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Alpha-lactalbumin isolated from camel milk on hyperlipidemia and hyperglycemia in experimental mice

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ABSTRACT: This study investigated the effect of α-lactalbumin which was isolated from camel milk (a-Lac) at 200 and 400 mcg/day against metabolic disorders hyperlipidemia and hyperglycemia in cholesterol-induced lipidemia for mice. Monitor vital signs such as body weight, fasting glucose in blood level was observed every week until 8 weeks (1st 4wk adaptation and abnormality 2nd, 4wk during treatment investigated period), oral glucose tolerance test (OGTT) level and biochemical parameters were measured after the second 4wk in blood and serum samples, like lipid profiles, insulin resistance, Liver enzymes including ALT, AST, and ALP. The results showed that camel α-La contributed effectively to maintaining vital indicators within healthy limits, and caused decreases in the level of hyperlipidemia and hyperglycemia. It gave activity to liver enzymes. The results were a clear statistical difference. Recommend using camel whey proteins and α-La in particular due to its abundance in camel milk and its therapeutic properties.
Keywords: camel milk; a-lactalbumin; purification; hyperglycemia; hyperlipidemia.

Alfa-lactalbumina isolada do leite de camelo na hiperlipidemia e hiperglicemia em camundongos experimentais

RESUMO: Este estudo investigou o efeito da α-lactalbumina isolada do leite de camelo (a-Lac) nas doses de 200 e 400 mcg/dia contra distúrbios metabólicos, hiperlipidemia e hiperglicemia na lipidemia induzida por colesterol, em camundongos. Foram monitorados os sinais vitais, como peso corporal, glicemia de jejum no nível sanguíneo todas as semanas, até 8 semanas (sendo a adaptação de nas 4 primeiras semanas, e, a segunda etapa de anormalidade, nas 4 semanas restantes), nível de teste oral de tolerância à glicose (OGTT). Os parâmetros bioquímicos foram medidos após as 8 semanas, em amostras de sangue e soro, quantificando os perfis lipídicos, resistência à insulina, enzimas hepáticas, incluindo ALT, AST e ALP. Os resultados mostraram que α-Lac contribuiu efetivamente para manter os indicadores vitais dentro dos limites saudáveis e causou reduções no nível de hiperlipidemia e hiperglicemia, gerando atividade às enzimas hepáticas. Os resultados apresentaram diferenças estatísticas evidentes, permitindo recomendar o uso de proteínas de soro de camelo e α-Lac, em particular, devido à sua abundância no leite de camelo e às suas propriedades terapêuticas.
Palavras-chave: leite de camelo; a-lactalbumina; purificação; hiperglicemia; hiperlipidemia.

1. INTRODUCTION

The term "hyperlipidemia" typically refers to an increased serum triglyceride (TG) and total cholesterol levels (TC), both of which are associated with metabolic disorders and a wide range of chronic conditions, including hypertension, diabetes, cardiovascular disease (SPERLONGANO et al., 2018). Naturally, all forms of hyperlipidemia result in hyperglycemia and vice versa; consequently, there is a correlation between the lipid and sugar metabolic processes.

One of the biggest exponentially increasing is Diabetes, which is now a global health concern. It is known also as a lifestyle-related disease or a complex metabolic disorder, mainly due to insulin resistance, and begins with postprandial hyperglycemia. As mentioned in the International Diabetes Federation Atlas (8th ed.), regions of the Middle East and Africa have the second highest incidence of diabetes (WILLIAMS et al. 2019). Death cases related to diabetes frequently happen because of complications such as oxidative dysfunction, organ failure, hypertension, hyperlipidemia, and cardiovascular disease; Hyperlipidemia has secondary effects and complications on liver function, causing an imbalance in metabolic systems that may lead to hepatotoxicity (SADDAM, 2021).

Numerous studies in recent years have shown that natural bioactive chemicals have a vital function in obesity prevention (RAHEEM et al., 2022; QADIR and SHNAWA, 2022; KHOSRAVI et al., 2023). The bioactive compounds identified in the whey proteins include lactoferrin, glycomacropeptide, β-lactoglobulin, and A-lactalbumin (A-Lac), and has numerous protective properties against cancer, infection, and inflammation. Clinical studies and examinations have revealed that overweight and obese people who consume whey protein eliminate much more body fat while also reducing cholesterol and insulin levels.

The first of the major proteins in whey protein, its properties and physiological actions of (WP) are greatly affected by A-Lac (PAL et al., 2010). Inhibiting peptidases dipeptidyl 4 activity, lowering oxidative stress, enhancing insulin resistance, and decreasing inflammation are only a few of the bioactivities that have been linked to a-La (LIU et al., 2018). In addition, recently, a large number of studies indicated that α-LA has effective vital roles against metabolic disorders, hyperlipidemia and hyperglycemia, and acts as a therapeutic nutritional supplement for obesity and its pathological complications as a result, a-Lac might be utilized as a dietary treatment for obesity and other metabolic diseases (CHEN et al., 2020).

Given the importance of a-Lac as mentioned above, this current study was conducted and aimed to isolate alpha protein from camel milk whey and use it to treat hyperlipidemia and hyperglycemia, and some other nutritional indicators.

2. MATERIALS AND METHODS

2.1. Chemical

Cholesterol (from Sigma-Aldrich purchased), standard α-lactalbumin purchased from Sigma Aldrich, and all of the other chemical reagents utilized in the investigation were of analytical grade.

2.2. Animals

Healthy forty male BALB/C mice albino white (5 wk old) and weighed 26 ± 4 gm, were obtained from Al-Razi Center for Pharmaceutical and Veterinary Research - Ministry of Industry and Minerals Baghdad. The rats were housed inside a well-ventilated room in metal wire cages with a 12-hour dark–light cycle, the temperature is 27°C ± 2°C, relative, Humidity is 50% ± 15%, and free to access water and food throughout the period in the experimental procedure (MUHAISEN; SADDAM, 2023).

The experiment was not approved by an Ethics Committee of Scientific-Research of Al-ayen University (Iraq) for the use of animals.

2.3. Camel milk

The camel milk was obtained at the peak of the lactation period, the sample was transported under standard sterile conditions and refrigeration. Camel milk was analyzed In the Department of Food Sciences – University of Baghdad.

2.4. Isolation, purification and detection of α-Lac

2.4.1. Preparation of skim milk and whey

Camel milk was centrifuged with 5000 x g for 35 minutes at 4C° to produce skim milk, and the fatty top layer was removed. After that, skim milk was pasteurized at 63°C and 1 M of hydrochloric acid was added to retain the pH at 4.3, maintaining it for 1 hour at 36 C°, and the casein was separated by centrifuge with 11,000xg 30 min. The remaining liquid is whey.

2.4.2. A-Lac isolation and purification

A-Lac was neutralized by chromatographic methods, which included anion exchange chromatography (AEC) using chromatography (column DEAE cellulose and gel filtration using a column Sephadex G-100, A-LacI was isolated and purified by the hemodialysis through a dialysis bag against 20 mM phosphate buffer and 35mM EDTA, pH7.6 Dialysis was performed for 24hours unwanted liquids eluted from the A-Lac. procedure described in NEYESTANI et al. (2003).

2.4.3. A-Lac detection and characterization

Purified A-Lac has a run by SDS-PAGE showed Weight 14.4 kDa is confirmed by the gel in ladder bands marked and Compared to the stander α-LA in lane 5 which purchased from Sigma Aldrich, has significant match was found. as shown in (Fig 1), and used HPLC assay as an additional confirm purification and detection step shown in (Fig 2). This result agrees with what was found by UVERSKY et al. (2016).

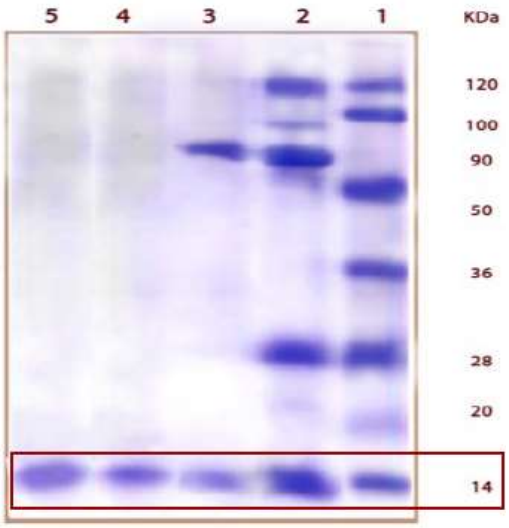


Figure 1. SDS-PAGE for α-Lac steps purification.
Lane 1: Lader Mark; Lane 2: whey camel milk after salting-out by 45% ammonium sulfate; Lane 3: (ion-exchange) fraction from eluted by DEAE–cellulose column; Lane 4: a-Lac from column Sephadexs G100; - Lane 5: purified stander a-LA from Sigma.
Figura 1. SDS-PAGE para purificação de etapas de α-Lac.
Faixa 1: Lader Mark; Faixa 2: soro de leite de camelo após salga com 45% de sulfato de amônio; Faixa 3: fração (troca iônica) eluída pela coluna DEAE-celulose; Faixa 4: a-Lac da coluna Sephadexs G100; - Faixa 5: stander a-LA purificado da Sigma.

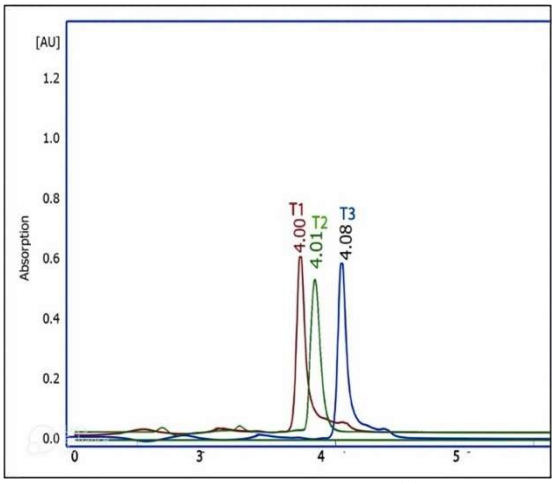


Figure 2. HPLC assay pattern of α-La stander (T1), α-Lac from Ion exchange using DEAE-cellulose column (T2), α-Lac from Gel filtration chromatography Sephadex G-100 column (T3)
Figura 2. Padrão de ensaio HPLC de α-La stander (T1), α-Lac de troca iônica usando coluna DEAE-celulose (T2) e α-Lac de cromatografia de filtração em gel coluna Sephadex G-100 (T3).

2.5. Experimental design

Forty normal healthy male BALB/C Albino mice were kept separately in 4 random groups (n = 10).

Group 1: Negative Control (C $^{-}$) fed a normal diet without cholesterol;

Group 2: positive Control (C $^{+}$) **(**fed 2% cholesterol-enriched diet);

Group 3:(T1) oral dose of a-Lac at 200 mcg/day (fed2% cholesterol-enriched diet);

Group 4: (T2) oral dose of a-Lac 400 mcg/day (fed 2% cholesterol-enriched diet).

The total duration of the study was 56 days. During the first 28 days - induced abnormal lipidemia to the groups (2, 3, and 4). During the subsequent 28 days, elevated blood lipid and glucose levels were confirmed. Oral dosing of groups 3 and 4 began by a-Lac with 200 and 400 mcg/day, respectively.

2.6. Hyperlipidemia and hyperglycemia induction

The Animals were acclimatized on a standard diet as [American Institute of Nutrition, 1993, (AIN-93M)], which included the following components: corn starch (44%), fiber (20.0%), casein (15.0%), sucrose (10.0%), oil from soybean (7.0%), group minerals (3.0%), vitamin group (1.0%). After acclimatization, hyperlipidemia and hyperglycemia were induced by a 2% cholesterol-enriched diet in all groups except (Group 1) the normal control group continued with a normal diet. Throughout the experiment (8) wk, feeding Animals a 2% cholesterol-enriched diet. Induction hyperlipidemia and hyperglycemia according to KAFI (2014).

2.7. The treatments α-Lactalbumin

Mice in groups 3 and 4 were dosed with a-Lac solution with distilled water as 200 - 400 mcg/day gavage protein a-Lac 0.5 ml orally daily dose.

2.8. Blood collection

After treatment for 28 days and fasting for 24 hours, mice were sacrificed (AST), (ALT), (TC), and (TG) were estimated by using reagent kits (Chengxinde reagent c). The blood samples were taken by heart in two types of tubes. In the first tube, the blood centrifuged to obtain a serum when was stored at 70°C, and in the second tube, the blood mixed with EDTA to obtain a plasma, Estimation of Serum lipoproteins high density of (HDL) followed methods for enzyme hydrolysis, Serum LDL and VLDL Level Estimation Freid's mathematical equation was used to calculate the levels LDL and VLDL, serum glucose level (CBG) was determined using an Adaltis reagent kit and the procedure described by oral blood glucose test (OGTT) insulin resistance.

2.9. Statistical analysis

Utilizing a complete random design (CRD) and the statistical program the Statistical Analysis System, the data were analyzed to determine the impact of different coefficients on the traits under study. The significant different between results were subsequently evaluated with the mean significant difference (LSD) test.

3. RESULTS

3.1. Study the effects of a-Lac against the body weight of mice

The following table shows the effect of gavage a-Lac on the daily weight gain rate at the start of the experiment and the final body weight gain rate after 28 days for groups of mice that were fed a conventional diet (group C-negative control), a fat-rich diet (group C+ positive control) and a diet rich in fat with oral administration of the a-Lac at a concentration of 200 and 400 micrograms of a-Lac/day, respectively. The results show that the positive control mice C + group showed the fastest daily weight increase. Which was 0.2870 g/day. The total weight gain after 28 days was 8.038 g, which is greater than the C- group saw daily gains of 0.189 g and 5.31 g overall. The obvious differences in findings between treatment, C-, and C+ can be attributed to the type of diet provided to each group. The positive control C+ treatment's diet was high in fat, which caused a larger weight gain. This result was in line with what was found by KALAIVANISAILAJA et al. (2003).

Table 1. Average weight gain in groups against different treatments after 28 days.
Tabela 1. Média de ganho de peso nos grupos frente aos diferentes tratamentos após 28 dias.

Group	Body-weight (gm)		Increase or decrease body weights after28 day (gm)	Average daily increase body-weights (gm)
	Average of starting weights (gm)	Average weights after 28 days (gm)		
Control negative group-fed standard diet C-	31.36a	36.67a	5.316a	0.1898a
Control positive groups fed diets rich in fat 2% cholesterol C+	32.83a	40.87c	8.038c	0.2870c
Treatment groups were fed diets rich in fats, 2% cholesterol, and oral dose a-Lac at 200 mcg/day T1 treatment.	33.75b	38.99b	5.241a	0.1871a
Treatments group feeding diets rich in fats 2% cholesterol and an oral dose of a-Lac at 400 mcg/day T2	31.688a	35.69a	4.007b	0.1603b
L.S.D value (P ≤ 0.05) NS	2.87 ^{NS}	3.79 *	3.05 *	0.077 *

*(P 0.05) significant difference. NS (non-significative difference). Averages with different letters inside the same column indicate they differ significantly.

As shown in Table 1 final weight of the group that fed on a diet with high-fat content (C+) increased the group compared that is treatment T1 and T2, where the final gain weight after 28 days was 5.241 gm, 4.007 gm with an average 0.187gm and 0.1603gm respectively this weight analytical showed significant difference more than (P ≤ 0.05), a-Lac decrease body weight compared with C+ group were significant. Furthermore, all groups had the same food consumption and calorie intake, demonstrating that a-lac may relieve obesity without changing energy intake (Table 2), These findings reveal that a-Lac is a considerably safe and potential therapy for weight gain and digestive disorders, The effect was ascending with increasing dose of a-Lac when

comparing results with T1 and T2. Dosing with a-Lac contributed to curbing unhealthy weight levels that cause fat accumulation in the body, the result, as agreed with Ushida et al. (2003). Likewise, as reported by Freedman et al., when body weight declines, the lipid profile (TG, TC, and LDL-C) also does (FREEDMAN et al., 2001). As a result, -LA may prevent stout mice from gaining as much weight, leading to higher blood and liver fat levels.

3.2. Effect of gavage A-Lacon levels of total cholesterol, triglycerides, HDL, LDL, and VLDL

After 28 days, the impact of gavage a-Lac on cholesterol, triglycerides, HDL, LDL, and VLDL for C-, C+, T1 and T2 mice treated is shown in Table 2. This is related to the type of meal offered to this group, which is high in fat compared to its level of 127.4 mg/100 mL in the C-treatment. It had a higher fat content than mice on a regular diet, with 207.70 and 127.45 mg/100mL, respectively.

Table 2. Percentages of TC, TG, HDL, LDL, and VLDL in the serum of experimental mice after 28 days.
Tabela 2. Porcentagens de CT, TG, HDL, LDL e VLDL no soro de camundongos experimentais após 28 dias.

Group	TC	TG	HDL	(LDL)	(VLDL)
			mg / 100 mL		
C- control Negative group-fed standard diet	127.45	127.85	72.20	45.60	23.84
C+ control Positive group-fed a diet rich in fat 2% cholesterol	207.70	177.66	68.44	77.55	34.74
Treatment group fed on a diet rich in fat 2% cholesterol and oral dose of a-Lac at 200 mcg/day T1 treatment	154.01	137.25	69.45	56.19	25.20
Treatment group fed on a diet rich in fat 2% cholesterol and oral dose of a-Lac at 400 mcg/day T2 treatment	121.40	111.50	71.81	44.80	24.85
L.S.D value (P ≤ 0.05)	41.66 *	36.05 *	6.021 NS	8.452 *	8.724 *

TC is the total cholesterol, TG; triglyceride, HDL; high-density lipoprotein, LDL; low-density lipoprotein cholesterol, VLDL; very low-density lipoprotein; * (P 0.05) indicates a significant difference. Averages with different letters within the same column indicate substantial differences.

After 28 days, Table 2 illustrates the effect of gavage -Lac on the TC, TG, HDL, LDL, and VLDL in C-, C +, T1, and T2 treated mice. This is related to the type of meal offered to this group, which was high in fat compared to its level of 127.4 mg/100 mL in the C-treatment. It had a higher fat content than mice on a regular diet, with 207.70 and 127.45 mg/100 mL, respectively. When the dosage of -Lac was increased, there was a significant decrease ($P < 0.05$) in levels of TG, TC, LDL and vLDL in a-Lac group, except the level of HDL results, which showed a significant increase ($P < 0.05$) compared to the control group. High doses of A-L corrected the lipid levels, which is consistent with those findings by CHEN et al. (2020). It was discovered that both a-Lac doses (200 and 400 mg/kg) significantly increased blood HDL cholesterol levels while lowering cholesterol levels, triglyceride, and LDL cholesterol levels ($P < 0.001$).

Where the value of total cholesterol for the individuals of the C+ treatment was 187.70 mg/100 mL, and this figure was high compared with the value of TC of the C- treatment, which amounted to 127.45 mg / 100 mL. These thoughts are congruent with the findings of Hamid; Doosh et al. (2021), which indicated an increase in the percentages of both total cholesterol and LDL, VLDL, triglycerides and HDL decreased in a group of rats fed on a high-fat diet, and as it agreed with what was found by Huang et al. (2004) who discovered that mice fed a high-fat diet had blood levels of total cholesterol that reached There was a significant difference ($p < 0.05$), based to statistically significant results in Table 4.

High blood lipid profile TC and LDL levels may hasten the progression of atherosclerosis. High blood HDL-C levels, on the other hand, have been found to have a favorable influence on the progress of vascular disease. In this investigation, we discovered that a-Lac significantly reduced blood TC and LDL levels while increasing HDL levels (Figure 4D). Additionally, it was shown that circulating leptin levels are closely related to adipocyte hypertrophy and may be used as a biomarker to assess fat of body mass (SKURK et al., 2001).

3.3. Measure the level of glucose in the blood during fasting

Fasting blood glucose or CBG levels were tested 3 times to confirm sustained hyperglycemia during the test at 0, 2, and 4 wk, using a blood glucose meter (plus-Check, boost Diagnostics, Greece). Blood was collected from the tail vein by using a sterilized needle, and mice with FBG levels highest up to 260 mg/dL were considered diabetic.

Table 3. The results of Serum glucose after treatments 28 days.
Tabela 3. Resultados da glicemia sérica após tratamentos de 28 dias.

Group	glucose mg/100 ml
C - Negative control group with standard diet -fed	123.35 ±7.52
C + Positive control group with fed a rich fat diet 2% cholesterol	276.77 ±23.50
Treatment group was fed a rich fat diet with 2% cholesterol and an oral dose of a-Lac at 200 mcg/day (T1 treatment)	106.45 ±6.20
Treatment group fed rich a fat diet of 2% cholesterol and an oral dose of a-Lac at 400 mcg/day (T2 treatment)	97. 90 ±7.15
L.S.D value (P ≤ 0.05)	46.724 *

The effects of ingesting a-Lac at doses of 200 and 400 mcg per day contributed to the normalization of blood sugar levels. This might be explained by an increase in intestinal viscosity and a thickening of the intestinal layer, especially in those who are fasting, which would obstruct or restrict sugar absorption (KIM; SHIN, 1998).

Insulin resistance is frequently linked to obesity, which increases the risk of type 2 diabetes (QU et al. 2019). Contrarily and insulin resistance rises. Levels of plasma (FFA), which are afterward delivered to the liver, increase the creation of cholesterol and fat storage, ultimately resulting in higher blood (TG) levels and lower (HDL-C) levels. Although the effects of a-Lac on blood glucose control were also carefully examined, this study found that fast blood

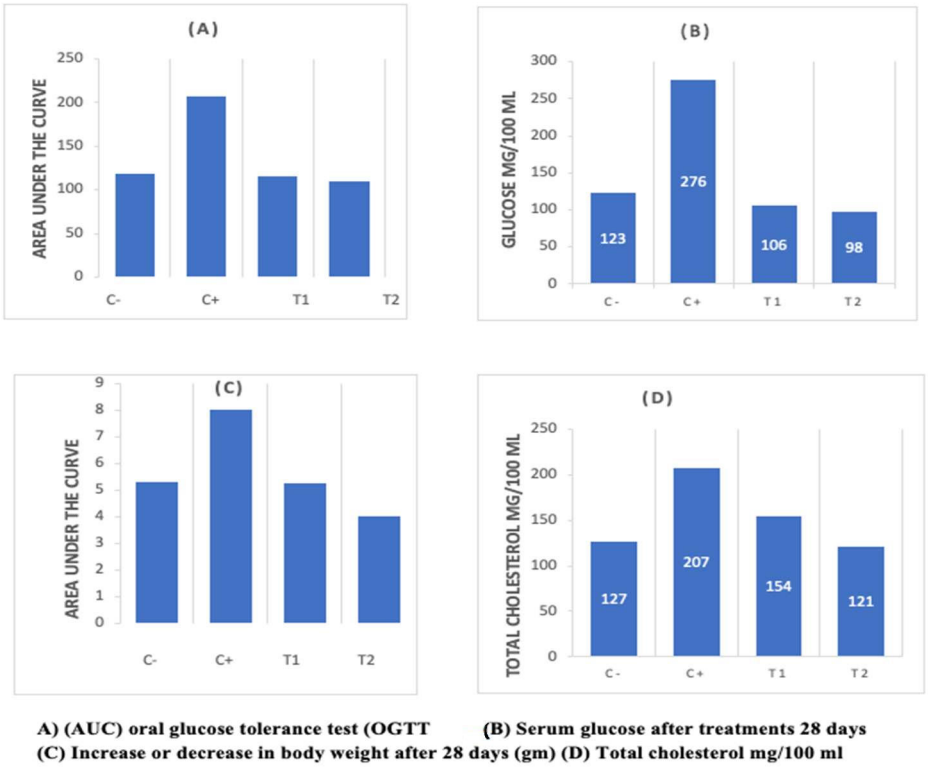
glucose, serum insulin levels, and glucose tolerance were all enhanced in obese mice, suggesting that insulin sensitivity increased, as shown in (Figures 4A and 4C).

3.4. The oral glucose tolerance test (OGTT)

OGTT was tested after the end of the experiment (after 56 days). Mice in all groups were orally given sugar (Glucose 2 g/kg of BW) after the overnight fast. before 30 min of Glucose dose, they gavage protein a-Lac orally to groups 3and4 and then test (FBG). Glucose blood levels were measured in follow-up times 0,15,30, 60, and 90 minutes after gavage, with blood drawn from the head of tail veins using a blood glucose meter (plus-Check, boost Diagnostics, Greece). To assess the efficacy of a-Lac on glucose tolerance. The glucose level of mice is calculated as the following math: AUC0-120 = [(Glucose 0m + Glucose 15m) + (Glucose 15m + Glucose 30m) +(Glucose 30m + Glucose 60m) + (Glucose 60m + Glucose 90m)] × 30/2.

As shown in Figure 5A - AUC of group C+ was the highest level of than other group, Equivalent to double the value of the rest group, noticed a decrease in the value of AUC with increasing the dose for the treated groups, and a decrease in for these groups compared to the control group, Studies has shown that whey proteins, especially a-Lac, improve insulin resistance (ZAPATA et al., 2017).

Numerous studies indicated the ability of A-Lacto to increase the vital and protective capacities of the liver; it was observed that all readings of the C+ group increased significantly from the rest of the treatment group T1 and T2 or C- group, and this is due to the system their diet rich in cholesterol that results agreed with MUSSO et al. (2013), compared C+ group to the C- group we note, there is a significant difference (P ≤ 0. 0 5). Likewise, there is a significant difference between treated groups in an ascending manner proportional to the increase in the dose, and this indicates that the increase in the dose and concentration of protein helped stimulate the liver and its enzymes.



A) (AUC) oral glucose tolerance test (OGTT (B) Serum glucose after treatments 28 days
(C) Increase or decrease in body weight after 28 days (gm) (D) Total cholesterol mg/100 ml

Figure 3. Effect of gavage α-Lactalbumin AST, ALT, ALP liver enzymes, and GPx enzyme activity.
Figura 3. Efeito da gavagem α-lactalbumina AST, ALT, enzimas hepáticas ALP e atividade da enzima GPx.

Table 4. Levels of liver enzymes AST, ALT, ALP and for groups for mice after 28 days.
Tabela 4. Níveis das enzimas hepáticas AST, ALT, ALP e para grupos de camundongos após 28 dias.

Group	AST IU/ml	ALT IU/ml	ALP IU/ml	GPx IU/ ml
C-Negative control group fed standard diet -	49.80	67.36	73.66	15.52
C +Positive control group with fed a rich fat diet 2% cholesterol.	70.24	79.89	108.72	11.22
Treatment group with fed a rich fat diet 2% cholesterol and oral dose of a-Lac at 200 mcg/day (T1 treatment).	57.64	70.84	69.56	22.52
Treatment group with fed a rich fat diet 2% cholesterol and oral dose of a-Lac at 400 mcg/day (T2 treatment)	50.33	65.45	60.88	26.80
L.S.D value (P ≤ 0.05)	8.923 *	7.645 *	17.865 *	0. *

AST=aspartate aminotransferase, ALT=alanine transaminase, ALP-alkaline phosphatase, GPx -Glutathione peroxidase* Significant difference (P ≤ 0.05). A considerable difference between two averages is shown by their having distinct letters in the same column.

4. DISCUSSION

Serum from HCV patients is tried pre-and post-supplementations of the CM. A critical lessening in the degree of (HCV) boundaries, including liver function (ALTs and ASTs) and the development of overall exhaustion, has been noticed. Numerous studies indicated the possibility of camel whey proteins to enhance the functioning of liver functions and liver enzymes, by more effective suppression of biological factors linked to immunity, which include the markers of inflammation, factor-A tumor necrosis, monocyte-chemotactic protein-1, ALT, and AST, with raising serum albumin levels, the anti-apoptotic protein BCL-2, interleukin-10, Vitamin D levels, also antioxidant status (MOHAMED et al., 2015; KHAN et al., 2021; DU et al., 2022).

With the help of the modified purification procedure, it was possible to extract high quantities of extremely pure a-La from camel milk samples (> 90%, depending on total protein). Using a straightforward two-step chromatographic purification method, valuable protein may be obtained.

5. CONCLUSIONS

The purest A-Lac samples were also useful for studying the α-lactalbumin isolated from camel milk effects (a-Lac) at 200 and 400 mcg/day against metabolic disorders such as hyperlipidemia and hyperglycemia in cholesterol-induced lipidemia for mice.

The results showed that camel α-La contributed effectively to maintaining vital indicators within healthy limits, and caused decreases in the level of hyperlipidemia and hyperglycemia. It gave activity to liver enzymes.

The results showed a clear statistical difference, recommending the use of camel whey proteins and α-La in particular due to its abundance in camel milk and its therapeutic properties.

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