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Gross morphological and physiological evaluation of hepatic alterations induced by whey protein supplementation in healthy adult male albino rats

Zaid Ali Zaidan
Department of Anatomy and Histology, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq. E-mail: zaidali@vet.uoqasim.edu.iq

Ekhlas Abid Hamza Al-Alwany
* Corresponding author. Department of Anatomy and Histology, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq. E-mail: Ekhlas.alalwany@vet.uoqasim.edu.iq

Abstract

Objective

The liver is a highly complex visceral organ that provides integrated metabolic, digestive, detoxifying, endocrine regulatory and immune functions and thus, is indispensable for the maintenance of homeostasis in the whole body. This study evaluated the impact of nutritional whey protein supplementation on liver-related morphophysiological alterations in healthy adult male albino rats.

Materials and methods

Thirty-six rats were randomly allocated into three equal groups: a control group, a low-dose whey protein group (5 g/kg body weight), and a high-dose whey protein group (10 g/kg body weight), with oral supplementation continued for six weeks. At the end of the experiment, body weight, liver weight, liver to body weight ratio, and selected serum biochemical markers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), superoxide dismutase (SOD), malondialdehyde (MDA), and triglycerides (TG), were evaluated. Receiver operating characteristic (ROC) analysis was also performed to assess the discriminatory value of the investigated markers.

Results

The results demonstrated significant reductions in final body weight and liver weight in both whey protein-treated groups compared with the control group, whereas the liver to body weight ratio showed a declining trend without reaching statistical significance. Biochemically, ALT and AST showed mild elevations that were not statistically significant. In contrast, SOD, MDA, and TG were significantly increased in the treated groups, indicating enhanced oxidative stress, lipid peroxidation, and disturbed lipid handling. ROC analysis further revealed limited diagnostic utility for ALT and AST, whereas SOD, MDA, and TG showed strong discriminatory performance, with MDA exhibiting the highest overall predictive accuracy. Collectively, these

findings indicate that whey protein supplementation at the tested doses in healthy rats induced a measurable hepatic metabolic and oxidative burden rather than clear enzyme-defined hepatocellular injury.

Conclusions

The study suggests that oxidative and lipid-related biomarkers may provide greater sensitivity than conventional transaminases for detecting early liver-related responses to excessive protein supplementation.

Keywords: albino rats, biomarkers, liver, oxidative stress, whey protein supplementation

Paper Type: Research Paper.

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Introduction

The liver is a highly complex visceral organ that provides integrated metabolic, digestive, detoxifying, endocrine regulatory and immune functions and thus, is indispensable for the maintenance of homeostasis in the whole body (Gan et al., 2025). It consists of an organized spatially heterogeneous tissue with hepatocytes distributing metabolic functions along the porto-central axis**.** It is efficient coordination of the role of nutrient, protein turnover and biotransformation of xenobiotics (Martini et al., 2023). Recent evidence shows that hepatocytes are not functionally identical, since distinct populations with differing hepatocyte populations experience in respect of their molecular identity, metabolic specialization, and their contribution to tissue renewal during homeostasis and regeneration (Chen et al. 2023). In the liver, apart from the hepatocytes, there are some distinct non-parenchymal cells, especially liver sinusoidal endothelial cells that control the vascular exchange, inflammatory and tissue maintenance under physiological conditions (McConnell et al., 2023). The biological significance of the liver stems from its pivotal role in homeostasis equilibrium in the body and the protection of the body from endogenous and exogenous toxic insults through carefully controlled detoxification pathways (Wang et al., 2025). The liver is also an immunological center of the body and a compromise of hepatic activity can cause significant disruptions in the both the innate and adaptive regulation of immunity (Dąbrowska et al., 2024). Because hepatic function is tightly connected with the neuroimmune and systemic signaling networks indicating that changes in liver's condition can affect the functionality of a distant organ and have consequences for the pathophysiologic

consequences beyond the hepatobiliary structure itself (Zou et al., 2024). This broad functional relevance is the reason that liver disorders are a huge burden on worldwide health and why the liver is considered a critical target organ in physiological, toxicological and nutritional studies (Gan et al., 2025). The liver is a major metabolic hub that provides centralized nutrient sensing, nuclear receptor signaling, and autophagy using an environment from which to maintain the homeostasis of biochemical tissues at system levels during varying nutritional conditions (Kim & Lee, 2025). Recent mechanistic work has added to this perspective that hepatic physiology requires the tightly coordinated adaptation of metabolism in stress and repair measures witnessing the center of the role of the liver on maintaining whole body metabolic equilibrium (Ma et al., 2025). The interaction between phenolic compounds and proteins lead to alteration in the carbonyl group composition and the thiol group's oxidation. The liver regulates carbohydrate homeostasis through glycogenesis, glycogenolysis, and gluconeogenesis and for the first time, recent experimental evidence showed that hepatic glycogen directly controls gluconeogenic activity through an AMP-Activated Protein Kinase/CREB-Regulated Transcription Coactivator 2 (AMPK/CRTC2) regulatory axis in mice (Zhang et al., 2025). In addition to this, hepatic gluconeogenesis from lactate and glycerol is directly responsible for systemic supply, of different intensities, during exercise, therefore, it confirms that the liver plays a main role in managing blood glucose availability during greater potentiation of metabolic demand (Horiuchi et al., 2025). The liver also regulates systemic lipid transport by assembling and secreting very-low-density lipoproteins, and thus Very-Low-Density Lipoprotein (VLDL) biogenesis represents a fundamental physiological pathway between hepatic), triglycerides. equal functions and whole-body distribution of lipid. (Van Zwol et al., 2024) Protein metabolism in the liver involves amino acid metabolism and amino acid transamination, nitrogen metabolism, as well as coordination of cellular metabolism with the mitochondrion in pathways involved in ureagenesis and metabolic homeostasis (Erdal et al., 2025). The liver also is the major site of albumin synthesis, and this synthetic function is one of the most obvious physiological manifestations of hepatic ability for protein synthesis (Baral, 2025). The significance of hepatic nitrogen metabolism is also shown by recent evidence indicating that dysfunction of nitrogenous waste removal from the body leads to impaired amino acid metabolism and contributes to the progression of disease, indicating the physiological importance of the hepatic urea cycle (Han et al., 2026). Although several studies have investigated the antioxidant and hepatoprotective effects of whey protein under pathological conditions, limited information is available regarding its potential hepatic effects in healthy animals receiving high-dose supplementation**.** Therefore, this study was designed to investigate whether chronic oral supplementation with two different doses of whey protein induces dose-dependent morphological and physiological alterations in the liver of healthy adult male albino rats.

Materials and methods

Experimental animals: A total of 36 adult male albino rats (10-12 weeks old, 200-250 g) were used. They were obtained from Babylon Province in Iraq and kept under normal laboratory conditions and fed ad libitum. Animals were housed for 7 days prior to the experiment and care

was carried out in accordance with accepted guidelines for the care of laboratory animals and the ARRIVE 2.0 guidelines.

Experimental design: The rats were then randomly allocated into three groups of 12 rats each. The control group was gavaged with distilled water and the second and third groups were gavaged with 5 and 10 g/kg body weight of whey protein, respectively, for 6 weeks. All the animals were morphologically and physiologically evaluated at the end of the treatment.

The sacrifice of animals and samples collected: At the end of the 6 weeks, animals were fasted for 8–12 hours and then sacrificed after being anesthetized with ketamine and xylazine. The blood was taken from the heart, centrifuged and the serum was kept at -20°C for analysis. This liver was then dissected and gross morphological assessment was made.

Preparing and administration of whey protein: Commercial whey protein powder was used as the experimental supplement. It comprised whey protein isolate, whey protein concentrate, hydrolyzed whey protein, and bioactive compounds like lactoglobulin, lactalbumin, immunoglobulins, lactoferrin and BCAAs. The powder was freshly dissolved in distilled water each day and fed to the rats for 6 weeks by gastric gavage at a dose of 5 g/kg and 10 g/kg body weight.

Morphological evaluation: Body weight was determined at the end of the experiment prior to slaughtering. Liver was removed and cleaned and weighed on digital weighing machine. Liver to body weight ratio was obtained by the following formula:

Liver to body weight ratio **(%) = Liver weight/Body weight × 100.**

Body weight, liver weight and liver/body weight ratio were evaluated as parameters.

Physiological evaluation: Serum levels of liver function, oxidative stress and lipid metabolism markers were evaluated. The parameters measured were ALT, AST, SOD, MDA and Triglyceride.

Determination of AST: The activity of AST in serum was determined by the colorimetric assay kit purchased from Ray Biotech. The serum samples were mixed with the working solution, incubated at 37°C and the absorbance was measured at 340 nm. AST activity was determined based on the manufacturer's instructions and expressed in U/L.

Determination of ALT: A Ray Biotech colorimetric assay kit was used to measure the activity of serum ALT. The change in absorbance at 340 nm after treatment with the working solution was used as the assay. The activity of ALT was determined by the kit formula and was shown in U/L.

Determination of SOD: The activity of SOD in serum was determined by a Solarbio SOD assay kit. The method was based on the inhibition of the xanthine–xanthine oxidase reaction and the absorbance were measured at 560 nm. The results were determined based on the manufacturer's formula and given in unit per milliliter (U/mL).

Determination of MDA: The level of serum MDA was determined by Solarbio MDA assay kit. The assay was carried out following the reaction of MDA with thio-barbituric acid which produces a coloured product which can be measured at 532 nm and 600 nm. The concentration of MDA was expressed as nmol/mL.

A serum triglyceride test is used to measure the level of triglycerides in the blood: The BIOLABO GPO enzymatic method was used for the measurement of serum triglycerides. The serum or standard was mixed with the reagent, then incubated at 37°C and the absorbance read at 505nm. All results were reported in mg/dL.

Statistical analysis: The data were analyzed with SPSS version 31 and presented as mean ± SE. Groups were compared by one way ANOVA with Tukey's post hoc test. $P \leq 0.05$ was considered significant and $P \leq 0.01$ highly significant. Relationships between variables and sensitivity of the markers were also tested using correlation and ROC analyses.

Results and discussion

Figure 1 in this study was the ventral view about the thoracoabdominal cavity of group 1 rats (control group). Liver was in the usual location within the apparatus of the cranial segment of the abdomen and had the usual anatomical association with the rest of the organs. Clear distinction of the left lateral lobe, left median lobe and right median lobe were seen which is a sign of normal hepatic lobulation. Its outer aspect of the liver was smooth, glossy and uniformly dark reddish-brown, as would be expected with a normal gross morphology of a healthy rat liver. There were also no instances of the stomach, jejunum, mesentery, caecum, left kidney, and left lung being noticed without apparent displacement. These results show that normal topographical and morphological appearances of the liver were preserved in the control animals. The Figure 2 shows the ventral appearance of the abdominal cavity in rats belonging to group 2 when the dose of whey protein is 5 g/kg body weight. The liver was not removed, and controlled its overall anatomical connections with the other organs of the abdominal cavity. Nevertheless, the liver seemed to be a little bit larger than in the control group, especially its visible cranial range. The hepatic mass has appeared as taking a bit more space of the abdominal cavity, and the small intestine and cecum are seen as relatively displaced towards the caudal part. With this augmented prominence, the overall arrangement of the abdominal entrails was retained. These observations indicate that when whey protein was administered at 5 g/kg body weight, there was mild gross morphological change, which was predominantly characterized by slight loss of prominence of the hepatic shape. In the Figure 3 the liver of group 2 has been isolated followed by the addition of whey protein at a body weight of 5 g/kg The lobules observed in the hepatic lobes were distinct which indicates maintenance of overall lobular structure. The left lateral and median lobes were slightly larger and smoother as compared to control group, implying the slight increase in hepatic mass. However, there was relatively smooth surface and lobes still were anatomically differentiated. There was no gross nodularity, gross deformity or gross discoloring present. Consequently, the gross observations in this group reveal that there are slight morphological alterations in the absence of destruction of the general hepatic structural configuration. The ventral view of the abdominal cavity in the rats of group 3 was taken at a dose of 10 g/k g body weight of whey protein as shown in Figure 4. Where the liver was more dominant in this group as compared to the control group and group 2, it was more dominant in the cranial abdominal cavity. The conspicuity of the left lateral and median lobes was greater, and the tissue of the liver took up a larger area of the field of cranial abdomen. Consequently, the usual physical position

of the liver in relation to other abdominal organs, such as stomach, spleen, jejunum, mesentery and caecum, was more distorted. These observations reveal that increased dose of whey protein was related with more apparent gross hepatic enlargement or prominence indicating that there was an increase in the gross hepatic effect at the high dose. The gross look of the liver in group 3 fed on whey protein at 10g/kg body weight, is further represented in Figure 5. The large hepatic lobes were prominent although the liver was larger as compared to the other groups. Moreover, the outer appearance was less homogenized in terms of color and a less smooth surface than that was seen among the control group and a little more pale and finely mottled. The hepatic size had grown rather large and had shifted the stomach and caecum. Despite the general lobular outline, the appeared gross appearance of the liver in the group indicates that there were more morphological changes altered compared to group 2. This possibly implies that the increased dose of whey protein had a greater influence on the liver morphology.

Figure 1. Gross photograph (ventral) view of the thoracoabdominal cavity in rats (control group) showing the liver in situ and its relationship with adjacent organ: 1- left lateral lobe, 2 left median lobe, 3 right median lobe, C- Caecum. M- Mesentery. J- Jejunum. ST-stomach. k - left kidney, L- left lung

The gross examination of the control group revealed that the morphology of the rat liver was normal in terms of the location in the abdomen, the distinct lobulation, smooth capsule, reddish-brown color, and normal anatomical relationships with other organs, consistent with previous anatomical description of the rat liver. Whey protein at 5 g/kg in group 2 resulted in mild prominence and slight enlargement of the left lateral and median lobes with no change in the normal lobular pattern. These changes could be interpreted as an early adaptive response of the liver, because high protein feeding enhances the metabolic requirements of the liver, due to amino acid deamination, ureagenesis and nitrogen metabolism. Mitochondrial and metabolic adaptations

of the liver have been observed in rats fed with high-protein diets and supplemented with whey protein (Morifuji et al., 2005).

Figure 2. Gross photograph (ventral) view of the abdominal cavity in rats (group 2) demonstrating the liver and its relationship with major abdominal organs: L. Liver (predominantly left lateral lobe), S. Small intestine, C. Caecum

Figure 3. Gross photograph (ventral) view of the liver in rats (group 2) showing :1-left lateral lobe, 2- median lobe, 3- right lateral lobe, 4- Caudate lobe, 5-Quadrate lobe

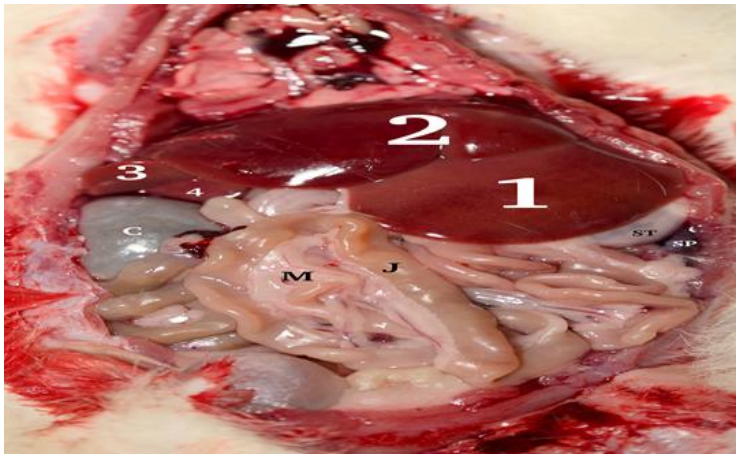

Figure 4. Gross photograph (ventral) view of the abdominal cavity in rats (group 3) demonstrating the liver and its relationship with abdominal organs, 1-left lateral lobe, 2-median lobe, 3-right lateral lobe, 4- Quadrate lobe, C- Caecum, M- Mesentery, J- Jejunum, ST-stomach, SP- spleen, M- Mesentery, J- Jejunum, ST-stomach, SP- spleen

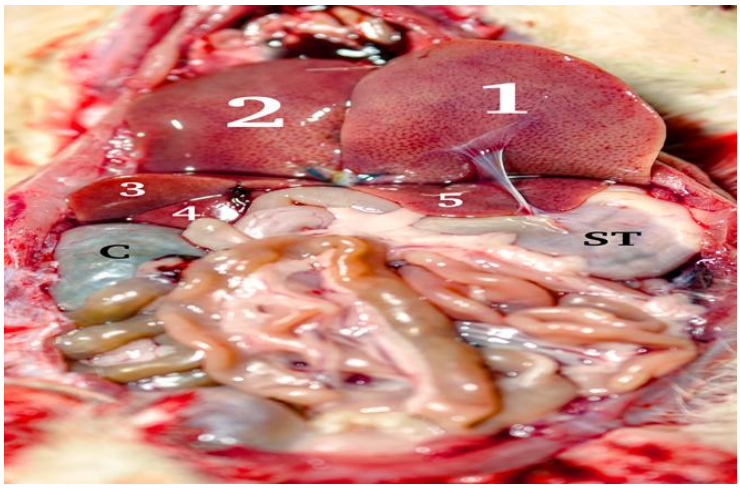

Figure 5. Gross photograph (ventral) view of the abdominal cavity in rats (group 3) demonstrating the liver and its relationship with abdominal organs, 1-left lateral lobe, 2-median lobe 3-right lateral lobe, 4-Quadrate lobe of Caudate lobe, C- Caecum, ST-stomach

However, group 3 (10 g/kg whey protein) exhibited more distinct gross liver changes such as liver prominence, increased liver space in the cranial abdominal cavity, mild color variation of the liver and displacement of adjacent organs. These results indicate a greater dose-dependent hepatic metabolic stress. While, hepatomegaly is an adaptive feature in isolation, if associated with colour or structural changes, it could be a sign of early adverse hepatic modification (Hall et al., 2012). The dose dependent interpretation is corroborated by studies demonstrating that high protein or whey protein intake can lead to hepatic inflammatory, apoptotic, biochemical or histological changes, depending on the dose, duration, metabolic status and physical activity (Yiğit Ziolkowski et al., 2024; Hamad & Alalwany, 2024). On the other hand, other studies showed the increase in liver weight without obvious histomorphological damage, which indicated

that liver enlargement sometimes might be a metabolic adaptation rather than a permanent damage (Ghazal & Al-Alwany, 2025). Systematic evidence also suggests that exercise can mitigate the negative effects of high protein diets while sedentary lifestyle may promote metabolic and inflammatory modifications (Medeiros et al., 2021). Table 1 represented the morphological changes of the groups of animals untreated with the dose concentrations in the study experiment. The results indicated that a significant decrease (P=0.005) in the weights of the experimental animals were found in both groups treated with the concentration (5 g/kg and 10 g/kg) (428.00 ± 13.263 and 448.00 ±16.861, respectively) as compared to the control group (500.40 ±4.320). Regarding the weights of the livers of the animals made anatomically after the end of the experiment, a significant reduction in the liver weights of the treated animals was observed compared to the liver weights of the control animals. The function of the mean liver weight of control group was 15.66480 ± 0.4220, while liver weight of other groups was decreased to 12.7162 ± 0.09886 and 12.0834 ± 1.0827 and there wasn't no statistically significant difference between each other (P<0.05).

Table 1. show relationship of liver weight to weight of rats treated of different doses of protein (Mean ± S.E.)

Morphological data	Experimental Animals Groups Mean ± S.E.			*P value*
	Control Without dose	Group 1 Dose (5g/Kg)	Group 2 Dose (10 g/Kg)	
Animals weight (Kg)	500.40 ± 4.320[a]	428.00 ± 13.263[b]	448.00 ± 16.861[b]	0.005*
Liver weight (g)	15.66480 ± 0.4220[a]	12.7162 ± 0.09886[b]	12.0834 ± 1.0827[b]	0.006*

*Significant under p ≤ 0.05 by One way – ANOVA test, Different small letters refer to significant differences.

From the present results, it is observed that whey protein supplementation in both the doses significantly decreased the final body weight as compared to the control group. This could be due to changes in whole-body metabolism, appetite control, nutrient distribution, and/or energy expenditure, and protein-rich diets have been shown to affect satiety, gastric emptying, thermogenesis, and anorexigenic gut pathways (Cava et al., 2024). In metabolic models, clinical or dietary-intervention settings, whey-based preparations have been shown to cause similar reductions in body-weight gain (Sepandi et al., 2022; Al-Rawhani et al., 2024; Shao et al., 2025). The liver weight was considerably decreased indicating that liver was an important target organ of the supplementation. As the liver plays a main role in amino acid metabolism, lipid oxidation and detoxification, chronic exposure to high protein may trigger adaptive or stress responses in the liver, such as glycogen depletion, lipid mobilization, protein turnover, or liver damage. This aligns with the context-dependent nature of whey protein effects, potentially being beneficial or detrimental depending on the dose, physiological state, and experimental model used. Past research suggests that whey protein could be beneficial in the recovery of damaged or metabolically compromised livers as it shows promise in reducing steatosis, lipid accumulation,

inflammation, oxidative stress, and biochemical injury (Wang et al., 2022; Yiğit Ziolkowski et al., 2024; Shao et al., 2025;). Other studies have also shown antioxidant and mitochondrial protective effects against chemically-induced liver injury (Aydin et al., 2022; Tufan et al., 2023). Thus, the observed liver weight reduction in the present study could represent a metabolic stress of high dose whey protein in healthy rats. The lack of a significant difference between the two treatment doses could be due to a threshold or plateau response, as organ level responses do not always increase with dose. The liver to body weight ratio did not show statistical significance, but its results pointed to a decreasing trend that may have biological significance and should be supported by the results of histological, histochemical, and biochemical examinations (Yiğit Ziolkowski et al., 2024; Helvacıoğlu et al., 2026). Table 2 shows the levels of some physiological parameters in the study population including ALT, AST, SOD (U /mL), MDA (nmol/mL) and TG. Statistical analysis indicated that ALT and AST were non- significant difference (p= 0.379, 0.122) in all animal's groups with simple rise in treated animals compare to the control group. Other parameters tested were significantly higher in both groups (1 and 2) as compared to control. SOD (µl/ml) was a high concentration with significant difference (P=0.011) in the group treated with low dose (318.40 ±32.666) and (251.40 ± 31.024) in the groups with the highest dosage compared with the control group (181.80 ± 5.757). MDA (nmol/mL) and TG were also found to be significantly (P=0.049 and 0.020, respectively) higher than control, but differences between them were not significant.

Table 2. Evaluate of some physiological parameters for rats treated by different doses of protein (Mean ± S.E.)

Physiological parameters	Experimental Animals Groups Mean ± S.E.			*P value*
	Control Without dose	Group 1 Dose (5 g/Kg)	Group 2 Dose (10 g/Kg)	
ALT	23.00 ± 1.483	30.80 ± 6.733	31.00 ± 3.421	0.379^{NS}
AST	28.60 ± 1.749	30.60 ± 4.915	38.60 ± 2.462	0.122^{NS}
SOD (U/mL)	181.80 ± 5.757^{c}	318.40 ± 32.666^{a}	251.40 ± 31.024^{b}	0.011*
MDA (nmol/mL)	$2.1000 \pm .08367^{b}$	$3.6820 \pm .77306^{a}$	$3.7500 \pm .25199^{a}$	0.049*
TG	134.60 ± 2.768^{c}	206.00 ± 31.204^{a}	282.40 ± 44.739^{b}	0.020*

*Significant under $p \leq 0.05$ by One way – ANOVA test, Different small letters refer to significant differences.
NS: Non-Significant difference under $p \leq 0.05$

The biochemical results indicated that there was a trend of increasing the levels of ALT and AST in the whey protein treated groups, however, such changes were not significant. This indicates a slight or initial hepatocellular stress and not liver injury. This effect could represent context-dependent hepatic effects of whey protein, as these can be dose-, time-, and physiology-dependent (Yiğit Ziolkowski et al., 2024). SOD activity, on the contrary, was significantly raised in both the treated groups as it is an indication of antioxidant defense mechanism against the

increase in the level of ROS. But, the less SOD response in the high-dose group might indicate that the antioxidant compensation is less efficient or that the antioxidant system is exhausted. Whey protein has been shown to have a similar antioxidant interaction in experimental models of oxidative injury (Aydin et al., 2022; Tufan et al., 2023; Al-Zharani et al., 2024). Lipid peroxidation was also reflected by an increase in MDA levels in treated groups, which also significantly increased. The result in this study is different from the results of other studies where whey protein has been found to be a protector against oxidative damage in toxic or metabolic disease models (Aydin et al., 2022; Ghafil & Al-Taee, 2020; Tufan et al., 2023; Al-Zharani et al., 2024; Wang et al., 2022). The high levels of SOD and MDA suggest that the cells are in an active oxidative stress state in which there is only partial antioxidant compensation. This aligns with the notion that the effects of whey protein are not necessarily positive, but rather depend on the formulation, dosage, disease model, and metabolic background. There was a significant rise in the levels of triglycerides in both the treated groups, particularly at high dose, indicating lipid metabolism disturbances. This is in contrast to the lipid-lowering effects reported with whey protein in human and disease models, which may be due to the difference in species, health status, formulation and dose (Wang et al., 2022; Gataa et al., 2025; Shao et al., 2025). Whey protein supplementation overall resulted in mild changes in hepatic enzyme levels, however, clear effects on both oxidative and lipid metabolic levels were observed. The results of nonsignificant ALT and AST changes suggest that there was limited leakage of the hepatocellular enzyme and increased SOD, MDA and triglycerides represent antioxidant activation, lipid peroxidation and altered hepatic metabolism. This result suggests a dose- and context-dependent effect of whey protein instead of a hepatoprotective action per se (Aydin et al., 2022; Wang et al., 2022; Tufan et al., 2023; Shao et al., 2025). Table 3 illustrates the correlation of physiological parameters with other studied variants as morphological change in experiment animals, the results of the correlation analysis showed that SOD had a significant positive correlation with both Liver weight and weight ratio ($r=0.894$, $p=0.041$) and GP130 ($r=0.984$, $p=0.002$), while it had a significant negative correlation with weight ($r=0.492$, $p=0.017$) in control group. MDA and TG, appears significant revers correlation to the weight in control group only ($r=0.927$, $p=0.023$) and ($r=0.913$, $p=0.031$) respectively. Its correlations with the other studied parameters did not show a statistically significant correlation in both treated animals' group. On the other hand, ALT showed only a significant positive correlation with weight ratio in control and first treated animal group ($r=0.943$, $p=0.016$) and ($r=0.888$, $p=0.044$) respectively. Under normal conditions, body and liver morphometric indices were found to be well correlated with biochemical and oxidative stress markers but this correlation was altered after whey protein administration. Among control, body weight was negatively correlated with SOD, MDA and TG and liver weight and liver to body weight ratio were positively correlated with SOD and ALT, reflecting coordinated metabolism, redox balance, and morphometry of the liver (Thakur et al., 2024; Pădureanu et al., 2025). The negative correlation between MDA and body weight indicates that untreated animals with a lower body weight might experience higher lipid peroxidation or lipid metabolism, which is consistent with the relationship between oxidative stress, lipid metabolism, and liver dysfunction (Pădureanu et al., 2025).

Table 3. Correlation of physiological parameters with morphological change in rats treated by different dodes of protein (Mean ± S.E.)

Correlations			Physiological parameters				
Animals Groups	Morphological data		ALT	AST	SOD U/mL	MDA nmol/mL	TG
Control	Weight	Pearson Correlation	-0.874	0.660	-0.942*	-0.927*	-0.913*
		Sig. (2-tailed)	0.053	0.225	0.017	0.023	0.031
		N	5	5	5	5	5
	liver Weight	Pearson Correlation	0.795	-0.403	0.894*	0.717	0.823
		Sig. (2-tailed)	0.108	0.502	0.041	0.173	0.087
		N	5	5	5	5	5
	Weight ratio%	Pearson Correlation	0.943*	-0.534	0.984**	0.818	0.963**
		Sig. (2-tailed)	0.016	0.353	0.002	0.090	0.009
		N	5	5	5	5	5
Animals group1 (Dose 5g/Kg)	Weight	Pearson Correlation	-0.831	-0.678	-0.039	0.353	0.321
		Sig. (2-tailed)	0.081	0.208	0.950	0.560	0.599
		N	5	5	5	5	5
	liver Weight	Pearson Correlation	-0.326	-0.014	0.463	0.538	0.067
		Sig. (2-tailed)	0.592	0.982	0.433	0.350	0.914
		N	5	5	5	5	5
	Weight ratio%	Pearson Correlation	0.888*	0.809	0.206	-0.234	-0.338
		Sig. (2-tailed)	0.044	0.098	0.739	0.705	0.578
		N	5	5	5	5	5
Animals group2 (Dose 10g/Kg)	Weight	Pearson Correlation	0.544	0.310	0.455	-0.152	0.706
		Sig. (2-tailed)	0.344	0.612	0.442	0.807	0.183
		N	5	5	5	5	5
	liver Weight	Pearson Correlation	0.753	0.572	0.583	0.127	0.782
		Sig. (2-tailed)	0.142	0.314	0.302	0.839	0.118
		N	5	5	5	5	5
	Weight ratio%	Pearson Correlation	0.861	0.740	0.674	0.352	0.792
		Sig. (2-tailed)	0.061	0.153	0.212	0.561	0.110
		N	5	5	5	5	5

* Correlation is significant at the 0.05 level (2-tailed), ** Correlation is significant at the 0.01 level (2-tailed).

Most significant relationships were lost after whey protein treatment indicating that the normal relationship between morphometric and physiological markers has been disrupted (Alalwany, 2019; Hamzah et al., 2023). This aligns with the notion that whey protein benefits are dose-, time-, and physiological state-dependent and can turn either antioxidant or hepatoprotective or stress-inducing when given in different amounts for different durations in various physiological contexts. The positive correlation between ALT and liver to body weight ratio remained unchanged in control and low dose groups, which might suggest that there is an early relationship between liver size and hepatocellular enzyme activity. However, its absence in the high dose group indicates that the response of enzymes may be separated from gross morphometric indices at higher doses of whey protein exposure, particularly at early/mixed hepatic stress (Thakur et al., 2024). The lack of any significant correlations in the high dose group could be due to the simultaneous and complex changes in oxidative stress, lipid disturbance, activation of antioxidants and early tissue damage. Comparable dose-, and condition-dependent hepatic responses to whey protein have been observed in metabolic, or toxin-induced models (Yiğit Ziolkowski et al., 2024; Aydın et al., 2022; Tufan et al., 2023; Yiğit Ziolkowski et al.,

2024). Table 4 displayed the outcomes of Receiver Operating Characteristic (ROC) analysis that performed to estimate the diagnostic ability of the studied markers. The ALT yielded AUC value of 0.700, with sensitivity of 0.6 with perfect specificity (1) at the cutoff value of 29, which indicated moderate predictive performance without statistical significance (P= 0.221). Similarly, AST yielded an AUC of 0.680, sensitivity of 0.6, and specificity of (1) at a cutoff of 35.5 (p= 0.270), which reflected limited diagnostic ability. In contrast, SOD (U/mL), MDA (nmol/mL), and TG revealed excellent predictive precision; SOD (U/mL) showed an AUC value at 0.900, sensitivity was 0.8, and specificity of 1 at a cutoff of 202, with a statistical significance (p=0.014). MDA (nmol/mL) displays a similar performance (AUC= 0.920, sensitivity = 0.9, specificity = 1, cutoff= 2.55, p=0.010). Finally, TG also displayed potent discrimination ability, where it yielded AUC of 0.91, sensitivity of 0.9, perfect specificity (1), cutoff at 165, and a significant p value at 0.012. A specificity value of 1 means that these markers (100%) correctly detected all control animals (negative cases), and none of these animals were misclassified as positive (animals that have been given 5 g/Kg, and 10 g/Kg doses). This indicates excellent ability to exclude animals did not have treated. While, the sensitivity values represent proportion of true positive animals (treated animals) that are correctly detected by this test. These findings underscore the potential utility as reliable diagnostic markers.

Table 4. ROC curve for physiological parameters in treatment Animals groups

Parameters	AUC	Sensitivity	Specificity	Cut-off	P-value
ALT	0.700	00.6	1	29	0.221^{NS}
AST	0.680	0.6	1	35.5	0.270^{NS}
SOD (U/mL)	0.900	0.8	1	202	0.014*
MDA (nmol/mL)	0.920	0.9	1	2.55	0.010*
TG	0.910	0.9	1	165	0.012*

AUC: area under curve; *Significant difference at the 0.05 level by ROC analysis

ROC analysis revealed that ALT and AST were less sensitive to changes associated with whey protein, with AUC values of 0.700 and 0.680, respectively. This could be due to the fact that ALT and AST are primarily markers of hepatocellular membrane leakage and may not be elevated during early, mild or predominantly metabolic hepatic stress (Nahm, 2022; Thakur et al., 2024). SOD, MDA and TG on the other hand exhibited good discriminatory power. The AUC for SOD was 0.900, MDA 0.920 and TG 0.910 with high sensitivity and perfect specificity in this data-set. These results suggest that the oxidative and lipid-metabolic signals were more intensive in the whey protein treatment than in the classical enzyme-based liver injury signals. MDA was found to be the best discriminator and thus the most sensitive treatment-related alteration in the present study, as suggested by Pădureanu et al. (2025) and Al-Taee (2018). The high ROC value for TG also indicates that there is a lipid metabolic disruption following whey protein ingestion. This is consistent with the studies that revealed the ability of triglyceride-related indices to

distinguish liver-related metabolic dysfunction, with most of these studies focusing on composite indices in humans with fatty liver disease (Khan et al., 2025). Specificity of 100% was obtained for some of the markers, but these cut-off values must be interpreted with care, given the limited number of individuals studied. Hence, the suggested cut-off values of ALT, AST, SOD, MDA and TG are experimental and can be validated in the context of larger studies (Nahm, 2022).

Conclusion: Hepatic morphological and physiological disturbances were observed in healthy adult male albino rats after six weeks whey protein supplementation in a dose dependent manner. These changes comprised decrease in body and liver weight, increase in SOD, MDA and triglyceride levels, but no significant changes in ALT and AST levels. This indicates that oxidative stress and lipid markers are more sensitive than traditional liver markers in the early detection of hepatic stress. In general, excess whey protein intake can be a metabolic and oxidative burden to the liver, which is why the dosage is important.

Author contribution

The author was equally responsible for study conception and design, experimental work, data collection, data analysis, and manuscript preparation.

Data availability statement

Not applicable.

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Ethical approval

All experimental procedures involving animals were conducted in accordance with institutional guidelines for the care and use of laboratory animals and after obtaining ethical approval from Al-Qasim Green University. Every effort was made to minimize animal suffering and to use the minimum number of animals required to achieve the objectives of the study.

Conflict of interest

The author declares that there is no conflict of interest regarding the publication of this study.

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ارزیابی مورفولوژیک ماکروسکوپی و فیزیولوژیک تغییرات کبدی ناشی از مصرف مکمل پروتئین وی در موش‌های صحرایی آلبینوی نر بالغ سالم

زید علی زیدان

گروه کالبدشناسی و بافت‌شناسی، دانشکده دامپزشکی، دانشگاه سبز القاسم، بابل ۵۱۰۱۳، عراق. ایمیل: zaidali@vet.uoqasim.edu.iq

اخلاص عابد حمزه العلوانی

نویسنده مسئول. گروه کالبدشناسی و بافت‌شناسی، دانشکده دامپزشکی، دانشگاه سبز القاسم، بابل ۵۱۰۱۳، عراق. ایمیل: Ekhlas.alalwany@vet.uoqasim.edu.iq

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چکیده

هدف: کبد یک اندام احشایی بسیار پیچیده است که با ایفای نقش‌های متابولیکی، گوارشی، سم‌زدایی، تنظیم غدد درون‌ریز و عملکردهای ایمنی، نقش اساسی در حفظ هموستاز (تعادل داخلی) بدن ایفا می‌کند. ازاین‌رو، عملکرد طبیعی آن برای سلامت کل بدن ضروری است. هدف این مطالعه، ارزیابی تأثیر مصرف مکمل غذایی پروتئین وی بر تغییرات مورفولوژیک و فیزیولوژیک مرتبط با کبد در موش‌های صحرایی آلبینوی نر بالغ سالم بود.

مواد و روش‌ها: تعداد ۳۶ سر موش صحرایی به‌صورت تصادفی در سه گروه مساوی تقسیم شدند: گروه شاهد، گروه دریافت‌کننده دوز پایین پروتئین وی (۵ گرم به ازای هر کیلوگرم وزن بدن) و گروه دریافت‌کننده دوز بالای پروتئین وی (۱۰ گرم به ازای هر کیلوگرم وزن بدن). مکمل به‌صورت خوراکی به مدت شش هفته تجویز شد. در پایان آزمایش، وزن بدن، وزن کبد، نسبت وزن کبد به وزن بدن و تعدادی از شاخص‌های بیوشیمیایی سرم شامل آلانین آمینوترانسفراز (ALT)، آسپارتات آمینوترانسفراز (AST)، سوپراکسید دیسموتاز(SOD) ، مالون‌دی‌آلدئید (MDA) و تری‌گلیسرید (TG) اندازه‌گیری شدند. همچنین، برای ارزیابی قدرت افتراق شاخص‌های مورد مطالعه، تحلیل منحنی مشخصه عملکرد گیرنده (ROC) انجام گرفت.

نتایج: نتایج نشان داد که وزن نهایی بدن و وزن کبد در هر دو گروه دریافت‌کننده پروتئین وی در مقایسه با گروه شاهد به‌طور معنی‌داری کاهش یافت. در مقابل، نسبت وزن کبد به وزن بدن گرچه روند کاهشی داشت، اما این کاهش از نظر آماری معنی‌دار نبود. از نظر بیوشیمیایی، مقادیر آنزیم‌های ALT و AST افزایش خفیفی را نشان دادند که از نظر آماری معنی‌دار نبود. در مقابل، سطوح SOD، MDA و TG در گروه‌های تیمار به‌طور معنی‌داری افزایش یافت که بیانگر تشدید استرس اکسیداتیو، افزایش پراکسیداسیون لیپیدی و اختلال در متابولیسم چربی‌ها بود. نتایج تحلیل ROC نیز نشان داد که ALT و AST ارزش تشخیصی محدودی برای شناسایی تغییرات ایجادشده دارند، در حالی که شاخص‌های SOD، MDA و TG عملکرد افتراقی مطلوبی از خود نشان دادند و در

میان آن‌ها، MDA بالاترین دقت پیش‌بینی را داشت. به‌طور کلی، یافته‌های این پژوهش نشان می‌دهد که مصرف مکمل پروتئین وی در دوزهای مورد بررسی، در موش‌های سالم بیشتر موجب ایجاد بار متابولیکی و استرس اکسیداتیو در کبد شده است تا اینکه آسیب آشکار سلول‌های کبدی همراه با افزایش قابل توجه آنزیم‌های کبدی را ایجاد کند.

نتیجه‌گیری: نتایج این مطالعه نشان می‌دهد که شاخص‌های مرتبط با استرس اکسیداتیو و متابولیسم لیپیدها، نسبت به آنزیم‌های متداول کبدی (ترانس‌آمینازها)، حساسیت بیشتری برای شناسایی پاسخ‌های اولیه کبد به مصرف بیش از حد مکمل‌های پروتئینی دارند و می‌توانند به عنوان نشانگرهای مناسب‌تری برای ارزیابی تغییرات اولیه عملکرد کبد مورد استفاده قرار گیرند.

کلمات کلیدی: استرس اکسیداتیو، کبد، مکمل پروتئین وی، موش‌های صحرایی آلبینو، نشانگرهای زیستی

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