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PROTECTIVE EFFECTS OF URINE AND MILK OF CAMEL ON ISONIAZID AND RIFAMPICIN INDUCED HEPATOTOXICITY

S. Benhizia[1], N. Arzour[1], F. Bouldjenib[2] and A.B. Lakehal[3]

[1]Animal Pathology Research Laboratory PADESCA. Institute of Veterinary Sciences, El Khroub, Constantine, Algeria
[2]Department of Pathological Anatomy, [3]Department of Epidemiology,
University Hospital Centre IBN BADIS, Constantine, Algeria

ABSTRACT

The objective of the present study was to study the hepatoprotective effects of camel milk and urine in 5 groups of 8 rabbits. The first group I received saline solution (control), animals in group 2 received daily isoniazid (50 mg/Kg/d) with rifampicin (100 mg/Kg/d) orally during 10 days. Rabbits in groups 3, 4, 5 received isoniazid (50 mg/kg/day) and rifampicin (100 mg/kg/d) with added milk, urine, and a mixture of camel milk and urine, respectively. Camel milk was administered @ 33 ml, kg bodyweight, day with oral gavage using a nasopharyngeal catheter. Urine was administered @ 20 ml, kg bodyweight, day. Plasma levels of bilirubin, and activities of ALAT, ASAT and PAL were measured. Histological variations on liver tissues were also described. Group 2 rabbits showed a non-significant increase in plasma ALAT and ASAT levels and a very significant increase in bilirubin and PAL. Histological sections of liver of rabbits in group 2 showed signs of hepatocyte suffering, these biochemical changes were reversed in groups 3, 4 and 5 animals compared to the group 2. Histological variations were also reduced in animals receiving camel milk and urine mixture. Camel milk and urine thus have protective effects on hepatotoxicity induced by isoniazid-Rifampicin combination.

Key words: Camel, hepatotoxicity, isoniazid-rifampicin, milk, urine

Camel milk and urine together is used extensively in traditional medicine in Sudan and they claimed that this combination cures a lot of diseases including liver disease and jaundice (Elhag *et al*, 2017). Researchers validated these in rats with ethanol induced hepatotoxicity and beneficial hepatoprotective effects of using camel's milk and urine mixture were evidenced which could be attributed to antioxidant activity or to its chelate effects on toxicants (Elhag *et al*, 2017). Camel urine is known to contain many active components and essential inorganic elements which play a protective role as antibacterial, antifungal, antiviral and anticancer agents. Protective role of camel urine (CU) against CCl4-induced liver damage in rats was also studied. Camel urine showed to play a promising anti-oxidative and anti-free radical scavenging mechanism against hepatic dysfunction (Hany *et al*, 2019). The possible protective role of both camel milk and urine on CCL4 induced liver damage was studied and found that camel urine has protective effect against CCL4 induced liver damage more than camel milk (Khan *et al*, 2019). The thioacetamide-induced liver cirrhosis in rats was ameliorated by administration of camel milk and urine in a ratio of 2:1 (Mohamed *et al*, 2016). Histopathological and biochemical changes were also reported by rats intoxicated by carbontetrachloride and were treated by camel milk (Althnaian *et al*, 2013).

Isoniazid (INH) and Rifampicin (RMP), first-line drugs used as anti-tuberculosis chemotherapy, are associated with hepatotoxicity (Mohamed *et al,* 2016). However, hepatotoxicity has been shown to be increased in case of association with RMP. The latter playing the role of enzyme inducer (Perriot *et al,* 2011; Sahli and Rim, 2015).

In order to protect from the toxic metabolites, the liver is equipped with an endogenous protection system; antioxidant enzymes and reduced glutathione. Strengthening this system requires the use of antioxidant molecules of vegetal or animal origin.

Biochemical and histological variations related to the use of milk and urine of camels as a protective treatment against anti-tuberculosis chemotherapy has

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not been studied previously. Therefore, we studied the protective effects of camel milk and camel urine against hepatotoxicity induced by Isoniazid and Rifampicin in rabbits.

Materials and Methods

Camel milk and camel urine samples were taken from desert camels from Biskra in south-eastern of Algeria. Milk was collected in sterile vials and kept in coolers during transport to the laboratory. Milk and urine bottles were frozen at -20°C until use.

Animals

The experiment was carried out on 40 sexually mature female rabbits obtained from the Technical Institute of Livestock "ITELV" in the city of Constantine. Their live weight varied from 2 to 2.8 kg. They were individually kept stainless steel cages with a 12/12 hours light/darkness cycle. Food and drinking water were given *ad libitum*. Animals were brought 7 days before the starting of the experiment to stabilise their mood and behaviour.

Experimental protocol

The study was carried out in February 2019. Female rabbits were randomly divided into 5 groups of 8 animals on each:

- Group 01 animals represented negative controls; they did not receive treatment.
- Group 02 animals were treated with Isoniazid and Rifampicin in accordance with a standard protocol for the induction of hepatotoxicity (Bhupinder *et al,* 2007).
- Groups 03, 04 and 05 animals received treatment with Isoniazid-Rifampicin with added milk, urine and a milk/urine mixture, respectively.

Camel milk was administered at a rate of 33ml/kg bodyweight/ day by oral gavage using a nasoesophageal tube (Hassan and Emam, 2012).

Urine was administered at a rate of 20 ml/kg bodyweight/ day (Mohamed *et al,* 2014) (Table 1).

Three rabbits died during the experiment, i.e. a group 2 rabbit (on the 6th day of treatment), a group 04 rabbit (the first day of treatment following a confirmed enterotoxemia after autopsy) and a group 05 rabbit (following a false swallowing).

Blood sampling and tissue manipulation

After 10 days of treatment, 5 ml of blood were drawn directly at the time of sacrificing the animals by puncturing the jugular vein. Blood was collected in heparinised tubes, and it was quickly sent to the laboratory under cold conditions for analysis to carry out some hepatobiochemical parameters.

Table 1. Experimental protocol.

Groups	Treatment	Dose
Control 01	• No treatment (distilled water)	/
02	• Isoniazid • Rifampicin	• Isoniazid (50 mg/kg/d) • Rifampicin (100 mg/kg/d)
03	• Isoniazid • Rifampicin • Camel milk	• Isoniazid (50 mg/kg/d) • Rifampicin (100 mg/kg/d) • 33 ml milk/kg/d for 10 days
04	• Isoniazid • Rifampicin • Camel milk	• Isoniazid (50 mg/Kg/d) • Rifampicin (100 mg/kg/d) • 20 ml milk/kg/d for 10 days
05	• Isoniazid • Rifampicin • Camel milk/ urine mixture	• Isoniazid (50 mg/Kg/d) • Rifampicin (100 mg/kg/d) • 16,5 ml milk and 10 ml urine /kg/d for 10 days

Histopathological examination

Liver tissue was cut into small pieces and immersed in 10% neutral buffered formalin for 24h. The fixed tissues were treated, incorporated into paraffin, sectioned, dewaxed and rehydrated using standard techniques (Sahli and Rim, 2015). The histopathological examination was carried out at the department of pathological anatomy- University hospital centre IBN BADIS, Constantine –Algeria.

Biochemical analyses

Separated plasmas were subjected to the determination of concentrations of bilirubin, and the measurements of the activities of ALAT, ASAT and PAL.

The assay of bilirubin is carried out by a colorimetric technique with dimethylsulfoxide (DMSO) (Bergmeyer *et al,* 1978).

The evaluation of alkaline phosphatase activity is a kinetic p-Nitrophenylphosphate photometric test in accordance with the recommendations of the International Federation of Clinical Chemistry and Laboratory Medicines (IFCC) (Rosalki *et al,* 1993).

ALAT and ASAT activities are measured by enzymatic and kinetic tests with lactate dehydrogenase and malate dehydrogenase, respectively (Maloy and Evelyn, 1937).

Statistical analyses

The 2019 version of the XL-STAT software was used for statistical analysis. Data were analysed by a one-way analysis ANOVA test. The Tukey HSD

(Honestly Significant Difference) test was used to compare the means. The differences were considered significant when P-value <0,05. The results were expressed as mean ± standard deviation (mean ±SD).

Results

Biochemical analysis results

The combination of anti-tuberculosis drugs (Isoniazid and Rifampicin) caused a considerable degree of hepatotoxicity and tissue damage in the liver. There has been an increase in the activity of plasma enzymes ALAT, ASAT and PAL. We found in the present study that the group that received only suspensions of anti-tuberculosis drugs, i.e. group 02 showed a non-significant increase in the activity of ALAT and ASAT compared to the control group, a significant increase in the concentration of bilirubin (p <0,05) and a very significant increase in PAL activity (p <0,001). In the groups which were treated with milk and camel urine associated with anti-tuberculosis drugs (groups 03, 04, 05), we noticed a reversal in the levels of ALAT, ASAT, PAL and bilirubine compared to group 02 animals. However, the difference between the three groups that were treated with milk, urine, and milk/urine mixture (03, 04, 05, respectively) was not statistically significant (Table 2).

Table 2. Plasma concentrations (mean ± standard deviation) of ASAT, ALAT, PAL and Bilirubin in rabbits in the control group, intoxicated rabbits, and treated rabbits.

Group	ASAT	ALAT	PAL	Bilirubin
Control 01	69.4± 25.1	77.7± 32 .0	48.25± 17.8	0.96± 0.09
02	174± 115.9	161.01± 86.6	617± 286.4***	8.57± 7.55*
03	110.1± 62.6	97.2± 45.4	389.57± 92.0$^{***.\#}$	4.57± 3.91
04	61.1± 44.1$^{\#}$	71.14± 59.1$^{\#}$	392.71± 133.7$^{***.\#}$	5.57± 5.77
05	71.85± 55.2	68.28± 12.6$^{\#}$	461.14± 160.6***	4.42± 1.99

Values were expressed as mean±SD, (* , # = p<0.05 ; ** , ## = p<0.01 ; *** , ### = p<0,001) *when compared with normal control group (NC) , #when compared with treated group (03).

Histopathological analysis

Untreated rabbits liver (control group) showed a well-preserved histological architecture. Hepatocytes were arranged in radiating spans from the central lobular vein and surrounded by vascular sinusoids (Fig 1).

Liver of rabbits treated with anti-tuberculosis drugs (INH / RMP) during 10 days, showed signs of hepatocyte suffering, namely; clarification of the cytoplasm, increase in the size of the nuclei and hepatocytes, binucleation, presence of Councilman's bodies, macrovesicular and microvesicular steatosis, predominantly microvesicular, slight cholestasis and, a significant inflammatory infiltrate of lympho-plasmocytic nature with eosinophilic polynuclear cells (Fig 2, 3, 4, 5).

Liver histopathological sections of rabbits treated with anti-tuberculosis drugs (INH/RMP) associated with milk, urine and milk/urine mixture of camels showed recovery to a normal appearance. Hepatocytes with discrete microvesicular steatosis were seen for the group treated with camel urine (Fig 6, 7, 8).

Discussion

The results of our study demonstrate that the 10-days treatment with anti-tuberculosis drugs causes an alteration in the biochemical balance of intoxicated animals. These findings are in agreement with the findings of several studies (Ravinder *et al,* 2006; Bouchentouf *et al,* 2011; Shih *et al,* 2013; Vandana *et al,* 2007).

ALAT and ASAT transaminases are hepatocyte enzymes whose function is to catalyse transfer reactions of an amino group from an alpha-amino acid to an alpha-ketonic acid. ALAT is present mainly in the liver and incidentally in muscles and kidneys. ASAT has a much wider distribution, in the liver but also in the heart, skeletal muscles, kidneys and brain. PAL is found especially in liver and bones. It is increased in case of cholestatic liver damage (Sahli and Rim, 2015).

In present study, liver damage was manifested as high liver enzymes (ASAT, ALAT, PAL) and increased concentrations of bilirubin.

In animals intoxicated with anti-tuberculosis drugs, the liver parenchyma showed a less organised appearance, presence of cholestasis and signs of hepatocyte suffering. These disturbances could be due to an oxidative stress produced following the administration of Isoniazid and Rifampicin. Similar results were reported by Khan and Al Zohairy (2011) and Khan *et al* (2019) using carbon tetrachloride (CCL4) protocol in rats, by Mahrous *et al* (2016) using acetaminophen and by Elhag *et al* (2017) using ethanol in rats. Additionally, the treatment with milk, urine, and the mixture of milk and urine produced a noticeable improvement in the values of hepatic enzymes mainly of alkaline phosphatase (PAL), and also a decrease in the concentrations of bilirubin. A return to normal liver architecture was evidenced

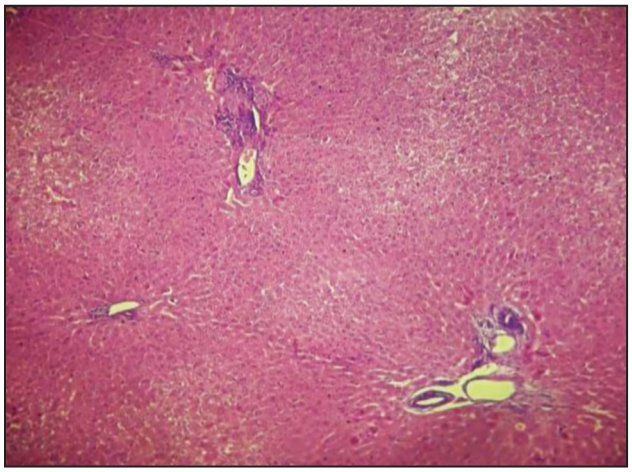

Fig 1. Normal architecture of the liver in rabbits of the control group (H&E, X40).

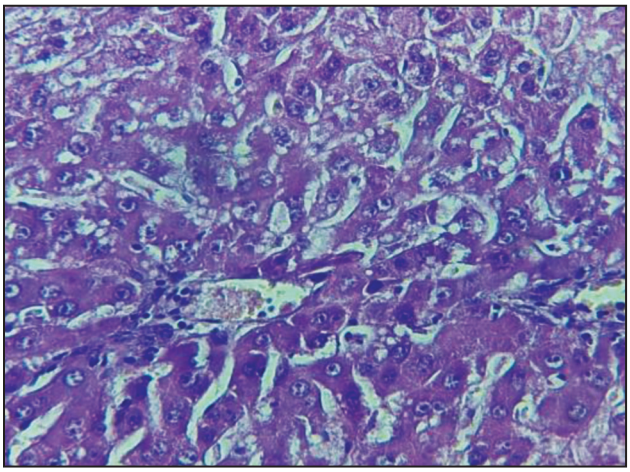

Fig 3. Clarification of the cytoplasm and microvesicular steatosis in group 01 rabbits (H&E, X400).

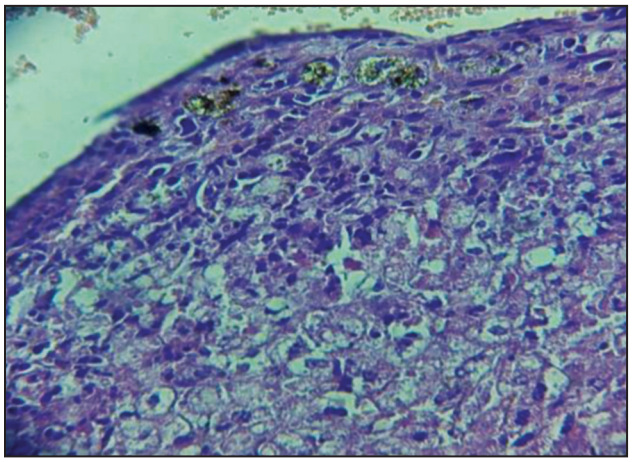

Fig 2. Cholestasis in group 01 rabbits (H&E, X100).

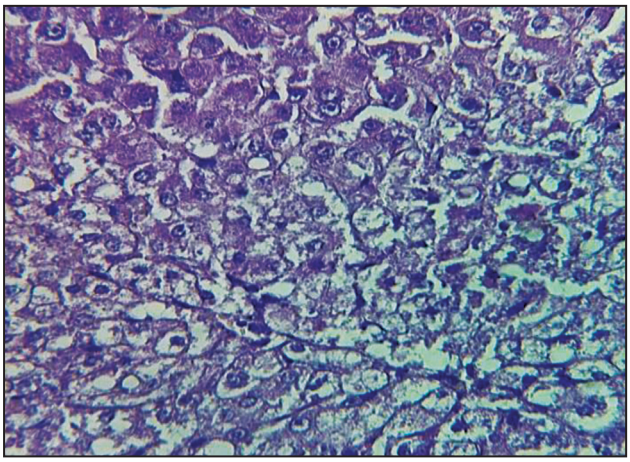

Fig 4. Presence of councilman bodies and binucleation in group 01 rabbits (H&E, X400).

that can be attributed to significant protection of the liver structure and preservation of liver function by camel milk and urine. Our results are in agreement with previous studies (Al-Hashem, 2009; Mohamed *et al,* 2016). Korish and Arafah (2013) investigated the effects of camel's milk on improving the hepatic biochemical and cellular alterations induced by a high-fat and cholesterol-rich diet. The same findings were obtained from studies with camel's urine against alcohol or CCL4 induced liver damage (Elhag *et al,* 2016; Hany *et al*, 2019).

Mahrous *et al* (2016) showed that oral administration of camel milk resulted in significant histopathological and biochemical antioxidant effect in mice, and this was indicated by a marked decrease in malondialdehyde levels. Also, ALT and AST levels were lower in the camel milk treated group.

High levels of antioxidant vitamins C, A and E and antioxidant minerals (zinc, copper and magnesium) in camel milk help reducing oxidative stress. Vitamin E and magnesium have been suggested to improve the biosynthesis of glutathione. Magnesium has been associated with the prevention of reactive oxygen species (Larrey, 2013; Wang *et al,* 2017; Mohamed, 2017; Mahrous *et al,* 2017).

Elhag *et al* (2017) showed a decrease in serum activities of liver enzymes AST, ALT and ALP especially in rats treated with camel milk and urine plus alcohol. The study suggested that camel milk intake may play an important role in ameliorating alcoholic liver injury.

Khorshid *et al* (2016) evaluated the hepato-protective effect of an extract of lyophilised camel urine in intoxicated rats.

The evaluation of the antioxidant activity of camel urine by the tests of ABTS, DPPH, FRAP and PPM showed a very important antioxidant activity (Hasni and Habita, 2015; Alebie *et al,* 2017).

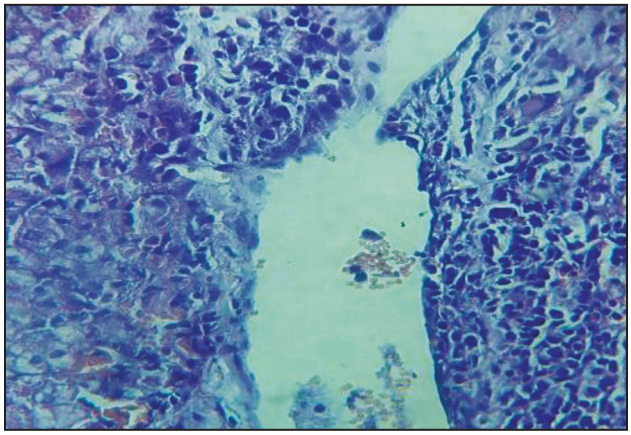

Fig 5. Portitis and lympho-plasmocyte infiltration in group 01 rabbits (H&E, X100).

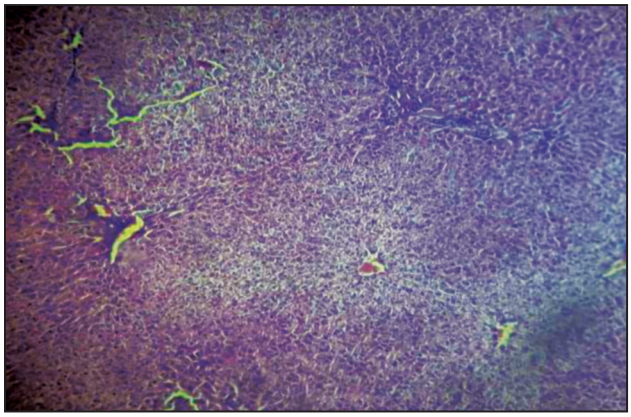

Fig 6. Portitis and lympho-plasmocyte infiltrate in group 01 rabbits (H&E, X100).

Camel urine is rich in potassium, phosphorus and magnesium, which are known for their therapeutic effects as well as by polyphenols and flavonoids. Our results were consistent with the findings of many authors (Al-Attas, 2008; Khorshid *et al*, 2016)

Conclusion

In present study, hepatotoxicity induced by the combination of anti-tuberculosis drugs was evidenced in histopathological and biochemical assessments.

Camel milk and urine administered with oral gavage were effective in reducing the severity of hepatotoxicity as reflected in histopathological lesions and biochemical changes in plasma levels of ALAT, ASAT, PAL and bilirubin.

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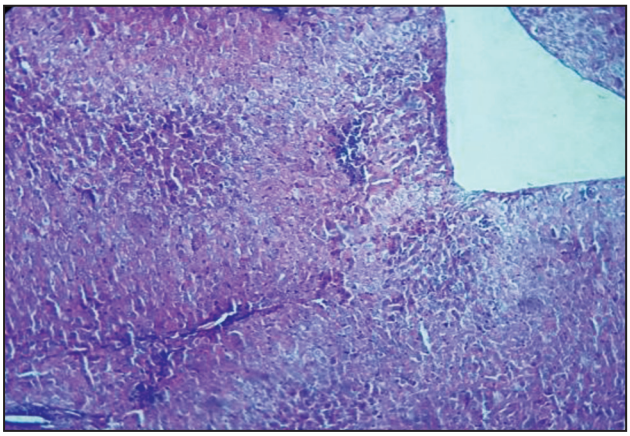

Fig 7. Discrete Microvesicular Steatosis in group 03 rabbits (H&E, X100).

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