International Journal of Pharmacology, 2008
ISSN 1811-7775

Preclinical Evaluation of PM 701 in Experimental Animals

F. Khorshid
Department of Medical Biology, Faculty of Medicine, King Abdul Aziz University (KAU),
P.O. Box 80216, Jeddah 21589, Saudi Arabia

Abstract: In this study, we addressed the toxicological effect of PM 701 on various animal species. Results showed that there was no mortality recorded to doses up to 10 g kg^{-1} body weights during the 4 weeks of observation. Function tests for Liver (SGOT-SGPT-Alk.Phos) and kidney (urea and creatinine) revealed that PM 701 have no hepatotoxic or nephrotoxic effects. No hematological toxicity was detected. Histological studies, showed no effect on gastric mucosa, no alteration in liver or kidney parenchyamatous architecture. Hepatocytes showed preserved cellular outline with no signs of necrosis. Few renal tubules showed degenerative changes. Splenic tissue showed activation and enlargement of germinal centers of white pulp lymph nodules indicating activation of immune defense without any effect on vital body organs. Therefore, compared to toxicity induced by well known chemotherapeutic agent, PM 701 could be considered safe as potentially anticancer agent with minimal or even negligible effects on vital organs such as liver and kidney and recommended to be subjected to clinical trial in human volunteers. PM 701 was categorized as practically non toxic.

Key words: PM 701, organ toxicity, histological examination, physiological function, toxicological effects

INTRODUCTION

Cancer is a disease in which the cells fail to respond to the fundamental rules governing cells proliferation and differentiation (Salman and Sartorlli, 2001).

There has been a gradual evolution in the philosophy of treatment of cancer (Crown, 1998). With progress in understanding its nature, many therapeutic anticancer have been developed and improved but it still remain far from the ideal treatment, which selectively kills the malignant cell and sparing the normal healthy tissue and the function of the vital organs (Grever and Chabner, 1997).

Traditionally, cancer drugs were discovered through a large-scale screening of synthetic chemicals and natural products against animal tumor system, but many new natural cytotoxic agents still have not yet been discovered.

In the last decades a considerable growth in scientific and medical interest for the use of traditional medicines has been observed. In the United States of America, it has been reported that one of three people is using one type of alternative therapy (Eisenberg *et al*., 1993). In Australia the use of alternative medicine reached up to 48.5% (Maclennan *et al*., 1996) and in Denmark and France the proportions were 23 and 49%, respectively (Fisher and Ward, 1994). In Saudi Arabia it has been reported that 24% of patients who attended a health center used an alternative medicine and of those, 25% were using Quran's reading, 28% used cautery and 45% were using medical herbs (Al-Rawais, 2002).

All ancient cultures of the world have involved their own medical lore's and practices to take care of their health problems. Over the millennia, the most effective remedies among them were selected by the trail and error, empirical reasoning and even by experimentation, which have now become part of the ethnomedical traditions. Chinese and Indian traditional medicine which used to treat cancer has been assessed in different kind of studies, many of these showed promising results as anticancer (Cai *et al*., 2004; Saha *et al*., 2004; Shoemaker *et al*., 2005; Malairajan *et al*., 2006; Zhao and He, 2006; Yang *et al*., 2007). Other studies have been undertaken on other traditional medicine from different countries, such as: African (Charlson, 1980), Iran (Amirghofran *et al*., 2006a, b), Europe and North America (Thole *et al*., 2006; Wang *et al*., 2006) and South America (Gonzales and Valerio, 2006).

The World Health Organization (WHO) has estimated that 80% of the world population relies on traditional medicine for primary health care. The WHO declaration of Health for all by year, 2000 emphasized the importance of Traditional Medicine in achieving primary health care.

Drug discovery from natural sources has played an important role in the treatment of cancer and indeed, most new clinical applications of natural materials over the last half century have been applied to combating cancer (Butler, 2004). Therefore, before any new compound is approved in testing in man, extensive toxicity testing is done in various animal species and with *in vitro* systems.

PM 701 is a natural product, readily available, cheap, sterile and non-toxic according to chemical and microbiological testing and proved effectiveness of this agent is reproducible on both *in vitro* and *in vivo* models (Khorshid *et al*., 2005).

PM 701 has been discovered in our laboratory to inhibit the growth of lung cancer and leukemic cells *in vitro* (Khorshid and Mosherf, 2006) and to increase life span of mice bearing leukemia cells by at least 3 folds, which means that it has a favorable antimitotic effect (Mosherf *et al*., 2006; Khorshid and Mosherf, 2006).

Before any new compound is approved for testing in man much extensive toxicity testing must be done in various animal species to investigate its safety or any side effects of it.

Therefore this study is directed to investigate the toxicological effect of PM 701 in animal models.

MATERIALS AND METHODS

PM 701 was prepared in the lab from natural product (unpublished data) PM 701, is a yellowish powdered form, pH 8.3 that has sharp (offensive) odor and insoluble in water, but has good suspension with Tween 80, which was stable for at least one month.

Measurement of toxicity

Determination of LD_{50}: LD_{50} were determined in male and female mice and rats according to the methods of Miller and Tainter (1944) using two routes of administration Oral and Intra peritoneal (i.p.). Rodents were observed for 4 weeks following a single and multiple doses.

Behavioural and physiological function: PM 701 in a dose of 0.35 g kg^{-1} (6.25-10 times human dose according to the method of Freireich *et al*., 1966), was injected i.p. into male albino rats for 1, 2 and 4 weeks. Control rats received equivalent volumes of control solution for the same periods. All animals received human care according to ethical requirements approves by the Animals Research Ethic Committee of KAU.

On experimenting dates rats were weighed on week one, two and four and observed for behavior and physiological function (primary observation) according to Irwin test in rodents (Irwin, 1968). The guideline of ICH S7A was observed. After initial observation, rats were anaesthesized by di-ethyl ether and blood sample was withdrawn by heart puncture. Part of blood was taken on EDTA for hematological investigation and other part was centrifuged for serum separation. Liver function, (GOT, GPT, alkaline phosphatase and bilirubin) and kidney function (urea and creatinine) were determined by ready made kit. Blood elements were determined by coulter counter. After withdrawal of blood, rats were killed by overdose of anesthesia and the following organs were dissected, heart, liver, kidney, lungs, spleen and testes. Organs were freed from fats and connective tissues, weighed and fixed in formalin 10% for histopathological examination.

Interaction with barbiturate: Male Wistar rats with weighing between 200 to 300 g were divided into two groups. Group A serves as control received saline with tween 80 and group B injected with PM 701 with tween 80 serves as test group.

Rats in each group were injected with pentobarbital in a dose of 60 mg kg^{-1} and observed for actual onset time and recovery time. SPSS program was used for the statistical evaluation.

Histological examination: The examination of the microscopic features with light microscope included the morphology of tissues, before and after administration of examined substrate.

RESULTS AND DISCUSSION

Ion selective electrode apparatus (ISE) assay showed that PM 701 contain Na^{+}, K^{+}, urea nitrogen, creatinine, glucose, Ca^{++}, Mg^{++}, phosphorus, uric acid, nitrogen, protein, ketones, amylase in variable quantities.

LD_{50}: Using Irwin check list, rats treated with PM 701 for 1, 2 and 4 weeks showed no mortality to doses up to 10 g kg^{-1} body weights during the 4 weeks of observation. Therefore, PM 701 was categorized as practically non toxic.

There was no change in movement, appearance, no hair erection or salivation, significantly different from that of rats treated with control solution for the same periods. Some animals treated with PM 701 showed sign of transient sedation; this was confirmed by PM701 interaction with barbiturate on sleeping time see below.

Effect of PM 701 on body weight: Rats treated with PM 701 up to 4 weeks showed no impairment in rat's growth rates. The relative changes in body weights were comparable with that of rats treated with control solution, p-value 0.3 (non significant) (Table 1).

Table 1: Effect of oral administration of PM 701 (0.35 g kg^{-1}) on the body weight of animal compared with controls

Experiments	1st week	2nd week	4th week
Control	234.250±7.25	271.0±17.26268	312.75±4.272
Test	247.166±18.166	259.4±10.8074	286.67±20.95
p-value	0.309	0.255	0.27

p-value ≠ 0.3

Effect of PM 701 on organs weight: No significant differences was seen in the relative weights of liver, kidney, heart, spleen, lung and testes in rats treated with PM 701 IP for 4 weeks, compared with that of rats treated with equivalent volumes of control solution (saline plus tween 80), p-value 0.2 to 0.9 (non significant) (Table 2, 3 and 4a, b).

Effect of PM 701 on kidney function biomarkers: No significant differences were seen in urea and creatinine levels in rats treated daily with PM 701 for 2 and 4 weeks, compared with that of rats treated with equivalent volumes of control solution for the same periods, p-value 0.3 to 0.9 (non significant) (Table 5-10).

Table 2: Effect of oral administration of PM 701 (0.35 g kg^{-1}) on the liver weight of animal compared with controls

Experiments	1st week	2nd week	4th week
Control	43.437±1.526	39.695±1.157	43.40±0.189
Test	40.660±3.016	38.249±0.677	40.63±0.422
p-value	0.20	0.59	0.2

p-value 0.2 to 0.5

Table 3: Effect of oral administration of PM 701 (0.35 g kg^{-1}) on the kidney weight of animal compared with controls

Experiments	1st week	2nd week	4th week
Control	3.18±0.1	6.948±2.612	3.48±0.18
Test	3.4±0.2	5.686±0.705	3.27±0.34
p-value	0.5	0.198	0.25

p-value ≠ 0.2

Table 4a: Effect of oral administration of PM 701 (0.35 g kg^{-1}) on the heart weight of animal compared with controls

Experiments	1st week	2nd week	4th week
Control	3.702±0.345	4.540±18.807	3.39±0.22
Test	3.912±0.420	4.227±0.35	3.32±0.301
p-value	0.5	0.32	0.70

p-value 0.3 to 0.7

Table 4b: Effect of oral administration of PM 701 (0.35 g kg^{-1}) on the spleen weight of animal compared with controls

Experiments	1st week	2nd week	4th week
Control	2.056±0.221	1.887±0.207	2.04±0.29
Test	2.067±0.746	1.785±0.2111	1.81±0.21
p-value	0.9	0.404	0.23

p-value 0.2 to 0.9

Table 5: Effect of oral administration of PM 701 on kidney function (BUN) analysis showed no significant differences between treated and non treated animals

Experiments	1st week	2nd week	4th week
Control	7.8250±0.55000	7.4000±0.78740	8.15±0.2
Test	8.0333±0.61536	8.9200±0.95499	7.7±0.8
p-value	0.6	0.3	0.3

p-value 0.3 to 0.6

Table 6: Effect of oral administration of PM 701 on Kidney function (creatinine) analysis showed no significant differences between treated and non treated animal's levels

Experiments	1st week	2nd week	4th week
Control	36.5000±1.73205	24.5000±10.34408	27.75±6.2
Test	32.6667±3.88158	25.0000±6.20484	32.3±7.34
p-value	0.9	0.9	0.32

p-value 0.3 to 0.9

Effect of PM 701 on serum electrolytes: There were no significant differences in serum electrolytes (Na, p- value 0.2 to 0.5; K, p-value 0.2 to 0.4 and Cl p-value 0.3 to 0.7 in rats treated with PM 701 for 1, 2 and 4 weeks and rats treated with equivalent volumes of control solution for the same periods (Table 7, Fig. 1a, b).

Effect of PM 701 on liver function test: Rats treated with PM 701 for 1-4 weeks showed no significant increase in alkaline phosphatase (Alk.Phos), p-value 0.08 to 0.5, during the period of treatment compared with that of rats treated with equivalent volumes of control solution. On

Table 7: Effect of oral administration of PM 701 (0.35 g kg^{-1}) on serum electrolytes (Cl level) of animal compared with controls: Cl levels

Experiments	1st week	2nd week	4th week
Control	100.0000±1.15470	100.2500±1.25831	100.25±0.47
Test	100.5000±1.151658	100.0000±1.00000	101.2±0.81
p-value	000.5	000.749	000.33

p-value 0.3 to 0.7

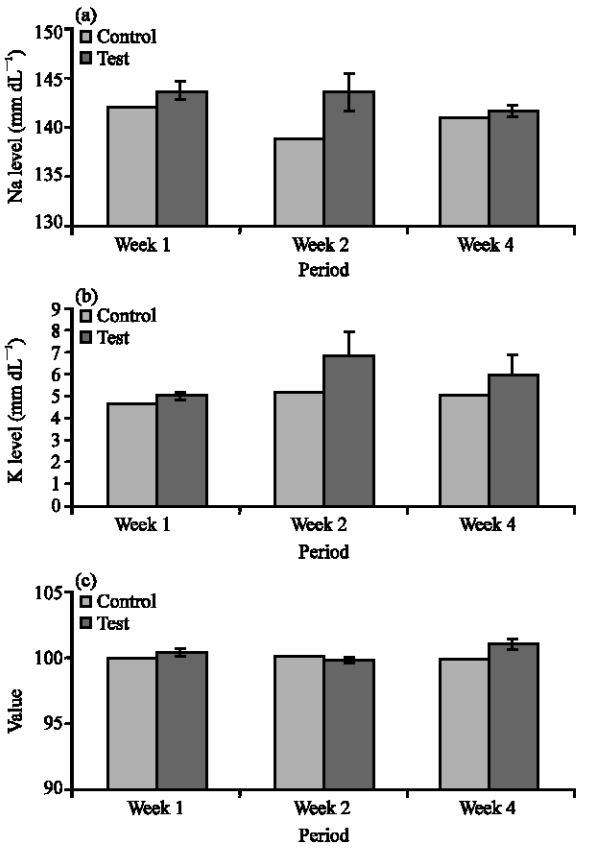

Fig. 1: Effect of oral administration of PM 701 (0.35 g kg^{-1}) on serum electrolytes (Na, K and Cl level) of animal compared with controls: (a) Na p-value 0.2 to 0.5 (b) K p-value 0.2 to 0.4 and (c) Cl levels p-value 0.3 to 0.7

the other hand, treatment with PM 701 showed 50% and 10% increase in alanine transaminase (SGPT) activity in the first and fourth weeks after PM 701 administration, (p-value 0.01 and 0.006), respectively, The aspartate transaminase (SGOT) level also increased in week one p-value 0.9 and week four after PM 701 administration, p-value 0.001 (Table 11-13).

Effect of PM 701 on blood components: There were no changes seen on Hemoglobin level (Hb), Red blood cells (RBC's), White blood Cells (WBC's) and Platelets in rats treated with PM 701 compared with that of rats treated with equivalent volumes of control solution), p-value 0.18 to 0.81 (Table 14a-b).

Interaction with barbiturate: Co-administration of PM 701 with barbiturate in rats slightly increases the onset time of barbiturate induced sleep. Yet, the recovery time was prolonged by 26.5% compared with that of rats treated with equivalent volumes of control solution.

Respiratory and cardiovascular system: Preliminary observation showed no adverse effects on respiratory and cardiovascular systems. Further experimentation is required to exclude the possibility of aspiration pneumonia due to tubular feeding and also to assess the QT interval according ICH S7B.

Acute dermal toxicity

Practically non toxic: Intradermal administration of PM 701 showed no signs of sensitivity to PM 701.

Reproduction: Initial observation on rat's males and females treated with PM 701 showed no signs of adverse effects on reproductive function. Experiments on progress to confirm effects on reproductive function in parent rats and their progeny.

Table 8: Effect of continuous treatment of PM 701 for 7 days on the kidney function and electrolytes of normal Swiss albino rats

Test	Na	K	Cl	BUN	CREN
Control	142.5000	4.7750	100.0000	7.8250	36.5000
	±2.08167	±0.12583	±1.15470	±0.55000	±1.73205
Test	143.5000	4.9833	100.5000	8.0333	32.6667
	±3.44964	±.44008	±1.51658	±.61536	±3.88158
p-value	0.5	0.3	0.5	0.6	0.9

Normal Swiss albino rats have been injected with 0.35 g kg^{-1} PM 701 for 7 successive days and on the 8th day, animal were anesthetized and blood withdrawn, serum was separated and kidney function

Table 9: Effect of continuous treatment with PM 701 for 14 days on kidney function and electrolytes of normal Swiss albino rats

Test	Na	K	Cl	BUN	CREN
Control	139.7500	4.8500	100.2500	7.4000	24.5000
	±1.50000	±1.06615	±1.25831	±0.78740	±10.34408
Test	143.0000	65.5600	100.0000	8.9200	25.0000
	±3.93700	±136.08741	±1.00000	±0.95499	±6.20484
p-value	0.166	0.408	0.749	0.3	0.9

Normal Swiss albino rats have been injected with 0.35 g kg^{-1} PM 701 for 14 successive days and on the 15th day, animal were anesthetized, blood withdrawn, serum was separated and kidney function

Table 10: Effect of continuous treatment for 28 days with PM 701 on the kidney function and electrolyte of normal albino rats

Test	Na	K	Cl	BUN	CREN
Control	141.50±1.3	5.0±0.22	100.25±0.47	8.15±0.20	27.75±6.20
Test	142.10±1.5	6.2±1.80	101.20±0.810	7.70±0.80	32.3±7.34
P-value	0.54	0.2	0.33	0.3	0.32

Normal Swiss albino rats have been injected with 0.35 g kg^{-1} PM 701 for 28 successive days and on the 29th day, animal were anesthetized, blood withdrawn, serum was separated and kidney function

Table 11: Effect of 7 days successive treatment with PM 701 on Liver function of normal albino rats

Test	ALP	SGOT	SGPT	Bill
Control	220.5000	113.5000	62.7500	1.5000
	±56.59505	±16.21727	±4.27200	±0.57735
Test	248.6667	138.0000	72.1667	1.8333
	±49.06187	±21.26970	±5.30723	±0.75277
p-value	0.4	0.08	0.01	0.4

Normal Swiss albino rats have been injected with 0.35 g kg^{-1} PM 701 for 7 successive days and on the 8th day, animal were anesthetized, blood withdrawn, serum was separated and liver function

Table 12: Effect of continuous treatment fro 14 days with PM 701 on the liver function of normal Swiss albino rats

Test	ALP	SGOT	SGPT	Bill
Control	250.5000	197.0000	14.72809	1.5000
	±31.04298	±103.59858	±7.36405	±1.00000
Test	264.8000	185.4000	57.36114	1.0000
	±41.20316	±156.20595	±25.65268	±0.70711
p-value	0.5	0.9	0.005	0.4

Normal Swiss albino rats have been injected with 0.35 g kg^{-1} PM 701 for 14 successive days and on the 15th day, animal were anesthetized and blood withdrawn, serum was separated and liver function

Table 13: Effect of continuous treatment with PM 701 for 28 days on the liver function of normal Swiss albino rats

Test	ALP	SGOT	SGPT	Bill
Control	218.25±24.7	169.75±12.7	73.25±5.4	1.0±0.00
Test	179.3±37.95	310.2±57.44	112.8±22.8	0.8±0.41
p-value	0.08	0.001	0.006	0.4

Normal Swiss albino rats have been injected with 0.35 g kg^{-1} PM 701 for 28 successive days and on the 29th day, animal were anesthetized and blood withdrawn, serum was separated and liver function

Table 14a: Effect of continuous treatment for 28 days with PM 701 on blood components

Type	WBC	NE	LY	MO	EO	RBC
Control	7.75	2.85	95.2	0.35	0.6	7.225
	±3.87	±4.97	±6.6	±0.25	±0.34	±0.25
Test	10.72	5.32	92.86	1.6	0.8	7.42
	±5.34	±5.8	±7.69	±2.21	±1.24	±0.37
p-value	0.38	0.52	0.65	0.33	0.7	0.39

Table 14b: Effect of continuous treatment for 28 days with PM 701 on blood components

Type	HGB	HCT	MCV	MCH	MCHC	PLT
Control	14.1	40.75	56.475	19.5	34.55	1131.75
	±0.13	±0.53	±1.42	±0.53	±0.17	±182.36
Test	14.5	41.68	56.16	19.58	34.9	1026.2
	±0.59	±2.25	±2.18	±0.64	±0.65	±206.17
P value	0.18	0.45	0.81	0.8	0.38	0.49

Normal Swiss albino rats have been injected with 0.35 g kg^{-1} PM 701 for 28 successive days and on the 29th day, animal were anesthetized and blood withdrawn on heparin and blood components were measured

Effects of PM 701 on gastro intestinal tract: Oral administration of PM 701 into 24 h fasting rats, has no effect on the gastric mucosa, no sign of ulceration or congestion were observed.

Gastro intestinal tract motility of rats injected with PM 701 IP showed 40% lower gastro intestinal tract motility compared with that of rats treated with equivalent volumes of control solution.

Histology examination: Most of the organs examined for histological changes showed no observable significant differences compared with that of control except testes.

Testicular lesions were seen in some of the tested rats. This lesion characterized by marked decrease of semini ferrous tubules containing mature sperms. The lumens of many tubules were full of non nucleated acidophilic elongated bodies. According to the pathologist the lesion seemed to be characteristic of apoptosis, as most cells appeared shrunken and showed chromatin condensation. Interstitial cell appeared normal, yet some exhibited highly acidophilic cytoplasm. Occasionally some blood vessels are congested with an increase in inter lobular tissue spaces. The same observations were seen in rats that treated with control solution and non treated rats from the same animal house.

No effect on gastric mucosa ,the lining epithelium showed no signs of inflammation or ulceration, mucopolysacharides content of surface mucous cells was also normal (Fig 2a, b).

On liver, insignificant changes were observed in the form of dilation and congestion of blood vessels, lymphocyte aggregation. No signs of necrosis or hepatocytes damage were observed. Only few cells showed enlargement and degenerative changes but this was within normal turnover rate of hepatocytes, most cells although looked shrunken, slightly dark, still keep its membranous integrity and vesicular active appearance of nuclei (Fig. 3a-f).

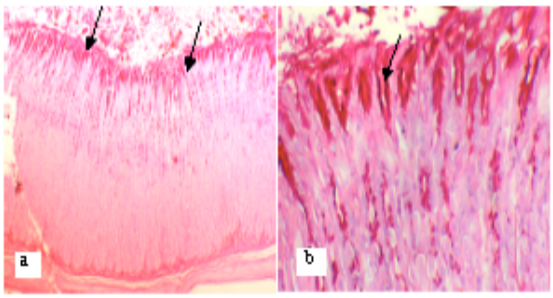

Fig. 2: (a) Effect of oral administration of PM 701 (0.35 g kg^{-1} every day for four weeks) on gastric mucosa and on cellular integrity or mucopolysacharides content of surface epithelium of stomach mucosa (arrow, X 100) and (b) Magnified part to show normal distribution of Mucopolysaccharides of gastric surface epithelium (arrow) in treated animal for four weeks (a and b with PAS stain, X400)

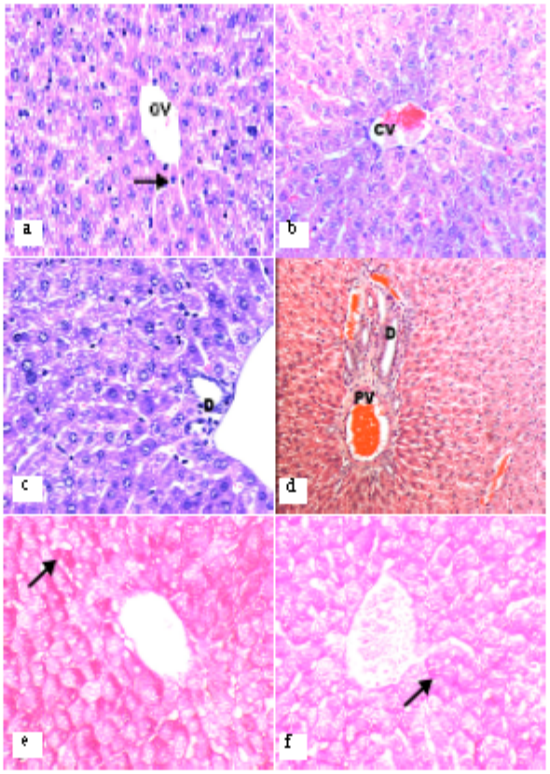

Fig. 3: Effect of oral administration of PM 701 (0.35 g kg^{-1} every day for four weeks) on the liver structure: (a) Control rat liver showing central veins (CV) and normal hepatocytes (arrow), (b) no marked changes in hepatocytes only slight congestion of central veins (CV) in the liver of treated group with PM 701 for four weeks,(c) control hepatocytes near portal area, showed small bile duct (D), (d) slight congestion of portal vessels (PV) and bile duct proliferation (D) in the liver of treated group with PM 701 for four weeks(a, b, c and d with HE stain , a, b, c X400 and d X200), (e) glycogen distribution in hepatocytes of control rat's liver around the central vein (arrow) and (f) more or less normal glycogen distribution (arrow) in hepatocytes of treated group with PM 701 for one week (e and f with PAS stain X400)

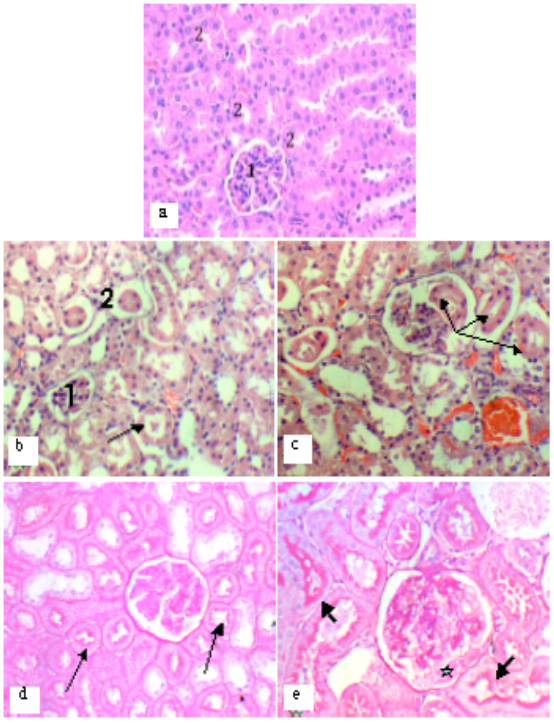

Fig. 4: Effect of oral administration of PM 701 (0.35 g kg^{-1} every day for four weeks) on the kidney structure: (a) Control rat kidney showing (1) normal renal corpuscles and (2) tubules, (b) kidney of treated animal with PM 701 for four weeks showing (1) mild focal changes in the form of atrophy and lobulation of glomerular tufts, (2) shrinkage and apoptosis (dark stained cytoplasm and dark pyknotic nuclei were observed in PCT which become surrounded by halo spaces. DCT Showed necrosis of the lining cells, widening of the lumen and reticulated casts (arrow), (c) kidney of treated animal with PM 701 for four weeks showing hypertrophy of renal corpuscle, degenerated renal tubules within Bowmen space (arrow). Mild vascular congestion was observed (a, b and c with HE stain, X400), (d) control animal kidney showed normal distribution of PAS+V material in the basement membrane and brush border of lining epithelium of PCT (arrow) (PAS, X100) and (e) Kidney of treated animal with PM 701 for four weeks showing increase PAS +V material in renal corpuscle (star) and tubular brush border (arrow) (PAS stain, X400)

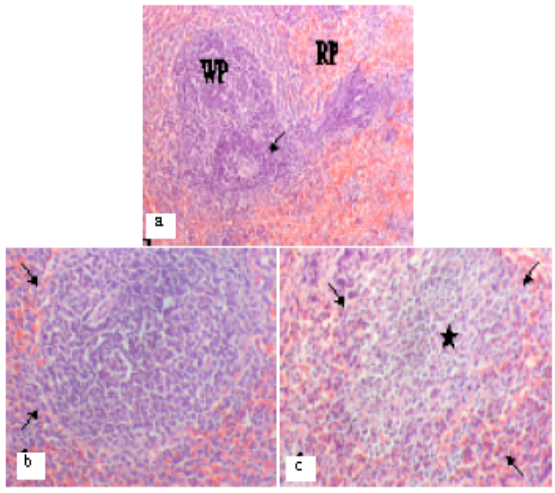

Fig. 5: Effect of oral administration of PM 701 (0.35 g kg^{-1} every day for four weeks) on the spleen structure: (a) control rat spleen shows white pulp (WP), with the central arteriole (arrow) within the lymphoid follicle. The red pulp (RP) contains numerous red corpuscles, (b) treated group with PM 701 for four weeks showing enlargement of white pulp(arrow) and (c) treated group with PM 701 for four weeks showing the increase size of germinal center (star) of white pulp (arrows) (HE stain, X400 for a, b and c)

On kidney, the changes were focal and could be considered insignificant. There was shrinkage of some proximal tubules, dilation of distal tubules, some showed degenerative changes of lining epithelium hypertrophy of renal corpuscles, slight vascular congestion (Fig. 4a-e).

On spleen, we notice the increase of the size of white pulp and enlargement of germinal canters of lymphoid nodules (Fig. 5a-c).

On the lung, focal changes in the form of inflammatory exudates were observed in some specimens also thickening of arteriolar walls accompanied bronchial tree was observed. The focal lymphocyte infiltration and hypertrophy of bronchiolar muscular layer in some animals required further experimentation to exclude the possibility of aspiration pneumonia due to tubular feeding (Fig. 6a-d).

Administration of PM 701 for 4 weeks had no marked effect on most body organs and could be considered safe compared to many chemical drugs used as antibiotics or cytotoxic medications. Apart from some testicular lesions that were observed even in animals treated with control solution and non treated animals from the same animal house. This may indicate another problem in the animal house is not related to our reagent.

PM 701 treatment showed slight increase in both AST (SGOT) and ALT (SGPT) in the first and 4th week. Although ALT is commonly used as a way of screening

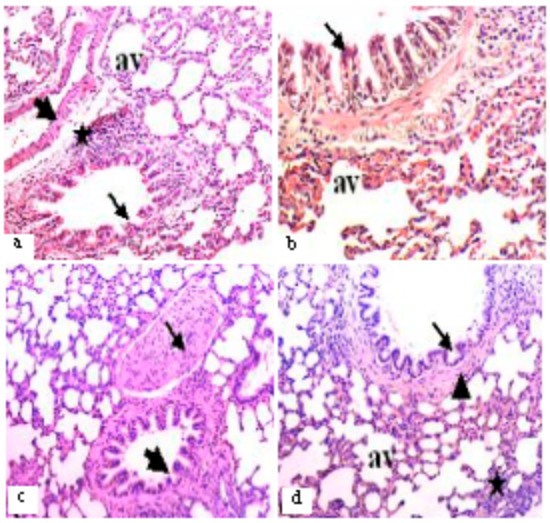

Fig. 6: Effect of oral administration of PM 701 (0.35 g kg^{-1}) on the lung structure: (a) Control rat lung parenchyma show a bronchiole with normal mucosal folds (arrow), thin muscle layer (thick arrow) and normal lymphoid aggregation (star). The accompanied blood vessel is of normal thickness. Most alveoli (av) are patent and separated by thin septa (HE stain, X100), (b) Magnified part of control animal to show detailed structure of normal bronchiole (arrow) and alveoli (av) (HE stain, X200), (c) Part of Lung of treated rat with PM 701 for four weeks showing slight decrease in bronchial folds (thick arrow), thickening of muscle layer in bronchioles and marked thickening of vascular wall (arrow) (HE stain, X100) and (d) Part of treated animals with PM 701 for four weeks showing slight thick bronchial wall, normal alveoli (av) and slight interalveolar cellular infiltrate (star) (HE stain, X100).

for liver function problems. It does not commonly mean that medical problems exist. It has been reported by Rosenzweig *et al.* (1999) that during drug development, ALT elevation to levels above the upper limit of normal can occur in subjects treated with placebo. The composition of the diet may play a role, Porikos and Van Itallie (1983) showed that combination of excess calories and a high sucrose intake was associated with enzyme elevation. In conclusion, the laboratory safe results of phase I trials or in experimental animals should be interpreted with caution to avoid premature discontinuation of development of NCEs (New chemical entities) wrongly believed to be hepatotoxic based on tansaminase elevation due to nondrug-related causes, such as experimental condition. Moreover, histopathological examination did not show significant changes which may support the above mentioned explanation.

The dilation and congestion of blood vessels, lymphocyte aggregation, in the liver could be good signs that for bringing more antibodies and active immune competent lymphocytes to deal with any viral infection in the vicinity of liver parenchyma. The changes in the kidney architecture were minimal compared to other cytotoxic or nephrotoxic drugs and could be avoided by modifying the dose or increase water intake. The increase of spleen weight after four weeks of PM 701 administration is consistent with the histological examination that indicate the increase of white pulp in the spleen, the part containing both T and B lymphocytes, enlargement of germinal centers of lymphoid nodules, are linked with their well known role in both humeral and cellular immunity. The histological changes in the lungs were most probably due to aspiration of the substance during tubular feeding and chemical irritations.

All the Preclinical toxicological tests conducted in this study showed no toxicity of PM 701, which may nominate this agent as non-toxic material. This may facilitate its entrance into clinical trial, which involve hundreds or thousands of patients at many centers.

CONCLUSION

From the present results, one could conclude that, PM 701 at the circumstance of the experiment is practically non toxic and had no significant observed adverse effects. Accordingly this tested material which is used in human non conventionally for years in treatment of many disease without significant harmful effects, is considerable interest for the purpose of the discovery of additional novel natural product with potentially anticancer activity which deserve to early phase I clinical trail on limited healthy volunteers to establish its kinetics. Although the effectiveness of PM 701 as therapy for certain diseases was beyond the scope of this work, we suggest that, the substance should be fractioned in order to search for active principle (S) and its safety should be redetermined to draw the last firm conclusion.

ACKNOWLEDGMENTS

The author gratefully acknowledged financial support of The Scientific Research Council,

King Abdulaziz University for Grant No. 001/427 and 2N0/ 427, I also would like to thank Prof. Mansor Soliman Prof, of Clinical Pharmacology, KAU for revising this study. Prof. Abdel-moneim Osman Prof of Clinical Pharmacology for helpful suggestions during the course of this study; Dr. Ahmed Shaker for the pharmaceutical advices for formulation and Dr. Soad Shaker for histopathology study.

REFERENCES

Al-Rawais, N.A., 2002. Herbal medicine in the treatment of diabetes mellitus. Saudi Med. J., 23: 1327-1331.

Amirghofran, Z., M. Bahmani, A. Azadmehr and K. Javidnia, 2006a. Anticancer effects of various Iranian native medicinal plants on human tumor cell lines. Neoplasma, 53: 428-433.

Amirghofran, Z., M. Bahmani, A. Azadmehr and K. Javidnia, 2006b. Induction of apoptosis in leukemia cell lines by *Linum persicum* and *Euphorbia cheiradenia*. J. Cancer Res. Clin. Oncol., 132: 427-432.

Butler, J.M., 2004. Short tandem repeat analysis for human identity testing. Curr. Protoc. Hum. Genet., Chapter 14: Unit 14.8-14.8.

Cai, Y., Q. Luo, M. Sun and H. Corke, 2004. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci., 74: 2157-2184.

Charlson, A.J., 1980. Antineoplastic constituents of some Southern African plants. J. Ethnopharmacol., 2: 323-335.

Crown, J., 1998. Evaluation in the treatment of advanced breast cancer. Semin. Oncol., 12: 12-17.

Eisenberg, D.M., R.C. Kessler, C. Foster, F.E. Norlock, D.R. Ca1kins and T.L. Delbanco, 1993. Unconventional medicine in the United States: Prevalence, cost and pattems of use. N. Engl. J. Med., 328: 246-252.

Fisher, P. and A. Ward, 1994. Complementary medicine in Europe. Br. Med. J., 309: 107-111.

Freireich, R.J., D.P. Gehan, L.H. Rall and H.E. Skipper, 1966. Quantitative comparison of toxicity of anticancer agents in mouse, rats, hamster, dog, monkey and man. Cancer Chemother. Rep., 50: 219-244.

Gonzales, G.F. and L.G. Valerio, 2006. Medicinal plants from Peru: A review of plants as potential agents against cancer. Anticancer Agents Med. Chem., 6: 429-444.

Grever, M. and B. Chabner, 1997. Cancer drug discovery and development in cancer propels and practice of oncology. Cytotoxicity of some medicinal plant extracts used in Tanzanian traditional medicine. J. Ethnopharmacol., 70: 143-149.

Irwin, S., 1968. Comprehensive observational assessment: 1a. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. Psychopharmacol. Berl, 13: 222-257.

Khorshid, F.A., S.S. Mushref and N.T. Heffny, 2005. An ideal selective anti-cancer agent *in vitro* I-tissue culture study of human lung cancer cells A549. J. KAU Med. Sci., 12: 3-18.

Khorshid, F.A. and S.S. Moshref, 2006. *In vitro* anticancer agent, I-tissue culture study of human lung cancer cells A549 II-tissue culture study of mice leukemia cells L1210. Int. J. Cancer Res., 2: 330-344.

Maclennan, A.H., D.H. Wilson and A.W. Taylor, 1996. Prevalence and cost of alternative medicine in Australia. Lancet, 347: 569-573.

Malairajan, P., G. Geetha, S. Narasimhan and K.J. Jessi, 2006. Analgesic activity of some Indian medicinal plants. Ethnopharmacology, 106: 425-428.

Miller, LC. and M.L. Tainter, 1944. Estimation of LD_{50} and its error means of logarithmic probit graph paper. Proc. Soc. Exp. Biol. Med., 57: 261-254.

Moshref, S.S., F.A. Khorshid and Y. Jamal, 2006. The effect of PM 701 on mice leukemic cells: I-tissue culture study of L1210 (*in vitro*) II - *in vivo* study on mice. J., KAU Med. Sci., 131: 3-19.

Porikos, K.P. and T.B. Van Itallie, 1983. Diet-induced changes in serum transaminase and triglyceride levels in healthy adult men. Role of sucrose and excess calories. Am. J. Med., 75: 624-630.

Rosenzweig, P., N. Miget and S. Brohier, 1999. Transaminase elevation on placebo during phase I trials: Prevalence and significance. Br. J. Clin. Pharmacol., 48: 19-23.

Saha, U.K., C. Liu, L.M. Kozak and P.M. Huang, 2004. Kinetics of selenite adsorption on hydroxyaluminum- and hydroxyaluminosilicate-montmorillonite complexes. Soil Sci. Soc. Am. J., 68: 1197-1209.

Salman, S. and A. Sartorlli, 2001. Cancer Chemotherapy. In: Basic and Clinical Pharmacology. 8th Edn. Katzang, B. (Ed.). Appleton and Large Publishers, USA., pp: 923-930.

Shoemaker, M., B. Hamilton, S.H. Dairkee, I. Cohen and M.J. Campbell, 2005. *In vitro* anticancer activity of twelve Chinese medicinal herbs. Phytother. Res., 19: 649-651.

Thole, J.M., T.F. Kraft, L.A. Sueiro, Y.H. Kang and J.J. Gills *et al*., 2006. Comparative evaluation of the anticancer properties of European and American Elderberry fruits. J. Med. Food Winter, 9: 498-504.

Wang, J., Q. Li, G. Ivanochko and Y. Huang, 2006. Anticancer effect of extracts from a North American medicinal plant-wild sarsaparilla. Anticancer Res., 26: 2157-2164.

Yang, H., G. Shi and Q.P. Dou, 2007. The tumor proteasome is a primary target for the natural anticancer compound withaferin a isolated from Indian Winter Cherry. Mol. Pharmacol., 71: 426-437.

Zhao, C. and C. He, 2006. Preparative isolation and purification of atractylon and atractylenolide III from the Chinese medicinal plant atractylodes macrocephala by high-speed counter-current chromatography. J. Sep. Sci., 29: 1630-1636.