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HEPATOPROTECTIVE ACTIVITY OF ALPHA-MELANOCYTE STIMULATING HORMONE (α-MSH) AGAINST CARBON TETRACHLORIDE (CCL_4) INDUCED HEPATOTOXICITY IN RATS

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Abstract- This study was designed to evaluate the hepatoprotective effect of Alpha-melanocyte Stimulating Hormone (α-MSH) against Carbon tetrachloride (CCl_4) induced hepatotoxicity in experimental rats in vivo. Sixty male Wister rats weighing 160-190 g respectively were used for the study and were divided into four groups, with 15 animals in each group. Group I consisted of nonhepatotoxic rats that received only the vehicle (0.5 mL/kg body weight) and served as a control group. Group II, the animals were injected intramuscularly with hormone α-MSH (sigma formula) 2mg/100g of body mass every alternate day for 15 days. Group III administered carbon tetrachloride (CCl4; 2 ml/kg body weight) rally every alternate day for 15 days to produce the hepatotoxity in rats. Groups IV (treated hepatotoxic group), the animals were administered CCl4; 2 ml/kg body weight and treated with hormone α-MSH (sigma formula) 2mg/100g of body mass every alternate day for 15 days. At the end of the experiment, the animals were sacrificed and blood was collected directly. Serum was separated after and analyzed for various biochemical parameters, i.e. Alanine transaminase (ALT), Aspartate transaminase (AST), alkaline phosphates (ALP), total and direct bilirubin, total protein and albumin. Our results showed that the administration of 2 ml/kg body weight of carbon tetrachloride (CCl_4) every alternate day for 15 days has enhanced the ALT, AST, ALP, total and direct bilirubin and liver weight, and reduced the total protein and albumin in comparison with the control group. The treatment with hormone α-MSH (sigma formula) 2mg/100g of body mass has brought back the altered levels of biochemical markers to the near normal levels. In our study, we observed that there is a significant ($P < 0.05$) increase in the concentration of total cholesterol, triglycerides, LDL cholesterol and a significant ($P < 0.05$) decrease in HDL cholesterol in the hepatotoxic rats. Also we investigated some hematological parameters and It was found that the RBC and WBC count, PCV, Hb, ESR, neutrophil percentage and lymphocyte percentage were significant decreased (($P < 0.05$) in the hepatotoxic rats and the treatment with alpha melanocyte stimulating hormone (alpha-MSH) cased significant increasing in these hematological parameters.

It was concluded from the result that the hormone α-MSH possesses hepatoprotective activity against carbon tetrachloride (CCl_4) induced hepatotoxicity in experimental rats.

Keywords- hepatoprotectiv, hepatotoxicity, carbon tetrachloride (CCl_4), Alpha-melanocyte Stimulating Hormone (α-MSH)

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Introduction

Liver is the largest organ in the body and the site for intense metabolism and an important organ actively involved in metabolic functions and it is a frequent target of number of toxicants [1]. Liver has a pivotal role in regulation of physiological processes. It is involved in several vital functions such as metabolism, secretion and storage Furthermore, detoxification of a variety of drugs and xenobiotics. Liver diseases are some of the fatal disease in the world today. They pose a serious challenge to international public health. In absence of a reliable liver protective drug in the modern medicine, there are number of medicinal preparations in recommended for the treatment of liver disorder. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. But there is not much drug available for the treatment of liver disorders [2]. Therefore; many folk remedies from plant origin are tested for its potential antioxidant and hepatoprotective liver damage in experimental animal model. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in liver. Enhanced lipidperoxidation produced during the liver microsomal metabolism of ethanol may result in hepatitis and cirrhosis [3].

Carbon tetrachloride (CCl_4)-induced hepatic injury is a very classic model widely used for hepatoprotective drug screening [4,5].The acute hepatotoxicity of free radicals which causes oxidative stress and membrane damage [6]. These free radicals cause lipid peroxidation which results in hepatocellular damage and enhances formation of inflamed tissues. The advantage of this model is that CCl_4 can fulminate hepatitis within a few hours, which specifically leads to necrosis and fatty liver, in a similar way as what happens in

the cases of acute hepatitis. Mean while, following an inflammatory response launched by resident inflammatory cells, CCl_4-induced acute liver injury also involves an intricately regulated process of hepatocyte regeneration when the dosage of CCl_4 is below lethal level which would lead to irreversible liver damage [7]. Among the various mechanisms involved in hepatotoxic effect of carbon tetrachloride, one is oxidative damage through free radical generation [8] and antioxidant property is claimed to be one of the mechanisms of hepatoprotective effect of indigenous substances. The antioxidant activity or the inhibition of the generation of free radicals is important in providing protection against hepatic damage. A number of plants have been shown to possess hepatoprotective property by improving antioxidant status. Medicinal plants play a key role in the human health care. About 80% of the world populations rely on the use of traditional medicine which is predominantly based on plant materials [9].

Previously it was reported that the more of different extracts of traditional plants used as hepatoprotictive activity, it is shown in the review on hepatoprotective activity of medicinal plant [10]. More studies showed the hepatoprotictive of extracts as the methanol leaves extract of Orthosiphon stamineus against acetaminophen as hepatotoxicity inducing agent [11].

The aqueous root extracts of *Hygrophila auriculata* possess significant hepatoprotective activity against carbon tetrachloride and paracetamol-induced hepatotoxicity in rats [12]. Kumar, et al. [13] had reviewed the articles of hepatoprotective activity of the medicinal plants and have arranged about sixty models of them in the systemic order. As the methanol and aqueous leaves extracts and fruits of *Phyllanthus nirur,* the aqueous of *Cochlospermum Planchoni* [14]*,* the ethanol whole plant extracts of *Saururus chinensis* and other extracts studied of different plants which possess significant hepatoprotective activity against hepatotoxicity inducing agents [15-18].

From the previous studies we can't find the using of hormones as treatment against hepatotoxicity inducing agents, so in our study we aimed to evaluate the hepatoprotective activity alpha-melanocyte-stimulating hormone (Alpha - MSH) against carbon tetrachloride (CCl_4) induced hepatotoxicity in experimental rats in vivo.

Alpha-melanocyte-stimulating hormone (α-MSH) is a tridecapiptide that was originally characterized a neuropeptide derived from a pituitary. α-MSH is hormone derived by post-translational processing from POMC and involved in stress and background adaptation. Alpha-MSH is synthesized from pro-opiomelanocortin (POMC) by the action of specific prohormone convertases that cleave into MSH, ACTH and Beta-endorphin. Alpha-MSH seems to be modulated by the release of two hormones within the hypothalamus: melanoncyte stimulating hormone releasing factor (MSHRF) and melanocyte stimulating hormone release-inhibiting factor (MSH-RIF). Five G-protein-coupled melanocortin receptors (MC (1)-MC (5) are expressed in mammalian tissues. The melanocortin receptors support diverse physiological functions, including the regulation of hair color, adrenal function, energy homeostasis, feed efficiency, sebaceous gland lipid production and immune and sexual function. The hormone is a neuroimmunomodulating peptide that was recently detected in many none pituitary tissues including the skin, accordingly, epidermal cells such as keratinocytes and melanocytes (as well as dermal cells such as fibroblasts and endothelial cells). Neuropeptide Alpha- Melanocyte stimulating hormone is known to suppress cytokine, suppressed the production of the proinflammatory cytokine interferon (IFN) mediated - gamma by antigen stimulated primed lymph node T cell [19]. More finding indicates that α-MSH is part of the mediator network that regulates cuteness inflammation and hyperproliferation skin diseases [20]. Alpha-MSH is known to have pleitrophic functions including pigmentary, anti-inflammatory, antipyretic and immunoregulatory roles in the mammalian body as reduction nterleukin-1 beta effects on rat stomach [20], inhibits lipolysccharide-induced biological responses by down regulating CD14 from macrophage, and suppresses antigen-stimulated T-cell production [21]. It is also reported to more influences, as treatment of various skin disorders including inflammatory dermatomes [22], modulates local and circulating tumor necrosis factor (TNF) in experimental brain inflammation [23], this hormone can inhibit the induction of contact hypersensitivity reactions and to induce hapten-specifictolerance [24], anti-inflammatory and anti-invasive effects in human melanoma cells [25], the anti-inflammatory actions of the peptide via peripheral and /or central melanocortin receptors might put the peptide into practice therapeutically in near future [26]. More studies suggest that α-MSH, decrease body fat in humans [27] as a potential neurotransmitter [28], regulate energy balance, appetite control, as well as glucose transport in rat adipocytes [29]. The neuropeptide, alpha-melanocyte stimulating hormone (α-MSH), is an endogenous antagonist of inflammation. Injections of α-MSH peptide into inflamed tissues have been found to be very effective in suppressing autoimmune and endotoxin mediated diseases. This study was designed to evaluate the hepatoprotective effect of Alpha-melanocyte stimulating hormone (α-MSH) against carbon tetrachloride (CCl4) induced hepatotoxicity in experimental rats in vivo.

Material and Methods

Animals

Sixty male Wister rats weighing 160-190 g respectively were used for our study**.** The rats were harbored in stainless steel cages under standard laboratory condition of 12 h light/dark cycle throughout the experimental periods. They had access to food (Top Fed, Sapele) and water *ad libitum.* The animals were carefully checked and monitored every day for any changes.

Experimental Design

The animals were kept inside cages at the house of animals at the faculty of science at Al al-Bayt University. Sixty rats ware used and grouped into 4 groups. Group I consisted of nonhepatotoxic rats that received only the vehicle (0.5 mL/kg body weight) and served as a control group; in group II the animals were injected intramuscularly with hormone α-MSH (sigma formula) 2mg/100g of body mass every alternate day for 15 days. Group III administered carbon tetrachloride (CCl4; 2 ml/kg body weight) for 15 days to produce the hepatotoxity in rats. Groups IV (treated hepatotoxic group), the animals were administered CCl4; 2 ml/kg body weight and treated with hormone α-MSH (sigma formula) 2mg/100g of body mass every alternate day for 15 days with free access to food and water *ad libitum*. Our experimental study complied with the guide lines of the animal ethics committee which was established in accordance with the internationally accepted principles for laboratory animal use and care.

Sample collection

Blood sample was collected from each animal by cardiac puncture and rats were sacrificed by cervical dislocation under light ether anesthesia. Part of the blood sample was put in EDTA bottles and

used for determining some hematological parameters. The remaining blood sample was put into test tubes and allowed to clot for 30 min before centrifuging using a bench top centrifuge for determining the biochemical parameters described. Serums, for biochemical determinations were stored at -20°C until the day of measurement.

Hematological Analysis

The CBC was performed on an automated hematology analyzer using well mixed whole blood to which EDTA was added to prevent clotting. (ESR) determined by Westengren method, differential WBC count was performed on Geimsa stained blood smears.

Biochemical Analysis

Liver enzymes A, total protein, albumin, total and direct bilirubin and lipid profile were determined by using commercial analytical kits from Sigma (Lab-Kit, Spain) and measured with spectrophotometer method by Automatic Analyzer [AU 2700 Olympus, OSR6121, Japan].

Statistical Analysis

The results were expressed as mean± SD. Differences between groups were analyzed with student's t-test. Differences between groups were considered at 95% confidence limit and probability level of 0.05. The results were taken as significant if $p<0.05$.

Results

The present study had been attempted to access the hepatoprotective effect of Alpha-melanocyte Stimulating Hormone (α-MSH) against carbon tetrachloride (CCl_4) induced hepatotoxicity in experimental rats in vivo.

The 2mg/100g of body mass of hormone α-MSH (sigma formula) were subjected to preliminary for their possible protective effects against hepatotoxic agent (CCl_4) in experimental rats in vivo. The hepatoprotective effect was studied further in rats, using carbon tetrachloride (CCl4)-induced hepatotoxicity as in vivo model. Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatotoxic effects of CCl4 are largely due to its active metabolite, trichloromethyl radical [31]. The administration of the hepatotoxic model to rats caused liver damage as indicated by a significant increase in serum liver enzymes ALT, AST, ALP activity and bilirubin level The levels of hepatic marker enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphates (ALP) were used to assess their hepatoprotective activity against carbon tetrachloride (CCl4)-induced hepatotoxicity in rats. The serum levels of these enzymes in the hepatotoxic animals were significantly higher ($p<0.05$) than those of the control group [Table-1]. Elevated levels of these enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver because they are cytoplasmic in location and released into circulation after cellular damages indicating development of hepatotoxicity The results revealed that treated rats with the 2mg/100g of body mass of hormone α-MSH (sigma formula) for 15 day significantly suppressed the plasma AST ($P < 0.05$) ALP and ALT ($P < 0.05$) when compared with the CCl4 intoxicated control. The administration of the hormone showed significant hepatoprotective activity. The liver weight was significantly decreased in the CCl4)-induced hepatotoxicity as in vivo model group when compared to control. The treatment with α-MSH has brought back the liver weight to the near normal.

Table 1- Effects of the hormone α-MSH (sigma formula) on the liver enzymes in the hepatotoxic rats

Group	Treatment	N	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Liver weight (g)
I	Control nonhepatotoxic rats	15	31 ± 1.22	19.14±0.	37 ± 5.73	10.9 ± 0.53
II	nonhepatotoxic rats with α-MSH	15	33 ±2.57	21.33± 3.6	38± 3.42	10.3 ± 0.73
III	hepatotoxic rats	15	128±12.6*	154 ±16.24**	141 ± 11.63**	8.73 ± 0.43**
IV	hepatotoxic rats treated with α-MSH	15	62 ± 3.93**	98 ± 10.44**	91 ± 5.16**	9.5 ± 0.51**

Our results in [Table-2] showed that the administration of carbon tetrachloride (CCl4)-induced hepatotoxicity alternate day for 15 days has enhanced the, total and direct bilirubin and liver weight, and reduced the total protein and albumin in comparison with the control group. The treatment with hormone α-MSH has brought back the altered levels of biochemical markers to the near normal levels. Hepatic injury caused by CCl4 administration showed significant increase in the lipid profile parameters as total cholesterol, triglycerides, LDL whereas HDL level was decreased as compared to that of control group of mice ($p<0.05$).

Table 2- Effects of the hormone α-MSH (sigma formula) on some Biochemical parameters

Group	Treatment	No	Total protein (g/l)	Albumin (g/dL)	Bilirubin Total (mg/ml)	Conjugated Bilirubin Direct (mg/ml)
I	Control nonhepatotoxic rats	15	6.23± 0.24	4.12 ± 0.53	0.79 ± 0.26	0. 274± 0. 013
II	nonhepatotoxic rats with α-MSH	15	6.84± 0.32	4.63 ± 0.72	0.62 ± 0.01	0. 238± 0. 012
III	hepatotoxic rats	15	3.52 ± 0.52	2.42 ± 0.82*	2.74 ± 0.36*	0.4306± 0. 018
IV	hepatotoxic rats treated with α-MSH	15	5.78 ± 1.03*	3.84 ± 0.36*	0.73 ± 0.14*	0. 282± 0. 016*

Table 3- Effects of the hormone α-MSH (sigma formula) on the parameters of lipid profile in hepatotoxic rats.

Treatment	N	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Control nonhepatotoxic rats	15	124.33 ± 6.4	73.6 ± 9.32	35.67 ± 4.2	26.5 ± 5.34
nonhepatotoxic rats with α-MSH	15	137.24 ± 8..72**	86.34 ± 7.4**	28.31 ± 4.54**	24.8 ± 3.3**
hepatotoxic rats	15	178.93 ± 14.56*	142.68±15.9*	19.43 ± 2.56*	56.9 ± 11.57*
hepatotoxic rats treated with α-MSH	15	144.66 ± 5.7**	109.73 10.74**	24.50 ± 5.82**	24.54 ± 4.65**

However, treatment with hormone α-MSH showed significant reduction in liver cholesterol, triglyceride, and LDL. On the other hand HDL level was increased compared to CCl4 treated group and also no change was observed in control. In this study, it has also been observed that there is a significant ($P < 0.05$) increase in the concentration of total cholesterol, triglycerides, LDL cholesterol and a significant ($P < 0.05$) decrease in HDL cholesterol in the hepatotoxic rats [Table-3]. We observed that the treatment with hormone α-

MSH significantly (P < 0.05) decrease in the concentration of total cholesterol, triglycerides, LDL cholesterol and a significant (P < 0.05) increase in HDL cholesterol in the hepatotoxic rats.

In our study we investigated some hematological parameters and It was found that the RBC and WBC count, PCV, Hb, ESR, neutrophil percentage and lymphocyte percentage were significant decreased ((P <0.05) in the hepatotoxic rats [Table-4] and the treatment with alpha melanocyte stimulating hormone (alpha-MSH) cased significant increasing in these hematological parameters.

Table 4- Effects of the hormone α-MSH (sigma formula) on some hematological parameters in hepatotoxic rats.

Parameters	Control nonhepatotoxic rats	nonhepatotoxic rats with α-MSH	hepatotoxic rats	hepatotoxic rats treated with α-MSH
RBC (x 10^6 µ	4.3±0.7**	0.8**± 3.8	5.9±0.5	5.8±0.7
WBC (x 10^3 µ	5.1±0.3**	4.4±0.6**	6.2±0.8	5.4±0.6
Hb (g dL	10.7±09**	9,8±0.5**	12.3±0.7	11.8±0.9
Neutrophils%	39±4.5*	32±3.6	40±8.3**	34±4.8
Basophiles%	3±0.8	3±0.4	2±0.7	2±0.1
Eosinophils	4±0.2	4±0.7	4±0.5	3±0.3
Lymphocytes%	61±3.6*	53±2.6*	68±4.6*	60±3.6
Monocytes%	5±0.3	6±0.3	6±0.8	4±0.4
PCV%	35±1.9	30±3.2*	38±2.2*	35±2.6
ESR (mm h)	18±3.5*	21±3.5*	14±2.1	13±2.3

Discussion

Literature review revealed that no research work have been done on hepatoprotective investigation of hormone α-MSH (sigma formula) 2mg/100g of body mass against hepatotoxicity in experimental rats in vivo. This study was carried out to evaluate the protective effect of hormone α-MSH on CCl4 induced hepatotoxicity. Liver damage induced by CCl4 is commonly used model for the screening of hepatoprotective drugs [32] which associated with increased amount of lipid peroxidation. The rise in serum levels of AST, ALT and cholesterol has been attributed to the damaged structural integrity of the liver because they are cytoplasmic in location and released into circulation after cellular damages when rats were treated with carbon tetrachloride it induces hepatotoxicity by metabolic activation [33]. Other studies suggested that the loss of membrane structure and integrity from the lipid peroxidation was accompanied with the elevated levels of marker enzymes like-AST, ALT, ALP, total protein and bilirubin.

Peroxidation during metabolism of hepatotoxic agent may result in development of hepatitis leading to cirrhosis. Our Results showed that the serum levels ALT, AST and Alkaline Phosphates (ALP) in the hepatotoxic animals were significantly higher (p<0.05) than those of the control group. The enhanced activities of ALT, AST and ALP observed in CCl4 -treated rats in this study corresponded to the extensive liver damage induced by toxin. The rise in the ALT is usually accompanied by an elevation in the levels of ALT, which play a vital role in the conversion of amino acids to keton acids [34]. Administration of hormone α-MSH (sigma formula) 2mg/100g of body mass every alternate day for 15 days was found to lower the enzymes levels and liver weight significantly in a dose dependent manner in treated hepatotoxic groups (p<0.05) when compared with those of the untreated group. The using of the hormone α-MSH in our study was potentially effective in blunting lipid peroxidation, suggesting that the hormone α-MSH possibly has antioxidant property to reduce hepatotoxic agent-induced membrane lipid peroxidation and thereby to preserve membrane structure might be due to the presence of glycosides, flavonoids, proteins, amino acids, tannins, saponins and triterpenoids [35].

Administration of CCl4 caused a significant (P<0.05) elevation of total bilirubin and decrease in total protein when compared to control. There was a significant (P<0.05) restoration of the enzyme levels and total protein on administration of the hormone α-MSH in a dose dependent manner 2mg/100g of body mass. The reversal of increased serum enzymes and other biochemical parameters in CCl4-induced liver damage by the hormone α-MSH may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes [36]. Effective control of ALP, bilirubin and total protein levels points towards an early improvement in the secretary mechanism of the hepatic cells. The oxidative damage of some amino acids is considered as the major cause of metabolic dysfunction in hepatic damage [37]. Administration of the hormone α-MSH enhanced the synthesis of proteins and albumin and accelerated the regeneration process, affording protection to liver cells. CCl4 intoxication also produced a significant elevation in the levels of serum bilirubin. Toxicants like CCl4 produce sufficient injury to hepatic cells causing elevation in serum bilirubin content. Hormone α-MSH treatment effectively reduced the elevated bilirubin levels to near normal values.

The results of this study revealed that the hormone α-MSH has significant hepatoprotective activity. This effect may be due to the ability of the hormone to inhibit lipid peroxidation and increase in the anti-oxidant enzymatic activity. In our study, we observed that there is a significant (P < 0.05) increase in the concentration of total cholesterol, triglycerides, LDL cholesterol and a significant (P < 0.05) decrease in HDL cholesterol in the hepatotoxic rats. Highly reactive free radicals formation directly attacks the poly unsaturated fatty acids of the endoplasmic reticulum and thus cause over production Cholesterol [38].

The injections of the hormone which has the ability of a hepatoprotective activity to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms which that have been disturbed by a hepatotoxin, is the index of its protective effects [39] and induced significant inhibition of the parameters of lipid profile and increase the HDL cholesterol as good cholestero related with the antioxidant activity or the inhibition of the generation of free radicals which is important in the protection against CCl4 -induced hepatopathylogy. The reduction of the levels of the lipid profile parameters as cholesterol, triglycerides, LDL cholesterol and a significant decreasing in HDL cholesterol in the hepatotoxic rats by hormone α-MSH is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage by CCL4.

The hepato- and nephrotoxicity of carbon tetrachloride (CCl4) in experimental animals are well characterized; little is known about the hematological effects of CCl4 poisoning. In this work found that the hematological parameters RBC and WBC count, PCV, Hb, ESR, neutrophil percentage and lymphocyte percentage were significant reduced in the hepatotoxic rats and the treatment with hormone (alpha-MSH) cased significant increasing in these hematological parameters. The liver plays a central role in haemopoiesis and synthesis of coagulation proteins; liver disease is associated with hematological abnormalities and causes alterations in red cell lipid

metabolism. Defects of platelet number and function arise due to the effects of liver disease, immune mechanisms and hypersplenism. Liver involvement is often observed in several hematological disorders, resulting in abnormal liver function tests, abnormalities in liver imaging studies, or clinical symptoms presenting with hepatic manifestations. In hemolytic anemia, jaundice and hepatosplenomegaly are often seen mimicking liver diseases. In hematologic malignancies, malignant cells often infiltrate the liver and may demonstrate abnormal liver function test results accompanied by hepatosplenomegaly or formation of multiple nodules in the liver. These cases may further evolve into fulminate hepatic failure. When the RBC membrane is severely damaged, immediate lyses occurs within the circulation (intravascular hemolysis). In cases of less severe damage, the cells may be destroyed within the monocyte-macrophage system in the spleen, liver, bone marrow, and lymph nodes (extravascular hemolysis) [40-42] CCl_4 administration also causes immunosuppressive effects as indicated by phagocytic capacity, chemotactic migration and cell adhesiveness of rat peritoneal macrophages.

In hemolysis, serum lactate dehydrogenase (LDH) levels (specifically the LDH1 and LDH2 isoforms) increase because of lysed erythrocytes [40]. Serum aspartate transaminase (AST) levels are also mildly elevated in hemolysis, with the LDH/AST ratio mostly over 30 [43]. Total bilirubin levels can uncommonly exceed 5 mg/dL if hepatic function is normal, except in the case of acute hemolysis caused by sickle cell crisis. Liver dysfunction can also be caused by blood transfusion for anemia in sickle cell disease (SCD) and thalassemia [44,45]. It was found that the RBC and WBC count, PCV, ESR and neutrophil percentage was decreased ($P < 0.05$). Moreover neutrophils from hepatotoxic rats have also shown to present functional abnormalities such as less phagocytizing capacity and chemotactic responses [25]. This might be extended to other inflammatory cells as those involved in allergic processes. The present study indicated that alpha-MSH treatment might ameliorate some disturbed hematological parameters of hepatotoxic rats. It has been suggested that anemia occurrence due to the increased non-enzymatic glycosylation of RBC membrane proteins, which correlates with hyperglycemia and hepaatotoxity [46]. Oxidation of these glycosylated membrane proteins and hyperglycemia in hepatotoxic rats cause an increase in the production of lipid peroxides causing a hemolysis of RBC.

In this experiment, we did not measure the RBC membrane lipid peroxide levels in hepatotoxic rats. However [47] demonstrated that serum lipid peroxide level increased in diabetic and hepatotoxic rats.

It was demonstrated that alpha-MSH treatment decreased the elevated lipid peroxide level to normal level. Thus increased RBC count of alpha-MSH treatment rats could be due to the lowered lipid peroxide level in RBC membrane leading to a decreased susceptibility of RBC to hemolysis [47]. Neutrophils ingest and kill bacteria and have been called the body's first line of defense against bacterial infections. It has been suggested that the body's defense mechanism against infections was disturbed due to the disturbed neutrophil function in diabetes [48]. In this experiment, we demonstrated that alpha-MSH treatment increased the lowered neutrophil percentage of WBC compared to control level. This result indicated that alpha-MSH treatment might also Increase the defense mechanism of the body against infections in experimental rats. CCl_4 administration also causes immunosuppressive effects as indicated by phagocytic capacity, chemotactic migration and cell adhesiveness of rat peritoneal macrophages. However, treatment with alpha-MSH deleted the immunosuppressive effect of CCl_4, since a significant increment in the functional capacities of rat peritoneal macrophages. The results of our experiment suggest that treatment by alpha-MSH may be the critical remedy for the adverse effect of CCl_4 in liver function as well as immune functions. The results of the present study indicated that under the present experimental conditions, the hormone alpha-MSH showed hepatoprotective abilities against carbon tetrachloride induced liver damage in experimental rats. It is concluded that alpha-MSH treatments might ameliorate the CCl_4-induced disturbances of anemia, and body's defense mechanism in CCl_4-treated rats.

Conclusion

The present work reveals the hepatoprotective activity of alpha-melanocyte-stimulating hormone (Alpha - MSH) against carbon tetrachloride (CCl_4) induced hepatotoxicity in experimental rats in vivo.

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