https://doi.org/10.31925/farmacia.2023.1.20 **ORIGINAL ARTICLE**

AMELIORATION OF HEPATIC INJURY BY CAMEL'S BIOLOGICAL FLUIDS THROUGH THE MODULATION OF NF-KB AND NRF2/HO-1 SINGLING PATHWAY

KHALID M. ALKHARFY [1]*, WAHAF B. ALDAHASI [2], AJAZ AHMAD [1], MODI A. ALKHARFI [3], MOHAMMAD RAISH [4], SYED RIZWAN AHAMAD [5,6], BASIT L. JAN [1]

[1]*Department Clinical Pharmacy College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia*
[2]*Pharma Pharmaceutical Industries, Riyadh 11351, Saudi Arabia*
[3]*College of Medicine, AlMaarefa University, Riyadh 11597, Saudi Arabia*
[4]*Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia*
[5]*Department Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia*
[6]*Central Laboratory, Research Centre, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia*

**corresponding author: alkharfy@ksu.edu.sa*

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Abstract

Camel's products are regarded historically as having a high-quality source of nutrients and used to treat various ailments. In particular, camel's milk and urine have been applied to treat disorders stemming from persistent liver dysfunction. The objective of this study was to explore the hepatoprotective effects and potential mechanisms of camel's biological fluids (*i.e.*, milk and urine) on D-Gal-induced liver injury in mice. Mice was allocated randomly into four groups of six mice each. Group 1 administered normal saline orally (p.o.) for 14 days. Group 2 were given normal saline for 14 days and D-GalN (800 mg/kg i.p.) 24 hr before the last saline administration and served as disease control. Group 3 and 4 were treated orally with camel's milk and urine (10 mL/kg) for 14 days, respectively, and 24 hr before the last dose D-Gal dose (800 mg/kg i.p.). At the end of the experiment, the blood samples were collected in heparinized tubes for biomarkers' analysis and liver tissues were harvested for histology. A marked increase in liver function tests (*i.e.*, AST, ALT, GGT, ALP, LDH and bilirubin) was observed in the D-Gal-treated mice compared with the control group ($p < 0.05$). Treatment with camel's milk and urine significantly ameliorated hepatic injury and improved liver histology findings. The protective mechanism of camel's milk and urine also appeared to be dependent on the downregulation of NF-κB and the reinstatement of antioxidant enzyme levels *via* the activation of the Nrf2/HO-1 pathway. The biological fluids or excreta of camel (*i.e.*, milk and urine) could have a potential application in the management acute hepatic injury and future clinical testing is warranted.

Rezumat

Încă din cele mai vechi timpuri, fluidele biologice ale cămilei, considerate o sursă de nutrienți de înaltă calitate, au fost folosite pentru a trata diverse afecțiuni. Laptele și urina de cămilă au fost utilizate în special pentru a trata disfuncții hepatice persistente. Scopul acestui studiu a fost de a explora efectele hepatoprotectoare și mecanismele potențiale ale fluidelor biologice de cămilă asupra leziunilor hepatice induse de D-Gal la șoareci. Animalele au fost repartizate aleatoriu în patru grupuri de câte șase șoareci fiecare. Grupului 1 i s-a administrat soluție salină normală pe cale orală (p.o.) timp de 14 zile. Grupului 2, grupul control, i s-a administrat soluție salină timp de 14 zile și D-GalN (800 mg/kg i.p.) cu 24 de ore înainte de ultima administrare. Grupurile 3 și 4 au fost tratate oral cu lapte de cămilă și urină (10 ml/kg) timp de 14 zile, respectiv cu 24 h înainte de ultima doză au primit D-GalN (800 mg/kg i.p.). La sfârșitul experimentului, au fost recoltate probe de sânge în tuburi heparinizate pentru analiza biomarkerilor, iar țesuturile hepatice au fost recoltate pentru histologie. La șoarecii tratați cu D-Gal s-a observat o creștere marcată a testelor funcționale hepatice (AST, ALT, GGT, ALP, LDH și bilirubină) în comparație cu grupul control ($p < 0,05$). Tratamentul cu lapte și urină de cămilă a ameliorat semnificativ leziunile hepatice și a îmbunătățit leziunile histologice hepatice. Este foarte posibil ca mecanismul de acțiune al laptelui și urinei de cămilă să implice căile NF-κB și Nrf2/HO-1. Fluidele biologice de cămilă (adică laptele și urina) ar putea avea potențial în gestionarea leziunilor hepatice acute.

Keywords: D-galactosamine, liver injury, camel's milk and urine, oxidative stress and inflammation, apoptosis, NF-κB and Nrf2/HO-1

Introduction

Liver injuries can be the result of exposure to certain viruses, chemicals, as well as autoimmune disorders. Non-alcoholic fatty liver diseases are highest in the Middle East and South America, with a 31% and 32% prevalence, respectively [50, 66]. For viral infections, World Health Organization estimated that the number of people exposed to Hepatitis B virus to be roughly 2 billion; 240 million of whom are chronic carriers [23, 38]. Moreover, it is estimated

that 71 million people suffer from chronic Hepatitis C virus infection worldwide, which resulted in 399,000 deaths in 2015, predominantly from complications such as liver cirrhosis and hepatocellular carcinoma (HCC) [42, 62].

Advanced fibrosis and conversion of normal architecture into structurally aberrant pseudo lobules are frequent clinical endpoints in all chronic liver disorders [34]. Liver cirrhosis is defined histologically by the formation of regenerating nodules surrounded by fibrous bands as a result of chronic liver injury that eventually leads to portal hypertension and end-stage liver disease [54]. Cirrhosis prevalence was estimated at 0.15% or 400,000 in the USA, where it accounted for more than 25,000 deaths and 373,000 hospital discharges in 1998 [24, 55]. Hepatocellular carcinoma is the fifth most common cancer in men and the seventh among women cancer in terms of incidence with more than 700,000 new cases being diagnosed each year, and the third leading cause of cancer death with chronic development and progression and over 600,000 deaths globally *per* year, accounting for 9.2% of all new global cancer cases (7.9% in men *vs.* 3.7% in women) [51].

Traditionally, the camel has played key role in the lives of desert dwellers. Camel's products are regarded historically as having a high-quality source of nutrients and used to treat various ailments [33]. In fact, camel's milk exhibits unique abilities in terms of availability of nutrients not seen in the milk of other mammals [58, 63]. In addition, compared to whey protein isolated from cow's milk, whey protein isolated from camel's milk contains significantly more antimicrobial agents such as lactoferrin, lysozyme, immunoglobulin and lactoperoxidase [2]. The high concentrations of insulin/insulin-like protein and peptidoglycan recognition protein in the camel's milk also give it a number of special health benefits [61]. Furthermore, camel's milk is easily absorbed by the body, because it lacks allergenic component β-lactoglobulin and can be safely taken by lactose-intolerant people and those with compromised immune systems [5]. Numerous chemical components in camel's urine have been found to exhibit antimicrobial, anti-fungal, antiviral and anticarcinogenic effects [8]. Some studies have reported the therapeutic benefits of camel urine based on clinical data [48, 49]. Contrary to human and bovine urine, camel urine has been shown to exhibit strong platelet inhibition activity and anti-cancer activity [10, 11]. Interestingly, camel's milk and urine have been reported to have some hepato-protective effects against hepatitis C virus [53]. Nevertheless, mechanisms underlying such effects are still unillustrated. Therefore, the objective of this study is to evaluate the potential effect of biological fluids of camel (*i.e.*, milk and urine) on oxidative stress, inflammatory mediators and apoptosis in a D-Gal hepatic injury model and explore the possible modulations of NF-κB and Nrf2/HO-1 in this process.

Materials and Methods

Chemicals and supplies

D-galactosamine (D-Gal) was purchased from Sigma, Aldrich (St. Louis, MO, USA). All other chemicals used were of at least analytical grade. Primers (Bax, caspase-3, Bcl-2, NF-κB (p65), IκBα, HO-1, Nrf2, TGFβ and β-actin were obtained from Macrogen, Inc. (Seoul, Korea). Trans AM Nrf2, NF-κB (p65) transcription factor DNA binding assay kits were procured from Active Motif (Carlsbad, CA USA). Tumour necrosis factor alpha (TNF-α) and interleukins (IL-1 α, IL-2, IL-6 and IL-10) ELISA kits were purchased from R&D Systems (Minneapolis, MN, USA).

Collection of camel's biological fluids (milk and urine)

Fresh milk samples from female lactating camels were collected at early morning in Riyadh, Saudi Arabia, in 2021. Approximately 150 - 200 mL of milk samples were collected into sterile containers and stored at -80°C. The camel urine samples were collected during normal urination or by Tashweel technique, which was performed by touching the abdominal side of the camel near the hide of the back leg. The milk and urine samples were freeze dried to obtain the moisture free powder. Briefly, the milk and urine samples (20 mL each) were frozen at -80°C for 3 - 4 h, and freeze-dried using a FreeZone-4.5 Freeze Dry System (Labconco Corporation, Kansas, MO, USA). The process was performed at -50°C for 24 h and at 0.12 mmHg of pressure to obtain a moisture free powder.

GC-MS analysis of camel's biological fluid samples

The analysis was conducted using a Perkin Elmer Clarus 600 gas chromatography and Clarus 600 T single quadrupole mass spectroscopy and Elite 5MS (30 m x 0.25 mm film thickness) column pumped with high quality helium at a flow rate of 1 mL/min. The injector temperature was set at 280°C with a splitless injector at 20:1. The temperature was raised from 40°C to 150°C in 10°C increments for 1 min, then to 300°C in 10°C increments for 1 min. The ion source was set to 200°C and the inlet line to 220°C. Camel milk and urine derivatized samples (1 µL each) were injected into the system with the split mode (split ratio 1:20). The mass scanning range was from 40 - 600 Dalton at 70 eV electron energy and a 7 min solvent delay (*i.e.*, the mass spectrometer started scanning from min = 7 onwards). The total run time of a single sample was 29 min. The sample peaks were identified by matching their spectra to the NIST 2008 (National Institute of Standard and Technology library 2008).

Animals and study approval

Twenty-four mice (Albino (BALB/c) of either sex weighing (25 - 30) gm were obtained from the Animal Care facility, College of Pharmacy, King Saud

University. The mice were kept for a week before the experiment under controlled temperature (23 ± 1°C) and relative humidity (60%) for acclimatization and given free access to food and water. The study was approved by College of Pharmacy Ethics Committee (Ethical Reference No: KSU-SE-21-16)

Hepatic injury model and study groups

At the end of the adaptation period, mice were divided randomly into 4 groups of 6 mice each. Group 1 served as a normal control and were administered orally normal saline (p.o.) for 14 days. Group 2 was given normal saline for 14 days and D-GalN (800 mg/kg i.p.) 24 hr before the last normal saline administration and served as disease control. Group 3 was administered camel urine (10 mL/kg p.o.) for 14 days, and 24 hr before the last urine dose animals were given D-GalN (800 mg/kg i.p). Animals in Group 4 received milk (10 mL/kg b.w) for 14 days orally, and 24 hr before the last dose animals were given D-GalN (800 mg/kg i.p). After the completion of the treatment protocol, the animals were anaesthetized with ketamine and xylazine (ketamine 100 mg/kg and xylazine 10 mg/kg, ip). The blood samples were collected in disodium EDTA tubes for the estimation various liver function and inflammatory markers.

Inflammation markers and histopathology

The resultant plasma samples were stored at -80°C for the testing. In addition, liver tissue samples were harvested for microscopic analysis for histopathological changes. Colorimetric techniques were used for the assessment of the liver function tests including AST, ALT, GGT, ALP, bilirubin, LDH and albumin (Human Diagnostic Worldwide, Wiesbaden, Germany). As an index of antioxidant enzyme activity, levels of various antioxidants (*i.e.*, MDA, SOD, CAT, GSH and MPO) were measured in plasma (Sigma Aldrich, St. Louis, MO, USA; Abcam, Cambridge, MA, USA). Furthermore, the levels of inflammatory cytokines, namely TNF-α, IL-1 α, IL-2, IL-6 and IL-10 were determined. For the histopathological studies, the liver tissues were fixed for 48 hours in 4% paraformaldehyde in 10 mM phosphate buffer (pH 7.4). Sections of 5 µM thickness were mounted on glass slides and stained with haematoxylin and eosin (H & E) for microscopical evaluation. The sections were inspected under the microscope for any abnormalities.

RNA isolation and quantification

Total RNA was isolated from liver tissue using Invitrogen™ TRIzol™ Plus RNA Purification Kit (Life Technologies, Carlsbad, CA, USA). A high-capacity cDNA reverse transcription kit was used to convert total RNA to cDNA (Applied Biosystems, Bedford, MA, USA). The ABI Prism 7500 instrument was used to conduct quantitative analysis of apoptotic, anti-apoptotic and fibrotic genes in 96-well optical reaction plates (Applied Biosystems, Bedford, MA, USA). Rat primers for Nrf2, HO-1, Caspase-3, Bax, Bcl2, iNOS, NF-κB (p65), IκBα and β-ACTIN genes were used according to the manufacturer's guide-lines.

Nrf2 and NF-κB (p65) activation assay

Nrf2 and NF-κB DNA-binding activity were evaluated with the Trans AM Nrf2 and NF-κB (p65) transcription factor ELISA assay kits. In brief, nuclear extracts were incubated in oligonucleotide-coated wells containing the consensus-binding sites for Nrf2 and NF- κB response elements. After washing, specific antibodies against Nrf2 and NF- κB (p65) were added to the samples, which were then incubated with HRP-conjugated secondary antibodies and measured with a spectrometer at 450 nm.

Statistical analysis

All data are presented as mean and standard error of the mean (±SEM). ANOVA and Dennett's post hoc test was applied to assess for significant variation among the groups. A p-value of ≤ 0.05 was considered significant. GraphPad Prism V8 was used to test all experiments (San Diego, CA, USA).

Results and Discussion

The GC-MS analysis revealed more several compounds in camel's milk and urine. The most prominent components found in the urine were hippuric acid (18.98%), adonitol (7.85), D-fructose (7.63%), benzene acetic acid (6.82%), D-glycero-D-gulo-heptonic acid (5.01%) followed by adonitol, hexa and hepta decanoic acid (Table I). The compounds that were present in higher concentrations in milk were D-fructose (36.80%), aminobutyrate (8.33%), glucose (7.13%), hexadecanoic acid (5.08%), 1H-indole carboxaldehyde (3.90%), L-proline (2.94%), phosphoric acid (3.66%), inositol (3.91%), hippuric acid (2.88%), citric acid (2.34%) and D-turanose (2.15%). Other components were also existing in the milk, but at the lower concentrations (Table II).

D-Gal administration caused a substantial increase in liver function tests (*i.e.*, AST, ALT, GGT, ALP, bilirubin, LDH) and albumin plasma concentrations in mice as compared with controls ($p < 0.05$), signifying a liver damage. Treatments with camel's milk and urine lead to a significant improvement in the liver functions and thus amelioration of hepatic injury (Table III). Similarly, a significant increase in oxidative stress markers including MDA and MPO levels and a reduction in anti-oxidant systems (*i.e.*, SOD, CAT and GSH levels) in the liver injury-induced group. Camel's biological fluids (*i.e.*, milk and urine) significantly decreased MDA and MPO concentrations and improved SOD, CAT and GSH activities (Table IV).

Table I

Composition and codification of metronidazole gel formulations

S. No.	Name	RT	Concentration
1	Urea	11.2	0.97
2	Gluconic acid	11.56	4.40
3	2-Amino benzoate	14.82	0.53
4	o-hydroxy benzyl alcohol	14.98	4.16
5	3-methyl adipic acid	15.43	2.02
6	3-hydroxy benzoic acid	15.87	3.38
7	b-phenyl hydracrylic acid	16.19	3.40
8	heptanedioic acid	16.37	3.67
9	benzeneacetic acid	16.83	6.82
10	octanedioic acid	17.55	2.21
11	adonitol	17.74	7.85
12	azelaic acid	17.93	3.25
13	D-glycero-D-gulo-heptonic acid	18.07	5.01
14	hippuric acid	19.75	18.98
15	N-phenyl acetyl glycine	20.13	0.53
16	benzene propanoic acid	20.33	1.87
17	2-isopropyl-3-ketobutyrate	20.61	2.44
18	hexanedecanoic acid	21.31	3.54
20	D-turanose	26.36	0.68
21	D-fructose	26.54	7.63
22	Glucopyranose	26.65	1.87
23	Allonic acid	27.18	1.72

Table II

Constituents present in camel milk identified by GC-MS analysis

Milk	Name	RT	Concentration
1	2-pyridine carboxylic acid	7.78	0.79
2	Benzyloxyamine	8.89	0.66
3	2,3-dihydro Benzofuran-2-one	9.92	0.20
4	aminobutyrate	11.39	8.33
5	glycine	11.93	1.20
6	methyl malonic acid	12.05	1.01
7	L-proline	15.25	2.94
8	2-butanedioic acid	15.76	0.33
10	benzene acetic acid	16.81	0.42
11	xylitol	17.74	0.41
12	phosphoric acid	18.27	3.66
13	citric acid	18.91	2.34
14	hippuric acid	19.31	2.88
15	D-galactose	19.65	1.17
16	D-glucitol	20.12	1.34
17	1H-indole carboxaldehyde	20.42	5.20
18	Prostaglandin 1A	21.09	0.20
19	Hexadecanoic acid	21.3	5.08
20	Inositol	21.66	3.91
21	Oleic acid	22.8	0.63
22	Octadecanoic acid	23.1	2.50
23	L-myoinositol phosphate	24.44	0.85
24	Prostaglandin F2 beta	24.76	0.52
25	D-turanose	24.94	2.15
26	Glucopyranose	25.11	1.84
27	Alpha D-glucopyranoside	25.51	0.74
28	Erytheropentonic acid	25.82	0.37
29	methyl alpha d-galactoside	26	0.36
30	D-fructose	26.6	36.80
31	Glucose	26.96	7.13
32	Allonic acid	28.7	1.35

Table III

Effect of camel's milk and urine treatment on liver function tests in study groups

Parameter	Control	D-GAL Induction	Milk Treatment	Urine Treatment
AST (U/L)	74.19 ± 1.99	322.84 ± 7.89*	151.54 ± 9.31*#	104.71 ± 7.00*#
GGT (U/L)	5.25 ± 0.20	13.22 ± 0.80*	9.92 ± 0.32*#	6.69 ± 0.34*#
ALT (U/L)	27.43 ± 1.29	229.82 ± 9.84*	186.36 ± 3.67*#	57.15 ± 8.80*#
ALP (U/L)	220.13 ± 7.41	428.59 ± 9.29*	315.00 ± 9.76*#	277.36 ± 12.62*#
Bilirubin (mg/dL)	0.55 ± 0.03	3.07 ± 0.15*	2.28 ± 0.10*#	1.33 ± 0.10*#
LDH (U/L)	74.28 ± 3.62	213.31 ± 7.53*	155.30 ± 3.80*#	128.66 ± 4.43*#
Albumin (g/dL)	3.32 ± 0.11	2.34 ± 0.10*	2.76 ± 0.06*	3.14 ± 0.04#

*denotes significant differences compared with the normal control group ($p < 0.05$); #denotes significant differences compared with the D-Gal group ($p < 0.05$)

Table IV

Effect of camel's milk and urine treatment on oxidative parameters in study groups

Parameter	Control	D-GAL Induction	Milk Treatment	Urine Treatment
MDA (nmol/g)	53.05 ± 3.07	127.93 ± 7.12*	92.93 ± 2.84*#	70.07 ± 3.12*#
SOD (U/mL)	96.49 ± 3.74	56.04 ± 3.36*	68.20 ± 4.25*#	75.62 ± 3.99*#
CAT (U/g)	293.58 ± 7.61	186.82 ± 7.08*	222.71 ± 4.03*#	247.38 ± 5.09*#
GSH (nmol/g)	2.16 ± 0.11	1.10 ± 0.08*	1.31 ± 0.07*	1.88 ± 0.06*#
MPO (nmol/g)	16.87 ± 1.76	45.19 ± 1.87*	33.71 ± 2.08*#	21.85 ± 2.13*#

*denotes significant differences compared with the normal control group ($p < 0.05$); #denotes significant differences compared with the D-Gal group ($p < 0.05$)

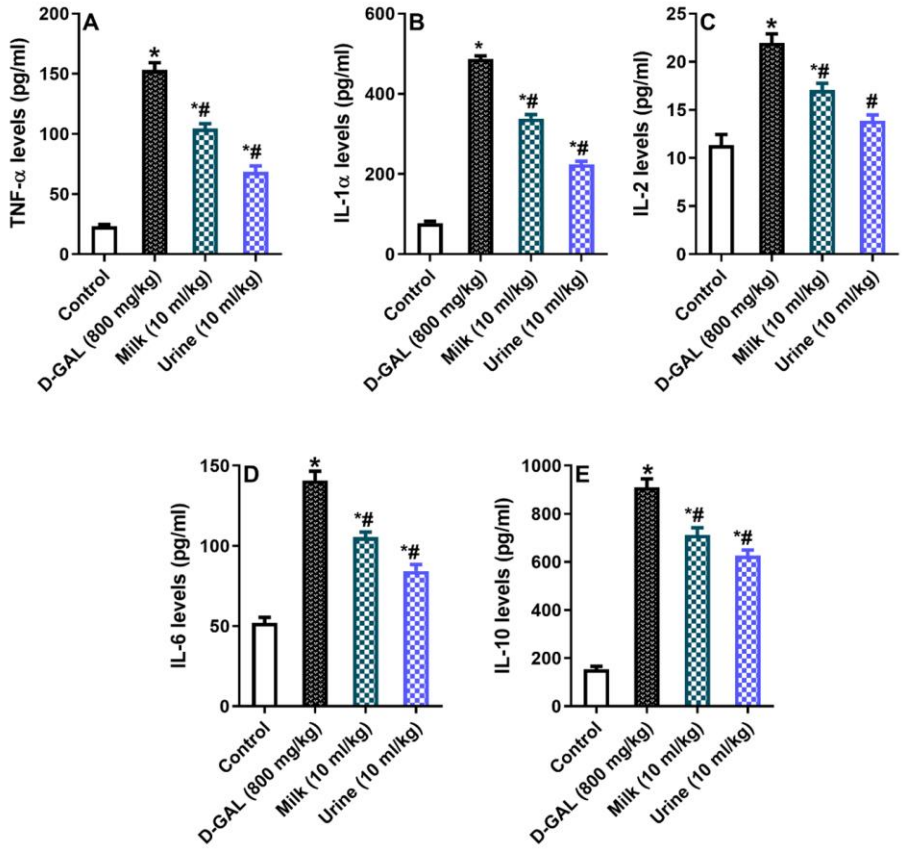

Figure 1.

Levels of inflammatory cytokines in treatment groups. (A) TNF-α, (B) IL-1α, (C) IL-2, (D) IL-6 and (E) IL-10.
*denotes significant differences compared with the normal control group ($p < 0.05$);
#denotes significant differences compared with the D-Gal group ($p < 0.05$)

To examine the modulatory influence of treatment with camel's milk and urine on the inflammatory cytokines (TNF-α, IL-1α, IL-2, IL-6 and IL-10) levels was determined. D-Gal administration increases the influx of pro-inflammatory cytokines. As shown in Figure 1, significant increases were observed in TNF-α level (153.16 ± 6.05 pg/mL from 23.30 ± 1.37 pg/ml, $p < 0.05$), IL-1α level (487.21 ± 18.87 pg/mL

from 77.88 ± 4.30 pg/mL, $p < 0.05$), IL-2 level (21.97 ± 0.94 pg/mL from 11.35 ± 1.11 pg/mL, $p < 0.05$), IL-6 level (140.63 ± 5.96 pg/mL from 52.11 ± 3.37 pg/mL, $p < 0.05$) and IL-10 level (910.77 pg/mL ± 34.83 from 153.42 ± 12.99 pg/mL, $p < 0.05$), when compared with normal controls. Conversely, treatment with camel's milk and urine at substantially restored the concentrations of TNF-α by 31.83% and 55.27%, respectively ($p < 0.05$). Similarly, IL-1α levels were reduced by 30.58% and 54.00%; IL-2 levels by 22.29% and 36.95%; IL-6 levels by 25.00% and 40.09%; and IL-10 levels by 21.82% and 31.21%, respectively ($p < 0.05$).

For mice with hepatic damage, liver iNOS mRNA expression was considerably elevated. This was reduced by camel's milk and urine treatment ($p < 0.05$). Likewise, caspase-3 and Bax apoptotic mRNA expression was dramatically enhanced whereas Bcl2 anti-apoptotic mRNA expression was significantly decreased ($p < 0.05$). The administration of camel's milk and urine lowered both caspase-3 and Bax while elevated Bcl2 mRNA expression as compared with untreated group ($p < 0.05$). Moreover, an upregulation of mRNA encoding TGF-β was observed in the liver tissues of mice challenged with D-Gal. Similarly, camel's milk and urine treatment had the opposite effect by downregulating TGF-β mRNA levels.

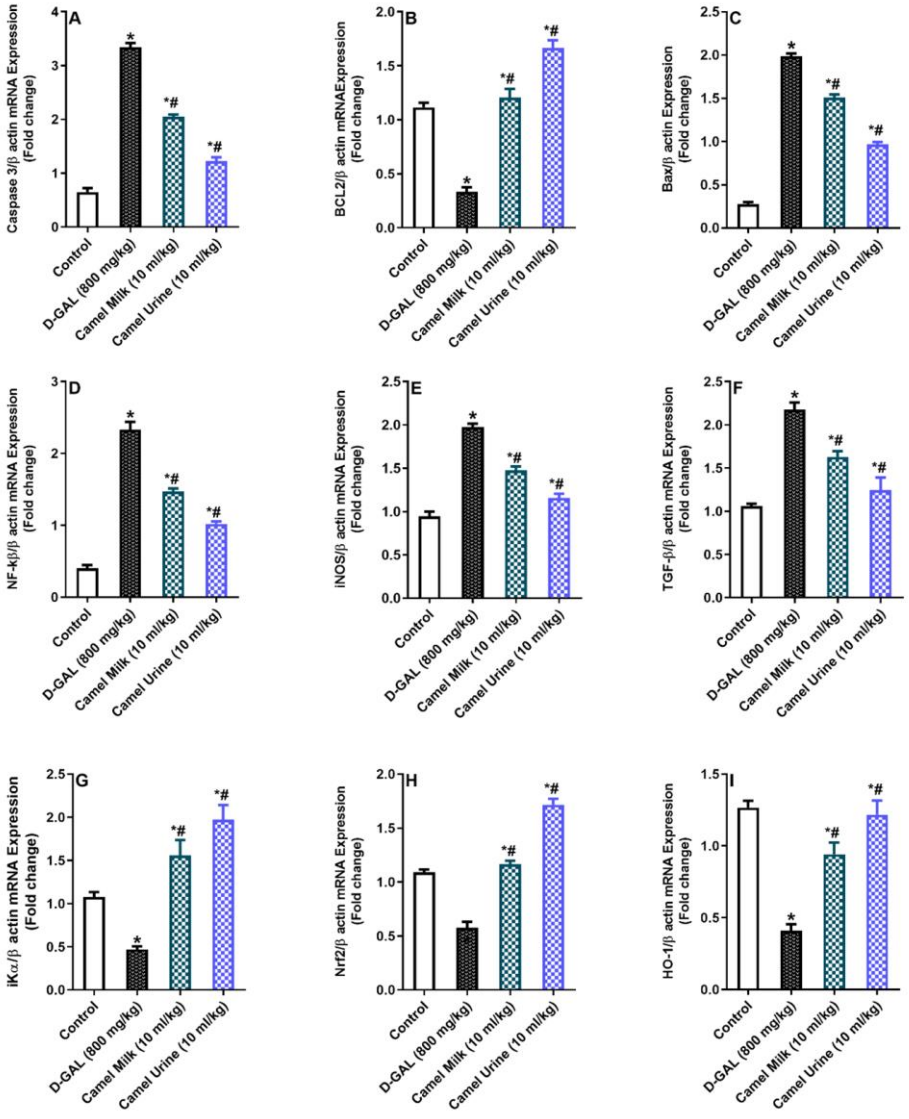

Figure 2.

The mRNA expression levels in treatment groups. (A) Caspase 3, (B) BCL2, (C) Bax, (D) NF-κB, I iNOS, I TGF-β, (G) IκBα, (H) Nrf2 and (I) HO-1 mRNA

*denotes significant differences compared with the normal control group ($p < 0.05$);

#, denotes significant differences compared with the D-Gal group ($p < 0.05$)

To further explore the molecular mechanisms of such effects, nuclear factor-kappa B (NF-κB) activation in the experimental groups was evaluated. The increase in nuclear translocation of the p65 subunit

of NF-κB and reduction in IκBα mRNA expression in mice received D-Gal is shown in Figure 2. Treatments with camel's biological fluids (*i.e.*, milk and urine) were effective in reducing p65 and increasing IκBα mRNA expression when administered orally ($p < 0.05$). Conceivably, inhibiting NF-κB translocation and therefore reducing apoptosis appears to be the mechanism of liver injury amelioration. As also illustrated in Figure 2, mRNA expressions of Nrf2 and HO-1 in liver tissues was considerably decreased. In accordance with other findings, camel's milk and urine administration significantly increased Nrf2 and HO-1 mRNA expressions ($p < 0.05$).

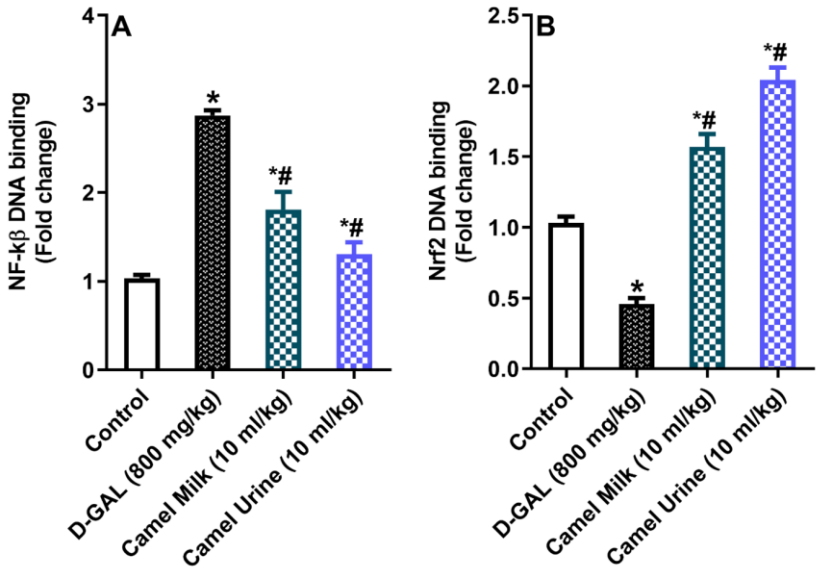

Figure 3.
Effects of camel's milk and urine on NF-κB and Nrf2 DNA-binding activity in treatment groups
*denotes significant differences compared with the normal control group ($p < 0.05$);
#denotes significant differences compared with the D-Gal group ($p < 0.05$)

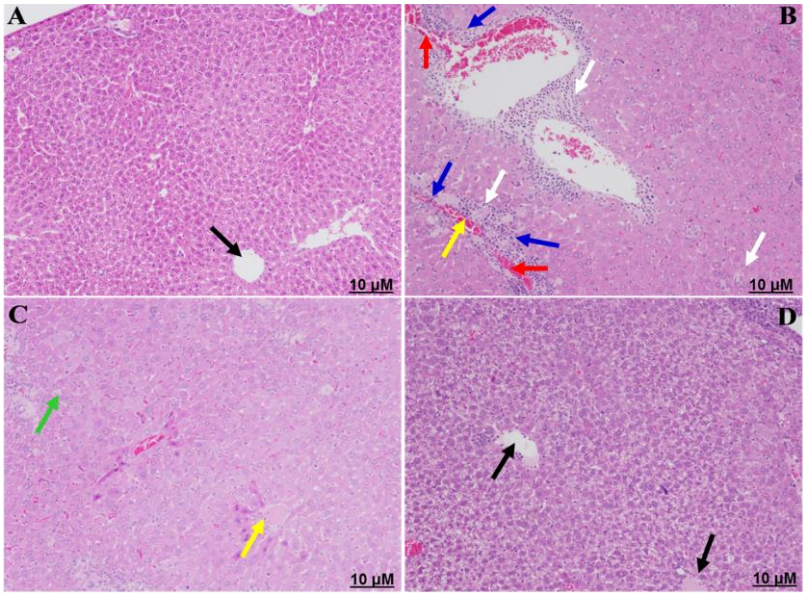

Figure 4.
Light photomicrographs of the liver tissues at (10X): (A) Hepatic tissue of normal control rat showing normal architecture of hepatic cord (black arrow); (B) Hepatic section of D-Gal treated mice exhibiting massive fatty changes, focal central vein congestion (yellow arrow), ballooning formation (white arrow), necrosis with inflammation (blue arrow) and loss of cellular boundaries, massive cellular infiltration (red arrow); (C) Liver tissue of mice treated with camel milk showing mild central vein congestion (yellow arrow), mild fatty changes (green arrow), mild ballooning, necrosis with sinusoidal dilatation and cellular infiltration; (D) Liver tissue of mice treated with camel urine exhibiting the absence of ballooning, inflammatory cells and regeneration of hepatocytes around central vein toward near-normal liver architecture

We also evaluated NF-*κ*B (p65) and Nrf2 DNA-binding activity in liver tissues in experimental groups. As compared to control animals, treatments with camel's milk and urine reduced NF-*κ*B (p65) DNA-binding in the nuclear fractions. Interestingly, hepatic injury induced by D-Gal reduced Nrf2 DNA-binding affinity as compared to untreated control mice, which was considerable restored following camel's milk and urine administration (Figure 3). Therefore, it can be assumed that these camel's products contain some antioxidant constituents as a defence barrier.

As for histopathological changes, liver tissues from the healthy controls demonstrated no alterations in liver histological architecture. However, in the D-Gal group, mice exhibited features of cellular infiltration, massive changes in lipid accumulation, sinusoidal dilatation, necrosis and acute vascular cramping. In contrast, camel's milk and urine greatly inhibited abnormal histological and morphological changes related to D-Gal administration (Figure 4).

Liver is a metabolically active organ which, in addition to biotransformation and clearance of endogenous compounds, is responsible for metabolizing xenobiotics [31]. Hepatitis is an inflammatory condition of the liver that can be caused by drugs, toxins, alcohol consumption, bacterial, viral infection and metabolic disorders, which can lead to liver damage that is difficult to treat [15]. Therefore, there is an urgent need for the discovery of both effective and nontoxic modalities to prevent and/or treat hepatitis because of the dearth of effective hepatoprotective therapies. The D-Gal model of hepatotoxicity, which is noted for its accuracy in simulating acute liver failure, is an excellent model for toxicity found in clinical settings [36, 39]. D-Gal specifically depletes uridine nucleotides in the liver, and this action is thought to reduce RNA synthesis in hepatocytes and enhance the acute toxicity [27, 41]. This study was conducted to examine the effect of camel's milk and urine in protecting against liver damage caused by from D-Gal and attempting to elucidate the role of the anti-oxidant, anti-inflammatory and apoptosis in the overall mechanism of action. The presented findings demonstrated that oral administration of camel's biological fluids (*i.e.*, milk and urine) at a dose of 10 mL/kg each can effectively provide a hepatoprotective effect and significantly reduces the levels of total inflammatory cytokines and biomarkers. It is also provided an important illustration of the antioxidant property of these biological fluids. These effects are potentially *via* the antioxidant property of different constituents present in camel's milk and urine and were identified by the GC-MS analysis, including gluconic acid, adonitol, azelaic acid, glucopyranose, allonic acid, methyl malonic acid, L-proline, xylitol, citric acid, hexadecanoic acid, hippuric acid, inositol, oleic acid, octadecanoic acid, L-myoinositol phosphate and α-D-glucopyranoside. In particular, hexadecanoic acid and hippuric acid, which are abundant in the camel's excreta are known to have anti-inflammatory effects. These compounds are chemically related to glycolysis, nucleosides and synthesis of fatty acids, mineralocorticoids, arachidonic acid, androgens and tyrosine inhibitors. Our data is in accordance with the other studies, which showed that camel's milk and urine components represent significant biological activities such as hepatoprotective, anti-inflammatory and anticancer properties [1-3, 14, 16, 19, 20, 30, 40, 44-46, 57, 64, 67].

From a biochemical perspective, camel's milk and urine help reduce the plasma concentration of liver enzymes, indicating that hepatic cell structure and function are maintained. The present results are corroborated with previous findings [2, 3, 6, 7, 45, 59]. D-Gal administration causes the release of reactive oxygen species (ROS), which damage liver tissues [36, 59, 69]. It is clearly demonstrated here the potential of effect of D-Gal to induce oxidative stress in the liver as evidenced by elevated levels of oxidative stress by MDA and MPO and a significant decline in the antioxidant defences by SOD, CAT and GSH [4, 12, 21] These results are concurrent with the results of Zeweil *et al*., [67] and Balkan *et al*., [13]. Conversely, the pre-treatment with camel's milk and urine maintained the plasma levels of MDA, SOD, CAT, MPO and GSH within normal ranges, which could be attributed to the presence of bioactive constituents in camel's milk and urine with powerful radical scavenging activity. D-Gal causes oxidative stress by generating free radicals, creating damage and inhibiting macromolecule synthesis. MPO activity provides a method for assessing the quantity and contribution of neutrophils [17]**.** Neutrophils are found to be a substantial contributor to the response to acute and chronic inflammation [52]**.** Oxidative stress can also be diminished with the help of a large amount of antioxidants, namely vitamins C, A and E, and other elements (*e.g.*, zinc, copper and magnesium) present in camel's milk and urine [2, 10, 22, 56].

Evolving proofs potentially propose that the substantial apoptosis of hepatocytes is a common symptom of acute liver damage induced by D-Gal [32, 43]. The *in vitro* and *in vivo* antiapoptotic effect of camel's milk and urine have been previously reported by [8, 54]. D-Gal has a potential to instigate inflammatory cytokines including TNF-α, IL-1α, IL-2, IL-6 and IL-10. Post-inflammatory cytokines stimulate the release of inflammatory molecules, which causes liver damage due to apoptotic injuries and inflammation [35]. Previous findings have also proposed that the stimulation of innate immunity by several factors (*e.g.*, D-Gal, LPS, complement and TNF-α) plays

key role in instigating and encouraging acute liver injury [28, 47, 65]. NF-κB is a transcription factor that controls proinflammatory cytokines mediated inflammatory response. Excessive activation of NF-κB signalling pathway plays an important role in the occurrence of acute liver injury [29]. In this study, both camel's milk and urine treatment attenuated the increases of TNF-α, IL-1α, IL-2, IL-6 and IL-10. In addition, a reduction in NF-κB expression was evident in the treatment groups. Therefore, camel's biological fluids (*i.e.*, milk and urine) can ameliorate the upregulation of cytokines in the D-Gal model; although, camel urine appears to exhibit more potent anti-inflammatory properties as compared to that of milk. TGF-β, proinflammatory cytokines and iNOS are known to contribute to liver regeneration [25, 26]. D-Gal induced mice displayed upregulation of TGF-β and iNOS mRNA compared with normal control mice. Treatment with camel's milk and urine downregulated TGF-β and iNOS mRNA expression in the liver injured mice.

The antioxidant response element (ARE)-dependent phase II enzyme expression, including that of HO-1, is mediated by Nrf2 [9, 18]. To maintain its inactive state, Nrf2 binds Keap1 and is ubiquitinated and subsequently proteolyzed. This process occurs while the cell is dormant. Keap1 and Nrf2 exert their repressive impact by sequestering and degrading Nrf2. The current results showed that HO-1 and Nrf2 were downregulated in liver tissues after D-Gal administration, but pre-treatment with camel's biological fluid (*i.e.*, milk and urine) enhanced their mRNA expressions, which support the notion that Nrf2/HO-1 signalling pathways are involved in the protective mechanism of camel's milk and urine. The data of NF-*κ*B (p65) and Nrf2 DNA-binding activity a showed an increase in binding activity in the nuclear fraction of hepatic tissue of the D-Gal group relative to the normal controls. Furthermore, camel's milk and urine significantly decreased NF-*κ*B (p65) and Nrf2 DNA binding and restored Nrf2 binding activity that was impaired by D-Gal treatment. In D-Gal-induced liver injury, apoptosis plays a significant role in pathogenesis [28, 29]. D-Gal therapy increased apoptotic and necrotic hepatocellular death by upregulating Bax and caspase-3 and decreasing Bcl-2, according to our findings. To reduce apoptotic cell damage in hepatocytes, pre-treatment with camel's milk and urine diminished Bax and caspase-3 expression while elevating Bcl2. Furthermore, the morphological and histopathological findings established that camel's milk and urine significantly ameliorated D-Gal induced acute liver damage.

Conclusions

Overall, our data demonstrate that treatments with the biological fluids of camel (*i.e.*, milk and urine) reduce hepatic dysfunction *via* amelioration of inflammation, oxidative stress and apoptosis. This protective effect depends on the downregulation of NF-κB and the reinstatement of antioxidant enzyme levels *via* activation of the Nrf2/HO-1 pathway. Thus, such camel's biological fluids (*i.e.*, milk and urine) could represent a potential therapeutic strategy for acute liver injuries.

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Conflict of interest

The authors declare no conflict of interest.

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