

## Biochemical and Immunological Study on the Effects of Barley and its Components as Hypoglycemic Agents in Diabetic Rats

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**Abstract:** The present study was carried out to investigate the hypoglycemic effect of barley (*Hordeum vulgare L*) and some of its components such as amino acids (L-leucine and L-glutamine) and chromium picolinate on some biochemical and immunological parameters of alloxan induced diabetic rats. Alloxan-diabetic rats were treated with barley water (10% w/v) at a dose 10 ml Kg<sup>-1</sup> b.wt., chromium picolinate at 15 µg Kg<sup>-1</sup> b.wt., L-leucine plus L-glutamine at 4.5 mg & 15 mg Kg<sup>-1</sup> b.wt., and/or the combination of barley plus chromium plus L-leucine and L-glutamine at the same previous doses in the same water volume, respectively. Rats received the treatments in their drinking water for four weeks. The levels of glucose, immunoglobulin G (IgG), total lipids (TL), cholesterol, triglycerides (TG), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were significantly (P<0.05) increased, while high-density lipoprotein (HDL) decreased in plasma of alloxan-diabetic rats compared to control group. The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid phosphatase (AcP) and alkaline phosphatase (AlP) were significantly (P<0.05) decreased in both plasma and liver of alloxan-diabetic rats. On the other hand, the activity of lactate dehydrogenase (LDH) decreased in plasma and increased in liver of alloxan-diabetic rats. Treatment of the diabetic rats with repeated doses of any one of the three treatments alone could restore the changes of the above parameters to their normal levels after four weeks of treatment. However, treatment with the combination of all together did not show complete restoration. Furthermore, the electron microscope results were supported biochemical and immunological findings. The present results showed that barley, amino acids and chromium picolinate exerted antihyperglycemic effects and consequently may alleviate liver damage caused by alloxan-induced diabetes.

**Key words:** Rats, diabetes, alloxan, barley, amino acids, chromium picolinate, IgG

### INTRODUCTION

The number of people diagnosed with type II diabetes mellitus is increasing at an alarming rate in western societies and has become a major health concern [1]. Eastman *et al.* [2] demonstrated that treating type II diabetic patients with the goal of achieving normoglycemia can be estimated to cost approximately \$16,000 per quality-adjusted life year gained. It is, therefore important to examine various strategies for improving glycemic control in patients with type II diabetes. With the appearance during the past few years of several new anti-diabetic agents, the task of choosing the most appropriate therapy for patients has become more complex. Efficacy, patient acceptability, side effects, and cost are important issues to be considered in developing therapeutic regimens [3].

Medicinal plants continue to provide valuable therapeutic agents, both in modern medicine and in traditional system [4-7]. Medicinal plants also offer good prospects to finding new drugs particularly against

conditions for which modern drugs are inadequate. Usually herbs are more accepted by the patients with less adverse effects. A wide variety of the traditional herbal remedies are used by diabetic patients, especially in the third world countries [8,9]. The folk medicine in Egypt has described several kinds of Egyptian herb and plant prescriptions, belonging to various families to be concerned with the treatment of diabetes mellitus [4,5,7]. Previous reports demonstrated that the administration of several herb extract could restore the changes in the activities of serum enzymes, lipid profiles and oxidative stress [4,5,7]. Also, Sheweita *et al.* [6] reported that hypoglycemic herbs could alleviate the deleterious effects of carcinogenic compounds in the liver of diabetic rats since these herbs reduced the hepatic content of cytochrome P450 and other associated enzyme activities compared to the diabetic group. Such alterations in the activity of phase I and II drug-metabolizing enzymes should be considered when therapeutic drugs are administered to diabetic patients

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since most of drugs are metabolized mainly by the cytochrome P450 system.

Barley, *Hordeum vulgare L.*, Family; Germinaceae, is the most nutritious food on earth, it contains a balance of many minerals, amino acids, fibers and enzymes. It is used to support the body's own self-healing mechanisms. The components of barley aid the body in maintaining cells in a healthy condition and work to correct abnormalities. Barley has been used as an aid in the treatment of a variety of conditions such as arthritis, digestive diseases, diabetes, skin abnormalities, weight loss, detoxifying and cancer [10]. In addition, it was postulated that the beneficial effect of barley might be explained by its high content of chromium [11].

Many studies serve to illustrate the beneficial effect of chromium in subjects consuming normal diet with normal life styles. Chromium has been shown to improve the signs and/or symptoms of diabetes in people with glucose intolerance, type I and type II, gestational and steroid induced diabetes [12-14]. Also, barley is rich with amino acids and it was reported that leucine with other amino acids can induce glycogen synthesis in muscle after exercise, thereby increasing glucose utilization. L-leucine in combination with L-glutamine and other amino acid acts as a non-glucose insulin secretagogue, increasing insulin secretion from diabetic pancreas [15].

Therefore, the present study aimed to investigate the hypoglycemic effect of barley and some of its components such as amino acids (L-leucine and L-glutamine) and chromium on some biochemical and immunological parameters of alloxan induced diabetic rats compared to control group.

## MATERIALS AND METHODS

### Preparation of barley water and tested compounds:

Pearl barley, *Hordeum vulgare L.*, was used in this study and it was obtained from the local markets, Alexandria, Egypt. Barley was washed in cold water and then boiled with distilled water at 10% (w/v) for about 20 minutes, then filtered through three layers of clean sterile Miracloth (Calbiochem, USA) with gentle press to obtain most of barley water. Chromium picolinate (CHROMIUM® capsules) was purchased from MEPACO Co., Cairo, Egypt, and was dissolved in distilled water at 250 µg L<sup>-1</sup>. Amino acids; L-glutamine and L-leucine; obtained from El-gomhoreya Co., Alexandria, Egypt. They were dissolved in distilled water at 75 gm and 25 gm L<sup>-1</sup>, respectively. Their combination was also prepared by dissolving chromium, L-glutamine and L-leucine in distilled water at 250 µg, 75 gm and 25 gm L<sup>-1</sup>, respectively. Barley water at a ratio of 1:25 (v/v) was added to 1 liter of the mixture.

### Animals and treatment:

Forty-eight rats weighing 200-250 gm in average were obtained from Abo-Rawash farm, Egypt. Rats were housed in standard cages, where feed and water were supplied *ad libitum*. After two weeks of acclimation, animals were divided into two groups. The first group (8 rats) was used as control and received double distilled water as vehicle. The second group (40 rats) was injected subcutaneously (s.c.) two times with alloxan at 60 mg Kg<sup>-1</sup> b.wt. in two successive days, and divided into five subgroups (8 rats per each) after stabilization of diabetes for 1 week. The first subgroup was kept as diabetic. The second subgroup was supplied with barley water (D+B) at 10 ml Kg<sup>-1</sup> b.wt. Third subgroup was received chromium picolinate (D+Cr) at 15 µg Kg<sup>-1</sup> b.wt. and fourth subgroup was supplied with L-leucine and L-glutamine (D+AA) at 4.5 mg & 15 mg Kg<sup>-1</sup> b.wt., respectively. The fifth subgroup was administered with the combination of barley, chromium, L-leucine and L-glutamine at the same previous doses in the same water volume (D+Comb.). Animals were received the treatment in their drinking water for 4 weeks.

### Enzyme assessments:

At the end of the experimental period, rats were fasted for 12 hours, and then sacrificed by cervical decapitation and fasting blood samples were collected from the sacrificed animals in tubes with heparin. Plasma samples were obtained by centrifugation at 860 ×g for 20 minutes and stored at -20°C till measurements. Also, liver was immediately removed, weighed and washed using chilled saline solution. Liver was minced and homogenized (10% w/v), separately, in ice-cold 1.15% KCl-0.01M sodium, potassium phosphate buffer (PH 7.4) in a Potter-Elvehjem type homogenizer. The homogenate was centrifuged at 10,000 ×g for 20 minutes at 4°C, and the resultant supernatant was used for different enzyme assays.

Plasma and liver aspartate aminotransferase (AST; EC 2.6.1.1) and alanine aminotransferase (ALT; EC 2.6.1.2) activities were determined with kits from SENTINEL CH. (via principle Eugenio 5-20155 MILAN-ITALY). The principle reaction of the colorimetric determination of AST or ALT activity is based on the reaction of aspartate or alanine with alpha-ketoglutarate to form oxaloacetate or pyruvate, respectively. The oxaloacetate or pyruvate formed is measured by monitoring the concentration of oxaloacetate or pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine. Alkaline phosphatase (AIP; EC 3.1.3.1) activity was measured at 405 nm by the formation of paranitrophenol from para-nitrophenylphosphate as a substrate [16]. For assaying acid phosphatase (AcP; EC 3.1.3.2) activity, the method of Moss [17] was used. p-Nitrophenyl phosphate is hydrolyzed in acid pH medium by the action of acid phosphatase. Liberated p-nitrophenyl is

spectrophotometrically quantified. Plasma and liver lactate dehydrogenase (LDH, EC 1.1.1.27) activity was determined by the method of Cabaud and Wroblewski [18].

#### Biochemical assays:

The concentrations of glucose were determined with kits from Biosystems, S.A. Costa Brava, 30-Barcelona (Spain). Plasma concentration of total lipids also was determined according to the method of Knight *et al.* [19]. Plasma concentration of cholesterol and triglycerides (TG) were determined according to the methods of Carr *et al.* [20]. High-density lipoprotein-cholesterol (HDL-c) and low-density lipoprotein-cholesterol (LDL-c) were determined according to the methods of Warnick *et al.* [21] and Bergmenyer [22], respectively. Very low-density lipoprotein-cholesterol (VLDL-c) was calculated by dividing the values of TG by factor of 5.

#### Immunoglobulin G measurement by ELISA:

Goat anti-mouse immunoglobulin G (IgG) antiserum, standard mouse IgG, goat anti-mouse IgG conjugated to HRP and tetramethylbenzidine were purchased from Sigma (Sigma-Aldrich Company Ltd., UK) and all other chemicals were supplied from BDH (VWR International Ltd., UK). Immunoglobulin G was measured using the method of Engvall [23].

#### Electron microscopy:

Transmission electron microscopy (TEM) was carried out on pancreas specimen taken immediately after animal scarification. The specimen was subsequently processed for electron microscopy [24, 25].

#### Statistical analysis:

Statistical analysis was performed using the SPSS statistical software package (Statistical Package for the Social Sciences, Salem, OR, USA). Data are presented as means with their standard errors. Normality and homogeneity of the data were confirmed before ANOVA; differences among the control, diabetic and treated groups were assessed by one-way ANOVA followed by Scheffe test to analyze specific differences between means.

## RESULTS

The effects of administration of barley, chromium, amino acids (L-leucine and L-glutamine) and/or their combination on plasma levels of glucose, immunoglobulin G (IgG), total lipids (TL), cholesterol, triglycerides (TG), high density lipoprotein-cholesterol (HDL-c), low density lipoprotein-cholesterol (LDL-c) and very low density lipoprotein-cholesterol (VLDL-c) are presented in Table 1. The experimentally induced-diabetes increased ( $P<0.05$ ) the level of plasma glucose by 97.5% of control level (Table 1), however, treatment of alloxan-diabetic rats with barley, chromium, amino acids and/or their combination reduced their plasma

glucose levels by 69, 63, 64.5 and 86%, respectively compared with the diabetic group.

Also, in alloxan-diabetic rats, the concentrations of plasma IgG, TL, TG, cholesterol, LDL and VLDL of alloxan diabetic rats were significantly ( $P<0.01$ ) increased by 52, 54, 89, 30, 28 and 82%, respectively compared to control group values, whereas HDL level of diabetic rats was significantly decreased by 42% (Table 1). While, after treatment of alloxan-diabetic rats with barley, chromium, amino acids (L-leucine and L-glutamine) and/or their combination, the levels of these parameters were significantly ( $P<0.05$ ) decreased as compared with the mean values of diabetic group (Table 1).

In alloxan-diabetic rats the activities of plasma AST, ALT, AIP, AcP and LDH were significantly ( $P<0.05$ ) decreased by 39, 11, 17, 19 and 40%, respectively, relative to their normal levels (Table 2). In addition, the activities of AST, ALT, AIP and AcP were also significantly ( $P<0.05$ ) decreased in the liver tissue of alloxan-diabetic rats by 35, 25, 8 and 41%, respectively compared to the control values (Table 3).

The electron microscope examination of pancreatic  $\beta$ -cells of control, diabetic, and diabetic treated with barley, chromium, amino acids and/or their combinations are shown in Fig. 1 a, b, c, d, e and f, respectively. The control group showed a normal beta-cell structure, the cells were well granulated and the cell organelles such as cisterna of the rough endoplasmic reticulum and the Golgi complex are of normal size and development. The mitochondrial structures are also well preserved. The intracellular spaces between the endocrine cells are not extended and the endothelial cells and the exocrine parenchymal cells remained intact (Fig. 1a). Injection with alloxan (diabetic group) caused several necrotic islets damages and beta cells showed characteristic changes as the granules disappeared malformation of endoplasmic reticulum and destruction of the mitochondria (Fig. 1b). Treatment of alloxan-diabetic rats with barley showed that  $\beta$ -cells were recovered its normal structure, and well granulated, while endoplasmic reticulum was slightly normal (Fig. 1c). The important feature was that the necrotic islets disappeared. Also, treatment of with chromium caused a promising effect on pancreatic  $\beta$ -cells (Fig. 1d). The cells looked like the normal one, where they appeared well granulated with normal endoplasmic reticulum but only few necrotic lesions still present. The effect of amino acids on pancreatic  $\beta$ -cells of diabetic rats is shown in Fig. 1e, where  $\beta$ -cells appeared well granulated but endoplasmic reticulum was slightly deformed compared to control group, with the appearance of few necrotic lesions compared to diabetic group. Amino acids could be helpful in cells regeneration as it help in protein synthesis. Fig. 1f presented the effects of the combination of barley, chromium and amino acids. The combination showed a well-granulated cell and slightly normal endoplasmic

reticulum, while it showed a bi-nuclei cell that may be indication of bad effect of the overdose.

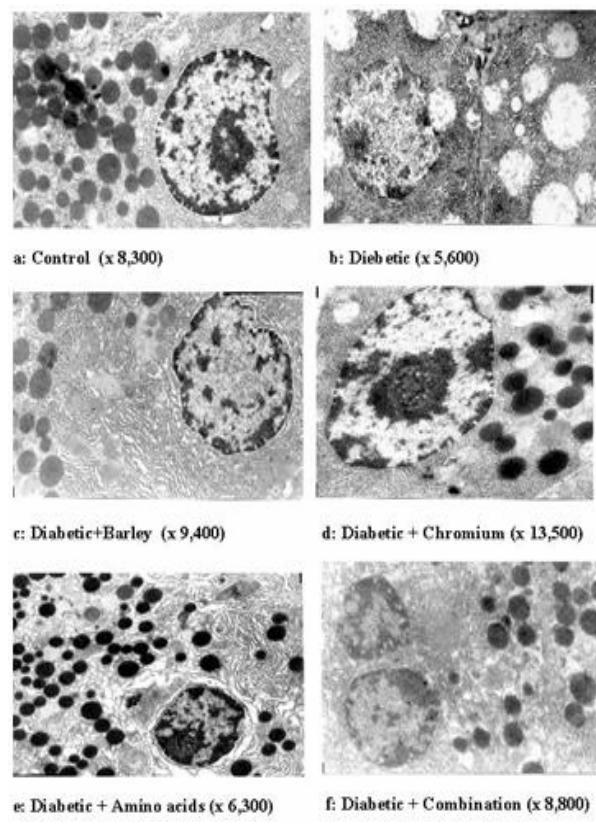
## DISCUSSION

Barley contains many different amino acids, so the hypoglycemic effect of barley (Table 1) may be explained by its content of amino acids and/or chromium. This finding is consistent with Naismith *et al.*<sup>[26]</sup> and Mahdi and Naismith<sup>[11]</sup> who reported that diet-containing barley had a modulating effect on the symptoms of diabetes when compared with a starch or sucrose based diet.

The present study also showed that chromium affect the blood glucose level in alloxan-diabetic rats, and this is in agreement with the results obtained by Ravian *et al.*<sup>[27]</sup> and Anderson *et al.*<sup>[28]</sup> in patients with type I and II diabetes mellitus. Chromium is a key component of glucose tolerance factor (GTF) which enhances the action and function of insulin by increasing its binding to cells, number of receptor, and phosphorelation of receptors leading to increase insulin sensitivity<sup>[29]</sup>.

The control of plasma glucose level caused by amino acids (Table 1) is supported by Vanloon *et al.*<sup>[15]</sup> who reported that the increase in plasma insulin concentration have been observed in rats after the infusion of free amino acids. Li *et al.*<sup>[30]</sup> supported these results, which found that leucine induced insulin secretion by augmenting glutaminolysis through activation of glutaminase and glutamine dehydrogenase in isolated rat islets. Also, L-glutamine a component of barley enhances insulin release acts as a non-glucose insulin secretagogue evoked by L-leucine in isolated rat pancreatic islets<sup>[31]</sup>. The combination of barley, chromium and amino acids presents an unexpected result. It reduced the plasma glucose level by lesser degree than each treatment alone (13.6% of diabetic group). This result may be explained by the high dose effect, due to addition of the excess of chromium and amino acids to already present in barley. Diabetic rats showed a significant ( $P<0.01$ ) increase in the levels of plasma IgG compared to normal control group and this finding is consistent with the finding of Isomaa *et al.*<sup>[32]</sup>. Treatment of alloxan-diabetic rats with barley, chromium and amino acids caused a significant decrease in the elevation of IgG compared to diabetic group. Chromium supplementation in mouse diets has been effective in reducing stress-induced losses of zinc, iron, copper and manganese in urine. The exact mechanism by which chromium enhances the immune system is not known. However, one consistent result of the studies was that chromium reduces serum cortisol levels. Glucocorticoids, which include cortisol, are known to suppress the immune<sup>[33]</sup>. Also, chromium supplementation increased proliferation of peripheral blood lymphocytes in terms of increased blastogenic activity of peripheral blood lymphocytes<sup>[34]</sup>. Change

and Mowat<sup>[35]</sup>, who reported an improvement of IgM and total Immunoglobulin levels reported additional evidence on the enhancing effect of chromium on humoral immunity. Treatment of diabetic rats with amino acids showed also immunity improvement, which explained by restore the normal level of plasma IgG. The lack of dietary amino acids results in diminished protein synthesis in all tissues. One of side effects of protein underfeeding is impaired immune system and susceptibility to infections<sup>[36]</sup>. This fact may explain why amino acids improved immune system. It's clear that barley has immune suppressive function as it contains chromium and many amino acids as source of protein. However, treatment with the combination showed significant increase in the levels of IgG compared to control group. This may reflect the over dose effect of the combination and may development of certain immunoglobulin against this trigger. Interestingly, this finding is consistent with Isomaa *et al.*<sup>[32]</sup> who reported that; the changes in total immunoglobulin concentrations at onset may reflect exposure to environmental triggers; such as viral infections or to (relative) insulinopenia prior to clinical disease onset.



**Fig. 1:** The structure of pancreatic beta-cell of male rats in control (a), alloxan diabetic-group (b), treatment with barley (c), chromium (d), amino acids (e) and the combination (f)

It was reported that hypertriglyceridemia, hypercholesterolemia and reduced HDL level were commonly seen in diabetes<sup>[5, 37]</sup>. It has been suggested that either the removal of triglycerides from the circulation or its entry into the circulation or both was impaired in non-insulin-dependent diabetics<sup>[38]</sup>. The present results showed high significant increases in plasma TL, TG, cholesterol, LDL and VLDL, also a decrease in HDL level (Table 1), and this is in agreement with those found in alloxan-diabetic rats<sup>[5]</sup>. Treatment with barley, chromium and amino acids and/or their combination resulted in improvements in plasma lipid profile (Table 1).

Barley is rich in soluble fiber beta-glucan and it is contributes to lowering blood cholesterol level<sup>[39]</sup>. The mechanism involved in reduction of diabetic hypercholesterolemia by treatment of barley has yet to be elucidated although several hypotheses have been advanced. There is strong evidence that the soluble polysaccharide beta-glucan present in barley was related to hypocholesterolemic effect by many mechanisms. The soluble beta-glucan is bind bile acids in the small intestine, these bile acids are synthesized in the liver from cholesterol and secreted into the small intestine. The fiber-bile acids complex prevents bile acids from being reabsorbed from the small intestine,

enhancing the secretion of bile acids, to replace the lost acids; cholesterol is drawn from the circulation for the production of bile acids, thereby reducing the blood cholesterol levels.

Other proposed mechanism for the reduction of blood cholesterol by the fibers include, the soluble polysaccharides were fermented in the colon. This molecule is absorbed and taken to the liver where it has inhibitory effect on the activity of hydroxymethylglutaryl-CoA-reductase, thus reducing de novo cholesterol synthesis<sup>[40]</sup>. Also, tocols (tocopherols and tocotrienols) have been identified as another minor component in barley with value-added potential. Weber *et al.*<sup>[41]</sup> reported that tocotrienols had ability to lower serum cholesterol. Other suggestion that the hypolipidemic effect of barley may contributed to presence of some saponins. Sidhu *et al.*<sup>[42]</sup> reported that saponins increased the cholesterol secretion to 65.8%, and stimulated lipoprotein lipase activity and might stimulate the enzymes relating to the metabolism of lipid including cholesterol. Other studies have found that saponins in the high cholesterol diet of rats reversed the hypercholesterolemia and increased both the rate of bile acid secretion and the fecal excretion of bile acids and neutral sterols<sup>[43]</sup>.

Table 1: Plasma glucose, immunoglobulin G and lipid profile in control, diabetic and diabetic treated rats with barley (B), chromium (Cr), amino Acids (AA) and their combination (Comb.)

Parameter	Experimental groups					
	Control	Diabetic	Diabetic + B	Diabetic + Cr	Diabetic + AA	Diabetic + Comb.
Glucose (mg dL <sup>-1</sup> )	86±2.1 <sup>e</sup>	169±2.2 <sup>a</sup>	117±1.1 <sup>c</sup>	107±4.2 <sup>d</sup>	110±2.1 <sup>cd</sup>	146±1.4 <sup>b</sup>
Immunoglobulin G (mg L <sup>-1</sup> )	0.52±0.02 <sup>c</sup>	0.79±0.03 <sup>a</sup>	0.70±0.01 <sup>b</sup>	0.62±0.01 <sup>b</sup>	0.63±0.01 <sup>b</sup>	0.84±0.01 <sup>a</sup>
Total lipids (mg dL <sup>-1</sup> )	224±15 <sup>c</sup>	345 ± 6 <sup>a</sup>	257± 10 <sup>b</sup>	199 ± 6 <sup>c</sup>	259±6 <sup>b</sup>	257±7 <sup>b</sup>
Cholesterol (mg dL <sup>-1</sup> )	91±3.8 <sup>c</sup>	118±1.1 <sup>a</sup>	99±2.9 <sup>b</sup>	99±3.7 <sup>b</sup>	94±0.6 <sup>bc</sup>	97±0.9 <sup>b</sup>
Triglycerides (mg dL <sup>-1</sup> )	106±3.0 <sup>e</sup>	201±8.4 <sup>a</sup>	135±5.0 <sup>c</sup>	129±6.5 <sup>cd</sup>	165±2.9 <sup>b</sup>	114±1.0 <sup>ed</sup>
LDL-c (mg dL <sup>-1</sup> )	47±1.5 <sup>d</sup>	60±1.0 <sup>a</sup>	49 ± 1.9 <sup>cd</sup>	55±1.9 <sup>ab</sup>	51.5±3.1 <sup>bcd</sup>	54 ± 1.3 <sup>cb</sup>
VLDL-c (mg dL <sup>-1</sup> )	22±0.86 <sup>c</sup>	40 ± 2.3 <sup>a</sup>	25±1.9 <sup>c</sup>	26±1.7 <sup>b c</sup>	30±2.7 <sup>b</sup>	23±0.36 <sup>c</sup>
HDL-c (mg dL <sup>-1</sup> )	18±0.58 <sup>a</sup>	7.6±0.35 <sup>d</sup>	13.5±0.6 <sup>c</sup>	13±1.30 <sup>c</sup>	14±0.53 <sup>bc</sup>	16±1.0 <sup>b</sup>

Values are the means of eight rats (Mean ± SE)

Within row, means with different superscript letter (a-e) differ significantly (P<0.01).

High density lipoprotein-cholesterol = HDL-c; Low density lipoprotein-cholesterol = LDL-c; very low density lipoprotein-cholesterol = VLDL-c

Table 2: Assay of plasma enzyme activities in control, diabetic and diabetic treated rats with barley (B), chromium (Cr), amino Acids (AA) and their combination (Comb.)

Parameter	Experimental groups					
	Control	Diabetic	Diabetic + B	Diabetic + Cr	Diabetic + AA	Diabetic + Comb.
AST (U dL <sup>-1</sup> )	43±2.2 <sup>a</sup>	26±0.62 <sup>d</sup>	32±0.24 <sup>c</sup>	38±0.59 <sup>b</sup>	30±0.35 <sup>cd</sup>	36±0.50 <sup>b</sup>
ALT (U dL <sup>-1</sup> )	79±0.74 <sup>cd</sup>	70±1.1 <sup>e</sup>	78±0.91 <sup>d</sup>	91±1.9 <sup>a</sup>	84±0.64 <sup>b</sup>	82±0.83 <sup>bc</sup>
AcP (U L <sup>-1</sup> )	48± 0.82 <sup>bc</sup>	40±0.2 <sup>d</sup>	47±0.29 <sup>c</sup>	50±0.29 <sup>ab</sup>	52±0.54 <sup>a</sup>	51±1.1 <sup>a</sup>
AlP (U L <sup>-1</sup> )	160±4.0 <sup>ab</sup>	130±2.0 <sup>d</sup>	148 ±1.5 <sup>bc</sup>	149±2.3 <sup>bc</sup>	142±8.2 <sup>cd</sup>	167±0.45 <sup>a</sup>
LDH (U L <sup>-1</sup> )	1187± 33 <sup>a</sup>	715±24 <sup>bc</sup>	1164±72 <sup>a</sup>	892± 42 <sup>b</sup>	1085± 54 <sup>a</sup>	676±116 <sup>c</sup>

Values are the means of eight rats (Mean ± SE)

Within row, means with different superscript letter (a-e) differ significantly (P<0.01).

Table 3: Assay of liver enzyme activities in control, diabetic and diabetic treated rats with barley (B), chromium (Cr), amino Acids (AA) and their combination (Comb.)

Parameter	Experimental groups					
	Control	Diabetic	Diabetic + B	Diabetic + Cr	Diabetic + AA	Diabetic + Comb.
AST*	178±3.5 <sup>a</sup>	116±0.93 <sup>c</sup>	113±1.7 <sup>c</sup>	127±1.4 <sup>b</sup>	129±1.3 <sup>b</sup>	111±1.0 <sup>c</sup>
ALT*	213±6.1 <sup>a</sup>	160±2.8 <sup>c</sup>	165±3.7 <sup>bc</sup>	175±1.3 <sup>b</sup>	163±2.9 <sup>c</sup>	167±6.4 <sup>bc</sup>
AcP*	87±1.2 <sup>b</sup>	80±0.55 <sup>c</sup>	82±1.5 <sup>c</sup>	88± 0.90 <sup>b</sup>	95±1.3 <sup>a</sup>	54±0.93 <sup>d</sup>
AlP*	225 ±16 <sup>a</sup>	133±12 <sup>d</sup>	168±7 <sup>bc</sup>	183± 10 <sup>bc</sup>	195±15 <sup>ab</sup>	151±2 <sup>cd</sup>

Values are the means of eight rats (Mean ± SE)

Within row, means with different superscript letter (a-e) differ significantly (P<0.01).

\* IU mg<sup>-1</sup>: international unit, the amount of the enzyme that under defined assay conditions will catalyze 1 mole of substrate per minute, per mg protein.

Chromium and amino acids showed a significant improvement in the lipid profile of alloxan-diabetic rats (Table 1). This observation is consistent with the results of Lien *et al.* [44] that indicated that insulin with its stimulated biological activity due to dietary chromium could increase the lipoprotein lipase activity and eventually decrease the content of triglycerides-rich lipoproteins. It also can increase liver LDL receptors, thereby reducing the LDL content. Therefore, the hypoglycemic effect of barley may be also due to its content of chromium. Also, Stone and Vanthiel [45] reported that the hypercholesterolemic effect of dietary amino acids is associated with down regulation of hepatic LDL receptors.

The decrease in the activities of plasma and liver AST, ALT, LDH, AlP and Acp (Tables 2 and 3)

indicated that diabetes may be induced hepatic dysfunction [4,7] and impaired synthesis of enzymes themselves from its store in liver. Thus the metabolic abnormalities caused by diabetes may result in disturbance of some metabolic enzyme synthesis. Supporting our finding it has been found by Larcan *et al.* [46] that liver was necrotized in diabetic patients. However, treatment of alloxan diabetic groups with barley water, chromium, amino acids and/or their combination for 28 consecutive days could restore the activities of the above enzymes to their normal levels. A possible explanation for the differential effects of barley and its components on the activities of these enzymes is that the treatments may inhibit the liver damage induced by alloxan.

The normal observation of beta-cell structure of the control group is consistent with the observation of Jorns *et al.* [47] for the same kind of cell. The changes in the

structure of beta-cells of alloxan-diabetic rats (Fig. 1 b) is consistent with studies of Lanzen and Paten [48] who reported that the injection of alloxan into experimental rats caused a selective specific necrosis of beta cells of the islets of Langerhans resulting in diabetes mellitus.

The recovery of  $\beta$ -cells of alloxan-diabetic rats treated with barley is support by the finding of Scheer [49], who stated that barley is used to support the body's own self-healing mechanisms and the components of barley aid the body in maintaining cells in healthy condition and work to correct abnormalities. The promising effect of chromium on pancreatic  $\beta$ -cells may be explained with the fact that chromium is directly affects certain genetic processes as ribonucleic acid (RNA) synthesis [50].

The present study demonstrated that treatment of diabetic rats with barley and some of its components (chromium and amino acids) could repair liver damage and restoring pancreatic  $\beta$ -cells deformation. This was manifested by the biochemical and immunoassay results and electron microscope study where the hypoglycemic and hypolipidemic action of barley may be due to its contents generally and in specific to its content of chromium and/or amino acids.

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