Effect of camel milk against renal toxicity in experimental rats

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Abstract: Most of the antibiotics are associated with considerable side effects. Gentamicin (GM) is one of the most commonly used antibiotics, but has significant nephrotoxic side effects. Hence, the current study is investigating the beneficial role of camel milk (CM) that ameliorate GM unwanted renal defects and dysfunctions in some experimental animals. Sprague-Dawely rats weighing (200-220g) were divided into groups (four) of six. Group 1 (Control) received normal saline (only). Group 2 was given oral administration of CM at the dose of 5ml/rat/day for fifteen days. Group 3 was injected with GM (80mg/kg b.wt., i.p.) for 10 days. Group 4 was first given oral administration of CM at the dose of 5ml/rat/day alone, for five days, and then followed with the administration GM for next 10 days, accordingly. The results show that administration of GM significantly enhanced the kidney weight and levels of renal toxicity markers like urea and creatinine, in addition to decreased levels of blood glucose. Treatment with CM ameliorated and reversed these drastic changes in levels of creatinine, urea and improved renal weight. Glucose levels were also reversed and increased significantly. Furthermore, GM induced renal histological anomalies like degeneration of glomeruli and tubules were suppressed by CM and showed better progress. The present study confirm that pretreatment with CM attenuates GM unwanted, induced renal dysfunction and cellular damage.

Keywords: Gentamicin, camel milk, kidney, rats, toxicity.

INTRODUCTION

The distinctive characteristics of the CM make it demanded as a medicinal aid by considerable numbers of diabetic patients and also used as anti-microbial hepato-protective agent (Althnaian *et al*., 2013). CM is traditionally used in Saudi Arabia, as it differs from other stock animals milk for, it contains several beneficial biological materials in addition to minerals and vitamins (Knoess 1979). The medicinal benefits were observed in treatment of some autoimmune defects and dysfunction s related to metabolism, especially those connected with some types of diabetes (Agrawal *et al*., 2002, 2007; Mohamad *et al*., 2009). Furthermore, CM can be stored for longer period at room temperature than other animals milk (Omer and Eltinary, 2009). CM is also consumed against some chronic diseases (Rao *et al*., 1970). Furthermore, many studies were reported CM has beneficial effect against toxic effects of Carbon tetrachloride, Paracetamol, Cisplatin, Cadmium chloride and Aluminum chloride (Khanand Al-Zohairy, 2011; Al-Fartosi *et al*., 2011; Afifi, 2010; Al-Hashem *et al*., 2009; Dallak *et al*., 2009; Al-Hashem, 2009).

Aminoglycoside antibiotic of GM is very effective in treatment of life threatening microbial infections (Ho and Barza, 1987) though, these useful clinical features are limited by its nephrotoxicity. Cuzzocrea *et al*., (2002) have shown this unwanted effect of GM in some treated patients. Humes, (1988) and Pedraza-Chaverrí *et al*., (2003) have referred to tubular necrosis that ensued after such induced renal toxicity. The correct mechanism of GM induced renal toxicity is unclear. Several antioxidants were employed to improve and enhance the effectivity of GM (Pedraza-Chaverrí *et al*., 2003; Maldonado *et al*., 2003; Yanagida *et al*., 2004). Our previous studies (Al Asmari *et al*., 2014) had experimentally proved the beneficial effects of CM against GM induced renal toxicity. The enriching role of CM against GM unwanted effects has still to be speculated. The objective of this current study is to determine the beneficial role of CM consumption on GM induced renal damages using experimental rats.

MATERIAL AND METHODS

Animals

Male albino rats (24) weighing 200 to 250g, were acclimated for 10 days (before running the experiment) in the animal house facility of the Research Center, Prince Sultan Military Medical City (PSMMC), Riyadh, Saudi Arabia. Animals were fed on normal laboratory diet with water *ad libitum* and kept in cages (six rats per each). The animal house facility rooms are air-conditioned and lights are kept at a 12h switch day and night cycles. Animals are maintained in accordance of the guide for the use of

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laboratory animals policy, approved by ethics of scientific research committee of PSMMC, Riyadh, Saudi Arabia.

Camel milk

CM was collected daily in the early morning after thoroughly cleaning and washing the teats and udders. Sterile steel containers were used in the collection of the milk, then samples were divided as portions in sterile screw capped bottles. They were put in boxes and stored in cool fridges for later processing in laboratories.

Drugs

GM 80mg (2ml vials) was obtained from Parkin Remedies.

Experimental design

Healthy acclimated four groups of rats (divided into six per each) were marked as:

Group 1 served as control and received orally normal saline alone and then along with i.p injection normal saline for next 10 days.

Group II animals were administered with camel milk at a dose of 5ml/ rat/ day orally for fifteen days associated with i.p injection of normal saline for the last 10 days.

Group III animals were administered normal saline orally for fifteen days associated with i.p. injection of GM at a dose of 80 mg/kg bw for the last ten days.

Group IV animals were given CM alone for first five days and then along with the GM for next 10 days.

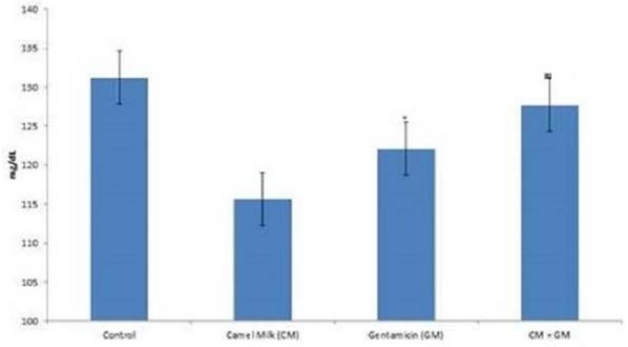

Fig. 1: Values are expressed as mean ± SEM (n = 6). *p≤0.05 shows significant difference in group III when compared with group I. #p≤0.05 shows the significant difference in the group IV when compared with group III.

Collection of blood serum

On day sixteen (last day), controls and test animals were fasted overnight, ether anaesthetized and killed to collect blood in vacutainers and separate sample sera. Centrifugation (1500g for 10min at 4°C) was done after clotting of the blood in the vacutainer tubes (20min/room temperature), then serum was stored at -80°C until used.

Biochemical analysis

The biochemical analysis parameters of Urea, Creatinine and Glucose were done using commercial kits purchased from local market (United Diagnostic Industry, UDI, Dammam, Saudi Arabia) and tests prescribed by manufacturer were done accordingly.

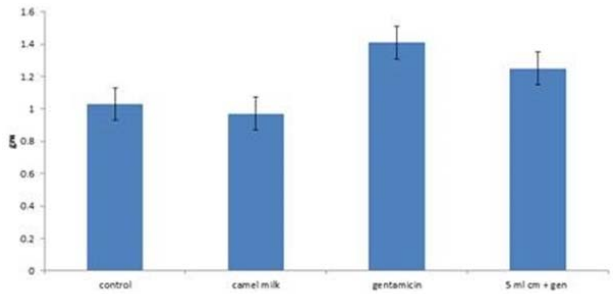

Fig. 2: Representative bar diagram depict the effect of CM on kidney weight in animals treated with GM and CM.

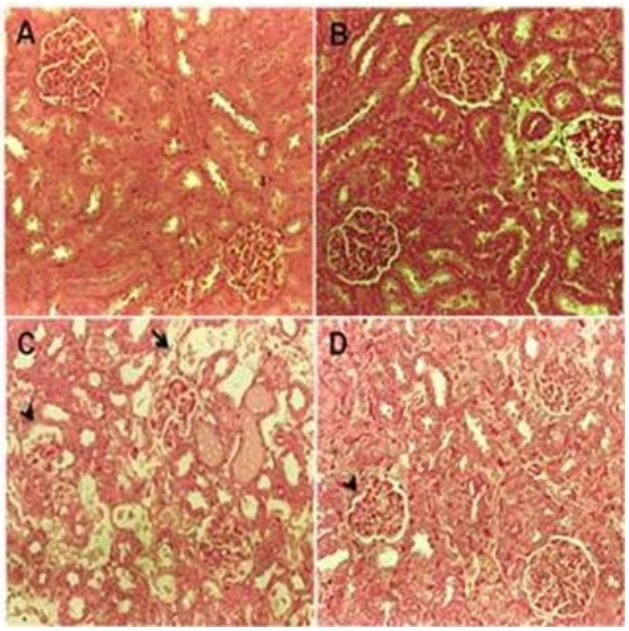

Fig. 3: Effect of CM administration on GM induced renal histopathological alterations. There was massive distortion in the tissue histology in group III animals (fig. 3C) as compared with the group I (fig. 3A). CM administration in group IV (fig. 3D) diminished GM - induced histological anomalies as compared to group III.

Histopathology

Each group of animals was sacrificed; kidneys were collected, cut and fixed in 10% neutral-buffered formalin that had been prepared fresh. This was followed by the dehydration process and clearing of samples in xylene. They were impregnated and embedded in paraffin wax, sectioned and stained in hematoxylin and eosin (H&E). Ready prepared slides were studied under light microscopes (BX51 microscope, Olympus).

STATISTICAL ANALYSIS

GraphPad Prism and Instat were used for analysis of the data. Results were expressed as Mean and standard errors (SEM). Mean data comparisons were done using student t-test, and an alpha value of 0.05 was considered to be statistically significant.

RESULTS

CM and GM effects on renal biomarkers levels

Kidney sera biomarker levels were shown in table 1. Significantly increased the level in the GM treated group 3, compared with the group 1 (65.41±1.78 to 97.57±4.42 ($p<0.001$); 0.81±0.07 to 1.23±0.07 ($p<0.001$). Whilst, in group 4, a significant reduction was observed in comparison with serum levels of group 3 (97.57±4.42 to 60.49±1.84 ($p<0.001$); 1.23±0.07 to 0.99±0.09 ($p<0.05$). fig. 1 showed a significant reduction in serum glucose of group 3GM treated (131.22±2.11 to 122.12±1.78 ($p< 0.05$) in comparison with controls. However, the levels of glucose increased significantly, in group 4 in comparison with group 3 (122.12±1.78 to 127.70±2.68 ($p<0.05$).

Effect of CM and GM on kidney weight

Administration of GM dose of 80mg/kg resulted in significant ($p<0.01$) increase in kidney weight (group 3) compared to controls. However, CM the treated kidneys in group 4, significantly restored their weight, compared with group 3 (fig. 2).

Histological effect of CM and GM on kidney

An orally administered CM effect was seen on renal histological changes induced by GM administration with reference to glomerular constriction and tubule damage. We found that GM administration induced damage in renal histo-architecture in group 3 (fig. 3C) compared with controls (fig. 3A). Treatment with CM in groups 4 (fig. 3D) reversed the GM caused histological anomalies. There were no significant changes observed between controls (fig. 3A) and group 2 (fig. 3Bwith respect to these histological alterations (fig. 3).

DISCUSSION

Aminoglycoside of GM is an important, therapeutic doses of this antibiotic commonly used against the life threatening infections in human. GM treatment produced markedly increase in the serum levels of renal function markers such as creatinine, urea, and reduced level of glucose as compared to group 1, indicating renal damages. These lesions may be due to the production of free radicals and association of oxidative stress to renal damage induced by GM treatment. This was confirmed by some recent studies (Agrawal *et al*., 2007; Khan and Al Zohairy 2011; Quan *et al*., 2008).

Earlier reports had mentioned similar changes, with respect to nephrotoxicity. GM application in test animals had markedly increased serum urea and creatinine levels in comparison with controls (Silan *et al*., 2007; Noorani *et al*., 2011). It could be believed that the accumulation of GM in the kidneys may increase deterioration of the renal tubules that lead to renal dysfunction and defects (Silan *et al*., 2007; Noorani *et al*., 2011). Confirmations that changes in serum urea might be related to metabolic disturbances secondary to renal dysfunction were provided by some investigators (Szilagyi *et al*., 1994). It was also speculated that animals treated with GM may suffer from alterations in in liver functions due to mediation activities attributed to GM, giving urea as byproducts of protein catabolism (Boroushaki *et al*., 2012). According to our present study, combined administration of CM with GM reversed the expected increase in levels of urea and creatinine to near normal status whilst, serum glucose levels in GM treated subjects were significantly reduced, compared to controls which, is in good agreement with previous reports (Hamad *et al*., 2011). It is also noted that some doses of administered GM could induce renal dysfunction in relation to increased urinary excretion of glucose and protein, elevated levels of serum urea and creatinine (Boroushaki *et al*., 2012). The contents of insulin/insulin like materials found in CM reflected a potential hypoglycemic effect in subjects like diabetics which, is consistent with the findings of Agrawal *et al*., (2002; 2007) and Hamad *et al*., (2012).

With respect the unwanted activity of aminoglycosides and its ability to induce nephrotoxicity, it was speculated that the signal transduction pathway could have been disrupted, leading to cellular permeability performance on membrane phospholipids (Schacht and Weiner, 1986). A decrease in rat body weight which, was associated with an increase in kidney weight compared with controls, and the significant induced renal toxicity, increase in serum activity and levels of urea and creatinine and damage, represent confirmed findings attributed to administration of the GM doses that were ameliorated by CM consumption. Histopathological findings further supported the anti-oxidative and anti-inflammatory potential of CM, in recessing and reversing the kidney inflammatory deterioration reflected in cellular infiltration and tubular edema, glomerular constriction and tubular damage, suggesting and supporting that CM consumption plays a protective effect on kidney disorders.

CONCLUSION

The present findings show that administration of CM exerts significant renoprotective effects in GM-treated rats. Further investigations are required to explore the underlying mechanisms involved, so, referring to our previous findings, we could suggest that CM may be identified as a new therapeutic agent.

Table 1: Effect of CM and GM on the levels of urea and creatinine

Parameters	Group I (Control)	Group II (CM)	Group III (GM)	Group IV (CM + GM)
Urea (mg/dl)	65.41±1.78	76.04 ± 1.92	97.57 ± 4.42[a]	60.49± 1.84[b]
Creatinine (mg/dl)	0.81±0.07	0.71 ± 0.06	1.23 ± 0.07[a]	0.99 ± 0.09[b]

Values are given as means ± S.E.M for groups of six animals each. Values are statistically significant between two groups $p \leq 0.05$. a-control group compared with GM group; b- GM group compared with CM & GM group.

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