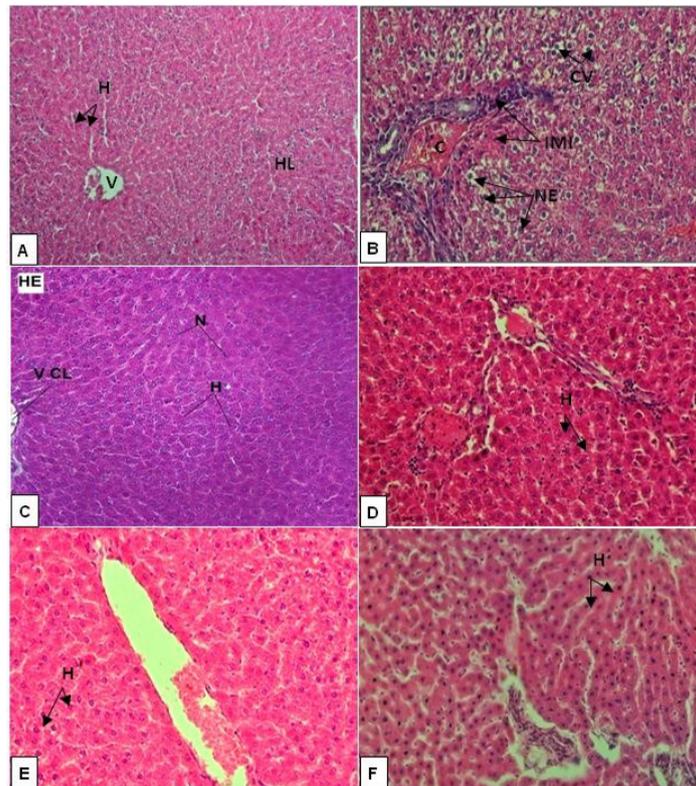
3.8. Liver Histological Analysis

Light microscopic inspection revealed a normal liver structure in control groups of rats ([Figure 4A](#)) with hepatic lobules consisting of a central vein surrounded by radiating hepatocytes separated by irregular blood sinusoids. Liver sections of dieldrin treated rats showed various histological changes ([Figure 4B](#)). Cytoplasmic vacuolization, focal necrosis and nuclear enlargement of hepatocytes were observed in intoxicated animals. Changes included also inflammatory mononuclear cellular infiltration and necrotic degeneration around the dilated and congested portal tract ([Figure 4B](#)). No histopathological changes could be observed in liver of rats treated with both doses of HGAE ([Figure 4C-D](#)). Animal's pretreated with HGAE before the toxic, showed normal hepatocytes ([Figure 4E-F](#)).

Table 3. Effect of Barley Grass on Hepatic and Renal Antioxidant System

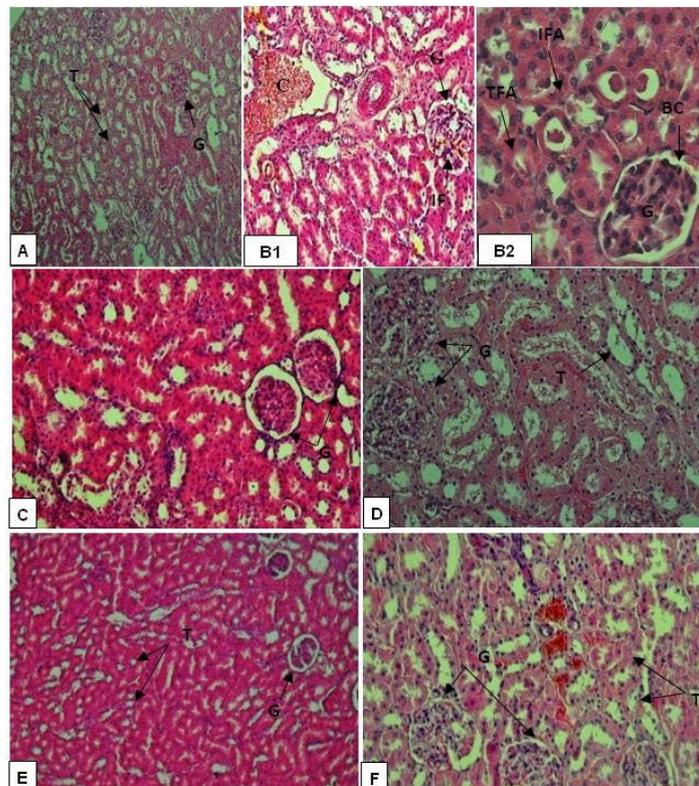
	Liver		Kidney	
	NPSH ($\mu\text{mol/g tissue}$)	CAT ($\mu\text{mol H}_2\text{O}_2/\text{min/mg protein}$)	NPSH ($\mu\text{mol/g tissue}$)	CAT ($\mu\text{mol H}_2\text{O}_2/\text{min/mg protein}$)
Control	0.45 \pm 0.02	705.55 \pm 30.82	0.20 \pm 0.01	3010 \pm 132
Dieldrin	0.25 \pm 0.01*	522.66 \pm 31.33*	0.14 \pm 0.01*	2350 \pm 111*
HGAEI	0.43 \pm 0.02#	683.03 \pm 33.82#	0.19 \pm 0.01#	2851 \pm 138#
HGAEII	0.44 \pm 0.02#	678.12 \pm 32.38#	0.19 \pm 0.01#	3009 \pm 123#
HGAEI+D	0.42 \pm 0.05#	651.20 \pm 30.81#	0.19 \pm 0.005#	2890 \pm 101#
HGAEII+D	0.43 \pm 0.02#	683.97 \pm 34.85#	0.20 \pm 0.008#	3007 \pm 145#

Values (means \pm standard errors of the mean (SEM), n=10) are significantly different ($p < 0.05$) in Tukey's multiple comparison post hoc test. *: $p < 0.05$ compared to control group and #: $p < 0.05$ compared to dieldrin group. HGAE, *Hordeum vulgare L.* grass aqueous extract; D, dieldrin; NPSH, non-protein sulfhydryl; CAT, catalase.



Photomicrography of hematoxylin-eosin-stained sections of normal rat liver (A) (200×): HL: hepatic lobules; H: hepatocytes; V: vein; liver of a dieldrin-intoxicated rat (B) (400×): CV: cytoplasmic vacuolization, NE: nuclear enlargement, IMI: inflammatory mononuclear infiltration, C: congested portal tract; liver from rats treated with HGAEI (10 ml/kg) (C) (200×): N: nuclear, VCL: central vein; liver from rats treated with HGAEII (20 ml/kg) (D) (400×); liver of a rat pretreated with HGAEI+D (E) (400×) and liver of a rat pretreated with HGAEII+D (F) (400×).

Figure 4. Effect of Barley Grass Extract Pretreatment on Dieldrin-Induced Liver Damage in Rats



Photomicrography of hematoxylin-eosin-stained sections of normal rat kidney (A) (200×): T: tubular, G: glomeruli; kidney of a dieldrin-intoxicated rat (B1) (400×) and (B2) (1000×): C: congestion, TFA: tubular focal alteration, IF: inflammatory infiltration, BC: Bowman's capsules; kidney from rats treated with HGAEI (10 ml/kg) (C) (400×); kidney from rats treated with HGAEII (20 ml/kg) (D) (400×); kidney of a rat pretreated with HGAEI+D (E) (200×) and kidney of a rat pretreated with HGAEII+D (F) (400×).

Figure 5. Effect of Barley Grass Extract Pretreatment on Dieldrin-Induced Kidney Damage in Rats

3.9. Kidney Histological Analysis

The kidney in the control group showed a normal structure (Figure 5A). In the dieldrin-intoxicated group, the tubular architecture of kidney tissue was altered and showed evidence of necrosis, loss of tubular details, degeneration of glomeruli and Bowman's capsules, as well as infiltration of inflammatory cells (Figure 5B1-B2). No changes could be observed in kidney tissues of rats treated with HGAE alone (Figure 5C-D). Rats pretreated with HGAE and exposed to dieldrin exhibited dose-dependent correction of renal injury as demonstrated by improved tubular and glomerular architecture and reduced inflammatory cells (Figure 5E). A better correction was attained by the higher dose of extract comparable to the control (Figure 5F).

4. Discussion

Our investigation showed that *Hordeum vulgare* L. possesses an important amount of polyphenols followed by condensed tannins. The phytochemical studies of HGAE revealed the presence of a total phenolic content of 168.33 ± 0.34 mg TAE.g⁻¹FW, which appeared higher than that the total phenolic content of green tea (3.066 ± 1.911 mg TAE/g) [27]. In addition, a higher total proanthocyanidins content (6.66 ± 0.2 mg TAE.g⁻¹FW) had been detected in HGAE when compared with the level 3.18 ± 0.02 mg CE/g detected in aqueous peel extracts of pomegranate recommended to have an anticancer effect [28]. Our results are similar to the previous data showing higher phenolic contents in barley extract [29]. Therefore, the abundant content of phenolic compounds in barley supported its use as a potential source of antioxidants for disease prevention and health promotion [3,30]. An extraordinary feature of the products derived from young barley is their well-established ability to degrade pesticides [31].

The results of present study clearly show that the administration of dieldrin significantly increased serum TG, TC and LDL-C, but decreased serum HDL-C. Interestingly, the alteration in TC, LDL-C and HDL-C, were found to be greatly ameliorated upon pretreatment with the barley phenolic extract. These changes may be attributed to bioactive compounds that were demonstrated in phytochemical studies. Thus, according to Kothari et al. [19] bioactive plant compounds flavonoids and triterpenoids are reported to modulate lipid levels in hypercholesterolemic rats treated by aqueous wheat (*Triticum aestivum*) grass extract.

The mechanisms by which HGAE improves blood lipid metabolism have not been identified clearly. Montagut et al. [32] demonstrated that proanthocyanidins may be largely responsible for inhibiting TG and apolipoprotein B secretion (a marker of VLDL) by hepatic cells. It also was found that proanthocyanidins repress the expression of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, HMG-CoA synthetase, and other enzymes involved in cholesterol biosynthesis [33]. Proanthocyanidins also increase the cytochrome P450 7A1 activity, which has a role in the transformation of cholesterol into bile acids [33]. Condensed tannins called

proanthocyanidins are also reported to increase in action of the endothelium bound lipoprotein lipase activity, which hydrolyzes triglycerides [33]. In addition, besides anthocyanidins, the presence of saponins in *Hordeum vulgare* L. might have contributed in increasing fecal cholesterol excretion [19]. Furthermore, the significant decrease in the serum levels of bilirubin in our study may be consecutive to the rapid and selective taken up of this metabolite into the liver as a function of healthy hepatocyte membranes maintained on pretreatment with barley extract relative to that of intoxicated groups [33].

Dieldrin is highly lipophilic and therefore accumulates in lipid-containing tissues, so liver is the first organ to be exposed to such hazards via the portal circulation [34]. Hence, several findings concluded that dieldrin affects liver membrane structure and functions [15,34]. Results obtained in the present study showed that liver relative weight increased significantly in rats that had been treated with dieldrin. This seems to be in agreement with earlier observations which found that many organochlorine compounds caused liver hypertrophy, which may be related to the rise in hepatic cellular hyperplasia manifested by an enhancement of nuclear DNA synthesis and the rise of mitotic activity [34,35]. However, this hepatomegaly almost disappeared and liver weight returned to its normal level in rats pretreated with the higher dose of HGAE.

Data of histopathological assessment of the hepatic tissue were found to be correlated with the above-mentioned relative weight findings. In the dieldrin-intoxicated group, the lobular architecture of liver tissue was deformed and showed evidence of extensive pericentral vein necrosis with ballooning of hepatocytes and infiltration of inflammatory cells. On the contrary, pretreatment of dieldrin-intoxicated rats with HGAE preserved the integrity of hepatocyte in a dose-dependent manner. Our findings are in agreement with those reported by previous barley studies [3,5].

On the contrary, the present results revealed that the kidney relative weight was markedly decreased after dieldrin challenge. This result is supported by previously data showing a decrease in kidney relative weight in dieldrin intoxicated animals [35]. Better, kidney relative weight has been achieved by administration of HGAE in dieldrin intoxicated animals. Histopathological evaluation of the renal tissue in the dieldrin-exposed group showed further necrosis, loss of tubular details, shrinkage of glomeruli and Bowman's capsules, and infiltration of inflammatory cells. Importantly, rats pretreated with HGAE exhibited dose-dependent correction of renal injury as demonstrated by improved tubular and glomerular architecture and reduced inflammatory cells. These findings concord with those of Avramovic et al. [36] who showed that a specific proanthocyanidin component of grape seed proanthocyanidin extract, proanthocyanidin-BP1, is protective in a rat model for oxidative renal injury.

It has been argued that proanthocyanidins possess powerful antioxidant and anti-inflammatory properties, and consequently therapeutic benefit against oxidative stress-related diseases [37]. Flavanols (catechin, procyanidin B, prodelfinidin B, procyanidin C), were identified as constituents of the fraction with the highest antioxidant capacity by flash chromatography in barley according to Gangopadhyay et al. [38].

Therefore, inhibition and/or scavenging of intracellular ROS through HGAE would play a critical role in preventing liver and kidney diseases. The anti-inflammatory activity of proanthocyanidins isolated from *Polypodium feei* has been proposed to function through inhibition of prostaglandin biosynthesis according to Subarnas and Wagner [39]. Other investigation affirms that flavonoids such as lutomarin and saponarin witch are abundant in barley primary leaves are able to preserve DNA structure from degradation by free radicals maintaining the stability of the cell wall [40].

Smida et al. [41] have shown a relationship between induction of lipid peroxidation and membrane-attack by ROS. Indeed, increased lipoperoxidation impairs membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors.

Our study revealed that dieldrin significantly enhanced lipid peroxidation, which is marked by high levels of liver and kidney MDA in comparison with untreated rats. Tissue MDA contents are significantly normalized after pretreatment with barley grass extract. These results are in line with those obtained by Abulnaja et al. [5] which illustrate the antioxidant potential of barley against ethylene glycol induced toxicity and hypercholesterolemic diet in male rats. Accordingly, this hepatorenal protective effect could be explained by the antioxidant capacity of high phenolic content of HGAE against ROS generated by dieldrin.

Alternatively, oxidative stress can also occur when there is a change in the antioxidant capacity of a cell. Production of glutathione (GSH) is considered to be the first line of defence against oxidative damage and free radical generation where GSH functions as a scavenger and cofactor in metabolic detoxification of ROS [42]. GSH is the major cytosolic low molecular weight non-protein sulfhydryl compound (NPSH) that acts as a cellular reducing and a protective reagent against numerous toxic substances through its SH groups [42].

Previous studies indicate that both of the no enzymatic (NP-SH system) and enzymatic (SOD, GPX, and CAT) antioxidants decrease in the tissues due to their rapid consumption after combating free radical induced oxidative stress [5]. In our study, dieldrin treatment significantly reduced the total content of NP-SH as well CAT will also be expected to be negatively affected by the toxic in liver and kidney tissues. The level of NP-SH content was notably restored in the liver and kidney on pretreatment with HGAEII. Besides, the pathological levels of CAT-antioxidant enzyme were remarkably rehabilitated, indicating the ability of barley extract to protect liver and kidney against the deleterious effect of dieldrin-derived free radicals.

The antioxidant activity of HGAE may be related to its richness on phenolic hydroxyl groups, which serve as electron or hydrogen donors to terminate the free radical chain reaction yielding a stable phenolic radical [43].

5. Conclusion

In conclusion, our data clearly demonstrated the protective effects of barley grass extract against hyperlipidemic effect and oxidative stress induced by dieldrin-treatment in rat. Administration of fresh aqueous

extract of barley grass was experimentally demonstrated to be safe and remarkably protected against dieldrin-induced hepatorenal injury. This protection could be attributed to the phenolic contents, which can suppress the oxidative stress caused by dieldrin-generating free radicals with the subsequent restoration of the histological and physiological parameters.

Declaration of Interests

The authors report no conflicts of interest in this work.

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References

- [1] Gladine, C., Morand, C., Rock, E. and Durand, D., "The antioxidative effect of plant extracts rich in polyphenols differs between liver and muscle tissues in rats fed n-3 PUFA rich diets", *Animal Feed Science and Technology*, 139 (3-4). 257-272. Dec.2007.
- [2] Van Hung, P., "Phenolic compounds of cereals and their antioxidant capacity", *Critical Reviews in Food Science and Nutrition*, 56(1). 25-35. Jul. 2014.
- [3] Lee, Y.H., Kim, J.H., Kim, S.H., Oh, J.Y., Seo, W.D., Kim, K.M., Jung, J.C, and Jung, Y.S., "Barley Sprouts Extract Attenuates Alcoholic Fatty Liver Injury in Mice by Reducing Inflammatory Response", *Nutrients*, 8(7). 440. Jul. 2016.
- [4] Shah, J.G., Patel, B.G., Patel, S.B, and Patel, R.K, "Antiuro lithiatic and antioxidant activity of *Hordeum vulgare* seeds on ethylene glycol-induced urolithiasis in rats", *Indian Journal of Pharmacology*, 44(6). 672-677. Nov-Dec. 2012.
- [5] Abulnaja, K.O, and El Rabey, H.A, "The Efficiency of Barley (*Hordeum vulgare*) Bran in Ameliorating Blood and Treating Fatty Heart and Liver of Male Rats", *Evid.-Based Complementary and Alternative Medicine*, 13p. Mar. 2015.
- [6] Gul, S., Ahmed, S., Kifli, N., Uddin, Q.T., Tahir, N.B., Hussain, A., Jaafar, H.Z., Moga, M, and Zia-UI.-Haq, M, "Multiple pathways are responsible for anti-inflammatory and cardiovascular activities of *Hordeum vulgare* L", *Journal of Translational Medicine*, 12(1). 316-319. Nov.2014.
- [7] Lahouar, L., El-Bok, S, and Achour, L, "Therapeutic potential of young green barley leaves in prevention and treatment of chronic diseases: An Overview", *American Journal of Chinese Medicine*, 43(7). 1311-1329. Oct.2015.
- [8] Ehrenbergerová, J., Vaculová, K., Paulícková, I., Brezinová Belcredi, N., Macuchová, S., Kopáček, J., Gabrovská, D., Holasová, M., Ouhřabková, J., Rysová, J., Fiedlerová, V., Winterová, R, and Horáčková, S, "Different barley cultivars as a source of green mass for improving nutrient balance in human diet", In: A. Brandstetter (ed.) Raumberg, Austria, 2007. 91-94.
- [9] Nishiyama, T., Hagiwara, Y., Hagiwara, H, and Shibamoto, T, "Inhibitory effect of 2'-o-glycosyl isovitexin and α -tocopherol on genotoxicglyoxal formation in a lipid peroxidation system", *Food and Chemical Toxicology*, 32(11). 1047-1051. Nov.1994.
- [10] Seo, W.D., Yuk, H.J., Curtis-Long, M.J., Jang, K.C., Lee, J.H., Han, S.I., Kang, H.W., Nam, M.H., Lee, S.J, and Lee, J.H, "Effect of the growth stage and cultivar on policosanol profiles of barley sprouts and their adenosine 5'-monophosphate-activated protein-kinase activation", *Journal of Agricultural and Food Chemistry*, 61. 1117-1123. Jan.2013.
- [11] Shibamoto, T., Hagiwara, Y., Hagiwara, H, and Osawa, J.A," flavonoid with strong antioxidative activity isolated from young

- green barley leaves", *Journal of American Chemistry Society*, 547. 153-163. 1994.
- [12] Kamiyama, M, and Shibamoto, T, "Flavonoids with potent antioxidant activity found in young green barley leaves", *Journal of Agricultural Food and Chemistry*, 60(25). 6260-6267. Jun. 2012.
- [13] Halliwell, B, "Mechanisms involved in the generation of free radicals", *Pathologie Biologie*, 44(1). 6-13. Jan.1996. PMID: 8734294.
- [14] Lozano-Paniagua, D., Parron, T., Alarcon, R., Requena, M., Gil, F., Lopez-Guarnido, O., Lacasana, M, and Hernandez, A.F, "Biomarkers of oxidative stress in blood of workers exposed to non-cholinesterase inhibiting pesticides", *Ecotoxicology and Environmental Safety*, 162. 121-128. Oct.2018.
- [15] Hallegue, D., Tebourbi, O., Kacem, K., Sakly, M, and Ben Rhouma, K, "Impact of dieldrin on liver morphological and biochemical parameters of rats", *Toxicology and Industrial Health*, 26(3). 131-137. Feb.2010.
- [16] Hfaiedh, N., Alimi, H., Murat, J.C, and Elfeki, A, "Protective effects of fenugreek (*Trigonella foenum graecum* L.) upon dieldrin-induced toxicity in male rat" *General Physiology and Biophysics*, 31(4). 423-430. Dec.2012.
- [17] Slotkin, T.A., Skavicus, S., Card, J., Levin, E.D, and Seidler, F.J, "Diverse neurotoxicants target the differentiation of embryonic neural stem cells into neuronal and glial phenotypes", *Toxicology*, 372. 42-51. Nov. 2016.
- [18] Trush, M.A, and Kensler, T.W, "An overview of the relationship between oxidative stress and chemical carcinogenesis", *Free Radical Biology and Medicine*, 10(3-4). 201-209. 1991.
- [19] Kothari, S., Jain, A.K., Mehta, S.C, and Tonpay, S.D, "Hypolipidemic effect of fresh *Triticum aestivum* (wheat) grass juice in hypercholesterolemic rats", *Acta Poloniae Pharmaceutica*, 68(2).291-294. Mar-Apr.2011. PMID: 21485304.
- [20] Singleton, V.L., Orthofer, R, and Lamuela-Raventos, R.M, "Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent", *Methods in Enzymology*, 299. 152-178. 1999.
- [21] Scalbert, A., Monties, B, and Janin, G, "Tannins in wood: comparison of different estimation methods", *Journal of Agricultural and Food Chemistry*, 37(5) 1324-1332. Sep.1989.
- [22] Friedewald, W.T, Levy, R.I., Fredrickson, D.S, "Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge", *Clinical Chemistry*, 18(6). 499-502. Jun. 1972.
- [23] Buege, J.A, and Aust, S.D, "On the solubilization of NADPH-cytochrome c reductase from rat liver microsomes with crude pancreatic lipase", *Biochimica et Biophysica Acta*, 286(2). 433-436. Dec.1972.
- [24] Hu, M.L, "Measurement of protein thiol groups and glutathione in plasma", *Methods in Enzymology*, 233. 380-385.1994.
- [25] Aebi, H, "Catalase in vitro" *Methods in Enzymology*, 105. 121-126. 1984.
- [26] Lowry, O.H., Rosenbrough, N.J, and Randall, R, "Protein measurement with the folin phenol reagent", *Journal of Biological Chemistry*, 193(1). 265-275. Nov.1951. PMID: 14907713.
- [27] Kaur, A., Kaur, M., Kaur, P., Kaur, H., Kaur, S, and Kaur, K, "Estimation and comparison of total phenolic and total antioxidants in green tea and black tea", *Global journal of biosciences and Biotechnology*, 4(1). 116-120. Jan.2015. ISSN 2278-9103.
- [28] Abdel-Hady, N.M, "Quantitative diversity of phenolic content in peels of some selectegyptian pomegranate cultivars correlated to antioxidant and anticancer effects", *Research Journal of Applied Sciences*, 9. 4823-4830. Jan.2013.
- [29] Anwar, F., Qayyum, H.M.A., Hussian, A.I, and Iqbal, S, "Antioxidant activity of 100% and 80% methanol extracts from barley seeds (*Hordeum vulgare* L.): stabilization of sunflower oil", *GrasasAceites*, 61(3). 237-243. Sep.2010.
- [30] Giriwono, P.E., Hashimoto, T., Ohsaki, Y., Shirakawa, H., Hoka-zono, H, and Komai, M, "Extract of fermented barley attenuates chronic alcohol induced liver damage by increasing antioxidative activities", *Food Research International*, 43(1). 118-124. Jan.2010.
- [31] Durham, J., Ogata, J., Nakajim, S., Hagiwara, Y, and Shibamoto, T, "Degradation of organophosphorous pesticides in aqueous extracts of young green barley leaves (*Hordeum vulgare* L.)", *Jouranal of Science Food Agriculture*, 79. 1311-1314. Mar.1999.
- [32] Montagut, G., Baiges, I., Valls, J., Terra, X., Del Bas, M.J., Vitrac, X, and Richard, T, "A trimer plus a dimer-gallate reproduce the bioactivity described for an extract of grape seed procyanidins" *Food Chemistry*, 116(1). 265-270. Sep. 2009.
- [33] Tong, L.T., Zhong, K., Liu, L., Zhou, X., Qiu, J, and Zhou, S, "Effects of dietary hull-less barley β -glucan on the cholesterol metabolism of hypercholesterolemic hamsters", *Food Chemistry*, 169. 344-349. Feb. 2015.
- [34] Kolaja, K.L., Stevenson, D.E., Walborg, E.F, and Klaunig, J.E, "Selective dieldrin promotion of hepatic focal lesions in mice", *Carcinogenesis*, 17(6). 1243-1250. Jun. 1996.
- [35] Sonne, C., Wolkers, H., Leifsson, P.S., Jenssen, B.M., Fuglei, E., Ahlström, O., Dietz, R., Kirkegaard, M., Muir, D.C, and Jørgensen, E, "Organochlorine-induced histopathology in kidney and liver tissue from Arctic fox (*Vulpes lagopus*)", *Chemosphere*, 71(7). 1214-1224. Apr. 2008.
- [36] Avramovic, V., Vlahovic, P., Mihailovic, D, and Stefanovic, V, "Protective effect of a bioflavonoid proanthocyanidin-BP1 in glycerol-induced acute renal failure in the rat: renal stereological study", *Renal Failure*, 21(6). 627-634. Jul.1999.
- [37] Byun, A.R., Chun, H., Lee, J., Lee, S.W., Lee, H.S, and Shim, K.W, "Effects of a dietary supplement with barley sprout extract on blood cholesterol metabolism", *Evid.-Based Complementary Alternative Medicine*, 2015. 7p. Nov. 2015.
- [38] Gangopadhyay, N., Rai, D.K., Brunton, N.P., Gallagher, E, and Hossain, M.B, "Antioxidant-guided isolation and mass spectrometric identification of the major polyphenols in barley (*Hordeum vulgare*) grain", *Food Chemistry*, 210. 212-220. Nov.2016.
- [39] Subarnas, A, and Wagner, H, "Analgesic and anti-inflammatory activity of the proanthocyanidin shellegeain A from *Polypodium feei* METT", *Phytomedicine*, 7(5). 401-405. Oct. 2000.
- [40] Ojha, A, and Gupta, Y.K, "Study of commonly used organophosphate pesticides that induced oxidative stress and apoptosis in peripheral blood lymphocytes of rats" *Human and Experimental Toxicology*, 36(11). 1158-1168. Dec. 2017.
- [41] Smida, A., Ncibi, S., Taleb, J., Ben Saad, A., Ncib, S, and Zourgui, L, "Immunoprotective activity and antioxidant properties of cactus (*Opuntia ficus indica*) extract against chlorpyrifos toxicity in rats" *Biomedicine and Pharmacotherapy*, 88. 844-851. Apr.2017.
- [42] Milnerowicz, M., Bukowski, R., Jablonowska, M., Ścisalska, M, and Milnerowicz, S, "The antioxidant profiles, lysosomal and membrane enzymes activity in patients with acute pancreatitis" *Mediators of Inflammation*, 10. p9. Sep. 2014.
- [43] Chen, F., Zhang, L., Zong, S., Xu, S., Li, X, and Ye, Y, "Antioxidant capacity and proanthocyanidin composition of the bark of *Metasequoia glyptostroboides*", *Evid-Based Complementary and Alternative Medicine*, 2014. 11p. Mar.2014.

