Safety profile of PMF a fraction derived from camel urine on mice (acute study)

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Abstract**—** Camel urine, a rich source of natural products with medicinal properties, has been used traditionally for the treatment of various diseases in Middle East. To exploit the therapeutic potential of various ingredients present in camel urine, evaluation of efficacy and safety profile is required. In the present study, we have evaluated the potential of camel urine fraction [PMF) to cause hepatotoxicity and nephrotoxicity in mice. Biochemical tests and complete blood picture [CBC) were performed after oral administration with varying doses of PMF. Blood samples were collected from the orbital sinus of mice after 24hrs of drug treatment. Liver function tests such as measurement of ALT, AST, Alkaline phosphatase, Gamma-glutamyltransferase [GGT), total bilirubin and albumin levels were performed to assess the hepatotoxic potential of PMF. Kidney function tests such as measurement of Na, K, Cl, total protein, creatinine, uric acid, blood urea nitrogen [BUN) and phosphatase level were performed in order to evaluate nephrotoxic effects of PMF in mice. Our study indicated that PMF did not cause any damage to liver and kidney even after treatment with twenty times of the therapeutic dose. The present study clearly suggested that PMF does not have any potential to cause hepatotoxicity and nephrooxicity and it is safe for therapeutic use in human.

Keywords**—** Camel urine, drug safety, PM701, PMF.

I. Introduction

Camel urine has been used traditionally for the treatment of various diseases in many Middle East countries. It is known to be a rich source of several potent compounds with novel many structures and with wide variety of biological functions [1]. Drinking camel urine was shown to be effective in treating numerous cancers in human [2]. Previous studies have suggested that PM701 (dried camel urine), has cytotoxic activity and induces apoptosis in cultured human lung cancer cells. Induction of apoptosis was also observed in cultured leukemia cells. PM701 was also found to be effective in preventing metastasis in animal models [1,3]. Several studies by Khorshid et al., [1;4] identified that Prophet Medicine fraction [PMF] is the biologically active fraction isolated from PM701, which has the potent anticancer activity [4]. The apoptotic effect of PMF in human cancer cell was analysed by TUNNEL method and it was observed that PMF was able to induce apoptosis in dose dependent manner. Cell proliferation study using MTT assay revealed that PMF treatment resulted in dramatic decrease in cell proliferation and cell survival [4]. It was also observed that Cesium [Cs] and Rubidium [Rb] particles are present in PMF as detected by Energy Dispersive X-ray attached to Scanning Electron Microscope. Treatment of lung cancer cell line A549 with PMF induced pores in the nuclear membrane resulting in the internalization of Cs and Rb with subsequent cell death due to elevation of cellular pH [4,5]. These studies clearly suggested that PMF derived from camel urine has tremendous therapeutic potential to treat various types of cancer.

The safety assessment of drug compounds is essential for its therapeutic use in humans. Several drugs have been withdrawn from the market due to the severe adverse effects in patients [6-8]. Most of these drugs have shown potential to cause hepatotoxicity and nephrotoxicity [9-11]. The objective of the present study is to evaluate the safety of PMF administration at high doses in mice and investigate its potential to cause hepatotoxicity and nephrotoxicity.

II. Material and Methods

A. Animals

125 adult mice were kept and maintained at room temperature with an adequate ventilation and normal 12-hours light-dark cycle and free access to food and water. All the studies were conducted in accordance with the laws and regulation of local governing authorities. These animals were divided into five groups, each one having twenty mice.

B. Treatment of mice with PMF

Group one was administered with a therapeutic dose of PMF (0.75 µl + 75 µl sterile water) through oral gavage. The second group was administered 2 X of the therapeutic dose (1.5 µl PMF + 75 µl sterile water). The third group was given 4 X of the therapeutic dose of PMF (3 µl + 75 µl sterile water). The fourth group of mice was administered with 20 X of the

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therapeutic dose of PMF (15 µl PMF + 75 µl sterile water). The fifth group was the control group and was administered with 0.2ml sterile water only.

C. Biochemical Analysis

Animals were anaesthetized with ether and blood was collected after 24 hours post- administration from Orbital sinus and the sera of the animals were isolated. Erythrocyte count (RBCs), Haemoglobin concentration (Hb), packed cell volume (PCV), haematological indices {mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), total leukocytic count (TLC) and lymphocytic count; were estimated by using automated blood cells counter i.e Exigo haematology analyser. Liver function tests for Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were estimated spectrophotometrically by using the test kits supplied from Biodiagnostic Laboratories according to the method of Reitman and Frankel (12). Kidney functions tests including blood urea nitrogen (BUN), total protein (TP), uric acid (URCA) were determined according to Walker et al.,[13]. Multiple Comparisons dependent variable test was performed using software according to Howell [14].

III. Results and Discussion

The blood components of mice administered with varying doses of PMF did not show any significant differences in the RBC count and platelets count as compared to control group. Similarly there was no significant difference in the concentration of Hb, MCV, MCH and MCHC between PMF treated and control groups as shown in Fig.1 and Table 1.

The liver enzymes mainly ALT, AST, alkaline Phosphatase (ALP), albumin (ALB), total bilirubin (TBIL) and Gamma-glutamyltransferase (GGT) did not show any significant differences between treated mice with PMF and control groups as shown in Fig.2 and Tables 2,3 and 4.

In addition the kidney function tests including Na, K, Cl, creatinine, blood urea nitrogen, uric acid, phosphorus and total protein values did not show any significant difference between treated mice with PMF and control groups as shown in Fig.3 and Table 5.

Whereas studies have reported that complete blood cell count (CBC) is one of the most common laboratory test to evaluate the safety of a drug compound. The measurement of these parameters is useful in diagnosis of different types of anemia, autoimmune diseases, neoplasm and inflammation [15]. In the present study, administration of PMF in mice using the doses higher than the therapeutic dose did not show any effect on the blood components as shown by the Multiple Comparisons dependent variable statistical test.

Deursen et al., [2010] showed that liver function abnormalities are frequently observed in patients with heart failure; in particular high levels of bilirubin, LDH and GGT have been detected [16]. Alteration in liver function tests including bilirubin concentration, AST, GGT and alkaline phosphatase activity were related to liver injury during heart failure. Thus, abnormalities in liver function tests have prognostic importance in chronic heart failure. Although we have no alterations in liver enzymes activity, we could reveal that these results showed the safety of PMF on heart function.

Kluwe [1981] analysed the nephrotoxic potential of various compounds including biphenyl, carbon tetrachloride and mercuric chloride in male rats [17]. Several renal function tests such as measurement of urine specific gravity, pH & volume, urinary excretion of glucose, protein, electrolytes & various enzymes, serum concentrations of urea nitrogen, creatinine & electrolytes, creatinine clearance and kidney weight, were performed after administration of four different doses of each compound. Ability to concentrate urine after water deprivation, kidney morphology and accumulation of organic ions by renal cortical tissue *in vitro* was also examined. In general, it was observed that in vitro accumulation of organic ions, urinary concentrating ability and kidney weight were the most sensitive and consistent indicators of nephrotoxicity [17]. On the other hand, standard urinalyses, serum analysis and pathological changes in renal morphology were less sensitive and less consistent indicators of renal injury. The most advantageous tests, therefore, appeared to be those that measured total, functional renal capacity. The administration of different doses of PMF showed none of the pervious mentioned signs on the treated animals.

Various studies have indicated that increase in uric acid level is generally associated with the development of hypertension, abdominal obesity, insulin resistance, cardiovascular disease and renal failure [18]. These studies have shown a correlation between increased uric acid concentrations with the oxidative stress, endothelial dysfunction, inflammation, subclinical atherosclerosis and increased risk of cardiovascular disease.

Our study clearly showed that there was no significant difference in liver and kidney function tests in PMF treated group as compared to the control group using the multiple comparisons dependent variable statistical test, suggesting that PMF does not cause liver or kidney injury in mice.

Although our previous work indicted the efficiency of PMF in fighting cancer cells [19-24], this work improved the safety of PMF for administration for human in clinical trial.

Conclusion

In conclusion, our study clearly suggests that PMF derived from camel urine, is a safe drug and does not have potential to cause hepatotoxicity and nephrotoxicity. Hence, PMF has tremendous therapeutic potential as a safe and potent anticancer agent for treatment of various types of cancer in human.

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FIGURES AND TABLES

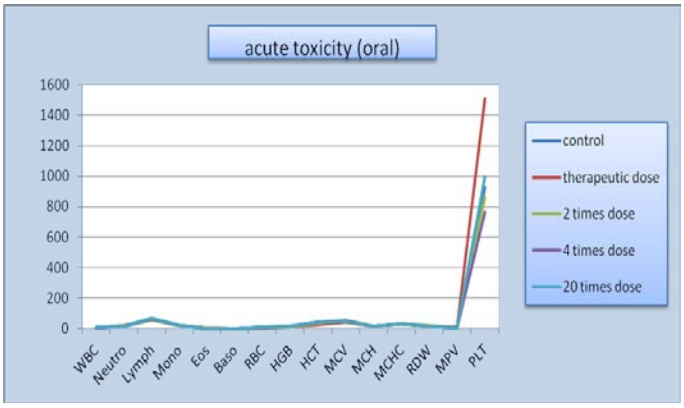

Fig.1. Complete blood picture showing no significant difference in the numbers of different components of blood after oral administration with different doses of PMF in comparison with the control group.

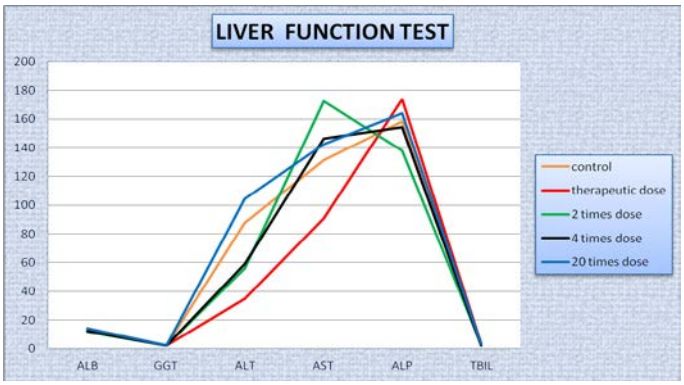

Fig.2. Liver function tests indicating no significant difference in liver enzymes between PMF treated and control groups.

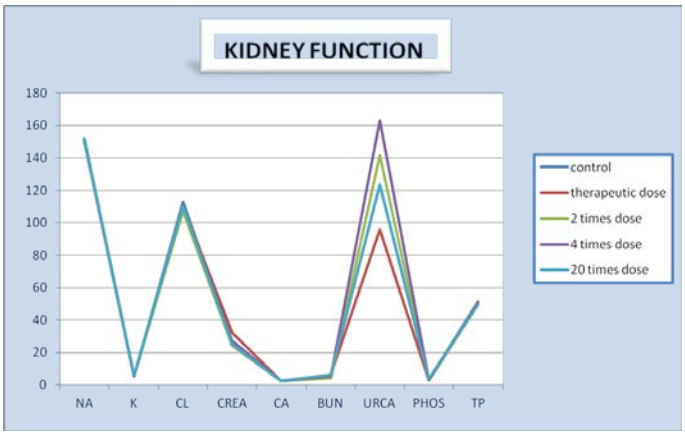

Fig.3: Analysis of Kidney function tests revealed no significant difference in kidney functions parameters between PMF treated and control groups.

Control Group	Exp. Group	Mean	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	therapeutic dose	-583.500	202.5998	.035	-1104.2995	-62.7005
	2 times dose	64.000	202.5998	.765	-456.7995	584.7995
	4 times dose	159.000	202.5998	.468	-361.7995	679.7995
	20 times dose	24.5000	202.5998	.908	-496.2995	545.2995

Table 1. Multiple Comparisons dependent variable of PLT showing that the differences between all groups treated with PMF are not significant as compared with control, whereas the significant is (0.005 – 0.0005).

Control Group	Exp. Group	Mean	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Therapeutic dose	52.66667	33.58703	.148	-22.1699	127.5032
	2 times dose	31.66667	33.58703	.368	-43.1699	106.5032
	4 times dose	28.66667	33.58703	.413	-46.1699	103.5032
	20 times dose	-17.0000	33.58703	.624	-91.8366	57.8366

Table 2. Multiple Comparisons dependent variable of ALT showing that the differences between all groups treated with PMF are not significant as compared with control.

Control Group	Exp. Group	Mean	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Therapeutic dose	73.6666	59.1307	.241	-58.0848	205.4182
	2 times dose	-7.6666	59.1307	.899	-139.4182	124.0848
	4 times dose	18.3333	59.1307	.763	-113.4182	150.0848
	20 times dose	22.0000	59.1307	.718	-109.7515	153.7515

Table3. Multiple Comparisons dependent variable of AST showing that the differences between all groups treated with PMF are not significant as compared with control, whereas the significant is (0.005 – 0.0005).

Control Group	Exp. Group	Mean	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Therapeutic dose	73.66667	59.13074	.241	-58.0848	205.4182
	2 times dose	-7.66667	59.13074	.899	-139.4182	124.0848
	4 times dose	18.33333	59.13074	.763	-113.4182	150.0848
	20 times dose	22.00000	59.13074	.718	-109.7515	153.7515

Table 4. Multiple Comparisons dependent variable of ALP showing that the differences between all groups treated with PMF are not significant as compared with control, whereas the significant is (0.005 – 0.0005).

Control Group	Exp. Group	Mean	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Therapeutic dose	28.0000	15.25487	.096	-5.9900	61.9900
	2 times dose	-18.0000	15.25487	.265	-51.9900	15.9900
	4 times dose	-39.3333	15.25487	.027	-73.3233	-5.3434
	20 times dose	.00000	15.25487	1.000	-33.9900	33.9900

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Table. 5: Multiple Comparisons dependent variable of URCA showing that the differences between all groups treated with PMF are not significant as compared with control, whereas the significant is (0.005 – 0.0005).

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