

Hepatoprotective Effect of Camel Milk on Poloxamer 407 Induced Hyperlipidaemic Wistar Rats

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Abstract

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AIM: To investigate the effect of oral administration of camel milk on liver enzymes, total proteins and histology of poloxamer 407 induced hyperlipidaemic wistar rats.

MATERIAL AND METHODS: Thirty male wistar rats weighing between 150-200 g were randomly assigned into six groups of five each; group I: administered distilled water, group II: induced with P407, group III: induced with P407 and treated with atorvastatin (20 mg/kg) and groups IV, V and VI: induced with P407 and treated with camel milk 250 mg/kg, 500 mg/kg and 1000 mg/kg respectively. After three weeks, blood samples and liver tissues were collected for the determination of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin, globulin, albumin/globulin ratio and histological studies respectively.

RESULTS: All camel milk treated groups showed significant ($p < 0.05$) decrease in ALT and AST. Camel milk treated groups; 250 mg/kg and 1000mg/kg showed significant ($p < 0.05$) decrease in total protein, globulin with all camel milk treated groups having significant ($p < 0.05$) increase in A/G ratio. Histological examination of liver tissues showed that camel milk at a dose of 250 mg/kg had slight adipocytes infiltration.

CONCLUSION: The results of our findings highlight the hepatoprotective effect of camel milk in poloxamer 407 induced hyperlipidaemic wistar rats.

Introduction

Dyslipidaemia has been implicated as the major risk factor of cardiovascular diseases. The World Health Organization holds it responsible for more than four million deaths annually [1]. Accumulation of triglycerides and other fats in the hepatocytes, if not treated results in inflammatory conditions of the liver. It is characterized by varying degree of liver injury ranging from steatosis to steatohepatitis, fibrosis and necrosis [2]. Poloxamer 407 (P407) is a ubiquitous manmade surfactant and non-ionic detergent with a molecular weight of 12,600 and liquid at room temperature. It assembles into micelles at body temperature and then aggregate into a gel. These temperature-dependent micellization and gelation properties have led to its widespread use in

mouthwashes, deodorants, and skin care products and also as an excipient in a variety of pharmaceutical preparations [3]. P407 has a major hyperlipidaemic effect as observed in experimental animals [4-6]. Induction of hyperlipidaemia using P407 has contributed towards understanding the impact of hyperlipidaemia on several tissue markers. The advantage with the P407 model is the production of hyperlipidaemia of the hereditary type [7]. Recent reports have shown the ability of P407 induced hyperlipidaemia to produce early and late stages of atherosclerosis [7-9] and alterations of liver transaminases and plasma proteins in rodents [7-11]. Consumption of synthetic hypolipidaemic drugs have been reported to cause hyperuricemia, diarrhea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function [12].

The unique composition and nutritional

values of camel milk are well known from ancient times for its beneficial health effects. Camel milk is a good substitute for human milk as it does not contain β -lacto globulin. Many research findings have proven that camel milk is easily digested by lactase deficient individuals. It contains disease-fighting immunoglobulins which are small in size, thus allowing penetration of antigens and boosting the effectiveness of the immune system [13]. Recently, researchers have reported the presence of vitamins, proteins, insulin like peptides, minerals and glycosides in camel milk [13-16]. Several works have displayed the ameliorative effect of camel milk on clinical and experimental animal models of diabetes, hyperlipidaemia and hepatic damage [14, 16-18].

Hence the aim of the current study is to investigate the effect of oral administration of camel milk on serum liver enzymes, total protein, albumin/globulin ratio and histology of the liver tissues in poloxamer 407 induced hyperlipidaemic wistar rats.

Materials and Methods

Experimental Animals

This study was conducted in the department of pharmacology, Ahmadu Bello University Zaria, Nigeria. Thirty healthy adult male wistar rats weighing 150-200 gm were obtained from the animal house of faculty of pharmaceutical sciences, Ahmadu Bello University Zaria. All the rats were kept in the same animal house. Housing was in well ventilated steel wire cages (5 rats per cage) with normal photoperiod of 12 h light/dark cycle and constant temperature of 25-27°C. The animals were maintained on standard animal feed (growers mash from vital feeds company Kaduna-Nigeria) and allowed access to water ad libitum. Experimental protocols were in accordance with the guidelines for animal research, as stated in the NIH guidelines for the care and use of laboratory animals (National Academy of Sciences and National Institute of Health Publications, 2011). The rats were allowed to acclimatize under laboratory conditions for two weeks before commencement of the experiment.

Camel milk collection

Milk collection was done every day from camel herds (*Camelus dromedaries*) in Kaura Namoda farms, Zamfara state Nigeria. The milk was stored in screwed bottles under ice. The milk was transferred and refrigerated at the department of pharmacology laboratory Ahmadu Bello University Zaria.

Administration of camel milk

Camel milk was administered orally at doses

250 mg/kg, 500 mg/kg and 1000 mg/kg respectively according to Zuberu et al., [16].

Induction of hyperlipidaemia

Poloxamer 407 (Lutrol F127; BASF, Ludwigshafen, Germany) was used to induce hyperlipidaemia. Administration of poloxamer 407 was at a dose of 500mg/kg intra peritoneally twice a week for 3 weeks [5, 11, 19]. Prior to the administration, poloxamer 407 was dissolved in distilled water and refrigerated overnight to facilitate its dissolution. Needles and syringes used for administration were cooled to prevent gelation within the syringe during injection as described by Johnston and Palmer [5].

Preparation of Standard Drug

Atorvastatin was purchased in a tablet form at strength 20 mg (Strovas Tablet 20 mg/kg, Ranbaxy Laboratory Ltd, Paonta Sahib Distribution, Sirmour H.P. 173025 India). Tablets were dissolved in distilled water and administered orally once daily [11].

Groupings of Animals

Group I: Normal control animals fed with a standard diet and orally administered 1 ml/kg distilled water for 21 days.

Group II: Hyperlipidaemic control animals induced with 500 mg/kg of poloxamer 407 intraperitoneally twice a week without treatment for 21 days

Group III: Induced with 500 mg/kg of poloxamer 407 intraperitoneally twice a week and treated with atorvastatin tablet (AT) orally at 20 mg/kg body for 21 days.

Groups IV: Induced with 500 mg/kg of poloxamer 407 intraperitoneally twice a week and co supplemented with oral administration of 250 mg/kg of camel milk once daily for 21 days.

Group V: Induced with 500 mg/kg of poloxamer 407 intraperitoneally twice a week and co supplemented with oral administration of 500 mg/kg of camel milk once daily for 21 days.

Group VI: Induced with 500 mg/kg of poloxamer 407 intraperitoneally twice a week and treated with oral administration of 1000 mg/kg of camel milk once daily for 21 days.

Biochemical Estimations

At the end of the 21-day experimental period, the animals were fasted overnight. All the animals were anaesthetized under chloroform vapor in an

anesthetic box with lid cover. Blood samples were collected via cardiac puncture into anticoagulant free tubes, centrifuged at a speed of 3000 rpm for 15 minutes.

The resultant serum was harvested into plain sample bottles for biochemical analysis. Serum total protein and albumin were measured by biuret reaction and colorimetric estimation respectively by using Agappe diagnostics total protein and albumin kits (Agappe Diagnostics Switzerland, GmbH). Globulin was measured from the difference between total protein and albumin. Serum alkaline phosphatase (ALP) activity was determined using Biolabo ALP reagent (Biolabo SA, 02160, Maizy, France). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using ALT and AST assay kits of Randox Laboratories Limited United Kingdom. Assay was performed according to manufacturer's manual.

Histopathological Studies

The Liver of the experimental animals were harvested and fixed in 10% formal saline solution containing plain sample bottles. This is followed by embedding of the liver tissue sections in melted paraffin wax and staining using hematoxylin and eosin. Histopathological assessment and photomicrography of the prepared slides was done by using an Olympus light microscope with attached Kodak digital camera.

Statistical Analysis

Data obtained was expressed as mean (\pm SEM). The result was analysed using one way analysis of variance (ANOVA), followed by Tukeys post hoc test to compare the level of significance between groups using statcato software version 0.9.12. Values of $p < 0.05$ were considered significant.

Results

The effect of camel milk on serum ALP on P407 induced hyperlipidaemic wistar rats is presented in Figure 1. Serum ALT activity showed a significant difference ($p < 0.05$) between the normal control group (4.0 ± 0.70 U/L) and hyperlipidaemic untreated group (42.50 ± 4.54 U/L). There was significant difference ($p < 0.05$) between the normal control group and camel milk treatment groups 500 mg/kg and 1000 mg/kg (10.20 ± 2.26 U/L and 11.20 ± 1.39 U/L) respectively. There was no significant difference ($p > 0.05$) between the normal control group and camel milk treatment group 250 mg/kg (6.40 ± 1.03 U/L).

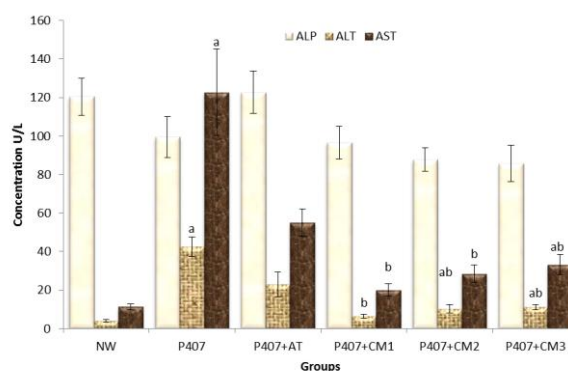


Figure 1: Effect of camel milk on serum liver enzymes of poloxamer 407 induced hyperlipidemic Wistar rats. *Results are expressed as mean \pm standard error of mean $n = 5$, Group DW: Normal control (1 ml/kg of distilled water) Group P407: P407 (500 mg/kg), Group P407+AT: P407 (500 mg/kg) + AT (20 mg/kg), Group P407+CM1: P407 (500 mg/kg) + CM (250 mg/kg), Group P407+CM2: P407 (500 mg/kg) + CM 500 mg/kg, Group P407+CM3: P407 (500 mg/kg) + CM (1000 mg/kg). Values with superscripts are statistically significant ($P < 0.05$); a = when compared to Group NW, b = when compared to Group P407, c = when compared to Group P407+AT

Serum concentration of total protein (TP) is shown in Figure 2. There was significant ($p < 0.05$) increase in the concentrations of serum TP between normal control (5.56 ± 0.26 g/dL) and hyperlipidaemic untreated group (11.23 ± 1.25 g/dL). There was significant ($p < 0.05$) decrease between hyperlipidaemic untreated group and camel milk treated groups 250 mg/kg (5.55 ± 0.56 g/dL) and 1000 mg/kg (6.42 ± 0.53 g/dL) respectively.

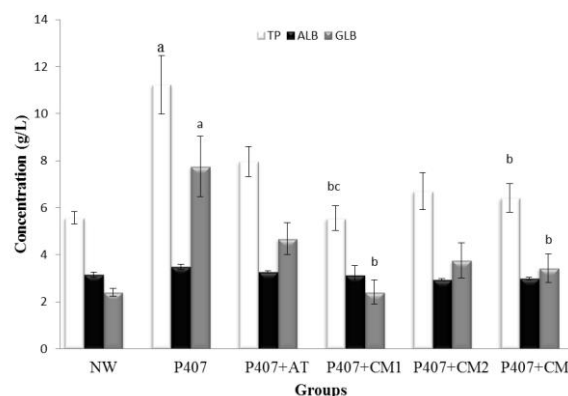


Figure 2: Effect of Camel Milk on Serum Total Protein, Albumin and Globulin of Poloxamer 407 Induced Hyperlipidemic Wistar Rats. *Results are expressed as mean \pm standard error of mean $n = 5$, Group DW: Normal control (1 ml/kg of distilled water) Group P407: P407 (500 mg/kg), Group P407+AT: P407 (500 mg/kg) + AT (20 mg/kg), Group P407+CM1: P407 (500 mg/kg) + CM (250 mg/kg), Group P407+CM2: P407 (500 mg/kg) + CM 500 mg/kg, Group P407+CM3: P407 (500 mg/kg) + CM (1000 mg/kg). Values with superscripts are statistically significant ($P < 0.05$); a = when compared to Group NW, b = when compared to Group P407, c = when compared to Group P407+AT

There was significant difference ($p < 0.05$) between all camel milk treated groups when compared to the hyperlipidaemic control group. In AST activity, there was significant difference ($p < 0.05$) between normal control group (11.40 ± 1.05

U/L), hyperlipidaemic untreated group (122.75 ± 22.45 U/L) and camel milk treated group 1000 mg/kg (33.20 ± 5.31 U/L) as shown in Figure 1. There was significant difference ($p < 0.05$) between all camel milk treatment group (250 mg/kg, 500 mg/kg and 1000 mg/kg) (20.00 ± 3.17 U/L, 28.40 ± 4.51 U/L and 33.20 ± 5.51 U/L) respectively, when compared to the hyperlipidaemic untreated group.

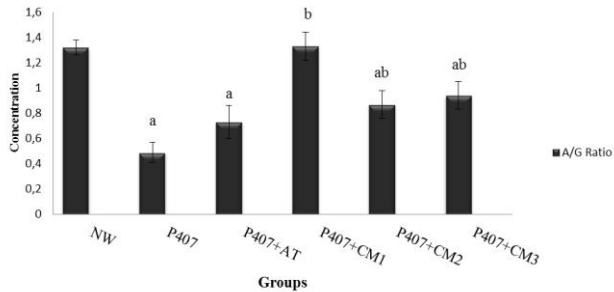


Figure 3: Effect of Camel Milk on Serum Albumin/Globulin Ratio of Poloxamer 407 Induced Hyperlipidemic Wistar Rats. *Results are expressed as mean \pm standard error of mean $n = 5$, Group DW: Normal control (1 ml/kg of distilled water) GroupP407: P407 (500 mg/kg), Group P407+AT: P407 (500 mg/kg) + AT (20 mg/kg), GroupP407 + CM1: P407 (500 mg/kg) + CM (250 mg/kg), GroupP407 + CM2: P407 (500 mg/kg) + CM500 mg/kg, Group P407 + CM3: P407 (500mg/k) + CM (1000 mg/kg). Values with superscripts are statistically significant ($P < 0.05$); a = when compared to Group NW, b = when compared to Group P407, c = when compared to Group P407+AT

Camel milk at a dose of 250 mg/kg also showed significant ($p < 0.05$) decrease when compared to atorvastatin treated group (7.96 ± 0.64 g/dL). There was significant ($p < 0.05$) increase in the concentrations of serum globulin between normal control group (2.41 ± 0.17 g/dL) and Hyperlipidemic untreated group (7.75 ± 1.28 g/dL).

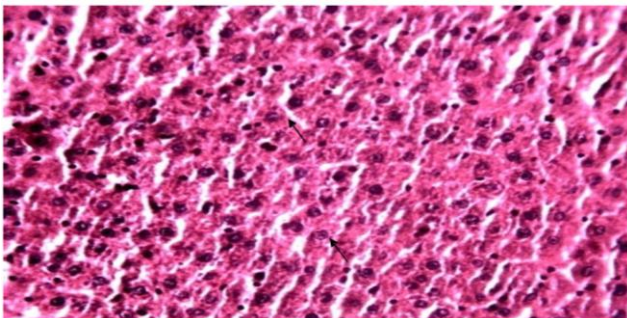


Figure 1: Photomicrograph of Liver Group I showing normal Hepatocytes (Longitudinal Section, H&E x250)

There was significant ($p < 0.05$) difference between Hyperlipidemic untreated group when compared to camel milk groups 1000 mg/k (3.43 ± 0.60 g/dL) and 250 mg/kg (2.41 ± 0.51 mg/dL) respectively. Serum Albumin/Globulin Ratio is shown in Figure 3. There was significant ($p < 0.05$) decrease in the Albumin/Globulin Ratio between normal control group (1.32 ± 0.06), Hyperlipidemic untreated group

(0.49 ± 0.08), atorvastatin-treated group (0.73 ± 0.13 g/dL) and Camel milk treated groups (500 mg/kg and 1000 mg/kg) (0.87 ± 0.11 and 0.94 ± 0.11). All Camel milk treated groups showed significant ($p < 0.05$) increase when compared to the hyperlipidemic untreated group.

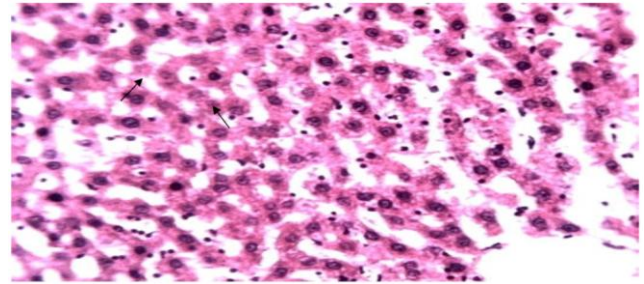


Figure 2: Photomicrograph of Liver of Group II with Intense Adipocytes Infiltration and Necrosis of Hepatocytes (Black Arrows) (Longitudinal Section, H&E x250)

Discussion

Poloxamer 407 (P-407) has been utilized as a model to induce hyperlipidaemia in rodents due to its convenience, reproducibility, low cost and the lack of undesirable underlying pathological conditions [20, 21]. P-407-induced hyperlipidaemia is associated with alterations in the activities of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, lecithin cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), hepatic lipase (HL) and lipoprotein lipase (LPL). It directly inhibits the heparin releasable fraction of LPL and HL and increases the biologic activity of CETP and LCAT indirectly [5, 6].

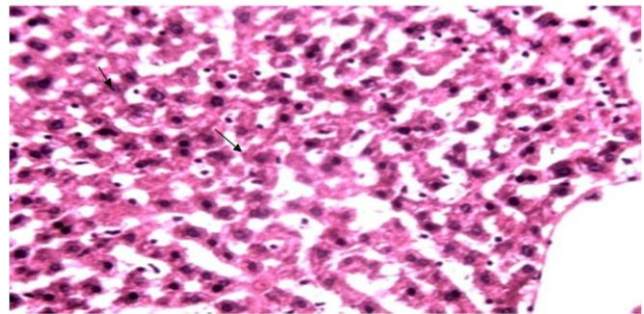


Figure 3: Photomicrograph of Liver Group III showing Moderate Adipocytes Infiltration and Necrosis of Hepatocytes (Black Arrows) (Longitudinal Section, H&E x250)

Fatty liver is an accumulation of triglycerides and other fats in the hepatocytes, if not treated leads to inflammation of the liver. It is characterized by varying degree of liver injury from steatosis to steatohepatitis, fibrosis and necrosis [2]. An elevated serum level of ALT and AST is indicative of liver disease as this enzymes are present in large quantities in the liver [14]. Serum ALT is thought to be more specific for hepatic degeneration [22].

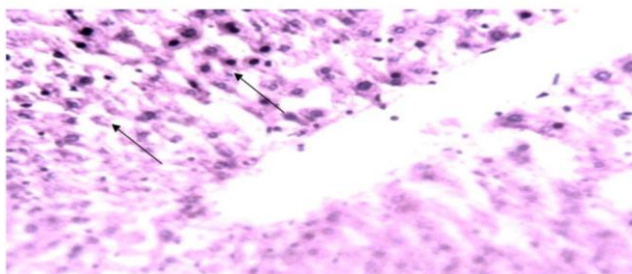


Figure 4: Photomicrograph of Liver Group IV showing Slight Adipocytes Infiltration of Hepatocytes (Black Arrows) (Longitudinal Section, H&E x250)

Following hepatocellular damage, hepatocytes alter their transport functions and membrane permeability thus leading to the leakage of these enzymes from their cells [11, 14]. Because it is a membrane-bound enzyme related to the transport of various metabolites, ALP is a sensitive biomarker of liver disease that is dependent on energy metabolism. A decrease in its activity may indicate impaired cellular energy processing [14].

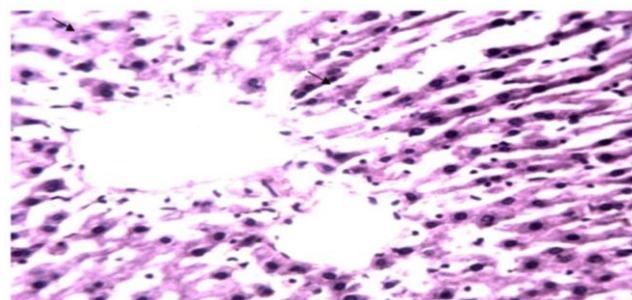


Figure 5: Photomicrograph of Liver Group V showing Intense Adipocytes Infiltration and Necrosis of Hepatocytes (Black Arrows) (Longitudinal Section, H&E x250)

There have been conflicting reports on the effect of poloxamer 407 induced hyperlipidaemia on liver enzymes (ALP, AST and ALT). Report on the effects of poloxamer 407 induced hyperlipidaemia on serum levels of the above enzymes showed that hyperlipidaemia elevated serum levels of ALT and AST [10].

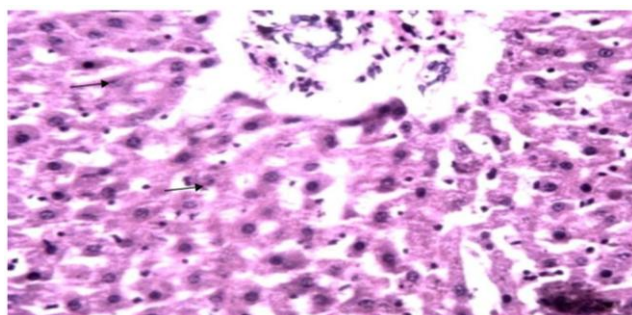


Figure 6: Photomicrograph of Liver Group VI showing Intense Adipocytes Infiltration and Necrosis of Hepatocytes (Black Arrows) (Longitudinal Section, H&E x250)

However, Ameh et al., [23] found no effect on ALT except on AST, while Johnston et al., [24] reported that P407 does not cause hepatic injury or damage.

Moreover, Victor et al., [11] showed elevation of all the three liver enzymes after induction of Wistar rats with P407. The discrepancies in the serum levels of the enzymes was attributed to the levels and duration of hyperlipidaemia [25]. In the present study, there was significant elevation in the serum ALT and AST but not ALP of hyperlipidaemic control group when compared to the normal control group. This may be due to injuries inflicted to the liver secondary to the accumulation of triglycerides and other fats in the liver cells, these findings conform with the work of Hyeung et al., [10]. There was a significant decrease in ALT and AST level of all the camel milk treated groups which is consistent with findings of Abbas et al., [26] and Al Hashem [14] but differs with the work of Helal et al., [27]. The reversal of these liver enzymes towards normal by the milk as observed in this study, maybe due to the prevention of the leakage of intracellular enzymes and increase membrane stabilizing activity. This is in agreement with the commonly accepted view that serum transaminases return to normal level upon healing of hepatic parenchyma and hepatocytes regeneration [28]. It therefore manifests the hepatoprotective effect of camel milk on P407 induced hepatic damage.

Evaluation of total protein or albumin status may be helpful in the assessment of disease progression [29]. Total protein is the measurement of all proteins in the blood serum. Majority of the proteins that are found in the blood are produced by the liver. By calculating the total amount of protein in the blood, performance of the liver in generating proteins can be understood. Total protein produced by the liver includes mainly globulin and albumin. Elevation of total proteins may be caused by dehydration and a decrease from overloading with water. The increase in disease arise mainly from an increase in total globulin, the albumin remaining normal or being reduced to a lesser extent. A decrease in total protein concentration is almost always the result of a drop in the level of albumin while the globulins do not change or increase by a smaller amount [26]. The results of our findings showed significant ($p < 0.05$) increase in total protein of hyperlipidaemic untreated group when compared to the normal control group. The increase total protein from our results differ from the works of Victor et al., [11] and Olorunnisola et al., [30] but are consistent with the works of Korolenko et al., [7]; who reported increased total protein in hyperlipidaemia. Camel milk significantly ($p < 0.05$) reduced the level of total protein of P407 induced hyperlipidaemic wistar rats which is in agreement with the work of Abbas et al., [26]. Albumin is important as nonspecific transport mechanism for many physiologic substances as well as drugs, antibiotics, various ions, amino acids and hormones. Albumin also serves as a precursor for

tissue proteins and in nutrition, proteins play a small part in maintaining the plasma pH. They are negatively charged at body pH and so act as bases, accepting hydrogen ions. Albumin is also an important component of plasma antioxidant activity that primarily binds free fatty acids, divalent cations and hydrogen oxychloride (HOCl) [30]. In chronic liver disease, levels of albumin are usually normal until a lot of liver is damaged [26, 31]. There was no significant ($p > 0.05$) change between all the groups. Globulin is a part of total protein and its raised level is noticed during chronic infection and liver disease such as liver necrosis [26]. Our findings showed significant increase ($p < 0.05$) in globulin level of hyperlipidaemic untreated group when compared to the normal control group. Increase in serum globulin level may have resulted from inflammatory or necrosis effect of P407 on the liver [7]. Camel milk significantly ($p < 0.05$) reduced the level of globulin of P407 induced hyperlipidaemic wistar rats which is in agreement with the work of Abbas et al., [26]. There was significant ($p < 0.05$) decrease of serum A/G ratio all treated groups except the group administered camel milk at a dose of 250 mg/kg. However all camel milk treated group showed significant ($p < 0.05$) increase in A/G ratio when compared to the hyperlipidaemic untreated group. This protective effect may be attributed to the high level of antioxidant vitamins, proteins and immunoglobulin present in camel milk [13, 14, 16, 26] or healing process which may have occurred in the liver.

Tissue sections from the hyperlipidaemic untreated group showed adipocyte infiltration and hepatocyte necrosis in the liver. This implies that the liver is a direct target for P407 as reported by Cogger et al., [32], whose findings showed that P407 has a marked effect on the ultrastructure of the liver sinusoidal endothelial cells (LSECs). Necrosis observed in liver tissues also agrees with the findings of Korolenko et al., [7, 9]. The liver of atorvastatin treated groups showed moderate infiltration and necrosis. This suggests that tissue repair occurred upon treatment with atorvastatin treatment. Group treated with camel milk (250 mg/kg) restored hepatocytes to normal. Thus, camel milk may be capable of ameliorating tissue damage such as hepatocytes injury that may occur in association with hyperlipidaemia.

In conclusion, camel milk showed hepatoprotective effect in poloxamer 407 induced hyperlipidaemic wistar rats. Drinking Camel milk may be helpful in ameliorating hazards caused by toxic substances.

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