SAFETY OF ORAL ADMINISTRATION OF PMF A FRACTION DERIVED FROM CAMEL URINE: ACUTE STUDY ON MICE

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Abstract - Camels researches have attracted interest of scientists since the beginning of the 20th century. Camel urine, a natural product, has been used long time ago traditionally in the treatment of many diseases in Arabic countries without scientifically tested. To exploit the therapeutic potential of various ingredients present in camel urine, evaluation of efficacy and safety profile is required. In the present study, Biochemical analysis and complete blood picture (CPC) were investigated after oral administration in mice with PMF (a fraction extracted from camel urine). PMF was administered orally with the therapeutic dose of (0.75ul), two-times, four-times, and twenty-times of the therapeutic dose. Blood samples were collected from the orbital sinus of mice after 24 hours post-administration. Complete blood picture, liver function testes (ALT, AST, ALP, GGT, TBIL and ALB). Kidney function testes (Na, K, Ca, Cl, TP, CREA, URECA, BUN, and PHOS) were measured and statistically analyzed. The results showed that PMF did not have any nephrototxic or hapatotoxic effects as indicated by the non-significant changes of values of both kidney and liver functions testes. Histopathology examination of liver and kidney tissues of mice administrated by PMF indicating that there are no alteration in the tissues architectures and pointed that PMF is a safe fraction even with high doses or long administration time of the therapeutic dose.

Keywords - Camel urine, drug safety, PM701, PMF

I. INTRODUCTION

Camel urine, a rich source of natural products with medicinal properties, has been used traditionally for the treatment of various diseases in the Middle East. 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 [3].

Induction of apoptosis was also observed in cultured leukemia cells. PM701 was also found to be effective in preventing metastasis in animal models [1,4]. Several studies carried out by Khorshid et al., [1,3;5] identified that Prophet Medicine fraction [PMF] is the biologically active fraction isolated from PM701, which was proved to has potent anticancer activity [1,3-5]. The apoptotic effect of PMF in human cancer cells was analysed by TUNNEL method and it was observed that PMF was able to induce apoptosis in dose dependent manner. Cells proliferation study using MTT assay revealed that PMF treatment resulted in dramatic decrease in cancer cells proliferation and cells 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 [5]. 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 [5]. These studies clearly suggested that PMF 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 based on its effect on the functions of liver, kidney and on the structure of their tissues.

II. MATERIAL AND METHODS

1. 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 (commercial laboratory mice's 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.

2. Treatment of mice with PMF

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

3. Biochemical Analysis

Animals were anaesthetized with ether and blood was collected after 24 hours post-administration from venous plexus and sera were stored at -80C^{0}for future analysis of liver and kidney functions. Erythrocyte count (RBCs). Haemoglobin concentration (Hb), packed cell volume (PCV), haematological indices {mean corpuscular volume} (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total leukocyte count (TLC) and lymphocyte count were estimated using automated blood cells counter i.e. Exigo hematology analyzer. Liver function tests for Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) Alkaline phosphtase (ALP), Gamma-glutamyltransferase (GGT), Total bilirubin (TBIL) and Total Albumin activities were estimated spectrophotometrically using the test kits supplied from Biodiagnostic Laboratories according to (12). Kidney functions tests including (Na, K, Cl; Ca), blood urea nitrogen (BUN), total protein (TP), creatinine (CREA), phosphate (Phos), uric acid (URCA) were determined according to (13). Multiple Comparisons dependent variable test was performed using statistical software according to [14].

4. Histopathology studies:

The routine processes were carried on the liver and kidney tissues at a thickness of 5mm that stained by Hematoxylin and Eosin (H&E) following Baker (15) method to conduct the examination using light microscope.

III. RESULTS AND DISCUSSION

Hematological examination done in the present study did not reveal any significant differences in the RBC count and platelets count at all doses of PMF administrated orally to experimental mice as compared to control group (did not receive PMF). 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.

In the present study, it could come to conclude that administration of PMF even in doses higher than the therapeutic dose did not show any effect on the blood components as shown Table 1, using the Multiple Comparisons dependent variable statistical test. In literature, complete blood cell count (CBC) was recommended as 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 [16].

To evaluate possible effect on liver function and exclude hepatotoxicity, liver function tests were done. Liver enzymes mainly ALT, AST, ALP, albumin (ALB), TBIL and 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.

Deursen et al., [17] 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. 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.

In addition, biochemical results of the present study showed insignificant alteration of kidney function tests including Na, K, Cl, creatinine, blood urea nitrogen, uric acid, phosphorus and total protein values in mice receiving oral PMF at various doses compared to control groups as shown in Fig.3 and Table 5.

Kluwe [18] analysed the nephrotoxic potential of various compounds including biphenyl, carbon tetrachloride and mercuric chloride in male rats.

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. 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 [18].

In the same time our results showed decreased of uric acids value in kidney test if mice administrated the PMF compared to control mice. 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[19]. 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.

The histopathology studies of vital organs (liver and kidney) from animals administrated by the therapeutic dose of the PMF showed no severe effect of PMF on the tissues architectures or structural alteration of studied organs comparing with control group even with long time of administration (60 days) as indicated in the pictures that represent in the Figs (4&5).

Although our previous work indicted the efficiency of PMF in fighting cancer cells [20-25], this work improved the safety of PMF for administration for human in clinical trial.

According to Tesfaye, et al. [26] the changes in the biochemical parameters of the liver enzymes might change the liver's capacity of drug detoxification especially after long-term use of these drugs. Beyond the knowledge of early phases of the clinical trial it is vital to make sure that before a new medicinal product is placed on the market, all available information on its safety and efficacy comes only from clinical trials.

Despite intensive research conducted in animals and during clinical trials in humans, some adverse reactions may be identified only after the product is used by a large number of people. So we submitted the PMF for approval for clinical trial, more over we will continue in studying its adverse effects. The conditions under which patients and medicines are studied may not necessarily reflect the way medicines are used in hospitals or outpatient practices following their placement on the market. For this reason it is very important to monitor the safety of medicinal products also after their marketing – and this is the subject of pharmacovigilance. Pharmacovigilance process dealing with the monitoring of use of medicinal products in everyday clinical practice, may enable identifying previously unidentified adverse reactions or a change in the nature of adverse reactions. Risk-benefit assessment of medicinal products, which helps to decide what action, if necessary, is essential for a safer use of medicinal products [27-28].

CONCLUSION

In conclusion, the present study clearly showed that PMF derived from camel urine, is a safe preparation and does not has any hepatotoxicity and nephrotoxicity in mice. Hence, it proved previously to work as anticancer agent so it can be used as a new alternative anticancer medicine with no or even minimum side effect on vital organs and could be submitted to clinical trail.

FIGURES AND TABLES

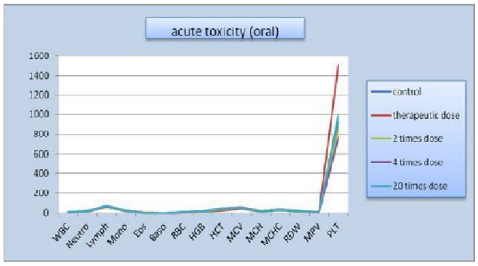


Fig.1. Complete blood picture showing no significant differences between all components of blood after oral administration of different doses of PMF in comparison with the control group.

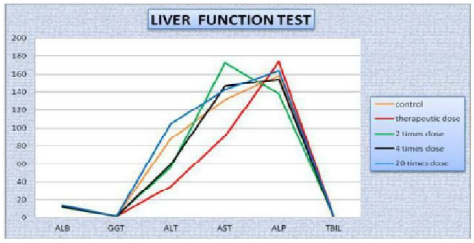


Fig.2. Liver function analysis indicating no significant differences in liver enzymes between treated and control groups.

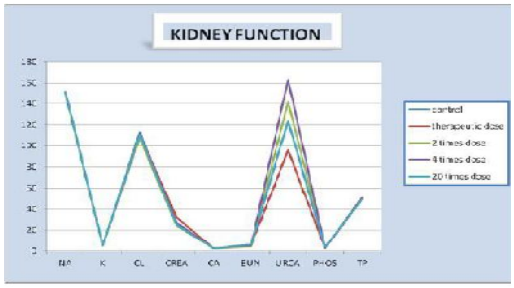


Fig.3: Analysis of Kidney functions revealed no significant differences in kidney functions parameters between treated and control groups.

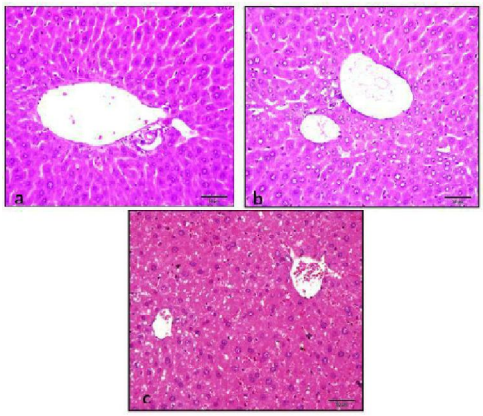


Fig.4. Sections from mice liver after of oral intake of therapeutic dose of PMF (a. control, b. treated for 7 days; c. treated for 60 days) note normal hepatocytes (binucleated) in treated mice's cells (even more active than control) (H&E X40).

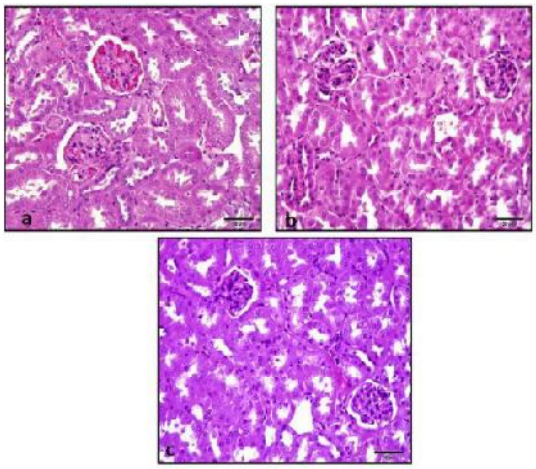


Fig.5. The effect of oral administration of PMF with a therapeutic dose on kidney structure showed normal renal corpuscles and tubules, whereas no architecture differences are seen between control (a, not administrated)

Control Group	Exp. Group	Mean	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Therap-eutic dose	583.50	202.5998	.035	-1104.2995	62.7005
	2 times dose	64.000	202.5998	.765	-456.7995	584.7995
	4 times dose	159.00	202.5998	.468	-361.7995	679.7995
	20 times dose	24.500	202.5998	.908	-496.2995	545.2995

Table I

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 II

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	Therapeu-tic 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

Table III

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	Therapeu-tic 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 IV

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

Table V

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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