ملخص البحث

استخدام بروتين الشرش فى تحسين وظائف الكبد فى الفئران المصابة

يهدف البحث الحالي إلي دراسة تأثير بروتين الشرش في تحسين وظائف الكبد في الفئران المصابة بالكبدو قياس الخواص الحسية للخبز المضاف إليه بروتين الشرش وذلك للتأكد من إمكانية الاستفادة الفعلية منه. ولهذا الغرض طبقت الدراسة علي ٤٠ فأر من ذكورالألبينو تم تقسيمهم إلي مجموعتين رئيستين ، المجموعة الرئيسية الأولي (٨ فئران) تم تغذيتهم علي الغذاء الأساسي فقط (مجموعة ضابطة غير مصابة) ،أما المجموعة الرئيسية الثانية (٣٢ فأر) فقد تم إصابتهم بالكبد عن طريق حقنهم بمادة رابع كلوريد الكربونبعدها تم تقسيم تلك المجموعة إلي أربع مجموعات فرعية متساوية العدد استمرت إحداهما كمجموعة ضابطة مصابة أما الثلاث مجموعات الباقية فقد أدخل الشرش ضمن غذائها بنسب ٥% ، ١٠% و ١٥% استبدال علي التوالي لمدة ٧ أسابيع . ولدراسة الخواص الحسية تم إدخال بروتين الشرش في الخبز بنسبة٥%، ١٠% و١٥% استبدال علي التوالي ، وتم تحكيم الخواص الحسية للخبز المنتج من خلال استمارة تحكيم تم تطبيقها علي عدد ١٠ من المحكمين . وقد أوضحت نتائج الدراسة أن الفئران المصابة بالكبد و التياستهلكت الشرش في غذائها بنسب ٥%،١٠%، و١٥% استبدال قد صاحبها انخفاض معنوى عند مستوي معنوية ٠,٠٥ في كل من مستوى الكولسترول الكلي ، الجليسريدات الثلاثية ، كولسترول البروتينات الدهنية المنخفضة الكثافة (LDL-c) ،البروتينات الدهنية المنخفضة جدا في الكثافة(VLDL-c)، مستوى جلوكوز الدم ووظائف الكبد (AST,ALT,ALP)، كما أظهرت النتائج ارتفاع معنوي عند مستوي معنوية ٠,٠٥ في كولسترول البروتينات الدهنية العالية الكثافة (HDL-c) وذلك بالمقارنة بالمجموعة الضابطة الموجبة،كماأظهر الفحص الهستوباثولوجى أن إضافة بروتينات الشرش لوجبات الفئران المصابة بالكبد لها تأثيرات وقائية على الكبد.كما أوضحتأيضاًدرجة تقبل عام مرتفعة للخبز المضاف إليه بروتين الشرش بنسب ٥% ،١٠%. ونستخلص مما سبق امكانية استخدام بروتين الشرش ضمن الاغذية المستخدمة فى تحسين وظائف الكبد ، واضافتها الى دقيق القمح فى صناعة الخبز بنسبة ٥%،١٠%.

الكلمات المفتاحية:بروتين الشرش – وظائف الكبد-فئران–خبز

ABSTRACT

USING WHEY PROTEIN to IMPROVE LIVER FUNCTIONS in INFECTED RATS

The current research aims to study the effect of why protein consumption on liver functions , biochemical analysis ,histopatological studies of hepatic male rats and also study the effect of sensory of whey protein bread. . Forty male albino rats were randomly divided into two main groups. The first main group (8 rats) was considered as negative control group (healthy rats) fed on basal diet while the second main group (32 rats) were infected with CCL4 to induced acute hepatic damage .The second main group consists of 4 subgroups each of (8 rats).One of these groups was chosen as a positive control. The rats in the positive control continued feeding on basal diet. however the other three groups has received whey protein in their diet at levels of 5%, 10% and 15% substitution, respectively for 7 weeks. Results revealed that all hepatic groups which fedon 5%,10% and15% whey protein resulted a decrease in body weight gain . The results also declared that all hepatic groups which treated with 5%,10% and 15% whey protein resulted in significant decrease ($p<0.05$) in the values of serum cholesterol, TG , LDL-c and VLDL-c but showed a significant increase ($p<0.05$) in the values of serum HDL-c comparing with thecontrol positive .On the other hand liver enzymes, results showed significant decrease in AST, ALT and ALP in hepatic groups treated with 5%, 10% and 15%whey protein. The results also showed that there was significant decrease in blood glucose in hepatic groups treated with 5%, 10% and 15%whey protein. Histopathological studies in hepatic rats fed on diets with whey protein has protective effect on liver .Results also showed high acceptance for whey protein powder bread at the ratios 5% and 10%.

The study recommended that the addition of whey protein to diet willimprove liver function and adding whey protein to bread wheat flour by 5%,10%.

Key words: whey protein -liver functions-rats-bread

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According to **Astrup(2005)** and **Luhovyy*et al.*,(2007)** whey protein have a role in contributing to the benefits of high protein diets as an inexpensive source of high nutritional quality protein.

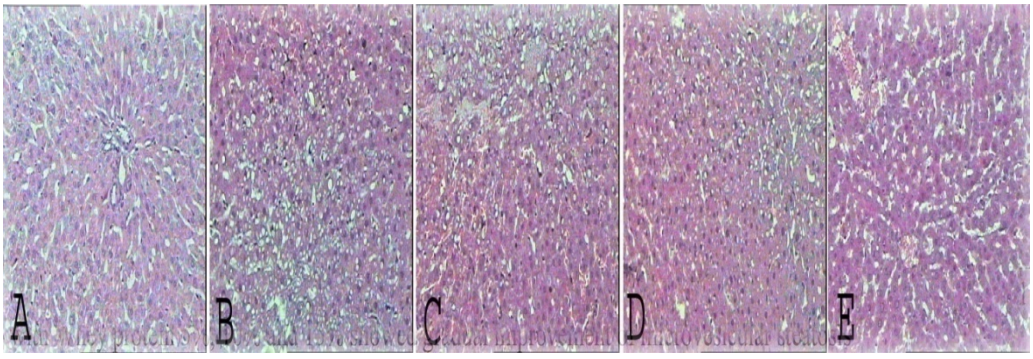
Table (4) : Effect of whey protein on sensory evaluation of produced bread.

Properties / Treatments	Aroma means of 10 scores	Taste means of 40 scores	Color means of 10 Scores	Tenderness means of 20 scores	Overall Acceptability means of 20 scores
WHB1	9.5	36.8	9.2	18.4	17.5
WHB2	8.8	34.6	8.8	17	16
WHB3	8.2	31	7.9	14	13.4

WHB1:whey protein bread (5% whey protein powder), WHB2 : whey protein bread (10% whey protein powder),WHB3 : whey protein bread (15% whey protein powder).

Histopathological Results:

Histopathological examination of liver section of normal rats showed in (PhotoA), While (photoB) positive control group which treated by CCL4 showed marked microvesicularsteatosis, on the other hand, (photoC,D,E) hepatic groups treated with 5%,10%,15% whey protein showed gradual improvement of microvesicularsteatosis.



Figure(3): Histopathological changes detected in the liver of (A) negative control,(B) positive control ,(C) hepatic rats treated with 5%whey protein, (D) hepatic rats treated with 10%whey protein ,(E) hepatic rats treated with 15 %whey protein.

Finally ,it could be concluded that the histopathological studies in rats fed on diets with whey protein declared protective effects on liver.

The results are in the same line with **Kume*et al.*,(2006)** who found that rats on a whey-containing diet demonstrated lower liver enzyme levels indicating liver damage (ALT and AST) and lower levels of traditional hepatitis markers (lactate dehydrogenase and bilirubin).

In this respect ,these results are in agreement with **Chitapanarux*et al.*,(2009)** who reported that a daily dose of 20 g whey protein given to fatty liver patients led to a significant reduction in ALT and AST .In addition ,**Hamad*et al.*,(2011)** stated that oral feeding of whey proteins lowered levels of ALT and AST in rats model of fatty liver.

Table (3): Effect of whey protein onliver functions of the experimental rat Suffering from acute hepatic damage

Groups	AST (U/L)	ALT(U/L)	ALP(U/L)
NC	40.36 ±0.58^{e}	26.98 ±0.91e	48.56±1.16e
PC	80.10 ±4.44^{a}	52.60 ±1.61^{a}	132.11±1.40^{a}
WH1	65.45±4.64^{b}	46.24 ±0.97^{b}	78.51 ± 1.16^{b}
WH2	60.65±3.32^{c}	40.21±2.90^{c}	71.95 ± 1.60^{c}
WH3	55.46±1.08^{d}	37.10 ±4.25^{d}	60.59 ± 1.38^{d}
LSD	3.33	2.52	1.73

NC: negative control group ,PC :positive control group(hepatic rats) ,WH1: hepatic rats treated with 5% whey protein, WH2: hepatic rats treated with 10% whey protein, WH3: hepatic rats treated with 15% whey protein

Different letters on same column represent statistically significant (P<0.05) difference between means, Values are means ± SD for8 rats.

Effect of whey protein supplementation on sensory evaluation of produced bread :

The effects of whey protein supplementation on the sensory characteristics of bread are presented in Table (4).

The results showed high acceptance for whey protein powder bread at the ratios of 5% and 10%·Concrning Table (4) results show the mean values of aroma, taste , color, tenderness , overall acceptability of bread 5% whey protein powderthe mean values were (9.5,36.8 ,9.2,18.4,17.5respectively), bread10% whey protein powder were (8.8,34.6,8.8,17,16 respectively)and bread15% whey proteinpowder(8.2,31,7.9,14,13.4 respectively).

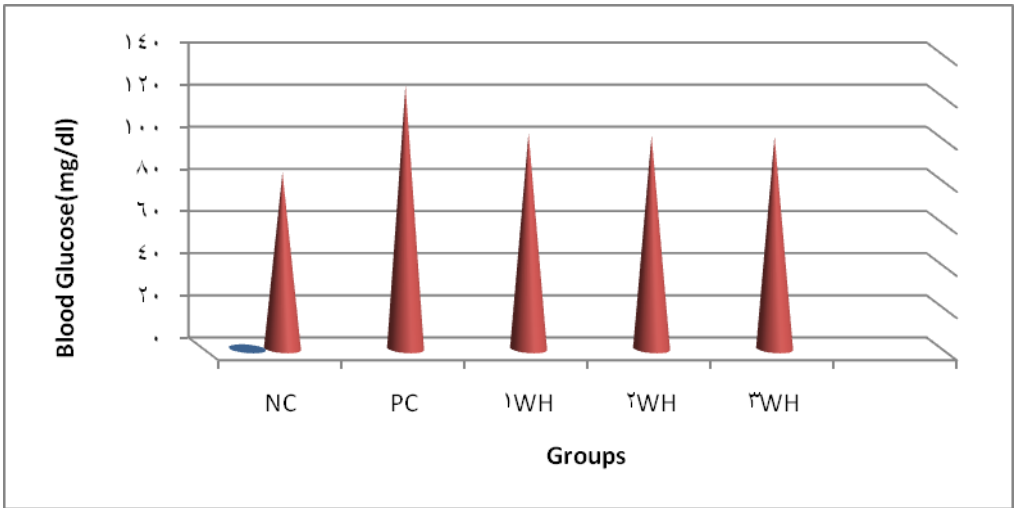


Figure (2): Effect of whey protein on blood glucose of the experimental rat Suffering from acute hepatic damage

NC: negative control group ,PC :positive control group(hepatic rats) ,WH1: hepatic rats treated with 5% whey protein, WH2: hepatic rats treated with 10% whey protein, WH3: hepatic rats treated with 15% whey protein.

Different letters on same column represent statistically significant ($P<0.05$) difference between means, Values are means $\pm$ SD for8 rats.

Effect of whey protein on liver functions of the experimental rat Suffering from acute hepatic damage

Results presented in Tables (3) demonstrate the effect of different levels of whey protein on the levels of liver enzymes AST , ALT and ALP .Results revealed that rats in the positive control group showed high level of liver enzymes AST , ALT and ALP compared to rats in the negative control group.

These results revealed that all rat groups ingested whey protein in the diet declared significant decrease ($P<0.05$) in the values of liver enzymes AST , ALT, ALP comparing with the control positive group .

These results also indicated that there are significant differences between all rat groups ingested whey protein in the diet WH1, WH2 , WH3 in liver enzymes AST , ALT and ALP .The best results of liver enzymes was at the ratio 15 % whey protein.

These results are in agreement with those of **Parekh and Anania (2007)** and **Hamad*et al.*,(2011)**who found that whey protein reduced serum cholesterol and triglyceride. In this respect, **Nagaoka (1996)**reported that whey protein concentration reduced the serum total cholesterol value and describedβ-lactoglobulin which inhibited cholesterol absorption through changes of micellar cholesterol solubility in the intestine, accompanied by an increase of fecal steroid excretion.

Table (2): Effect of whey protein on serum lipid profile of the experimental rat groups.

Groups	cholesterol (mg/dl)	TG (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
NC	107.79±2.21^{c}	54.18±10.69^{b}	85.00±18.39^{a}	11.95±18.38^{d}	10.84±2.14^{b}
PC	152.14±6.68^{a}	78.40±3.64^{a}	59.03±6.51^{d}	77.43±5.54^{a}	15.68±0.73^{a}
WH1	134.89 ±24.06^{b}	57.35±13.27^{b}	66.64±12.90^{bcd}	56.77±28.27^{ab}	11.47±2.65^{b}
WH2	132.16±22.70^{b}	57.24±6.34^{b}	74.50±16.17^{ac}	46.21±28.93^{bc}	11.45±1.27^{b}
WH3	115.21±11.75^{c}	55.34±7.20^{b}	77.43±9.35^{ab}	26.71±14.25^{cd}	11.07±1.45^{b}
LSD	16.25	9.03	13.59	21.33	1.81

NC: negative control group ,PC :positive control group(hepatic rats) ,WH1: hepatic rats treated with 5% whey protein, WH2: hepatic rats treated with 10% whey protein, WH3: hepatic rats treated with 15% whey protein

Different letters on same column represent statistically significant ($P<0.05$) difference between means, Values are means ± SD for8 rats.

Effect of whey protein on blood glucose of the experimental rat Suffering from acute hepatic damage

Data in figure (2) showed that significant decrease ($p<0.05$) in serum glucose between positive control group (PC)(125± 7.01mg/dl) and groups WH1, WH2 , WH3(102.38± 9.38, 101.21± 10.66, 100.72 ± 5.16mg/dl , respectively), (LSD = 8.27), while there are no significant differences between groups WH1, WH2 , WH3 in serum glucose concentration .The best result was at WH3 group (hepatic rats treated with 15% whey protein).

In this respect, **Belobrajdic et al .,(2004)**declared that the whey-fed rats demonstrated increased insulin sensitivity and reduced plasma insulin concentration are both associated with improved blood sugar control and reduced fat storage. On the other hand, **Hamad*et al.*,(2011)**declared that oral administration of all the tested whey proteins lowered the blood glucose levels of rats.

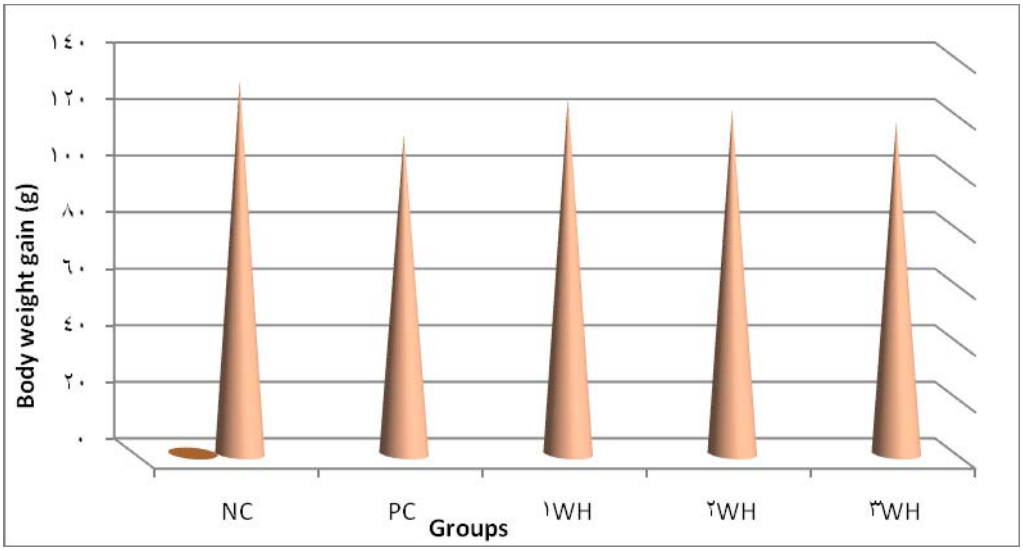


Figure (1): Effect of some levels from whey protein on body weight gain(BWG)of the experimental rat groups:

NC: negative control group ,PC :positive control group(hepatic rats) ,WH1: hepatic rats treated with 5% whey protein, WH2: hepatic rats treated with 10% whey protein, WH3: hepatic rats treated with 15% whey protein.

Different letters on same column represent statistically significant (P<0.05) difference between means, Values are means ± SD for8 rats.

Effect of whey protein on serum lipid profile of the experimental rats Suffering from acute hepatic damage

Results in Table (2) declared that, rats in the positive control group have higher cholesterol ,triglycerides(TG), Low density lipoprotein–cholesterol (LDL-c) and very low density lipoproteins (VLDL-c)levels, while have lower HDL level as compared to rats in the negative control group.

These results also showed significant decrease ($p<0.05$) in cholesterol ,low density lipoprotein–cholesterol (LDL-c) ,triglycerides(TG) and very low density lipoproteins (VLDL-c)between positive control group (PC) and hepatic rat groups which fed on basil diet supplemented with different levels of whey protein (WH1,WH2,WH3).However ,these results showed significant increase ($p<0.05$) in high density lipoprotein–cholesterol (HDL-c) between positive control group (PC) and WH1,WH2,WH3 .

Histopathological analysis:

The tissues of liver were fixed in 100% formalin and embedded in paraffin wax. Section of 4-5 microns thickness were made using rotary microtome and stained with haematoxylin-eosin and histological observation were made under light microscope **Carleton(1979)**.

Statistical Analysis:

Statistical analysis were performed by using computer of statistical package for social science (SPSS version 13.0). The results are presented as means $\pm$SD . One way analysis of variance (ANOVA) was used to test the differences between groups **(SPSS,1999)**.

RESULTS AND DISCUSSION

Effect of whey protein on body weight gain (BWG) of the experimental rats suffering from acute hepatic damage

Data in figure (1) showed that the highest body weight gain was observed for negative control group (NC) ,however the lowest body weight gain was observed for positive control group(PC),there was significant differences($p<0.05$) between positive control group(PC) and negative control group(NC) . In this respect there was significant differences($p<0.05$) between positive control group(PC) and hepatic rat groups which fed on basil diet supplemented with different levels of whey protein (WH1,WH2,WH3).On the other hand, hepatic rats treated with 5%,10%,15% whey protein (WH1,WH2,WH3) demonstrated lower values of body weight gain compared to negative control group (NC)and increased than that of the positive control group.

These results are in harmonization with **Belobrajdic*et al.*,(2004)**whodeclared that increasing the dietary density with whey protein led to reduced body-weight gain. Also**Hall *et al.*,(2003)**and **Luhovyy*et al.*,(2007)**found that whey consumption reduced appetite and decreased food intake which lead to lose weight by helping to limit the caloric intake.

Blood sampling:

At the end of experimental period(7 weeks) rats were fasted over night before sacrificing .Blood was collected and centrifuged (3000rpm), serum was separated for analysis .Serum was carefully aspirate, transferred in to clean cuvet tubes and stored frozen at -20◦C for analysis. Body weight gain was calculated by following formula:

BWG(g) =Final weight (g) -Initial weight (g)

Biochemical analysis:

For each group analyses included the following:

Total cholesterol (TC) was determined according to **Allen (1974).**The determination of serum triglycerides(TG) was done according to **Fassati and Prencipe(1982).** While high density lipoprotein–cholesterol (HDL-c) was determined according to **Lopez (1977)** , whereas Low density lipoprotein–cholesterol (LDL-c) were determined according to **Friedewable*et al.*,(1972)**.

LDL-c = TC-[HDL-c + (TG/5)]

VLDL-c = TG/5

Determination of GOT (AST) and GPT (ALT) were determinated according to **Reitman and Frankel (1957).**

Determination of serum alkaline phosphates (ALP) was carried out according to **Belfield and Goldberg(1971).**and determination of glucose oxidase was carried out according to **Tietz(1976)**.

Sensory Evaluation

Sensory properties for bread were evaluated by 10trained panelists from Home Economic Department ,Faculty of Specific Education, Damietta Univ., Egypt according to **Watts *et al.*,(1989).**

main group (32 rats) were subcutaneously administered a single dose of CCL4 (30%V/V) in paraffin oil (1ml/kg) for 2 daysat the beginning of the experimental period , to induced acute hepatic damage according to **Nadeen*etal.*,(1996).** Rats were divided into 4 subgroups continued feeding for 7 weeks as follows:

Group2: (positive Control group): 8 rats fed on basal diet only (PC)
Group 3: 8 rats fed on diet containing 5% whey protein (WH1)
Group 4: 8 rats fed on diet containing 10% whey protein (WH2)
Group5:8 rats fed on diet containing 15% whey protein (WH3)

The composition of different experimental diets is illustrated inTable (1)

Table(1):Composition of different experimental diets

Ingredients	Groups				
	Normal rats	Hepatic rats			
		Control(+)	WH1	WH2	WH3
Protein(casein)	10%	10%	10%	10%	10%
Corn oil	10%	10%	10%	10%	10%
Mineral mixture	4%	4%	4%	4%	4%
Vitamin mixture	1%	1%	1%	1%	1%
Cellulose	5%	5%	5%	5%	5%
Choline chloride	0.2%	0.2%	0.2%	0.2%	0.2%
Methionine	0.3%	0.3%	0.3%	0.3%	0.3%
Bile salts	-	0.25%	0.25%	0.25%	0.25%
Whey protein	-	-	5%	10%	15%
Corn starch	69.5%	69.25%	64.25%	59.25%	54.25%

Preparing of whey protein bread (WHB):

Bread was prepared from 86 g wheat flour extraction 72%, 8g dry yeast, 5 g sugar, 1g salt with the addition of water for kneading.

The used wheat flour was partially substituted by 5, 10 and 15 % of whey protein (WHB1,WHB2,WHB3).

that glutathione deficiency is an important factor contributing to liver damage. Thus, supplements that boost the body's production of glutathione indirectly benefit people with chronic hepatitis**(Cutler,2009)**.

The technological implications of whey products as an excellent additives to improve food formulation have been proposed for a broad range of foods due to their functional benefits in processing such as solubility, water-binding and viscosity, gelling, emulsification, whipping, foaming and aeration, dispersibility, edible film formation, antioxidant activity, adhesion property and heat-induced browning**(Burrington,2004)**.

This work aims to study the effect of whey protein on liver functions , biochemical analysis , histopatological studies of hepatic male rats and some sensory of whey protein bread.

MATERIALS AND METHODS

Materials:

1-Whey protein

whey protein powder was obtained from El Kasas co., for dairy products , Mansoura.

2-Chemicals and Kits

Vitamins, minerals, cellulose ,bile salts , choline chloride and diagnostic kits were purchased from El-Gomhoria Co., Sherief Street , Cairo, Egypt.

3-Carbon tetra chloride

Carbon tetra chloride (CCL_4) was obtained from scientific office co., Damiettain the form of 40% liquid dispensed in one litter plastic bottle.

Methods:

Experimental animals

Forty male albino rats (Sprague Dawley strain) weighing about165gwere obtained from (Zoology Dept., Fac. of Sci. , Damietta).All rats were fed on basal diet for one week, after one week period , the rats were divided into two main groups.The first main group (8 rats) were fed only on the basal diet (negative control group) (NC) according to **Reeves *et al.*,(1993)**The second

Whey, a by-product of cheese and curd manufacturing, was considered awaste product. The discovery of whey as a functional food with nutritional applications elevated whey to a co-product in the manufacturing of cheese (**Walzem*et al.*,2002)**.

The term "whey" actually refers to a complex, milk-derived substance made up of a combination of protein, lactose, and minerals, with trace amounts of fat. Protein is the most abundant component of whey and includes many smaller protein subfractions and minor peptides. Each of these subfractions has unique biological properties. Modern filtering technology has improved dramatically in the past decade, allowing companies to isolate some of whey's highly bioactive peptides, such as lactoferrin and lactoperoxidase, which occur in only minute amounts in cow's milk**(Hall *et al.,* 2003)**.

Whey, a protein complex derived from milk, is being touted as a functional food with a number of health benefits. The biological components of whey, including lactoferrin, betalactoglobulin, alpha-lactalbumin, glycomacropeptide, and immunoglobulins, demonstrate a range of immune-enhancing properties. In addition, whey has the ability to act as an antioxidant, antihypertensive, antitumor, hypolipidemic, antiviral, antibacterial, and chelating agent**(Marshal ,2001)** .

Effects of whey protein on human health are of great interest and this protein mixture is being investigated as a way of reducing disease risk or as a supplementary treatment for several diseases**(Krissansen, 2007)**.

The primary mechanism by which whey is thought to exert its effects is by intracellular conversion of the amino acid cysteine to glutathione, a potent intracellular antioxidant. A number of clinical trials have successfully been performed using whey in the treatment of cancer, HIV, hepatitis B, cardiovascular disease, osteoporosis, and as an antimicrobial agent. Whey protein has also exhibited benefit in the arena of exercise performance and enhancement**(Marshall, 2004).**

While some of whey protein's sub-fractions can help someone with hepatitis remain healthy, whey's promotion of glutathione delivers a specific benefit to those with liver disease. Whey protein contains high levels of the amino acid cysteine, which is needed for the body to produce glutathione. Glutathione is an antioxidant found in all tissues protecting against potential damage from wastes and toxins. Clinical studies have demonstrated that the level of glutathione is significantly depressed in many people with Hepatitis C. Experts also recognize

USING WHEY PROTEIN to IMPROVE LIVER FUNCTIONS in INFECTED RATS

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INTRODUCTION

Liver plays a pivotal role in regulating various physiological processes in the body such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesize useful principles. Therefore, damage on the liver inflicted by hepatotoxic agents is of grave consequences **(Shanmugasundaram and Venkataraman,2006).**

Unfortunately, liver is often abused by environmental toxins, poor eating habits and over counter drug use, which can damage and weaken the liver and evantually leads to hepatitis, cirrhosis and liver disease. Conventional medicine is now pursuing the use of natural products **(Treadway, 1998).**

Carbon tetrachloride is one of the most potent hepatotoxins (toxic to the liver), and is widely used in scientific research to evaluate hepatoprotective agents**(Seifert *et al.*,1994).**

Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of live)r diseases are inadequate and sometimes can have serious side effects**(Guntupalli*et al.*, 2006)**.

The major liver diseases that are responsible for the most morbidity and mortality are viral hepatitis (chronic hepatitis B and C), alcoholic liver disease, non-alcoholic fatty liver disease, cirrhosis and hepatocellular cancer. These conditions account for more than 95% of all deaths due to liver disease**(Federico *et al.*,2012).**

Nutrition has long been recognized as a prognostic and therapeutic determinant in patients with chronic liver disease **(Plauth*et al.*,1997).**

malnutrition is prevalent in all forms of liver disease; from 20% in compensated liver disease to more than 80% in those patients with decompensated disease **(Nompleggi and Bonkovsky, 1994).**