

Hepatocurative Potentials of Camel (*Camelus dromedarius*) Urine and Milk on Carbon Tetrachloride Induced Hepatotoxicity in Albino Rats

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Abstract

Damage to the liver cells caused by diseases such as hepatitis and cirrhosis can lead to dysfunction of the liver, which can later result in hepatic or liver failure. The present study was carried out to investigate the hepatocurative effects of camel urine and milk on carbon tetrachloride (CCl₄) induced hepatotoxicity in albino rats. Three different treatments (camel urine, camel milk and a 1:1 mixture of camel milk and urine) were administered to three different CCl₄-induced hepatotoxic rats (Groups A,B and C) and also to three different subgroups (D,E and F) of normal rats for two weeks. A positive control (Group G) was neither induced nor treated while negative control group (H) received no treatment after CCl₄-hepatotoxicity induction. Serum Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST) and Albumin (ALB) and histopathological findings all confirmed liver steatosis forty eight hours after CCl₄ hepatotoxicity induction on randomly selected rats. A significant weight gain was recorded for rats that received camel milk and urine mixture in the CCl₄ induced hepatotoxic group (P<0.05). A significant decrease in serum AST was observed in all test groups (P<0.05). Groups that received 1:1 urine and milk mixture showed a significant decrease at p<0.05 in serum ALT, AST and ALB than when treatments were administered singly. Camel urine resulted in periportal inflammation; camel milk resulted in vascular congestion while the 1:1 mixture of the two eliminated these side effects. In rats that received no treatment after hepatotoxicity induction, the condition of the liver deteriorated from liver steatosis to fibrosis and onset of cirrhosis. All these indicated that camel urine, camel milk and to a greater extent a mixture of the two, may have hepatocurative effects on CCl₄ induced hepatotoxicity in albino rats.

Keywords: Camel, Liver, Toxicity, Safety, Alternative medicine, hepatocurative.

1.0 Introduction

Arabian camel urine and milk were a standard prescription in Arabic medicine and remains a staple of Bedouin natural remedies to this day both as diuretic, snuff, and delousing hair wash (Jabbur, 1995) and also a cure for dropsy (Al-Azraq, 1999). It has been shown that urine has profound medical uses such as effectiveness against allergies, psoriasis and all skin problems (Martha, 2000), and on fertility, fever, burns and tuberculosis (Natalie, 2002) and even diabetes mellitus (Agrawal *et al.*, 2009).

Camel milk differs from other ruminant milk as it is low in cholesterol, sugar and protein but high in minerals (sodium, potassium, iron, copper, zinc and magnesium), vitamins A, B₂, C and E and contains a high concentration of insulin and immunoglobulins (Kamal *et al.*, 2007 and Al-Hashem, 2009). These novel results provide the first scientific evidence of the mechanism of the presumed therapeutic properties of camel urine.

Damage to the liver cells caused by diseases such as hepatitis and cirrhosis can lead to dysfunction of the liver, which can later result in hepatic or liver failure. Fibrous tissue is formed in response to inflammation or direct toxic insult to the liver. Unlike other responses which are reversible, fibrosis points towards generally irreversible hepatic damage (Kumar *et al.*, 2004). With continuing fibrosis, the liver is subdivided into nodules of proliferating hepatocytes surrounded by scar tissue, termed "cirrhosis" (Kumar *et al.*, 2004).

Due to the increasing prevalence and incidence of liver diseases globally (Lim and Kim, 2008; Williams, 2006) and with the availability of camels in Kano, a sub-Saharan town, this study was carried out to ascertain the scientific validity of use of a combination of camel milk and urine for the management/treatment of liver diseases.

2.0 MATERIALS AND METHODS

2.1 Collection of camel milk and urine samples

Fresh Camel milk and urine samples were obtained from Abattoir, Kofar Mazugal, Kano, Nigeria, out of which the 1:1 mixture was prepared. All the three samples (urine, milk and 1:1 mixture) were refrigerated at 2-8°C for the period of study.

2.2 Acquisition and Care of experimental animals

Twenty one (21) albino rats weighing between 100-120g were obtained from Veterinary Research Institute, Vom, Jos, Nigeria and were certified healthy by a veterinary doctor. They were kept in the animal house of the

Department of Biological Sciences, Bayero University, Kano for acclimatization. They were allowed food and water *ad-libitum* and exposed to twelve hour light-dark cycles and the experiments conducted according to the National Institute of Health Guide for the care and use of laboratory animals (NIH, 1996). The animals were also handled according to international guidelines, i.e. the Organization for Economic Cooperation and Development (OECD) Test Guidelines (TG407) (OECD, 2006).

2.3 Experimental design

Of the twenty one (21) animals, thirteen (13) animals received 150mg/kg body weight of carbon tetrachloride (CCl₄) intraperitoneally to induce hepatotoxicity - olive oil was used as a vehicle (Alhassan *et. al.*, 2009; Nagi *et.al.*, 1999).

2.4 Groupings

- A= Rats with CCl₄-induced hepatotoxicity that received 5ml/day camel urine treatment.
- B= Rats with CCl₄-induced hepatotoxicity that received 5ml/day camel milk treatment.
- C= Rats with CCl₄-induced hepatotoxicity that received 5ml/day camel milk and urine mixture (1:1).
- D=Non-CCl₄- induced non- hepatotoxic rats that received 5ml/day camel urine treatment.
- E= Non-CCl₄- induced non- hepatotoxic rats that received 5ml/day camel milk treatment.
- F= Non-CCl₄- induced non- hepatotoxic rats that received 5ml/day camel milk and urine mixtures treatment.
- G=Rats that received normal feed and water only (positive control).
- H= Rats with CCl₄-induced hepatotoxicity that received no treatment (negative control).

The animals received these treatments orally for two weeks after which they were sacrificed and the blood and liver samples obtained were subjected to liver function tests (serum activities of ALT, AST and concentration of ALB) and histopathological examinations respectively. All animals were weighed before commencement and after completion of experiment.

2.5 Liver function tests

The method of Reitman and Frankel (1957) was employed for the assay of serum AST and ALT. The procedure of Doumas, Watson and Biggs (1971) was applied for the estimation of serum albumin.

2.6 Histopathology

All histopathological examinations were carried out with the assistance of trained personnel at the department of histopathology, Aminu Kano Teaching Hospital, Kano. Haematoxylin and Eosin (H and E) staining method (Spector and Goldman, 2006) was used.

2.7 Statistical Analysis

All statistical analyses were carried out using SPSS software version 17.0. Student's t- test was used to compare means. Data are presented as mean \pm SEM.

3.0 Results

The result of assessment of hepatocurative effect of camel urine and milk on CCl₄ induced liver injury is presented in the table below:

Table 1: Liver function indices and weight gain of CCl₄ induced hepatotoxic rats administered camel urine and milk for two weeks

Groups/Parameters	A	B	C	D	E	F	G	H
ALT (U/L)	61.00 \pm	57.67 \pm	45.67 \pm	38.00 \pm	56.00 \pm	41.00 \pm	33.50 \pm	60.50 \pm
AST (U/L)	10.97 85.33 \pm	0.88 ^{c,f,h} 85.00 \pm	2.33 ^{c,l} 74.67 \pm	4.00 74.00 \pm	3.00 81.50 \pm	3.00 ^f 60.00 \pm	1.50 ^{h,l,w} 80.00 \pm	5.50 ^w 221.00 \pm
ALB (g/dl)	4.26 ^{a,b,d} 24.00 \pm	2.65 ^{e,g,j} 20.67 \pm	2.91 ^{d,n} 19.33 \pm	3.00 ^{s,p} 26.00 \pm	2.50 ^{q,r,t} 20.00 \pm	2.00 ^{a,g,q,v} 20.50 \pm	4.00 ^{r,x} 31.50 \pm	13.00 ^{b,j,n,p,t,v,x} 35.00 \pm
% weight gain	4.04 6.94 \pm	0.33 ^{i,k} 16.67 \pm	1.86 ^{m,o} 23.61 \pm	5.00 10.00 \pm	1.00 ^{s,u} 20.00 \pm	5.50 20.00 \pm	1.50 ^{i,m,s} 10.00 \pm	3.00 ^{k,o,u} 10.00 \pm
	1.39	0.00	1.39 ^y	0.00	0.00	0.00	0.00 ^y	0.00

Values are expressed as mean \pm standard error of the mean (SEM). Superscripts (under the same row) indicates significance at P<0.05; n=3.

3.1 Liver function tests

There was a clear reduction in serum activities of ALT, AST and concentration of ALB in all test groups during the two weeks treatment when compared to Group H.

3.2 Serum Activity of ALT

A significant decrease in the serum activity of ALT was observed in the CCl₄- induced hepatotoxic group that received camel milk and urine mixture (group C) when compared to the CCl₄-induced hepatotoxic group (B) that received only camel milk treatment ($P < 0.05$). A significant decrease was also observed in the non-CCl₄-non-hepatotoxic group that received the same treatment of camel milk and urine mixture (group F) when compared statistically to group B. The positive control group G (animals that received normal feed and water only) likewise showed a significant decrease in serum activity of ALT when compared to group B ($P < 0.05$). This same group (G) had the lowest mean serum ALT activity and was significantly lower than the negative control group, H (CCl₄-induced hepatotoxic group that received no treatment). ALT level in group G was also found to be significantly ($P < 0.05$) lower than that of group C (Table 1).

3.3 Serum activity of AST

Serum activity of AST was significantly higher in the negative control group, H (group that received CCl₄-induced hepatotoxic without treatment) than the entire test as well as the positive control groups ($P < 0.05$). Reduction in all groups was found to be highly significant with the highest significant reduction observed in groups F, D, C, G, E, B and A in that order. A significant decrease ($P < 0.05$) in serum AST activity was equally observed in group C as compared to group A; group F when compared to groups A, B, and E; group D compared to B; and group G compared to E (Table 1).

3.4 Serum Concentration of ALB

All test groups showed an observably less serum concentration of ALB than the negative control group, H with significant differences in groups B, C, and E ($P < 0.05$). Significant decrease in serum concentration of ALB was observed in group G with E and C at $p < 0.05$. Group B animals also showed significantly less serum concentration of albumin than the positive control group, G ($P < 0.05$) (Table 1).

3.5 Percentage weight gain

A significant increase in weight was observed in group C (hepatotoxic group that received milk and urine mixture) when statistically compared to the positive control group G. All other groups showed little increase in weight and even lesser weight gain was observed in group A, hepatotoxic group that received camel urine treatment (Table 1).

3.6 Histological appearance of the liver in rats studied

The histologic section of liver tissue after CCl₄ induction showed sheets and anastomosing plates of hepatocytes with round bland nuclei and abundant granular cytoplasm. Many of these cells demonstrated huge cytoplasmic vacuoles displacing the nuclei to the periphery in a diffuse pattern. The sinusoids were lined by endothelial cells but otherwise unremarkable. The central veins were mildly congested while the portal triads demonstrated sprinkles of lymphocytes. Also, there was accumulation of fat cells that appeared as empty spaces within the tissue (fatty liver). All these features are indicators of liver steatosis (Plate 1). Group G animals showed normal hepatocyte architecture (Plate 2). The test groups – A, B and C showed varying degrees of severe to mild indicators of liver damage for the period of treatment (Plates 3, 4 and 5). Plates 6, 7 and 8 are for the non-induced but treated animals showing almost normal liver histology but for some mild abnormalities. Group H animals presented severe indication of liver damage (fibrosis) (Plate 9) during the course of the study.

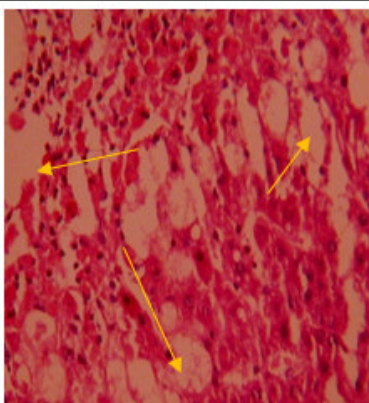


Plate 1: CCL₄ induced hepatotoxic liver of rat(Group H) showing liver steatosis. Arrows show accumulated fat cells.

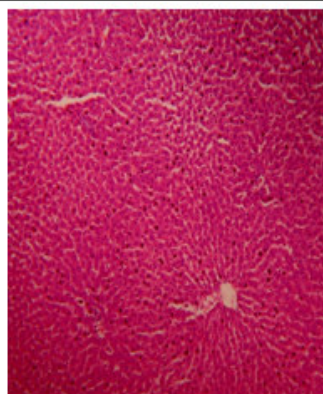


Plate 2: Liver of Group G rat showing normal histology.

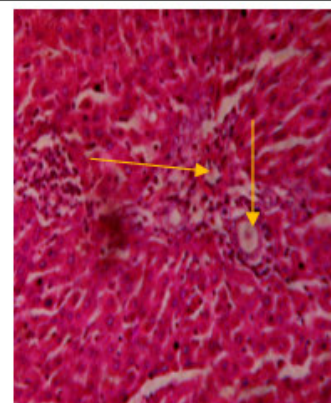


Plate 3: Liver of group A animal with arrows showing mild periportal inflammation.

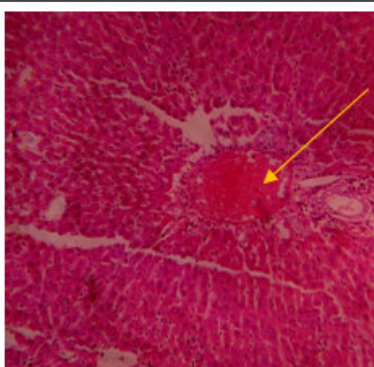


Plate 4: Liver of group B animal with arrow showing vascular congestion.

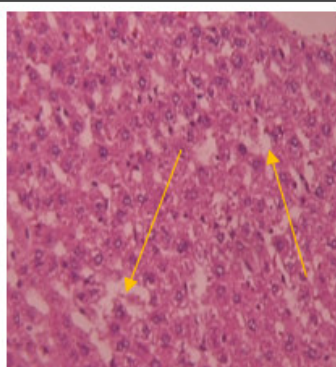


Plate 5: Liver of group C animal showing almost normal histology with mild steatosis.

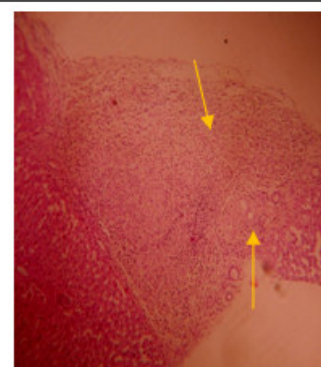


Plate 6: Liver of group D animal with arrow showing portal triad Xanthoma and central vein congestion



Plate 7: Liver of group E animal with arrows showing portal vein congestion (single arrow) and vascular dilatation (double 3D arrows).

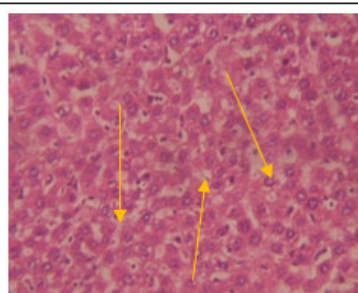


Plate 8: Liver of group F animal with arrows showing slightly reactive hepatocytes but otherwise normal histology.

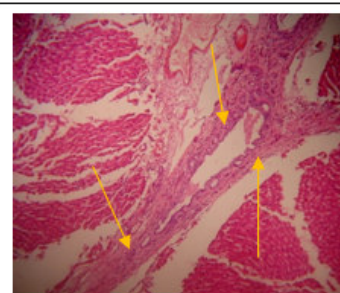


Plate 9: Liver of group H animal with arrows showing bridging fibrosis.

4.0 Discussion

The toxic effect of CCl₄ is due to its conversion by P-450 to the highly reactive toxic free radical CCl₃ (CCl₄ + e⁻ → CCl₃ + ·Cl⁻). The free radicals produced locally cause autoxidation of the polyenoic fatty acids present within the membrane phospholipids. There, oxidative decomposition of the lipid is initiated, and organic peroxides are formed after reacting with oxygen (lipid peroxidation). This reaction is autocatalytic in that new radicals are

formed from the peroxide radicals themselves. Thus, rapid breakdown of the structure and function of the endoplasmic reticulum is due to decomposition of the lipid. It is no surprise therefore, that CCl₄-induced liver injury is both severe and extremely rapid in onset (Kumar *et al.*, 2004).

The decreased serum activities of AST and the concentration of ALB in the different groups of animals that received treatments of camel urine, camel milk and a mixture of the two after CCl₄-hepatotoxicity induction are indicators that all three treatments possess hepatocurative effects on CCl₄-induced hepatotoxicity in rats. The treatments did not show adverse side effects.

Statistical analysis on the serum activities of ALT and AST and the concentration of ALB in the experimental animals indicate that the animals that received a mixture of camel milk and urine treatment after CCl₄-hepatotoxicity induction had the highest response to treatment and recovery from injury to the liver tissue. The serum activities of AST and ALT were significantly lower in this group of animals than in the groups that received the treatments separately. The non-hepatotoxic group that received the same treatment of camel milk and urine mixture also showed even lower activities of serum ALT and AST indicating that camel urine and/or milk in addition to possessing hepatocurative effects may also have hepatoprotective effects. This research is in accordance with the findings of Salawa *et al.* (2011), which showed the hepatocurative effect of camel urine against CCl₄ induced stress. On the bases of these results, it could be clearly suggested that camel urine has an active component(s) which may have an important role as an endogenous antioxidant and/or could act as cytoprotective agent against tissue damage mediated by the toxic substances (Salawa *et al.*, 2011). Salawa *et al.* (2011) also report that camel urine (in different forms) can penetrate sub epithelially and induce generation of mast cells with release of chemical mediators, followed by forceful peristaltic contractions caused by 5-HT and other newly formed mediators.

Serum concentration of ALB being highest in the negative control group may be regarded as an indicator of dehydration as stated by Philip, (1994). These animals displayed poor appetite for food and water. This also indicates that carbon tetrachloride poisoning does not appear to affect the ability of the liver to synthesize ALB; neither do any of the three treatments given. Whereas the relatively lower concentration of ALB in groups that received camel milk and camel milk and urine mixture may not necessarily indicate an impaired albumin synthesis in the liver, rather, possible reasons for that could be that camel milk is rich in potassium (Kamal *et al.*, 2007 and Al-Hashem, 2009), which helps to retain water in the tissues and thus balances albumin synthesis in the liver without causing overproduction or impairment of synthesis in the liver. These animals were well hydrated and appeared healthy.

It has been reported that camel milk contains high levels of vitamins A, B₂, C and E and is very rich in magnesium (Mg) and other trace elements (Al-Humaid *et al.*, 2010). These vitamins act as antioxidants that have been found to be useful in preventing tissue injury caused by toxic agents. High mineral content in camel milk (sodium, potassium, iron, zinc, copper and magnesium) as well as a high vitamin C intake may act as antioxidant, thereby removing free radicals, which may provide a stress free situation to the animals.

Percentage weight gain being highest in experimental animals that received camel milk and urine mixture and camel milk alone may have been predicted. This could be due to its high vitamin and mineral content (Anonymous 2008; Al-Humaid *et al.*, 2010), which contributed to the well-being of the animals by providing the requirements of a balanced diet in addition to what is in the normal feed. This helped in nourishing the subjects and providing a healthier atmosphere for growth.

Looking at the histopathological aspect, carbon tetrachloride poisoning caused fatty and hydropic vacuolation and necrosis of cells in parenchymal cells near the hepatic veins which is in accordance with the study of Cameron and Karunaratne (1935). Leaving the animals (negative control group) for two weeks without treatment resulted in the formation of fibrous tissue in the liver from one portal triad to another (bridging fibrosis). This, according to Kumar *et al.*, (2004) points towards generally irreversible hepatic damage as deposition of collagen has lasting consequences on patterns of hepatic blood flow and perfusion of hepatocytes. As fibrosis continues, it progresses to cirrhosis the end stage of chronic liver disease. All three characteristics of cirrhosis as stated by Kumar *et al.*, (2004) have been established in the negative control group.

However, the tremendous recovery observed in rats that received treatments of camel urine, camel milk and camel milk and urine mixture may be attributed to camel urine and/or milk as liver steatosis was completely or almost completely gone in most of the animals. The group of rats that received camel urine in the CCl₄-induced group appeared to have almost fully recovered from liver steatosis since all of its features are gone; only mild periportal inflammation which is not a feature of liver steatosis has been observed. The group that received the same treatment in their non-CCl₄-induced counterparts also showed no features of liver steatosis as well, but a mild periportal inflammation and a portal triad xanthoma was observed. Thus, periportal inflammation of the liver could be as a result of its reaction in response to the drug (camel urine).

The group of experimental animals that received camel milk in the CCl₄-induced group also showed a tremendous recovery without any feature of liver steatosis. Vascular congestion was however observed. Their non CCl₄-induced counterparts showed vascular dilatation and congestion of portal vein which may be attributed

to the liver's reaction to camel milk since both groups displayed this feature.

The group of rats that received a 1:1 mixture of camel urine and milk appeared to show the best recovery as there may exist in this mixture a "diluting" effect that eliminates the mild periportal inflammation side effect caused by camel urine alone and vascular congestion and dilatation side effect caused by camel milk alone. The only feature that was observed outside the ordinary architecture of the liver is that the hepatocytes appeared slightly reactive with mild steatosis which may be as a result of the liver's activities in processing the drug (camel milk and urine mixture). Features of liver steatosis in this animal have not progressed to fibrosis as seen in the negative control group which indicated that recovery is still on-going and it is suspected that perhaps a prolonged treatment or a higher dose may have shown a better result.

5.0 Conclusion:

Camel milk and/or urine do have hepatocurative effects as the results indicate as they have effectively cured liver steatosis and prevented its progression to cirrhosis and helped in reconstructing the liver back to its normal architecture within the two weeks treatment period. Camel urine treatment showed periportal inflammation and camel milk treatment showed vascular congestion whereas a mixture of the two eliminated these side effects. Moreover, biochemical markers of liver damage, i.e. ALT and AST were equally reduced.

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