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Evaluation of the Liver Function and Haematological Parameters of Wistar Rats Fed with Casein Protein

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ABSTRACT: Casein is the major protein found in bovine milk. Universally used in human nutrition for all ages and in the dairy industry, milk is regarded as whole food which provides high quality protein and indispensable micronutrients such as vitamins and minerals. The evaluation of the liver function test and haematological parameters of rats fed with casein protein was carried out. Graded levels of casein protein were incorporated into the diets of experimental rats at 20%, 30% and 50% concentrations. At the end of twenty-eight days, blood samples were collected and analyzed for liver function and haematological parameters. Statistically significant increases were observed in lactate dehydrogenase, gamma glutamyl transferase, alanine transaminase, aspartate transaminase and alkaline phosphatase activity levels when compared with the control. Also the granulocytes and platelets counts were significantly reduced. Consistent consumption of high concentrations of casein proteins may elevate liver enzyme activities and attenuate some haematological parameters.

Keywords: Liver function, Haematological parameters, Casein, Liver enzymes, Wistar rats

Introduction

Dietary choices directly affect health, longevity and quality of life (Cena and Calder, 2020). A healthy diet comprises the right proportion of nourishment that can help limit predisposition to diseases and promote wellbeing (Stark, 2013). Haematological parameters are modified by nutrition (Togun *et al.*, 2007), and values obtained can serve as measurable indices for disease prognosis and stress assessment (Olabanji *et al.*, 2007). Haematological studies aid understanding of the alterations in blood parameters which ensure proper diagnosis of many diseases (Bianco, 2011). Blood composition and the functionality of organs including the liver which is the main site of detoxification of drugs and nutrient metabolism can be affected by diet (Chiang, 2014). Dietary proteins are available in a variety of forms in diet and are needed in adequate amounts for growth and body maintenance (Lonnie *et al.*, 2018). According to Wolfe *et al.*, (2017), the recommended daily allowance of protein intake for adults is 0.8g /Kg body weight per day. Nowadays, consumption of excessive concentrations of protein for weight loss and building of muscles by athletes is common (Kreiger *et al.*, 2006; Carbone and Pasiakos, 2019). The liver plays a vital role in protein metabolism and excessive intake can impose a metabolic burden on it (Delinaris, 2013). In certain liver diseases, a high protein diet is recommended for adequate recovery (Poortsman and Dellalieux, 2000; Ikizler, 2003), also, protein hyper-nutrition is required to combat muscle wasting (Volpi *et al.*, 2003). Tome *et al.*, (2012) postulated that intake of high amounts of open chain amino acids may promote the development of some metabolic diseases. In some liver diseases like cirrhosis and encephalopathy, restriction in protein intake is advised (Mehli and Riggio, 2009). Protein nutrition is required in the synthesis of glutathione which helps to prevent oxidative stress and maintain a state of balanced homeostasis (Wu *et al.*, 2004).

Casein is the dominant protein which is typically found in mammalian milk, and is responsible for the white, opaque appearance (Sarode *et al.*, 2016). It comprises about 80 % of the proteins in cows' milk and between 20

% and 60 % of the proteins in human milk (Kunz and Lönnerdal 1990). Milk intake has been reported to reduce overall weight, body fat, waist circumference, insulin resistance, and blood pressure, while simultaneously increasing muscle mass, consequently reducing numerous cardio-metabolic risk factors (Abargouei *et al.,* 2012; Zemel *et al.,* 2005). Milk is widely consumed not only as the primary source of nutrition for any newborn in mammalian species, but also as excellent source of the nutrients for children's growth and most adults (WHO, 2003). The primary purpose of this study was to examine the effects of graded casein protein diet on the liver function and some haematological parameters of Wistar rats.

Materials and methods

Twenty-eight male rats of Wistar strain weighing between 180 g to 200 g were selected for this study. The rats were kept in groups, housed in standard cages and maintained under standard laboratory conditions.
The rats were acclimatized for two weeks during which they had free access to clean water and normal feed ration (Growers mash, containing 16 % protein). At the end of the acclimatization period, the rats were assigned randomly to three major groups based on diet, namely group 1 (control), group 2 (casein). Based on the dietary protein concentration, the experimental group (groups 2) was each divided into three subgroups of 7 rats each: 2a (20 %), 2b (30 %) and 2c 50 %). The rats in the control group were fed with normal rat chow (growers mash, containing 16 % protein) and clean water. All the animals had access to feed and clean water *ad libitum.* The experiment lasted for 28 days.
Blood collection: At the end of the experimental period, the rats were sacrificed and blood samples were collected by cardiac puncture. 4 ml of blood was transferred into plain containers, allowed to clot and thereafter serum was separated and stored at -20^{o}C until required for assay for the liver function test. Also, for the prothrombin time test, in a ratio of 9:1, 0.9 ml of blood was added to 0.1 ml of 3.2 % sodium citrate anticoagulant in a test tube. This was spun and the plasma was used for the prothrombin time test. 1 ml of blood was transferred into EDTA containers for hematological analyses via automation. The liver function tests namely: alanine transaminase (AST), Aspartate transaminase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), total protein (T BIL), direct bilirubin (D BIL), total protein (TP), albumin (Alb) and prothrombin time test (PT) were done using appropriate test kits following manufacturers' instructions.
Data analyses: Data obtained were analyzed using GraphPad Prism analytical software version 5.0. Results obtained were expressed as means ± SEM. Post Hoc using Tukey's least significant differences (LSD) showed statistically significant differences and a value of $P < 0.05$ was regarded as statistically significant.

Results

Results of liver function parameters of rats fed with graded concentrations of casein protein are presented in Table 1. ALT, ALP, LDH and GGT activities significantly increased in the 50% casein protein-fed rats when compared with the control.

Table 1: Liver function parameters of control and experimental rats

Parameters	Control	20% CAS	30% CAS	50% CAS
TP (g/dL)	6.81±0.44	6.91±1.54	7.94±0.57	8.04±0.80
Alb (g/dL)	3.73±0.20	3.74±0.84	3.79±0.93	4.52±0.67
T BIL (mg/dL)	0.30±0.05	0.37±0.05	0.49±0.02	0.52±0.03
D BIL (mg/dL)	0.08±0.02	0.09±0.04	0.18±0.03	0.20±0.03
PT (sec)	26.6±1.35	30.1±1.34	34.2±2.39	39.4±2.18
AST (u/L)	56.6±3.17	57.6±5.73	67.6±3.95	68.8±2.95
ALT (u/L)	33.2±3.07	46.6±2.26	57.2±6.11	69.8±4.24*
ALP (u/L)	81.4±9.85	94.4±8.91	109.2±11.92	137.6±9.40*
LDH (u/L)	79.6±15.04	93.0±16.99	109.6±11.78	129.8±13.30*
GGT (u/L)	21.8±4.77	23.0±6.96	31.6±6.40	59.0±3.20*

Key: $^{*}P < 0.05$ = statistically significant, CAS= Casein, TP = Total Protein, Alb = Albumin, T BIL = Total Bilirubin, D Bil = Direct Bilirubin, PT = Prothrombin Time, AST = Aspartate transaminase, ALT = Alanine amino transaminase, ALP= Alkaline Phosphatase, LDH= Lactate dehydrogenase, GGT= Gamma glutamyl transferase. n=7, means ± SEM; $P < 0.05$

Table 2 shows the results of the rats fed with graded concentrations of casein proteins. Granulocytes and platelets counts were significantly reduced in 50% concentration casein protein-fed rats when compared with the control.

Table 2: Haematological parameters of control and experimental rats

Parameters	**Control**	**20% CAS**	**30% CAS**	**50% CAS**
T WBC ($10^3/\mu L$)	6.4±0.26	7.50±0.20	9.02±0.33	9.1±0.50
LYM (%)	75.3±2.03	82.2±1.75	84.3±0.57	88.9±1.44
GRAN (%)	17.2±2.22	11.3±1.21	9.9±1.26	7.1±0.69*
MON (%)	7.6±0.98	6.1±0.45	5.2±0.42	4.2±0.68
RBC ($10^6/\mu L$)	7.7±1.10	7.9±0.26	7.9±0.13	8.2±0.16
HCT (%)	38.8±0.40	40.1±1.07	41.6±0.95	43.6±0.43
HGB (g/dL)	12.9±0.15	13.3±0.33	13.8±0.24	14.5±0.87
MCV ($fL\mu m^3$)	50.4±1.2	50.8.±0.51	52.6±0.52	53.2±2.47
MCH (pg)	16.7±0.39	16.8±0.15	17.4±0.25	17.6±0.83
MCHC (g/dL)	33.2±0.43	33.2±0.35	33.2±20.21	33.3±0.34
PLT ($10^3/\mu L$)	483.6±13.77	461.3±16.9	425.2±16.98	329.4±7.3*
PT (sec)	26.6±1.35	30.1±1.34	34.2±2.39	39.4±2.18

Key: $^*P < 0.05$ = statistically significant, CAS= Casein, T WBC= total white blood cells, LYM= lymphocytes, GRAN= granulocytes, MON= monocytes, RBC= red blood cells, HCT= haematocrit, HGB= haemoglobin, MCV= mean cell volume, MCH= mean cell haemoglobin, MCHC= mean cell haemoglobin concentration, PLT= Platelets. n=7, means ± SEM; $P < 0.05$)

Discussion

The observed increases in the liver enzymes function parameters which were concentration dependent clearly suggested that although the liver has ability to withstand stress and regenerate itself (Gilgenkrantz and Collin de l'Hortet, 2018): consistent consumption of increased concentrations of casein protein may become less beneficial and more detrimental to the liver.

The transaminases (aminotransferases) serve as excellent markers of hepatocellular injuries and liver necrosis (McGil, 2016; Oh *et al.*, 2017). The statistically significant elevated activities of ALT was in agreement with the findings of previous authors such as Delinaris (2013) and Mutlu *et al.*, (2004) who attributed the rise in transaminase activity to increased activity in liver hepatocytes as a result of the increase in protein intake. Due to the fact that ALT is more specific for liver function assessment (Lala *et al.*, 2022), the increased values recorded in this study raises concerns on the safety of a high casein protein diet on the liver. However, Mutlu *et al.*, (2004) further suggested that although there is an increase in the transaminases due to protein hyper-nutrition, the symptoms and abnormality may resolve after discontinuation of the high protein diet intake.

The significant elevations in the activity of ALP observed in this study may be due to increase in the activity of hepatocytes due to increase in protein metabolism: ALP plays an important role in the breakdown of protein in the body. Siddique and Kowdley (2012) reported that ALP measured in serum is used as a diagnostic tool for liver disease. The conjugation of enzyme ALP with bilirubin is normally used to monitor the formation and free flow of bile by the liver. Bile is very important in lipid metabolism. Aside from the liver, ALP activity is also found in the bones, intestine, kidneys and other organs (Sharma *et al.*, 2014, Song, 2017).

In this study, we observed a significant raise in the LDH and GGT enzymes activity in the 50% concentration group which is a pointer to possible liver damage. These tests, though not specific for the liver, are used to monitor liver damage thereby raising concern about the safety of the habitual consumption of high concentrations of casein proteins on the liver.

The reduction in the granulocytes counts in the experimental animals despite being fed high protein concentrations may be a pointer to the fact that consuming high concentrations of animal proteins may have similar detrimental effects on the immune system as a low protein diet. According to Daly *et al.*, (1990), low protein intake can impair the immune response by negatively impacting T-cell function, thereby resulting in increased susceptibility to opportunistic infections. Consequently, this study emphasizes the significance of consuming only an adequate amount of dietary protein in order to maintain healthy granulocytes counts. In addition to the major function of the leukocytes which is to fight infections and defend the body against foreign organisms, it has been documented in literature that leukocyte counts are also being considered as possible novel markers of immunotoxicity (Milla *et al.*, 2011).

Our finding of a significant reduction in the platelet count in this study showed that intake of a diet containing an excess amount of proteins may have a detrimental effect on thrombopoiesis. This finding is in agreement with the

report of Akoh and Hearnberger (1991), of a reduction in the platelet counts of healthy men fed a diet of catfish and salmon. Platelets as well as other blood coagulation parameters were reported to have been reduced following high protein nutrition in a recent study by Aikpitanyi-Iduitua and Ighoroje (2021). Platelets are important in blood clotting by helping to stop bleeding and prevent excessive loss of blood by swelling, clumping together, and forming a sticky plug. Thrombocytopenia (low platelet count) results in blood loss due to uncontrolled bleeding. Platelets play a role in inflammation by producing and releasing of prostaglandins and other substances causing either vasodilation or vasoconstriction (Ceresa *et al.,* 2007), and may be involved in hardening of the arteries (atherosclerosis). Thrombocytosis may predispose to thrombosis which is formation of a blood clot within a blood vessel, which can predispose to some cardiovascular challenges.

Conclusion

The findings of this study have demonstrated that the consumption of elevated quantities of casein protein may potentially exert detrimental effects on liver function as evidenced by marked elevations in liver enzymes activities. Moreover, this research revealed evidences of granulocytopenia and thrombocytopenia.

CONFLICT OF INTEREST

There is no conflict of interest

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