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Effect of Milk Protein Hydrolysate on Some Hematological Parameters of Type II Diabetic Rats

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Abstract: Milk protein-derived bioactive peptides could be used in human nutrition in both newborns and adults, since they are claimed to be health-improving components that can be used to reduce the risk of disease or to enhance certain physiological functions. This study investigated the effect of oral intake of milk protein concentrate (MPC) and milk protein concentrate hydrolysate (MPCH) on body weight, organ relative weight, and hematological parameters in alloxan-induced diabetes rats. The animals were divided into six major groups after stabilization of diabetes for one week. Diabetic and non-diabetic rats groups were received intra-gastric dose of MPC or MPCH (150mg/kg B.W) for six weeks. Feed treatment by MPC and MPCH significantly ($P<0.05$) increased the final body weight of normal rats and improved the body weight of diabetic rats and significantly protective ($p<0.05$) the liver, kidney, heart and lung of normal rats and reduced the fallers of these organs in diabetic rats. The diabetes groups treated by MPCH showed improvement in RBC, Hb, Ht and PLT values. Meanwhile WBC not affected. The oral intake of MPCH protected the normal range of Lymphocytes and S. Nutrophils in healthy and diabetic rats. The oral intake of MPCH caused significant ($P<0.05$) decrease of Monocytes in diabetic and non-diabetic rats, while the oral intake of MPC had no affected on Monocytes of diabetic and non-diabetic rats. MPC and MPCH reduced the harmful effect of T2D on body weight, organs weights of rats, and improvement most hematological parameters of normal and diabetes rats.

Keywords: Milk Protein, Protein Hydrolysate, Bioactive Peptides, T2 Diabetes

1. Introduction

Diabetes is prevalent in about 3% to 5% of the population in industrialized countries. Diabetes mellitus (DM) is a metabolic and chronic disease with a multifactorial etiology, caused by defects in insulin secretion and/or action and presenting hyperglycemia as a common manifestation [1]. Multiple metabolic disorders, including impaired lipid and lipoprotein metabolism, oxidative stress, subclinical inflammation, and vascular endothelial dysfunction, are common in DM [2].The major forms of diabetes, type 1 and type 2 diabetes, contribute at about 10 percent and 90 percent, respectively [3]. The worldwide incidence of type2 diabetes is increasing. It was estimated in 2000 that there were 171 million diabetics, while incidences for 2030 are estimated to reach 366 million people [4]. Type II diabetes (DM2) or non- insulin-dependent diabetes mellitus (NIDDM) is characterized by impaired insulin secretion or insulin resistance. DM2 is usually associated with obesity and hereditary disposition and it is the most common form of diabetes, affecting 90–95% of cases [5]. Loss of weight and increase in physical activity reduces the incidence of T2D and its vascular complications partially via anti-inflammatory mechanism. Diabetes is not only affected by the amount of dietary intake but also by the type of diet. The consumption of dairy products has been associated with a reduction of the

DM risk and an improvement in metabolic health [6]. The beneficial effects of milk have been related to its components such as calcium and whey proteins [7, 8]. Milk proteins are considered as the most important sources of bioactive peptides. Bioactive peptides show significant potential for use in health management strategies, particularly as components of drugs and functional foods for diabetes treatment [9]. The beneficial health effects of bioactive peptides are classified as cytomodulatory, mineral binding, antimicrobial, immunomodulatory, blood-pressure lowering (Angiotensin-converting enzyme ACE- inhibitory), antithrombotic, antioxidant and opioid like, in addition to cholesterol-lowering and enhancers of mineral absorption/bioavailability [10-14]. Bioactive peptides encrypted in major milk proteins are latent within the sequence of the parent protein molecule. Milk-derived bioactive peptides can be released during gastrointestinal digestion, food processing or by enzymatic and bacterial fermentation [15]. In relation to their mode of action, bioactive peptides may reach target sites at the luminal side of the intestinal tract, or after absorption, in peripheral organs [16]. Milk protein-derived bioactive peptides could be used in human nutrition in both newborns and adults, since they are claimed to be health-improving components that can be used to reduce the risk of disease or to enhance certain physiological functions [17]. Milk protein hydrolysates enhanced immunity in mice by stimulating host immunity, probably by increasing the weight of certain immune system organs, improving the level of hemolysin in serum, and enhancing the phagocytosis of macrophages [18]. The aim of this study was to investigate the biological effects of milk protein concentrate (MPC) and milk protein concentrate hydrolysate (MPCH) which contains bioactive peptides on body weight, relative organs weight and hematology alternation in alloxan-induced diabetic rats.

2. Materials and Methods

2.1. Animals

Sixty male healthy albino rats with weight ranged from 190 to 210 g were obtained from Institute of Graduate Studies and Research (IGSR), Alexandria University, Egypt. The local committee in Alexandria University, Egypt, approved the design of the experiments, and the protocol conforms to the guidelines of the National Institutes of Health (NIH).

2.2. Materials, Chemicals and Reagents

Cow milk protein concentrate (MPC) were obtained from Fonterra Ltd, Auckland, New Zealand, and its typical analysis was: Protein 69.89%, lactose 17%, minerals 7.2%, moisture 4.6% and milk fat 1.4%. Trypsin 2000 U/g (EX pancrease) from Loba Chemie, India. Alloxan was obtained from Alpha Co. India, dissolved in saline solution (0.9% sodium chloride, pH 7). The dose of alloxan used was 120mg/kg BW/day. The chosen dose of alloxan is well

documented to induce diabetes in rats, according to the previous studies of Mansour et al. [19]; Sheweita et al.[20].

2.3. Preparation of Milk Protein Hydrolysates

Preparation of milk protein hydrolysate was carried out according to the method of Otte et al, [21]. Protein solutions (2% w/w on protein basis) were made by dispersion of approximately 2.85 g of milk protein concentrate (according to the protein content) in 100 ml of distilled water, and stirring for 1 h at room temperature. Solutions were hydrated overnight at 5°C, after that, the pH was adjusted to 7.0 and 7.5 by 1% NaOH solution. A pretreatments using Trypsin enzyme solution (3.2 U mL^{-1}) was made in distilled water immediately prior to use as recommended by El-Sayed et al, [22]. For each hydrolysis experiment, 100 mL of protein solution was incubated at 40°C for 3, 6, 9, 20 and 24 h. Enzyme was inactivated by heating at 90°C for 15 min, and then cooling for 20 min in ice bath and then centrifugation (Sigma centrifuge 113 VWR International, Germany) for 10 min at 10,000 xg, the supernatant was used for further analyses as described below.

2.4. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE technique was carried out using the discontinuous buffer system described by Laemmli [23], and mentioned by Hames and Rickwood [24].

2.5. Analysis of Peptides by Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC)

Separation of the peptide extracts was carried out using a HPLC system (Agilent Technologies 1260) according to the method described by Awad, Lúthi-Peng, & Puhan [25].

2.6. Animals and Treatments

The animals were housed in cages under proper environmental conditions. Animals were housed 5 per cage and kept on commercial die (pellets). The chemical analysis of the pellets according AOAC [26] showed that they contained 17.5% crude protein, 14.0% crude fiber, 2.7% crude fat and 2200Kcal./kg diet), tap water provided ad libitum and kept for two weeks for acclimatization and maintained at 25 ± 1°C with 12 h dark and light cycle [27]. The protein concentrate hydrolysates were evaluated by RP-HPLC and bitter taste. Sample prepared by trypsin 8X at pH 7.5 and 40°C for 20h (Fig.4), was selected to be used in animal treatments. The experimental period was six weeks after stabilization of diabetes for one week and the animals were divided into six major groups as following:

Group I: ten rats were kept as a control.

Group II: ten rats were kept as a diabetic control.

Group III: ten rats were received intra-gastric dose of milk protein concentrate
800 mg/ kg B.W / day (MPC group).

Group IV: ten rats were kept as a diabetic received intra-

gastric dose of milk protein concentrate 800 mg / kg B.W/ day (MPCD group).

Group V: ten rats were received intra-gastric dose of milk protein concentrate hydrolysate 800 mg / kg B.W/ day (MPCH group).

Group VI: ten rats were kept as a diabetic received intra-gastric dose of milk protein concentrate hydrolysate 800 mg / kg B.W/ day (MPCHD group).

2.7. Body Weight and Organs Weight

At first and end of the experimental period, body weights of rats were recorded. Animals were anesthetized with ether and sacrificed. Liver, kidney, heart, lung, spleen, testes, brain and pancreas were immediately removed and weighted. Then the organs weight/body weight ratio was calculated. The relative weight of organs (%) was calculated as g/100g body weight.

2.8. Hematological Parameters

The blood samples were collected in two tubes: one containing EDTA (anti-coagulant) and the other containing Heparin (anti-coagulant). Non coagulated blood by EDTA was tested shortly after collection by Particle counter (from ERMA INC.-Tokyo. Model PCE-210) for measuring total erythrocyte count (TEC), platelets count (PLT), hemoglobin (Hb), hematorit (Ht), red cell distribution width (RDW), mean cell volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) total leukocyte counts (TLC), white blood cell count (WBC), lymphocytes, segmented nutrophils, monocytes and eosinophils.

2.9. Statistical Analysis

The data were analyzed by a general linear model procedure of the Fisher's protected least-significant difference (PLSD) test using SAS, 2004 (SAS Institute, Inc., Cary, NC) [28]. This test combines ANOVA with a comparison of differences between the means of the treatments at the significance level of $P \leq 0.05$. Correlations were calculated using Pearson's correlation coefficient.

3. Results

3.1. Poly Acrylamide Gel Electrophoresis (PAGE)

Thus in this study, SDS- PAGE technique was applied in analysis of MPC as well as in monitoring their proteolysis rate by trypsin. Figure (1, 2 and 3) illustrated the electrophoretic patterns of trypsin treated-milk protein concentrate at 2X, 4X, and 8X enzyme for 3, 6, 9, 20 and 24 h/ pH 6.8 and 7.5. It is clear that the behavior of MPC was not affected by enzyme action until nine hours. While after 20 and 24 hours of treatments, there were few and very small peptides, having MW less than 20 KD, are appearing on the gel. These peptides were differences in their intensities and migration positions. The obtained results concluded that the action of enzyme on MPC was affected by the time, pH and enzyme: substrate ratio.
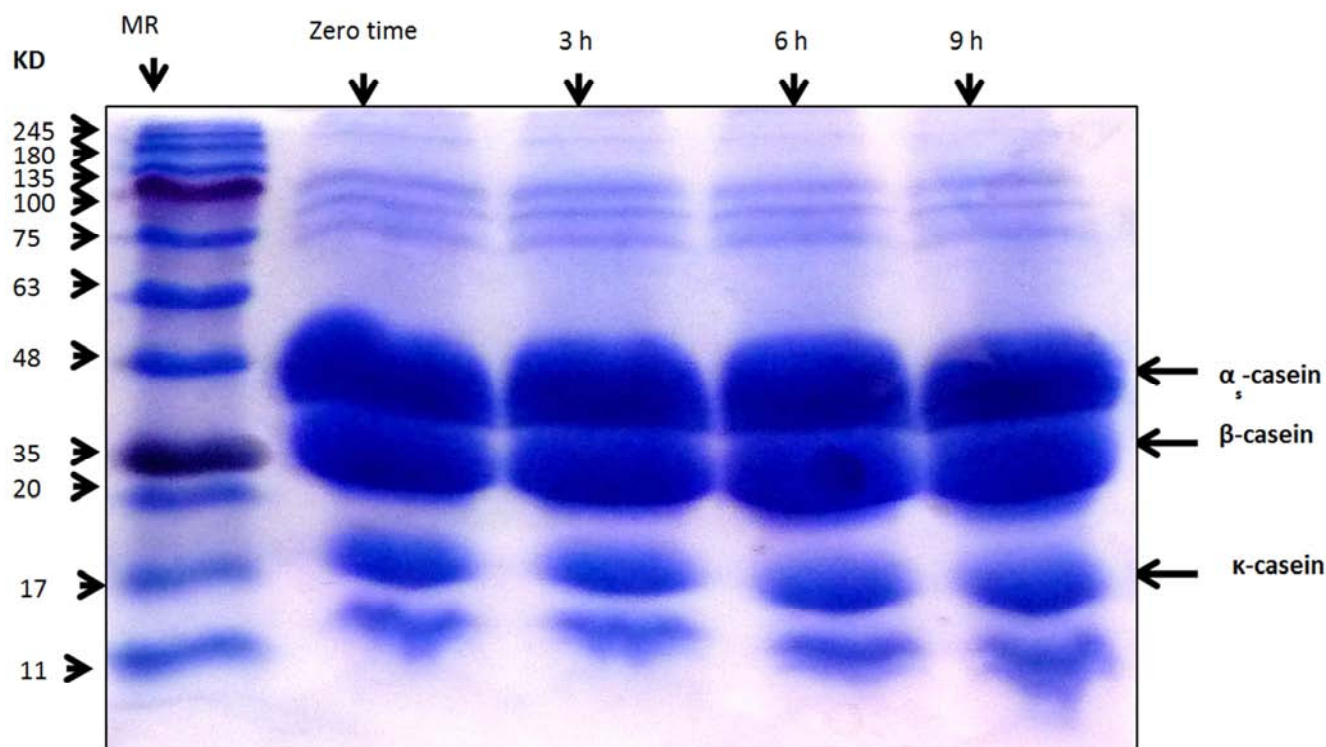

Figure 1. *SDS-PAGE of milk protein concentrate and milk protein concentrate treated by trypsin (2X) at pH7.5 for 3, 6 and 9 h/40°C.*
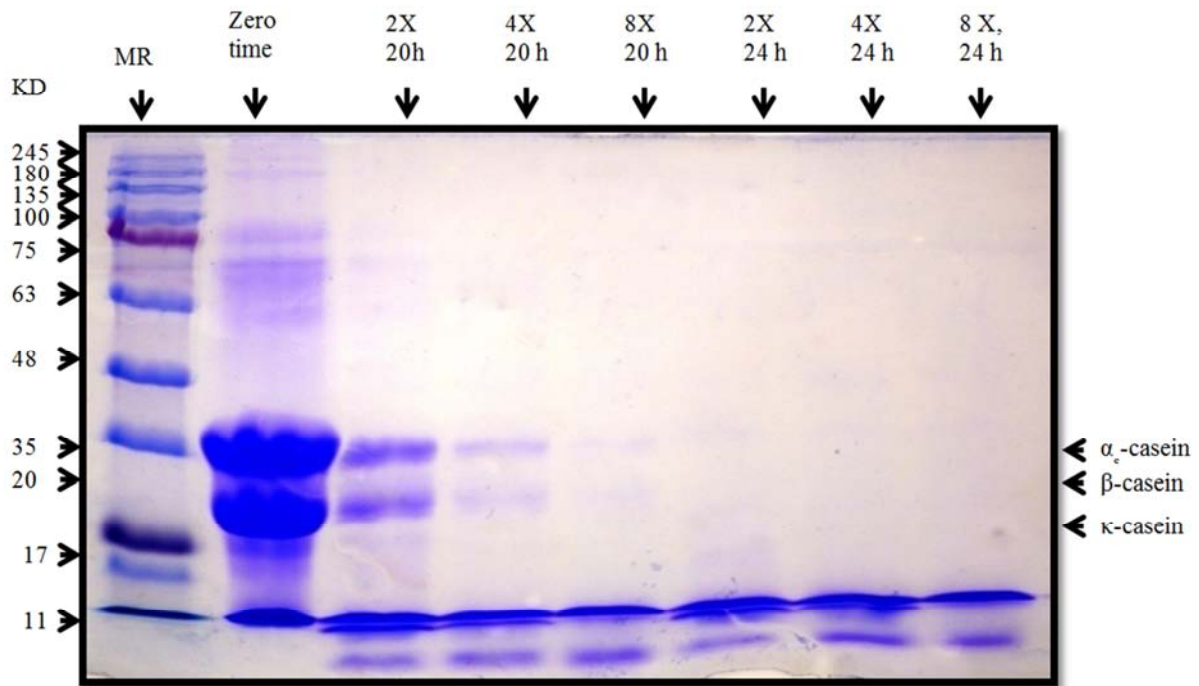
Figure 2. SDS-PAGE (12.5% T) of milk protein concentrate and milk protein concentrate hydrolysates no1, 2, 3, 4, 5 and 6 (samples treated by trypsin 2,4 and 8X at pH 6.9 and 40°C for 20 and 24 h respectively). MR: standar protein marker.
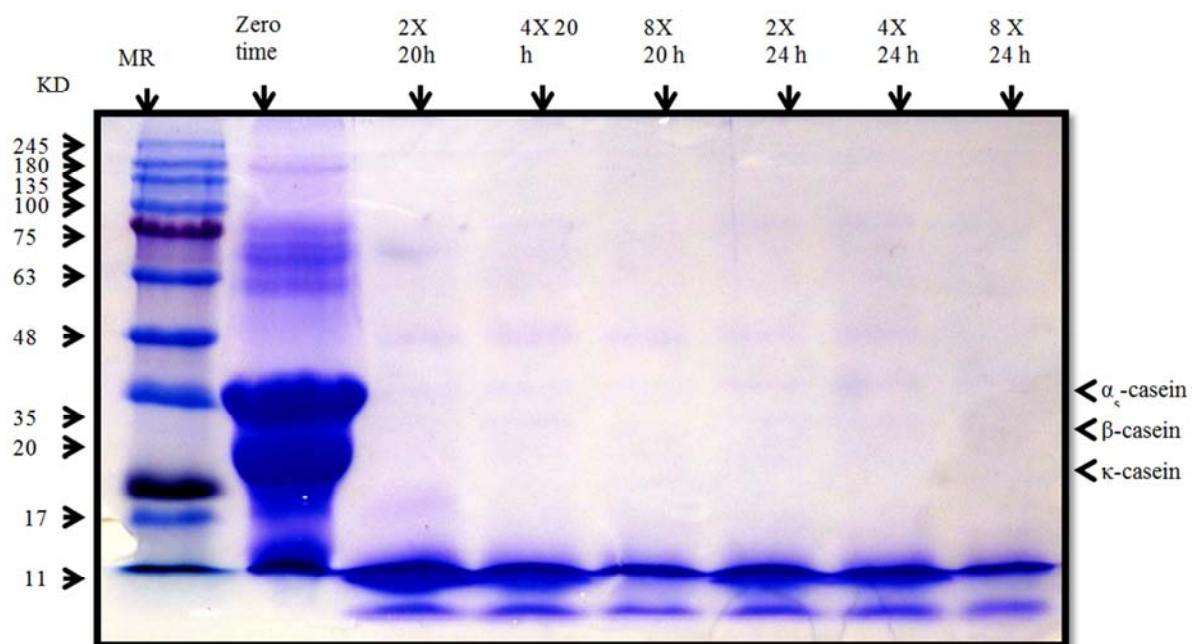

Figure 3. SDS-PAGE (12.5% T) of milk protein concentrate and milk protein concentrate hydrolysates no 7, 8, 9, 10, 11 and 12 (samples treated by trypsin 2,4 and 8X at pH 7.5 and 40°C for 20 and 24 h respectively). MR: standar protein marker.

3.2. Reverse Phase High Performance Liquid Chromatography (RP-HPLC)

In peptide chemistry, HPLC has gained importance as an analytical tool because of its exquisite sensitivity, speed, and resolving power. The most useful method for peptide separation is HPLC. Commercially available reversed-phase columns allow rapid separation and detection of their hydrophilic and hydrophobic characteristics. The hydrolysate of all MPC treatments were elated by RP-HPLC, chromatograms showed that the trypsin (2x, 4x and 8x) produced more peptides after 24 h than after 20h of incubation, and the hydrolysate at pH 7.5 produced small peptides than at pH 7. According to the evaluation of bitterness and RP-HPLC, the treatment of trypsin 8X at pH 7.5 and 40°C for 20h (Figure 4) was selected to use in the experiment.
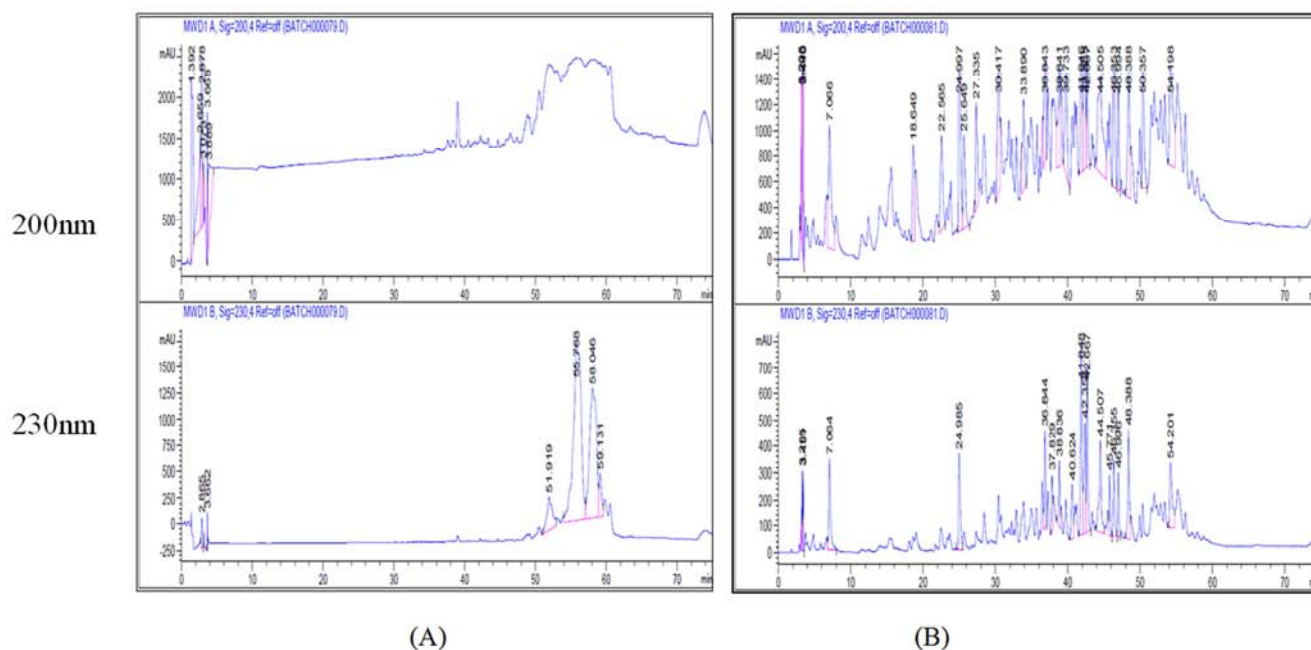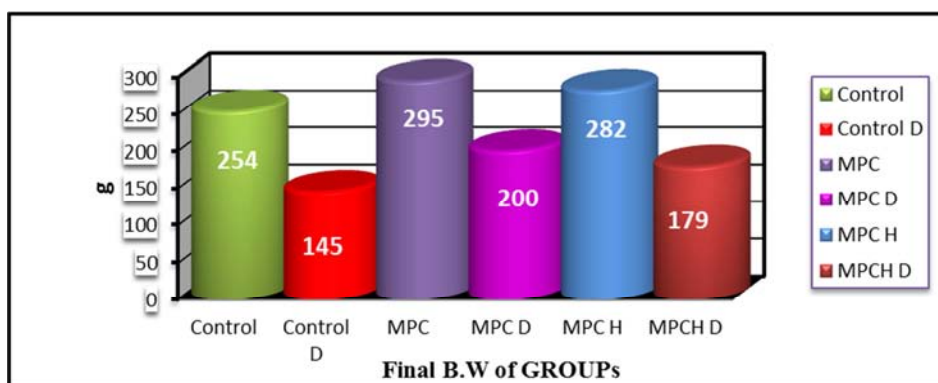
Figure 4. *RP-HPLC pattern of (A) milk protein concentrate, (B) milk protein concentrate hydrolysate MPCH treated by trypsin 8X at pH 7.5 and 40°C for 20h).*

3.3. Body Weight and Relative Organs Weight of Diabetic and Non-Diabetic Rats

Figure (5, 6 and 7) represent body weight and relative weight of liver, kidney, heart, lung, brain, testes, spleen and pancreas of male diabetic and non-diabetic rats treated by oral intake of MPC and MPCH. Treatment with alloxan alone caused a significant ($p<0.05$) decreasing in body, pancreas weights, and significant increasing in liver, kidney, heart and lung relative weights in comparison with control. Meanwhile, no significant ($p>0.05$) differences were found in brain, spleen and testes weights of diabetic rats. On the other side, the treatments with MPC and MPCH showed a significant ($P<0.05$) increasing in the final body weight of normal and diabetic rats when compared to its control, meanwhile the body weight of diabetic rats treated with MPC or MPCH was not reached the weight of normal control. The treatment by oral intake of MPCH significantly ($P<0.05$) reduced the liver weigh in diabetic rats when compared with diabetic control, however oral intake of MPC has no effect. On the other hand, oral intake of MPC and MPCH not affected the liver weight

in normal rats compared with the control group. The obtained results showed that the oral intake of MPCH caused significant ($P<0.05$) increase in pancreas weight of diabetic rats compared with diabetic control, while MPC has no effect. Also, there was no significant ($P>0.05$) difference in pancreas weight of normal rats treated with oral intake of MPC and MPCH. On the other side, the oral intake of MPCH significantly ($P<0.05$) reduced kidney and lung weights of diabetic rats compared with that in diabetic control, while there was no significant ($P>0.05$) difference of kidney and lung weights between diabetic rats treated by MPC and diabetic control. Treatments by MPC or MPCH have no effect on kidney and lung weights of normal rats compared to that of control. There were no significant ($P>0.05$) difference in heart, testes, brain, spleen of diabetic rats treated with MPC and MPCH when compared with that of diabetic control. Also, there were no significant ($P>0.05$) difference in heart, testes, brain, spleen of normal rats treated with MPC and MPCH when compared with that of normal control group.
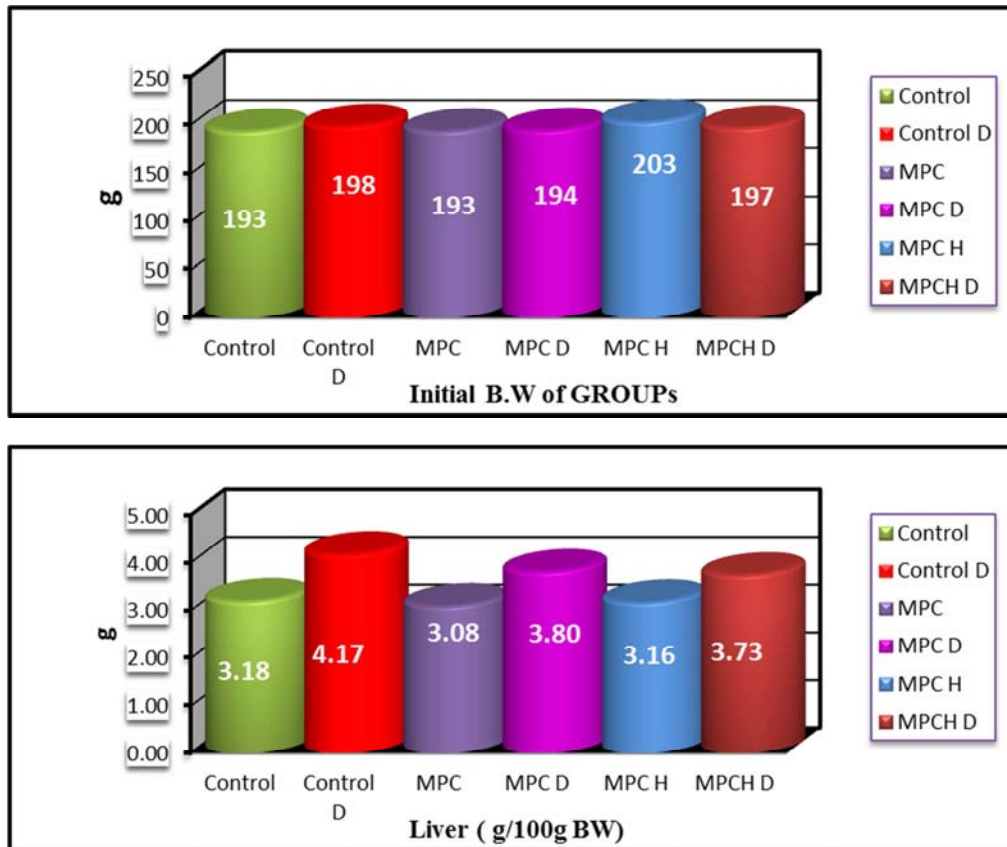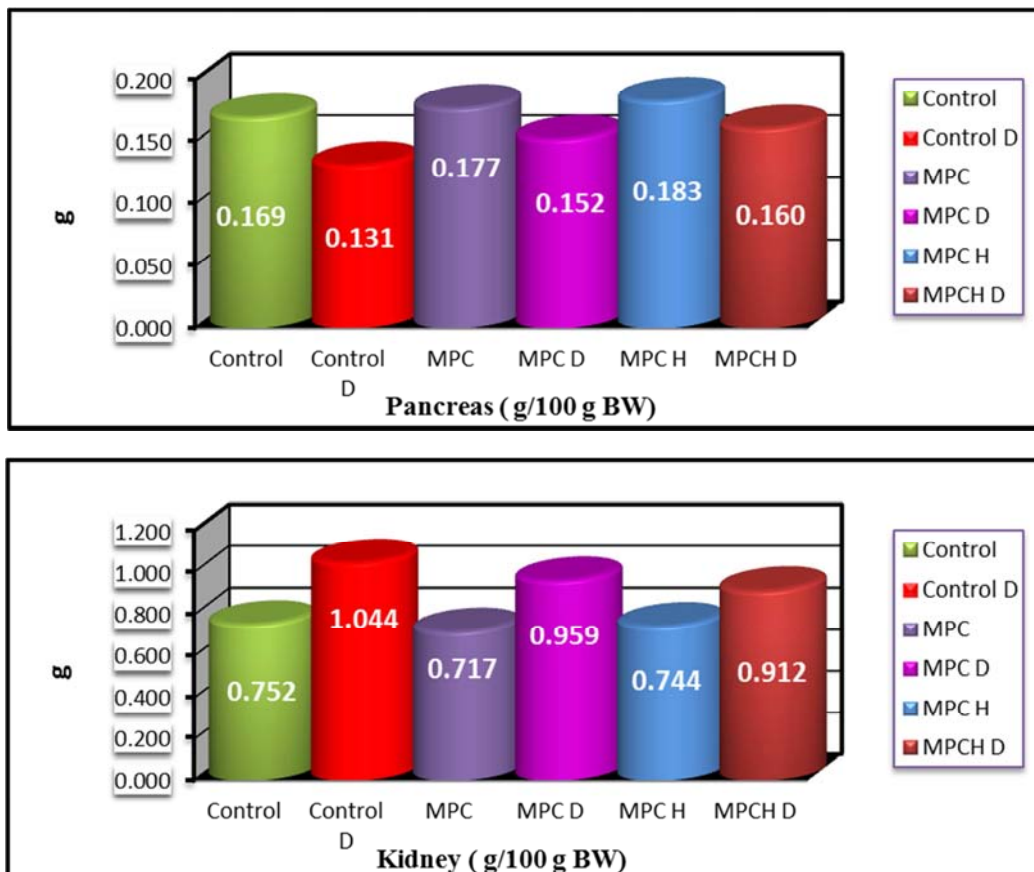
Figure 5. Changes in body weight (BW; g) and relative weight (g/100gm) of liver, of male diabetic (D) and non-diabetic rats treated with milk protein concentrate (MPC) and milk protein concentrate hydrolysate (MPCH).
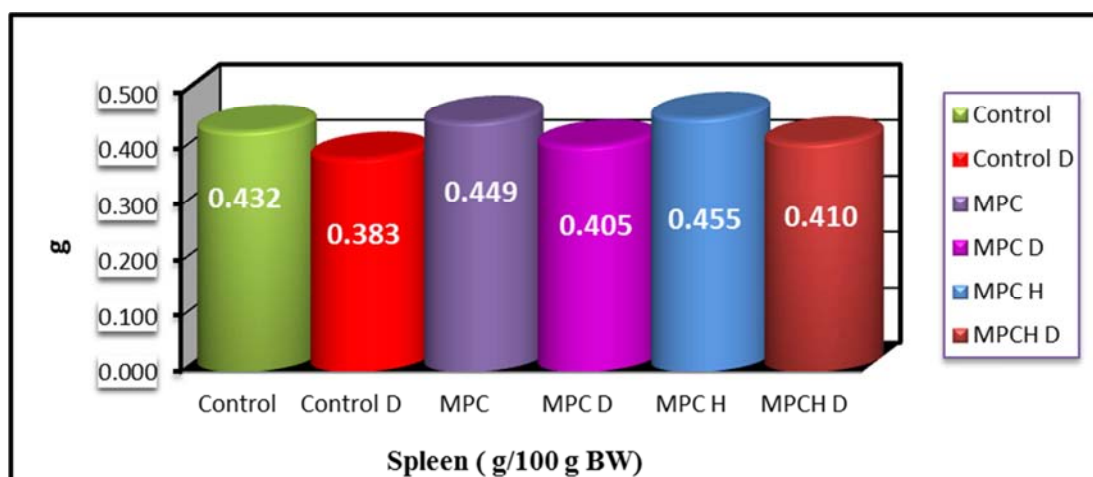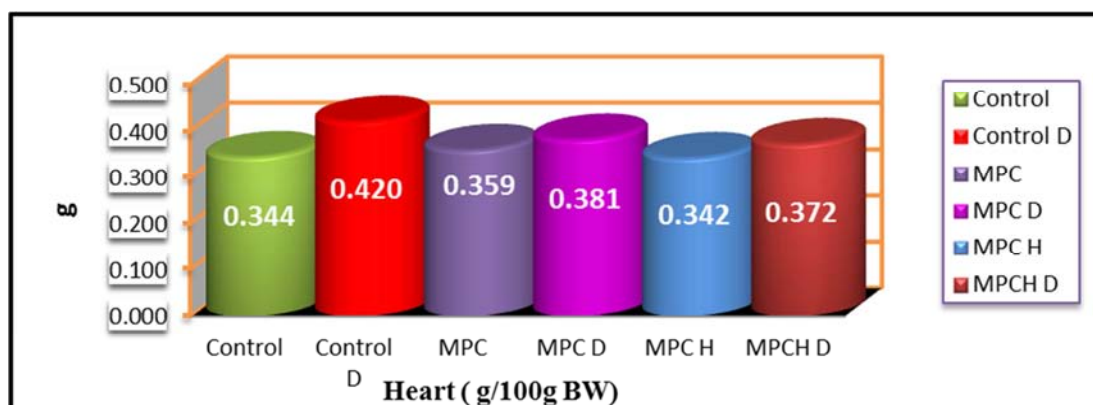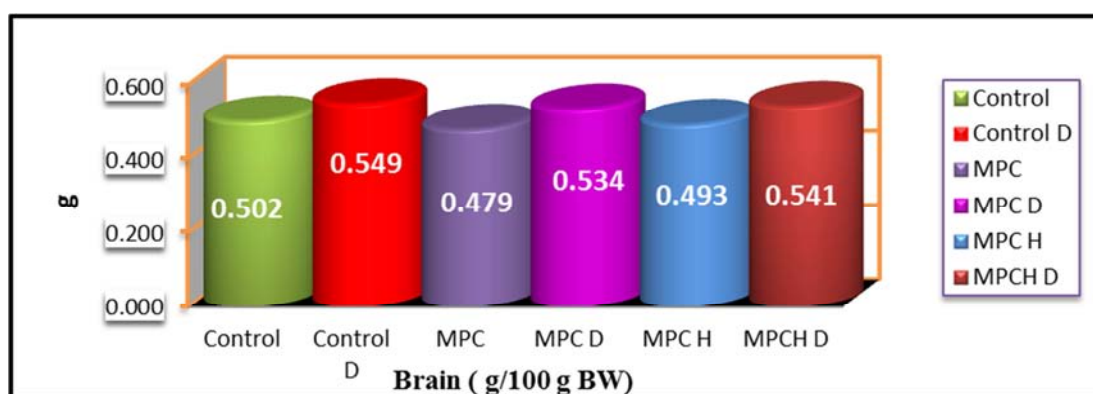
Figure 6. Changes in relative weight (g/100g BW) of pancreas, kidney and spleen of male diabetic(D) and non-diabetic rats treated with milk protein concentrate (MPC) and milk protein concentrate hydrolysate (MPCH).

Figure 7. *Changes in relative weight (g/100g BW) of heart, lung, brain and testes of male diabetic(D) and non-diabetic rats treated with milk protein concentrate (MPC) and milk protein concentrate hydrolysate (MPCH).*
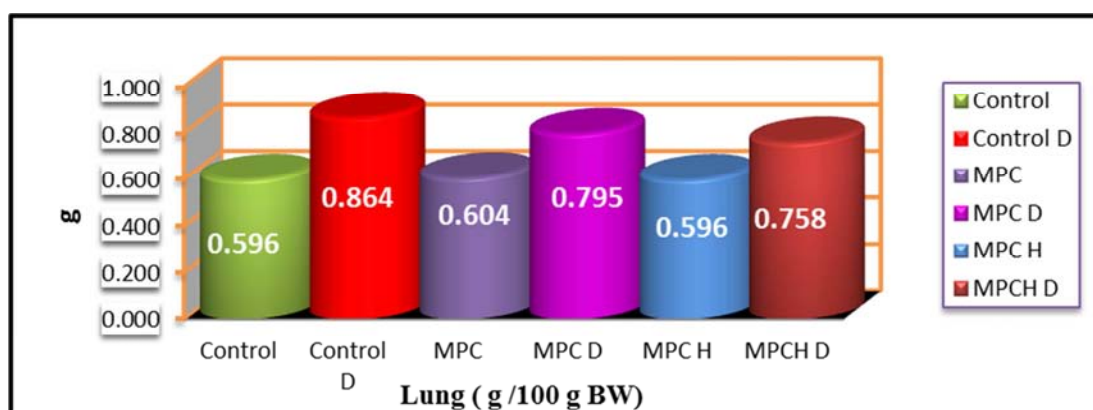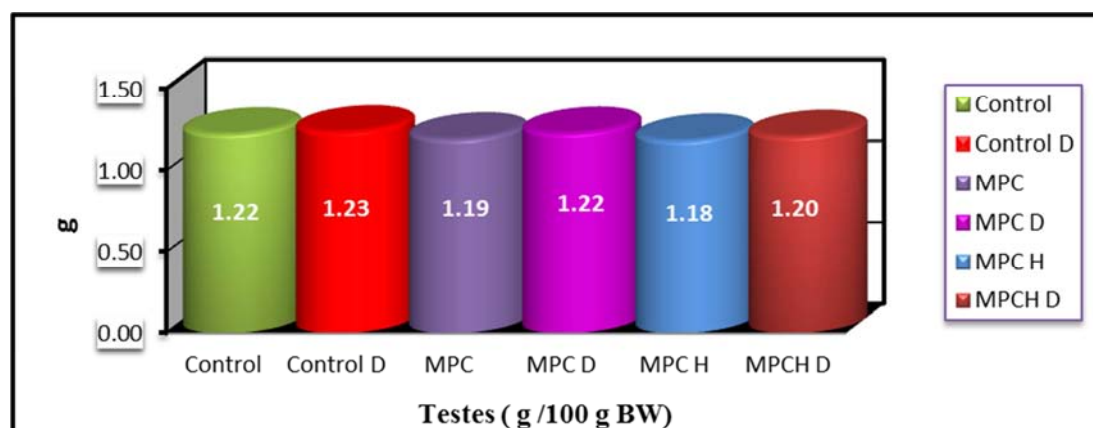
3.4. Changes in Hematological Parameters of Diabetic and Non-Diabetic Rats

Table (1) presents the distribution of red blood cells counts (RBC), Hemoglobin% (Hb g/dl) and Hematocrit (Ht%) in blood of diabetic and non-diabetic rats. The results showed that the RBC count, Hb% and Ht% of diabetic control rats were significant (P< 0.05) decreased when compared to normal control rats. Both treatment agents (MPC and MPCH) showed a detectable amelioration of RBC count of diabetic rats and returned the values to normal range. Meanwhile, there were no significant (P>0.05) difference in RBC and Ht of non-diabetic rats treated by oral intake of MPC and MPCH when compared to control normal rates. While the treatment by oral intake of MPC and MPCH significant (P< 0.05) increased the Hb% of non-diabetic rats. Concerning to the Platelets count (PLT), the data present in Table (1) showed significant (P<0.05) decreasing of PLT in diabetic control group than that in all other groups. On the other hand, treatments with oral intake of MPC and MPCH of diabetic rats returned the values of the PLT to near the normal range of the control group. In addition, treatment with oral intake of MPCH of normal healthy rats had no significant (P>0.05) difference in values of PLT, while the treatment with MPC

significant (P<0.05) decreased the PLT compared to control. Table (2) presents the distribution of red cell distribution width (RDW), mean cell volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration MCHC in blood of diabetic and non-diabetic rats treated by oral intake of MPC and MPCH. The results showed significant (P<0.05) increasing in red cell distribution width (RDW) with the diabetic control group when compared to normal control rats. Meanwhile, there were no significant (P<0.05) differences among normal control and all other treated groups. The mean cell volume (MCV) values are presented in Table (1). There is no significant (P>0.05) difference was observed between control and all other treated groups. Concerning to the mean corpuscular hemoglobin (MCH), there were significant (P<0.05) decreasing of MCH in the diabetic control group when compared to other groups. While the MCH was significant (P<0.05) increased in normal and diabetic rats treated with MPC and MPCH when compared to control rates. The results showed that there was no significant (P>0.05) difference in mean corpuscular hemoglobin concentration MCHC values between normal control and diabetic control groups (Table 1).

Table 1. *Changes in red blood cells count (RBC), hemoglobin% (Hb g/dl), hematocrit (Ht%), platelets count (PLT), red cell distribution width (RDW), mean cell volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) of male diabetic(D) and non-diabetic rats treated with milk protein concentrate (MPC) and milk protein concentrate hydrolysate (MPCH).*

Parameters	Experimental groups					
	Control	Control D	MPC	MPC D	MPCH	MPCH D
RBC*10⁶/µl	8.40 ± 0.076^{ab}	8.16 ± 0.08^{b}	8.44 ± 0.158^{ab}	8.34 ± 0.114^{ab}	8.49 ± 0.056^{a}	8.31 ± 0.207^{ab}
Hbg/dl	14.15 ± 0.184^{c}	13.27 ± 0.130^{d}	14.76 ± 0.234^{a}	14.29 ± 0.175^{bc}	14.55 ± 0.064^{ab}	14.15 ± 0.197^{c}
HT%	45.25 ± 0.479^{a}	42.75 ± 0.854^{b}	45 ± 0.707^{a}	44.91 ± 0.417^{a}	44.25 ± 1.031^{ab}	44 ± 0.577^{ab}
PLT	575 ± 6.45^{a}	453 ± 6.02^{d}	547 ± 4.93^{b}	530 ± 6.39^{c}	572 ± 6.46^{a}	548 ± 5.34^{b}
RDW	17.1 ± 0.147^{bc}	19.06 ± 0.306^{a}	16.5 ± 0.642^{c}	17.53 ± 0.417^{b}	16.6 ± 0.122^{c}	$17.3 \pm 0.523b^{c}$
MCV	53.25 ± 0.751^{a}	52.1 ± 0.443^{a}	52.1 ± 0.696^{a}	52.65 ± 0.312^{a}	52.13 ± 0.379^{a}	52.9 ± 0.780^{a}
MCH	16.85 ± 0.096^{bc}	16.45 ± 0.119^{d}	16.75 ± 0.21^{cd}	17.16 ± 0.062^{ab}	17.3 ± 0.212^{a}	17.32 ± 0.193^{a}
MCHC	31.5 ± 0.227^{b}	31.36 ± 0.12^{b}	32.33 ± 0.165^{a}	32.3 ± 0.227^{a}	32.43 ± 0.184^{a}	32.17 ± 0.347^{a}

Values are expressed as means ± SE; n = 7 for each treatment group.

Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different,

p<0.05.

While the oral intake of MPC and MPCH significant (P<0.05) increased the MCHC of normal and diabetic rats when compared to controls. Table (2) presents the distribution of white blood cell count (WBC), Lymphocytes, Segmented Nutrophils, Monocytes and Eosinophils in blood of diabetic and non-diabetic rats treated by oral intake of MPC and MPCH. There were significant (P<0.05) increasing of WBC in diabetic control group when compared to other treated groups. While there were no significant (P>0.05) difference between control normal group and all other treated groups. The percentage of Lymphocytes was a significant (P<0.05) decreased in the diabetic control and significant (P<0.05) increased in normal group treated by MPC. On the other hand, there were no significant (P>0.05) difference among normal control group and normal rates treated by MPCH, diabetic rats treated by MPCH and MPC. Treated

animals with alloxan alone showed a significant increasing (p<0.05) in percentage of blood segmented nutrophils and monocytes. Meanwhile, treatment with oral intake MPC and MPCH of diabetic rats returned these values to be near the normal value of control group. However, there is no significant (P>0.05) difference in blood S. Nutrophils level of normal rats treated by oral intake of MPC and MPCH when compared with control group. On the other hand, Eosinophils value was significant (P<0.05) decreased with diabetic control group when compared to normal control. On the other side, the treatment with oral intake MPC and MPCH of diabetic rats returned these values to be similar to the normal value of control group. In addition, the treatment with the oral intake of MPC and MPCH of normal healthy rats significantly (P<0.05) increased the Eosinophils of MPCH group and there is no significant (P>0.05) difference between MPC group and control group.

Table 2. Changes in white blood cell count WBC, lymphocytes, segmented neutrophils, monocytes, and eosinophils of male diabetic (D) and non-diabetic rats treated with milk protein concentrate (MPC) and milk protein concentrate hydrolysate (MPCH).

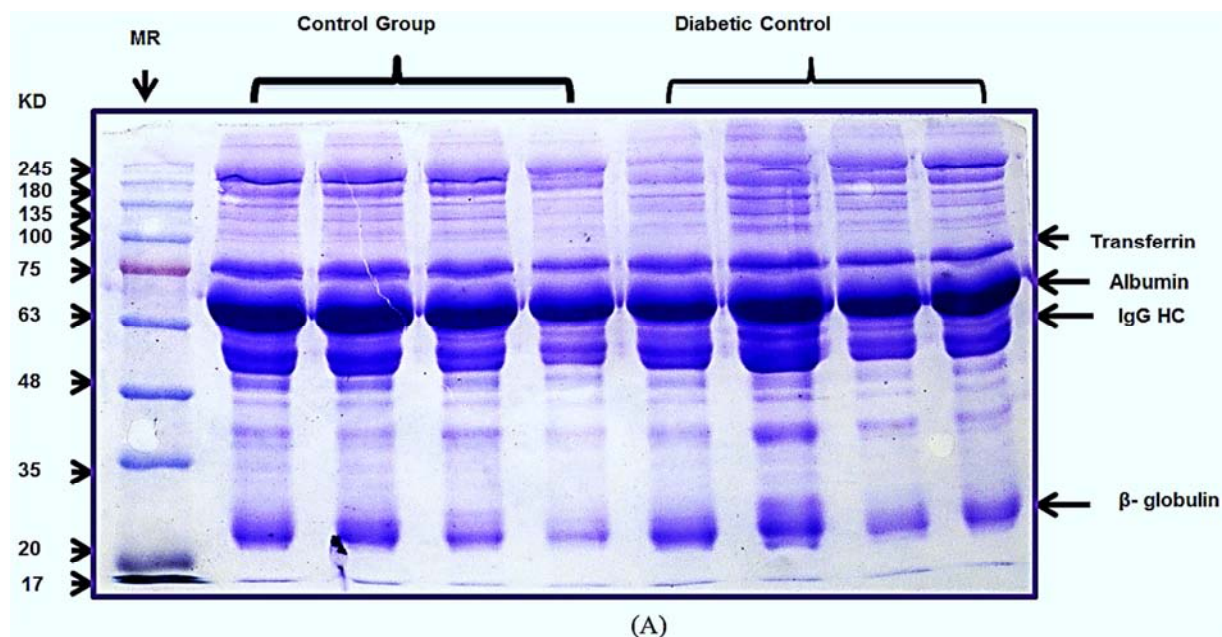| Parameters	Experimental groups					
	Control	Control D	MPC	MPC D	MPCH	MPCH D
WBC*10^3/μl	8.73 ± 0.411^b	10.65 ± 0.708^a	8.8 ± 0.730^b	9.25 ± 0.176^b	8.66 ± 0.478^b	8.96 ± 0.464^b
Lymphocytes%	76.5 ± 1.44^b	73.25 ± 0.479^c	80.25 ± 1.65^a	75.77 ± 2.11^{bc}	76 ± 0.816^{bc}	76.07 ± 1.08^{bc}
Segmented Neutrophils%	15 ± 0.408^c	17.32 ± 0.624^a	15.32 ± 0.624^{bc}	16.5 ± 0.645^{ab}	15 ± 0.707^c	15.75 ± 0.854^{bc}
Monocytes%	4.67 ± 0.236^{ab}	5.38 ± 0.375^a	5 ± 0.408^{ab}	5 ± 0.408^{ab}	4.5 ± 0.204^b	4.38 ± 0.239^b
Eosinophils%	2.75 ± 0.25^{ab}	2.3 ± 0.178^b	2.75 ± 0.25^{ab}	2.7 ± 0.071^{ab}	3 ± 0.204^a	2.75 ± 0.250^{ab}

Values are expressed as means ± SE; n = 7 for each treatment group

Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different, p<0.05.

SDS-PAGE of plasma proteins

The effect of oral intake of milk protein concentrate

(MPC) and milk protein concentrate hydrolysate (MPCH) on plasma proteins were mentioned by SDS- PAGE technique (Figure 8). The obtained results recommended that the all treatments have no effect on the behavior of plasma proteins, as there were no difference between the pattern of all treated groups in migration and concentration of albumin and globulin.
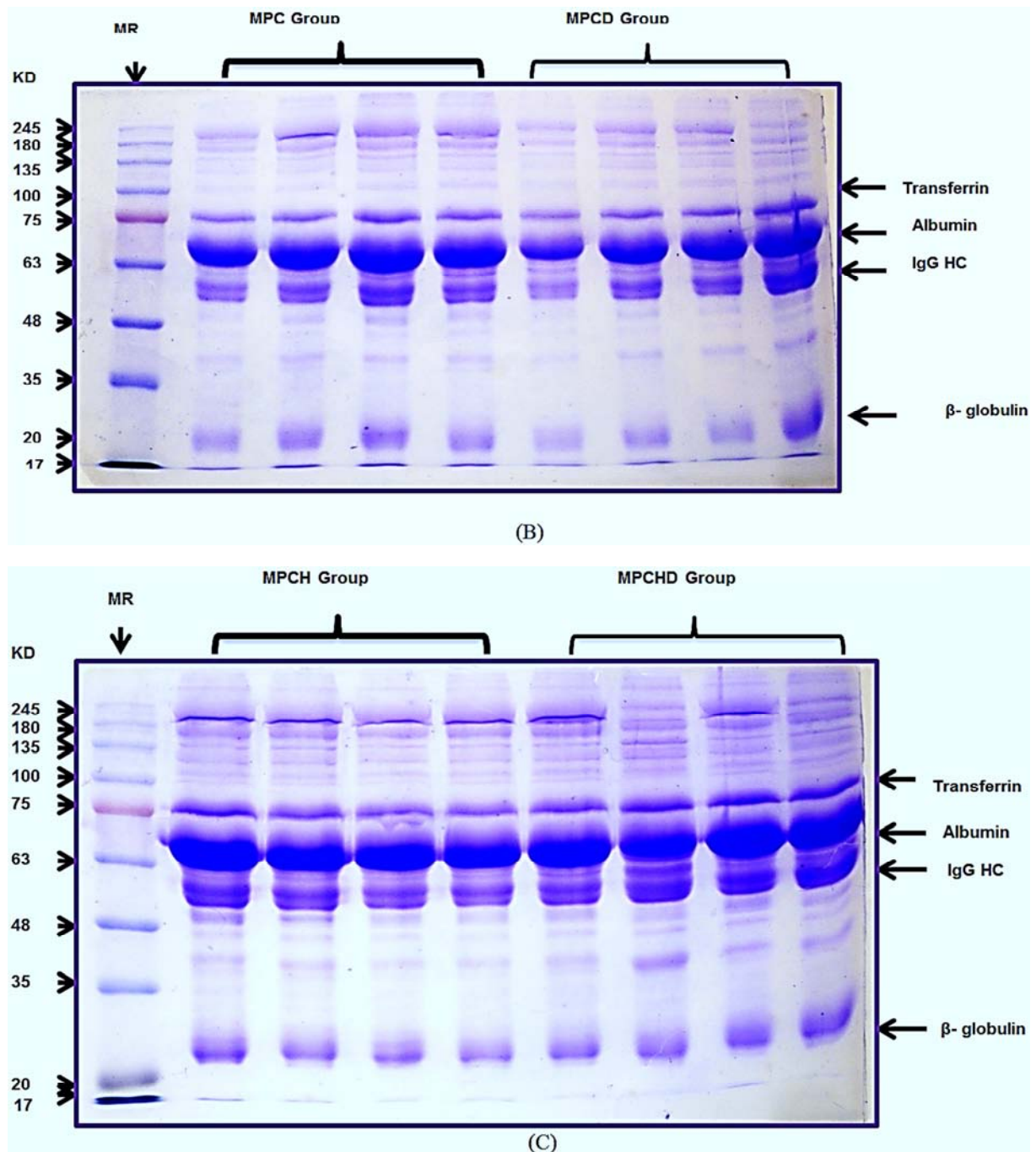

(A)

Figure 8. SDS-PAGE (10% T) of blood plasma (A) control and diabetic control group, (B) MPC and MPC D groups and (C) MPCH and MPCH D groups.

4. Discussion

Treatment of rats with alloxan alone caused a significant (p<0.05) decreasing in body, pancreas weights, and increasing in liver, kidney, heart and lung relative weights. These results were agreed with Zhang et al. [29]. Treated animals with alloxn alone showed a significant increasing (p<0.05) in percentage of blood segmented nutrophils and monocytes. Meanwhile, treatment with oral intake MPC and MPCH of diabetic rats returned these values to be near the normal value of the control group. However, there is no significant (P>0.05) difference in blood S. Nutrophils level of

normal rats treated by oral intake of MPC and MPCH when compared with control group. On the other hand, Eosinophils value was significant (P<0.05) decreased with diabetic control group when compared to normal control. On the other side, the treatment with oral intake MPC and MPCH of diabetic rats returned these values to be similar to the normal value of the control group. In addition, the treatment with the oral intake of MPC and MPCH of normal healthy rats significantly (P<0.05) increased the Eosinophils of MPCH group and there is no significant (P>0.05) difference between MPC group and control group.   Most of the obtained results are in agreement with Helal et al.[30], who found a

highly significant decrease (p<0.01) in RBCs count, Hb concentration and Ht value in diabetic rats compared with that in control group. Concerning to the white blood cell count, there were a significant increasing (p<0.01) in diabetic group when compared to control group. There were insignificant changes in the percentage of eosinophils, monocyte and staff neutrophils in diabetic group compared to the normal control. Lymphocytes showed a highly significant (p<0.01) decrease when compared with control group throughout the experimental period. Also, Mahmoud [31] found that diabetic group showed non-significant change (P > 0.05) in the percentage of eosinophils, however, there was a significant increase (P< 0.01) in neutrophils, monocytes and basophils. On the other hand, lymphocytes showed a significant decrease (P< 0.01) when compared with normal control group throughout the experimental period. In addition, he found that RBC, Hb, MCHC, MCH, PCV and MCV of control diabetic was the lowest that of control. The link between chronic diseases and anemia is well characterized [32]. The occurrence of anemia in diabetes mellitus has been reported due to the increased non-enzymatic glycosylation of RBC membrane proteins, which correlates with hyperglycemia [33]. Oxidation of these proteins and hyperglycaemia in diabetes mellitus causes an increase in the production of lipid peroxides that lead to hemolysis of RBC [34].

Our results showed that diabetic rats recorded a decrease in RBCs count, Hb content and Hct value.These rats suffered from anemia, which may be resulting from the toxic effect of alloxan used to induce diabetes in these rats. This anemia could be attributed to destruction of RBCs and reduced rate of its release from the bone marrow to the blood. Several studies attributed this anemia to increase in lipid peroxidation of the erythrocyte cell membrane [35, 36]. The increasing of neutrophil number may be due the engagement of these cells in the phagocytic process against different antigens. The increase in neutrophils also may be due to decrease its phagocytic activity in hyperglycemia and due to increase in hemopoitic activity after releasing the granules of neutrophil by exocytosis to lyses the antigens extra-cellularly [37].

5. Conclusion

Treatment of rats with alloxan alone caused a significant (p<0.05) decreasing in body, pancreas weights, and increasing in liver, kidney, heart and lung relative weights. However, brain, spleen and testes weights were not affected when compared with control. Intra-gastric treatment by MPC and MPCH significantly (P<0.05) increased the final body weight of normal rats and improved the body weight of diabetic rats and significantly protective (p<0.05) the liver, kidney, heart and lung of normal rats and reduced the failures of these organs in diabetic rats. The diabetes groups treated by MPCH showed improvement in RBC, Hb, Ht and PLT values. Meanwhile WBC not affected. The oral intake of MPCH protected the normal range of Lymphocytes and S. Nutrophils in healthy and diabetic rats. The oral intake of

MPCH caused significantantly (P<0.05) decrease of Monocytes in diabetic and non-diabetic rats, while the oral intake of MPC had no affected on Monocytes of diabetic and non-diabetic rats. From these results it can be concluded that the milk protein concentrate hydrolysate (MPCH) is characterized by a high biological value such as protective the hematological parameters in normal and diabetic rats and reduce the stress on various organs of the body because of diabetes.

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