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HEPATOPROTECTIVE AND ANTIOXIDANT ACTION OF WHEY PROTEIN, AND ITS FRACTIONS IN RATS AFTER ACUTE CCL_4 EXPOSURE

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ABSTRACT

Exposure to CCl_4 may increase the production of reactive oxygen species, which results in the damage of tissues, especially the liver. The purpose of this study is to evaluate the effects of whey protein (wp), α-lactalbumin(α-la)and β-lactoglobulin (β-lg) (100mg each) on the superoxide dismutase (SOD) status, liver and kidney functions after acute CCl_4 exposure in the rats. The results showed that with the feeding on WP, α-la and β-lg, the SOD level displayed a significant increase after acute exposure to CCl_4. The parameters of liver functions showed a significant decrease in the transferases (ALT, AST & GGT) and a significant increase in protein synthesis (TP & albumin). The kidney functions parameters (uric acid & createinine) are significantly reduced when WP, α-la and β-lg were administered. These results suggest that WP and its fractions should be a precursor agent to promote the production of SOD that enhance the antioxidant capacity and improve the liver and kidney functions.

INTRODUCTION

Whey is a by-product of cheese and casein manufacture. Its volume increased worldwide to reach ~72 54 0000 Tonnes at 2006. Whey includes biological components which demonstrate a wide range of immune enhancing properties. In addition, whey proteins have the ability to act as antioxidant, anticarcinogenic, antibacterial and antiviral agent. Also, whey proteins and its active peptides have the ability to lower cholesterol, reduce blood pressure, enhance bone growth and pain relief (Sipola *et al* 2002, Yamamura *et al* 2002, Ha & Zemel 2003, Marshall 2004 and Chatterton *et al* 2006). Liver is the largest organ in the body. It plays a vital role, performing many complex functions which are essential for life. Incorrect diet, excessive alcohol, adverse reactions to drugs and toxic chemical and viral hepatitis cause liver damage (Moreno *et al* 2005). Few informations are concerned with the use of whey proteins for treatment of liver damage caused by such factors. Therefore, this study focus on the use of total whey proteins, α-lactalbumin and β-lactoglobulin for treatment of rat liver damaged by CCl_4.

MATERIALS AND METHODS

Materials:

Sweet whey of Ras cheese was obtained from Dina Company for food and agricultural projects. It was ultrafiltered using a carbosep 252 UF system (SFEC, France) equipped with zirconium oxide membrane (CF6).

Carbon tetrachloride (CCL_4) (ADWEIC) was obtained from El-Nasr Pharmaceutical Chemical Co., Cairo, Egypt.

Gum acacia (5%) was obtained from El-Nasr Pharmaceutical Chemical Co., Cairo, Egypt.

Liquid paraffin was obtained from El- Gomhoria for Chemicals trade Co., Cairo, Egypt.

Commercial diagnostic kits for the determination of: serum gamma glutamyl (GGT), superoxide dismutase (SOD), activities of transaminases (ALT&AST), cholesterol, triglycerides, total protein and serum albumin, uric acid and creatinine were obtained from Biodiagonstic, Egypt.

Sprague dawley rats (of either sex average weight 120±20 gm each) were obtained from animal house Lab., National Research Center, Cairo,Egypt. Throughout the experimental period, animals were kept in stainless steel cages under good ventilation and hygienic conditions, both of water and food were available.

Basal diet: The animals were fed a balanced basal diet obtained from Meladco Company, Egypt.

Methods of analysis:

Total whey proteins were obtained by heating acid whey at 90 C º. The precipitated whey proteins were washed several times with dist. water then dried at 40 Cº.

α-lactalbumin and β -lactoglobulin were separated from whey retentate using the methods of Mailliart & Ribadeau-Dumas (1988). Both fractions were dialyzed then freez-dried, using Freeze-drying (LY-5M-R13Sinijders Scientific) Holland.

The purity of both fractions was examined using SDS-polyacrylamide gel electrophoresis according to the method of Laemmli (1970).

Dry matter and ash content were determined according to AOAC (1985).

Total nitrogen was determined by the kjeldahl method according to AOAC (1985). Nitrogen content was converted to protein using the factor of 6.38.

The amino acids content of total whey protein, α-lactalbumin and β-lactoglobulin was determined using Amino Acid Analyzer (LC3000 Eppendrof) Germany.

The activities of serum superoxide dismutase (SOD), γ-glutamyl transferase (GGT), aspartate and alanine aminotransferase (AST&ALT), cholesterol and triglycerides (TG) and serum total protein, albumin, uric acid and creatinine were measured using the colorimetric methods described in the kits from Biodiagonstic, Egypt.

Results were statistically analyzed by ANOVA single factor according to Snedecor and Cochran (1967) using SPSS v.10.0 program.

Experimental design

1-Hepatoprotective effect:

Four groups of rats (6 rats each) were submitted to the following treatments: Group 1: control: rats were fed on the basal diet. Groups 2, 3 and 4: rats were orally admisterd 0.1 gm of total whey protein, α-lactalbumin and β-lactoglobulin (as a suspension in 5% gum acacia). On the

16 th day of treatment, all groups were given 50%CCL_4 (v/v) in liquid paraffin (1.5 ml/kg b.w. as oral dose) to induce hepatic injury according to the method of Yadav and Dixit (2003).After 48 hr., and at end of the experimental time, animals were sacrificed and blood samples were collected from retro-orbital venus plexus from all animals in plain test tubes.

2-Treatment of injured liver hepatic damage rat:

24 rats received 50% CCL_4 in liquid paraffin (1.5 ml/ kg b.w. as oral dose). After 2 hr., all rats were fed the normal basal diet for 48 hr. then divided into four groups as above mentioned. After 2 weeks, blood samples were collected from retro-venus plexus, then rats were sacrificed as previously mentioned. Blood samples of all treatments were centrifuged at 3000 rpm/ 20 min. Serum samples were stored in Ependrof vials in freezer till the biochemical analysis.

RESULTS AND DISCUSSION

1- Composition and purity of whey protein, α-lactalbumin and β-lactoglobulin fractions

Table 1 shows the composition and purity of whey protein (WP) and the separated α-lactalbumin and β- lactoglobulin fractions using NaCl salting out at low pH-technique. Under these conditions, the resultant α-lactalbumin fraction had 90.79% TS, 66.06% protein and 23.56% ash while the β-lactoglobulin fraction had 95.08% TS, 52.00% protein and 41.5 % ash resp. The purity of the fractions was 77.30 % for the former and 93.70 % for the latter. As for the composition of the total whey proteins, it had 92.13 % TS, 63.97 % protein and 11.75 % ash.

Table 1: Chemical composition of the total whey proteins, α- lactalbumin and β- lactoglobulin.

Sample	TS %	Ash %	Total protein %	Purity %
whey protein	92.13	11.75	63.97	-
α- lactalbumin	90.79	23.56	66.06	77.30
β- lactoglobulin	95.08	41.50	52.00	93.70

Table 2 illustrates the amino acids composition of WP, α-la and β-lg. α-lactalbumin had higher content of amino acids than β-lactoglobulin. Relative to other protein sources, the amino acid profile comprises good quantities of essential sulfur-containing and branched-chain amino acids (BCAAs)**(**http://www.admworld.com/naen/mktcol/viewpdf.asp

BCAAs, particularly leucine, are important factors in tissue growth and repair. Leucine has been identified as a key amino acid in protein metabolism during the translation-initiation pathway of protein synthesis. Also, sulfur amino acids enhanced the immune function through intracellular conversion to glutathione, which referred to as the body's master antioxidant (Marshall, 2004).

Table 2: Amino acids composition of Whey protein (WP), α-lactalbumin (α-la) and β-lactoglobulin (β-lg).

Amino acid	WP	α-la	β-lg
		mg/ 100 mg	
Essential amino acids			
His.	1.59	1.63	0.72
Arg.	2.27	1.88	1.41
Ther.	3.62	3.34	2.62
Val.(BCAA)	3.85	3.14	2.27
Meth.	1.15	0.67	0.63
Isoleu.(BCAA)	3.11	2.59	2.47
Leu.(BCAA)	7.57	5.38	4.57
Phen.	2.67	2.56	1.14
Lys.	6.196	4.77	3.91
Non-essential amino acids			
Asp.	7.39	6.52	4.38
Ser.	3.48	3.31	1.69
Pro.	2.89	2.47	2.27
Ala.	3.09	1.83	2.20
Cys.	1.68	1.45	0.88
Glu.	12.7	9.50	8.75
Gly.	1.64	1.70	0.59
Total EAA	32.03	25.96	19.74
Total NEAA	32.87	26.78	20.76

BCAA: Branched chain amino acids.
EAA: Essential amino acids
NEAA: Non-essential amino acids

2- Biological value of whey proteins, α-lactalbumin and β-lactoglobulin

The effect of WP, α-la and β-lg on the biological activities of rats liver that exposed to carbon tetrachloride (CCl_4) was investigated.

Carbon tetrachloride is a manufactured chemical that does not occur naturally. It is used in the production of chloroflurocarbons and other chloronated hydrocarbons. Within the body, it breaks down to highly toxic free radical (CCl_3·) which causes brain, liver, kidney and skin injury. In some cases it can results in death.

Liver damage caused by CCl_4 is similar to that of acute viral hepatitis (Suja *et al.*, 2004).

2.1. Antioxidant effect and liver functions:

Efficient liver function is extremely important to the overall health and this depends on constant supplies of antioxidant nutrients.

Superoxide dismutase (SOD) enzymes serve to remove superoxide radicals by accelerating the formation of H_2O_2. Thus SOD out competes damaging reactions of superoxide protecting the cell from superoxide toxicity (www.wikipedia.org.).

Table (3) shows the effect of WP, α-la and β-lg on rats' serum SOD either before or after exposure to CCl_4.

Table (3):Effect of feeding whey proteins (WP), α-lactalbumin(α-la) and β-lactoglobulin(β-lg) on rats' serum SOD, transferasses, protein and albumin before and after exposure to CCl_4.

Treatment	Parameters					
	SOD	ALT	AST)	GGT	TP	Alb
	U/ml				g/dl	
A- Non-treatment with CCl_4						
Control	30.51^{A}	36.58^{A}	84.14^{A}	1.233^{A}	7.27^{A}	4.76^{A}
WP	44.55^{B}	28.50^{B}	64.62^{B}	0.83^{B}	7.23^{A}	4.31^{A}
α-la	49.98^{D}	30.03^{B}	66.20^{B}	0.75^{B}	7.75^{AB}	4.80^{A}
β-lg	77.11^{C}	24.54^{C}	56.45^{CE}	0.57^{B}	8.02^{B}	4.46^{A}
B- CCl_4-treatment before feeding						
Control	18.08^{E}	47.70^{D}	117.52^{E}	5.017^{C}	4.92^{C}	4.43^{A}
WP	22.93^{E}	40.52^{E}	93.19^{F}	3.732^{D}	6.00^{D}	4.48^{A}
α-la	29.83^{A}	42.67^{E}	98.93^{F}	3.500^{D}	5.92^{D}	4.50^{A}
β-lg	21.17^{E}	36.33^{A}	83.57^{A}	2.993^{E}	6.12^{DF}	4.75^{A}
C- CCl_4-treatment after feeding						
Control	17.55^{E}	49.12^{D}	114.39^{D}	4.867^{C}	4.88^{C}	4.43^{A}
WP	21.75^{E}	30.87^{B}	70.13^{B}	1.783^{D}	7.05^{A}	4.68^{A}
α-la	33.70^{A}	29.75^{B}	64.33^{BE}	1.655^{E}	7.65^{AB}	4.42^{A}
β-lg	40.88^{B}	25.58^{BC}	57.78^{E}	1.667^{E}	7.93^{AB}	4.44^{A}

Generally, it decreased as a result of CCl_4 administration. Feeding WP, α-la and β-lg caused a significant increase in SOD, but the increase caused by α-la was much higher than both of β-lg and WP. In addition, the level of SOD in the groups exposed to CCl_4 after 16 days of feeding on α-la was apparently greater than that of the groups exposed to CCl_4 before feeding.

Transferases (ALT, AST and GGT) are enzymes that catalyze the interconversion of keto acids and amino acids by transeferring the amino group. Transferases are normally present in the blood at low concentration and they increase with the liver damage (Thomas, 2005).

Levels of these serum marker enzymes are significantly increased in CCl_4 treated rats as compared to the control group.CCl_4 is known to cause hepatic damage with a marked elevation in serum levels of aminotransferases because these enzymes are cytoplasmic in location and released into the blood after cellular damage (Ko *et al*.,2006). Table (3) reveals that rats' serum transferases (ALT, AST and GGT) are significantly decreased in the CCl_4-treated groups fed on WP and its fractions especially α-la. Markedly reduction was observed in the groups exposed to CCl_4 after 16 days of feeding, which is in accordance with Kume *et al.,* (2006).

Concerning serum albumin, non significant differences were observed between treatments. It seems also that α-lactalbumin was more efficient than both of β-lactoglobulin and WP.

The liver is the site of protein synthesis. In advanced liver diseases, both of TP and albumin are decreased and albumin is a contributory factor in causing the odema present in such cases (Sarslimaz *et al* 2004). As shown in table (3) rats serum TP in CCl_4- treated rats fed on WP, α-la and β-lg is significantly higher than the control, especially when exposure to CCl_4 after 16 days of feeding.

2.2. Kidney functions:

Carbon tetrachloride causes tissue damage especially to the liver and kidney resulted in a rise both of serum uric acid and creatinine when administered to rats (Waterfield *et al.* 1993).

To evaluate the role of WP and its fractions in protection of renal function, the level of uric acid and creatinine was assayed and the results were showed in Table (4). Exposure to CCl_4 caused significant increase of serum uric acid and creatinine. Feeding on WP and its fractions, led to a significant reduction in both parameters.

No significant changes were observed between WP and its fractions.

Table (4): Uric acid and creatinine of rats fed on whey protein and its fractions before and after exposure to CCl_4.

Treatment	Kidney functions	
	Uric acid	Creatinine
	mg/dl	
A-Non-treatment with CCl_4		
Control	1.305 ^{AC}	0.453 ^{A}
WP	0.967^{B}	0.293^{B}
α-la	1.142^{BC}	0.300^{BC}
β-lg	1.267^{C}	0.238^{C}
B- CCl_4-treatment before feeding		
Control	2.58^{B}	0.753^{C}
WP	2.03^{CD}	0.542^{D}
α-la	1.78^{CD}	0.482^{AD}
β-lg	1.84^{C}	0.558^{D}
C- CCl_4-treatment after feeding		
Control	2.217^{D}	0.763^{D}
WP	1.177^{ABC}	0.315^{BC}
α-la	1.376^{AC}	0.373^{AB}
β-lg	1.336^{AC}	0.392^{AB}

2.3. Effect on serum cholesterol:

The effect of whey protein and its fractions in suppressing the increase in the serum cholesterol level is shown in Table (5). The levels of serum cholesterol (CHO) and triglycerides (TG) were significantly decreased in rats fed TWP and its fractions, as compared to control diet either treated with or without CCl_4, while no significant differences were observed in CHO and TG levels between rats fed with different WP fractions. These results are in agreement with Nagaoka *et al* (1991), Nagaoka *et al* (1992) and Kume *et al* (2006).The mechanism of action by which WP exerts its hypocholesterolmic

effect may be due to that whey proteins inhibited cholesterol absorption through the changes of micellar cholesterol solubility in the intestine, accompanied by an increase of fecal steroid excretion (Nagaoka 1996).

The obtained results indicate that WP and its fractions represent a potential antioxidant, which protect liver cells from CCl_4 damage, and the protection includes its capacity to stimulate SOD synthesis and decreased liver cells breakdown and transferases escape from the damaged tissue. α-lactalbumin showed manifest potentially greater antioxidant than both of the WP and β-lactoglobulin. This may be due to that α-la is a native rich source of the sulfur-containing amino acids cysteine and methionine has been related to its ability to deliver, in a biologically available form. Also, it acts as precursor, to the production of the tripeptide, glutathione (GSH), which, in turn moderates oxidative damage and improves immune function. The hepatoprotective effect of the used whey proteins can be arranged descendingly as follows: α-lactalbumin> β-lactoglobulin> WP which prepared by heating at low pH. These conditions reduced its biological value as compared to the native whey protein fractions.

Table (5): Serum cholesterol (CHO) and triglycerides (TG) of rats fed on whey protein and its fractions before and after exposure to CCl_4.

Treatment	Cholesterol (CHO)	Triglycerides (TG)
	mg/dl	
A-Non-treatment with CCl_4		
Control	61.55 ^{A}	130.17 ^{AE}
WP	52.50^{BDE}	95.67^{B}
α-la	53.67^{BD}	89.67^{B}
β-lg	40.17^{C}	91.50^{B}
B- CCl_4-treatment before feeding		
Control	78.17^{C}	176.17^{C}
WP	60.67^{A}	132.48^{A}
α-la	58.83^{AB}	123.00^{A}
β-lg	55.83^{BD}	129.68^{A}
C- CCl_4-treatment after feeding		
Control	79.33^{D}	166.17^{D}
WP	66.33^{A}	109.67^{E}
α-la	93.17^{A}	59.50^{E}
β-lg	51.83^{B}	95.00^{A}

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تـأثير بروتينـا ت الشـرش و مشـتقاتها الـواقي للكبـد والمضـاد للاكسـدة فـي الفئـران المعاملة برابع كلوريد الكربون

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يؤدى التعرض للمواد العضوية الكيميائية مثل رابع كلوريد الكربون (CCl_4)الى زيادة في جزيئات الاكسجين النشطة التي تسبب تلف فى الخلايا و خاصة خلايا الكبد. الهدف من هذه الدراسة هـو تقيـيم التـأثير البيولـوجى لبروتينـات الشـرش و مشـتقاتها (α-lactalbumin & β-lactoglobulin) بتركيز ١٠٠ مجم لكل منها على وظائف الكبد و الكلى و كذلك تقييم هذه المواد كمواد مضاده للاكسدة و ذلك بعد التعرض لرابع كلوريد الكربون في الفئران.

اشارت النتائج الى ان التغذية على بروتينات الشرش و مشتقاتها ادت الى زيادة ملحوظة فى SOD وذلك بعد التعرض المباشر لرابع كلوريد الكربون. كمااشارت ايضا تحاليل وظائف الكبد الى حدوث نقص ملحوظ فى انزيماته (ALT,AST & GGT) و زيادة ملموسة فتخليق البروتين (TP & Albumin).و بالنسبة لتحليل وظـائف الكلـى اظهرت النتـائج حدوث نقص معنوى فـى كل من حمض اليوريك و الكرياتينين و ذلك بعد تناول بروتينات الشرش و مشتقاتها.

وعليه فالدراسة تشير الـى ان تنـاول بروتينـات الشرش تحسن من وظـائف الكبد و الكلـى و تعتبر مواد مضـادة للاكسدة.