HEPATOPROTECTIVE EFFECT OF CAMEL MILK IN ANTITUBERCULOUS DRUGS INDUCED HEPATOTOXICITY IN MALE ALBINO RATS

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ABSTRACT

Background: Camel milk has been widely used as a dietry constituent in desert areas where camels are common. It has also been in use to cure a number of commonly occurring diseases. **Objective:** To investigate the hepato protective effect of camel milk in antituberculous drugs induced hepatotoxicity in rats. **Methodology:** This randomized control study was conducted on healthy male albino rats. 24 male albino rats (200–250gms) were obtained from National Institute of Health, Islamabad and kept in animal house of Pharmacology Department, Sheikh Zayed Medical College, Rahim Yar Khan. Rats were divided into 4-groups. Each group consisted of 6 rats. Rats were given nutritionally standard diet and recommended dosage of antituberculous drugs along with recommended quantity of camel milk. Data was entered and assessed by using SPSS version 17. **Results:** Hepatoprotective effect of camel milk was analyzed by liver function parameters as serum aminotransferases, alkaline phosphatases and lactate dehydrogenases. Data showed that antituberculous drugs given for 30 days developed severe liver damage. In biochemical study, there was significant raise in serum diagnostic liver marker enzyme (ALT, AST, ALP and LDH) levels in ATT treated rats. Co-administration of camel milk and antituberculous drugs led to significantly decreased enzyme levels. (P=0.01) **Conclusion:** This study concluded that co-administration of camel milk can reduce the toxicity and damage of liver caused by antituberculous drugs.

Key Words: Liver Marker enzymes, Antituberculous drugs, Camel milk.

INTRODUCTION

Tuberculosis is one of the major threatening diseases. Each year it is killing about 2 million people. It is estimated by world health organization that 1 billion people will be newly infected in the period 2000-2020, that will result in 35 million more deaths.[1] Detoxification and metabolism of various components that enter into the body takes place in liver. Liver is exposed to toxic substances and drugs that are absorbed from the intestine. In addition to toxins and drugs, microbial infections and viruses also cause damage to the hepatocytes.[2] In developing countries percentage of hepatotoxicity by use of antituberculous drugs (25-30%) has noted to be much higher as compared to that in advanced countries (2-3%) with similar dose schedule mainly due to oxidative stress of isoniazid and rifampicin.[3] In developing countries people were living with a poor health and nutritional status. Reduced glutathione removed most of the free radicals from liver and reduction in glutathione lead to the lipid per oxidation resulting in tissue injury.[3] Camel milk is reported to have antioxidant properties because it contains high concentration of vitamins A, B, C, and E and is very rich in magnesium and other trace elements. These vitamins have antioxidant property due to which they are useful in preventing tissue injury caused by toxins and were found to be effective in various models of oxidative stress. It prevents oxidative injury and cell damage by several mechanisms including scavenging free radicals and inhibiting lipid peroxidation.[4,5] In addition to that camel milk has antibacterial and antiviral activities. The camel milk has been used as drug against tuberculosis, auto-immune diseases, asthma and antitoxic effect.[6] By use of raw camel milk in type-1 diabetic patients, daily insulin dose requirement was reduced to 30 – 35 %.[7] Camel milk is found to be heratoprotective in hepatotoxicty induced by CCL_4 and also was found to be protective in aspirin drug induced hepatotoxicity.[8] The objective of this study was to investigate the hepatoprotective effects of camel milk, in antituberculosis induced hepatotoxicity in rats.[5]

METHODOLOGY

This randomized control study involved group of animals (albino rats) treated with antituberculous drugs and a combination of antituberculous drugs and raw camel milk. The changes in liver functions were studied in these groups. **Study Setting**: Study was conducted on 24 healthy male albino rats and they were kept in animal house of Pharmacology Department of Sheikh Zayed Medical College, Rahim Yar Khan from 1st September to November 30th 2016.

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Sample Size: A total of 24 male albino rats, obtained from National Institute of Health, Islamabad, weighing 200-250gms were divided into four groups, each group consisting of 6 rats.

All animals were kept for one week under the same laboratory conditions before commencement of the experiment, at temperature of (22 ± 2 degree Celsius) relative humidity (70 ± 4%), and 12 hour light/day cycle. They were given nutritionally standard diet and tap water.

Antituberculous drugs were taken in syrup form to make it easy to administer to rats orally. The drugs were purchased from pharmacy. The syrups were of following strength. Isoniazid 50mg/5ml, Rifampicin 20mg/ml, Pyrazinamide 250mg/5ml

Camel Milk: Raw camel milk was collected daily early in the morning from a herd of camels from a nearby desert area. Milk was collected from camels by traditional hand milking method. The samples were collected in sterile bottles with air tight screws and bottles were kept in cool boxes until transported to the animal house. Camel milk was given orally at a dose of (1ml/Kg) as prescribed.[8]

Drugs And Dosages: Following antituberculous drugs dosage was used.

Isoniazide, 7.5mg/kg body weight; Rifampicin, 10mg/kg body weight; Pyrazinamide, 35mg/kg body weight.[9] All these were administered orally once a day along with distilled water.

Experimental Procedure

After habituation period the rats were divided into four groups randomly, each of 6 rats.

The details of the groups were:

Group A: Received normal diet and fresh water orally.

Group B: Received antituberculous drugs (triple drug regimen) orally in doses mentioned above, Once daily for 30 days.

Group C: Received 1ml/kg body weight of camel milk, orally, once daily for 30 days.

Group D: Received 1ml/kg body weight of camel milk + Antituberculous drugs (triple drug regimen) orally, Once daily for 30 days.

Collection of Samples: A day after the last administration, the animals were anaesthetized using chloroform vapours. Blood samples of rats were collected by performing cardiac puncture in sterile vacutainers with gel. Blood samples were centrifuged at 3000 rev/min, using bench top centrifuge and serum samples were separated from clot for liver function tests. Serum samples were separated into sterile appendorf tubes and stored at -20°C until used for estimation. Within 24hrs of sample collection all analysis were completed.[10]

Liver function tests included; Serum Alanine Aminotransferase (ALT), Serum Aspartate Aminotransferase (AST), Serum Alkaline phosphatase (ALP),and serum Lactate dehydrogenase (LDH). Serum ALT, AST, ALP and LDH was determined by using ALT and ALP kit manufactured by diagnositic system Germany wit Lot No. 505 on Metro lab 2300 chemistry Analyzer by IFCC method.[11] Group A was taken as normal control and hepatotoxicity was induced in Group B rats by antituberculosis drugs, e.g Isoniazid, Rifampicin and Pyrazinamide. Group C was given camel milk as control and for evaluation of the protective effect of camel milk, group D rats were given antituberculous drugs in recommended doses and camel milk at a dose of 1ml/kg/body weight. After treating the rats for 30 days the biochemical analysis of Liver function tests including Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) was carried out.

Statistical Analysis: The data was entered and analyzed by using SPSS 17.0 (Statistical Package for Social Sciences). Mean ± S.E.M was used for quantitative variables. Frequencies, percentages and graphs were used for qualitative variables. One way Anova was applied to observe differences among groups. A p-value of <0.05 was considered as statistically significant.

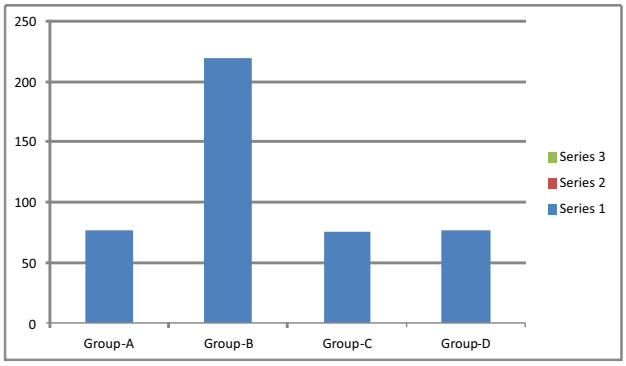
RESULTS

Table I: Biochemical Results of Hepatic Enzyme Markers in different Groups

Biochemical Parameters	**Group-A** (Normal)	**Group-B** ATT(Control)	**Group-C** Camel Milk (control)	**Group-D** ATT + Camel Milk
AST U/I	76.80 ± 7.93	219.13 ± 22.64	75.83 ± 8.22	77.13 ± 9.93
ALT U/L	53.40 ± 17.71	89.00 ± 17.40	43.00 ± 10.91	50.36 ± 13.82
ALP U/L	100.60 ± 39.76	392 ± 1058.02	98.09 ± 29.54	170.45 ± 25.69
LDH U/L	635.30 ± 80.08	1497.70 ± 314.04	649.45 ± 109.21	897.16 ± 116.33

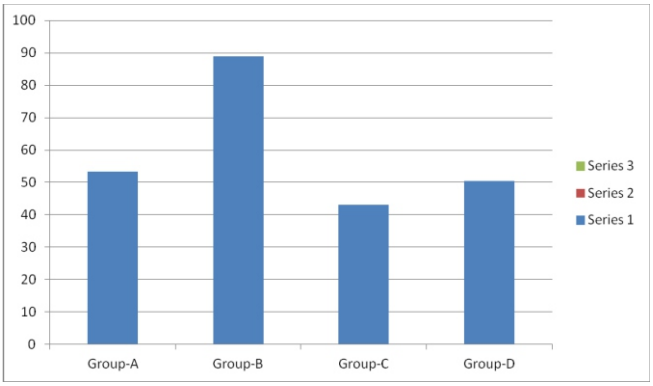
Table I shows the levels of serum diagnostic marker enzymes (AST, ALT, ALP and LDH) in normal and experimental groups of rats. There was a significant elevation noticed in the levels of serum diagnostic marker enzymes in group B antituberculosis drugs administered rats as compared to that of group A normal rats.

Figure I: Comparison of AST value among four groups.



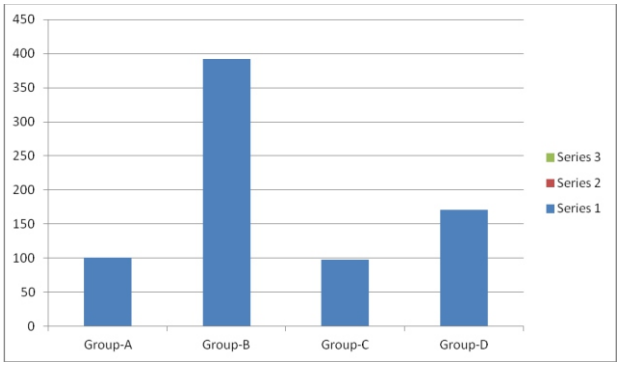
Significant difference were observed in AST level of four groups ($p < 0.01$). AST level was observed to be higher in group B rats as compared to group A rats(76.80 ± 7.Vs 219.13 ± 22.64). Significant difference was observed in AST level of group B and group D (219.13 ± 22.64Vs 77.13 ± 9.93) showed a significant difference ($p < 0.01$) in AST level. When normal group A was compared with group C rats (76.80 ± 7.93VS75.83 ± 8.22) no significant difference was observed in AST level.(Figuer I)

Figure II: Comparison of ALT value among four groups



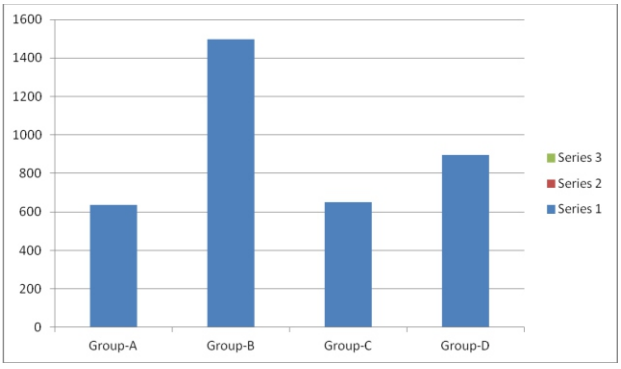
Significant difference was observed in ALT level of four groups ($p < 0.01$). ALT level was observed to be higher in group B rats as compared to group A rats (53.40±5.60 VS 89.00±5.50). Significant difference was observed in ALT level of group B and group D ($p < 0.01$) showing that ALT level was observed to be higher in group B than in group C. (89.00±17.40 VS 43.00±3.29) Similarly group B was compared with group D and showed a significant difference ($p < 0.01$) in ALT level ((89.00±17.40 VS 50.36±4.16). When normal group A was compared with group C and group D rats no significant difference was observed in ALT levels $p = 0.403$, $p = 0.967$, respectively. (Figure II)

Figure III : Comparison of ALP value among four groups.



Significant difference was observed in ALP level of four groups ($p < 0.01$). ALP level was observed to be higher in group B rats (100.60±12.57 VS 392±.18.34). Significant difference was observed in ALP level of group B and group C ($p < 0.01$) showing that ALP level was observed to be higher in group B than in group C (392±.10.58 VS 98.09±29.54). Similarly group B was compared with group D and showed a significant difference ($p < 0.01$) in ALP level (392±.10.358 VS 170.45±25.69). When normal group A was compared with group B the change in ALP was significant ($p = 0.01$) and group D rats showed no significant change in ALP ($p = 0.999$). (Figure III)

Figure IV: Comparison of LDH value among four groups.



Significant difference was observed in LDH level of four groups ($p < 0.01$). LDH level was observed to be higher in group A and group B rats (635.30±25.32 VS 1497.70±99.31). Significant difference was observed in LDH level of group B and group C ($p < 0.01$) showing that LDH level was observed to be higher in group B than in group C. (1497.70±99.31 VS 649.45± 109.21) Similarly, group B was

compared with group D and showed a significant difference ($p < 0.01$) in LDH level (1497.70±99.31 VS 897.16±116.33). When normal group A was compared with group D the change in LDH level was not significant ($p = 0.998$). (Figure IV)

DISCUSSION

Liver is a unique organ of the body in a sense that it regulates the internal chemical environment of the body. Through the process of detoxification of chemicals is its target.[12] Serum transaminases are cytoplasmic in location and are released into the circulation after cellular damage. Increase in their levels indicates liver injury. The levels of AST, ALT, ALP, total billirubin and LDH are increased in serum of rats treated with antituberculous drugs i.e. Isoniazid, Rifampicin and Pyrazinamide. These drugs cause toxicity by release of active intermediates such as 25-O-desacetyle rifampicin, acetylehydrazine and pyrazinoic acid respectively. Antituberculous drugs produce toxicity through these active intermediates. These free radicals could also be responsible for the degradation of phospholipids present in liver cell membranes.[9]

Isoniazid, that is first line antituberculous drug, is notorious for causing hepatotoxicity. Cytochrome *P*450 (CYP) 2E1, drug metabolizing enzyme, generates bioactive metabolites of isoniazid in humans, HepG2 (human hepatocellular liver carcinoma cell line) cells, rats and rabbits. Rifampicin and isoniazid that are commonly given in combination to patients, lead to potentiation of hepatotoxicity of isoniazid, due to its potent CYP 450 –enzymes induction effect.[13] In normal group mean AST with standard deviation was 76.80± 7.93 which raised to 219±22 in control group rats which were treated with antituberculosis drugs Isoniazid, Rifampicin and Pyrazinamide attributing the damaged structural integrity of the liver parenchyma because ALT, AST, ALP, LDH are normally located in the cytoplasm and are released into the circulation after cellular damage. The AST levels of group D demonstrated the co-administration of camel milk along with antituberculosis drugs, prevented drugs induced hepatotoxicity, decreasing AST values to 77.13± 9.93 at 1ml/kg dose of camel milk. This result shows a significant ($p<0.001$) difference.

The comparison of mean values of ALT in all groups indicates antituberculous drugs induced raise in ALT and then protection of camel milk. The comparison of effects of ALP, and the effects of LDH was seen in a similar pattern as described in AST.

The protective effects of camel milk, restores the harmful effects of liver parenchyma back to normal physiology through regeneration and protection of hepatocyte membrane integrity.[14] Studies on camel milk have shown that it is a high quality diet and people have used this product for treatment of a number of diseases. In addition to that camel milk has high quantity of zinc. It is one of the trace elements which are essential for living organisms. Zinc is required for the activation of more than 300 enzymes. Zinc influences cell division and differentiation through DNA replication, transportation and protein synthesis. It has been noted that zinc has a relationship with many different enzymes in the body and can prevent cell damage through activation of antioxidant system.[15] Camel milk could have protective effects through its antioxidant activity and it could give protection through its chelating effects on toxins also.[16]

CONCLUSION

It was concluded from this study that co-treatment of camel milk can prevent antituberculous drugs induced hepatotoxicity in rats. The overall hepatoprotective effect of camel milk is probably due to its antioxidant and radical scavenging property.

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