Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 5, Issue 4, 2012 ISSN - 0974-2441

Research Article

ACUTE AND SUBACUTE TOXICITY STUDIES OF *KODI PAVALA CHUNNAM* IN RODENTS

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Received:18 April 2012, Revised and Accepted:20 June 2012

ABSTRACT

Kodi Pavala Chunnam (KPC) a Siddha formulation which have so many pharmacological activities. The study was designed according to the OECD guidelines as per that the dose level used as 5, 50, 300, 2000, 4000 mg/kg. The aim of the present investigation was to evaluate acute and subacute toxicity of the KPC on wistar rats at the dose levels of 100, 200 and 400 mg/kg was fixed for study. The different sets of animal used for 28 days and results from the present study have elucidated that treatment of KPC exerts no significant signs of toxicity at any dose level used in the study. Physical, Biochemical, Hematological as well as histopathological parameters were unaltered throughout the study. The results of study have suggested there was no obvious toxicity observed with the treatment of KPC.

Keywords: Histopathology; Hematology; Kodi pavala chunnam; OECD guidelines.

INTRODUCTION

Among the system of medicine practiced all over the world the Siddha system in undoubtedly, the ancient and transcending system in many centuries Siddha system of medicine is a gift of the mankind by the '*Siddhars*' who were the greatest scientists in ancient times. Siddha system of medicine (SSM) is one such ancient traditional system of India and practiced mostly in its southern part for treating various diseases including even chronic conditions.[1] Siddha system is an impressive and ancient Indian medical system, which, historically, was not popularized due to the secrecy maintained by the *Siddharkal*. Medicine as everyone knows is not merely a science but an art as well. The traditional system of medicine became significantly more popular all over the globe because of the less toxic and has no side effects. The mode of preparation and plant used in traditional medicine varies from place to place and in addition acceptance of traditional medicines in development world is sharply increasing. [2, 3, 4]

The Siddha system of medicine is one of the oldest in India. The term '*Siddha*' means achievement and the *Siddharkal* were saintly figures that have contributed to the development of this medical system. In particular the marine environment provides a remarkable source of many animal species including corals, which is used in Siddha medicine. [5] The therapeutic uses of corals were firstly cited as medicine in ancient Indian Ayurvedic literature. KPC according to the *Siddharkal*, the human body (microcosm) is a replica of the universe (macrocosm), and so are foods or drugs irrespective of their origin. The *Siddharkal*, through enumeration, implied that the herbs and minerals are used as 'special foods', serving to eliminate excesses and to strengthen deficiencies. They have a powerful nutritive impact on a weakened body and their primary action is to stimulate particular organic functions, thereby acting more effectively than normal food. Siddha medicine uses an extensive pharmacopoeia that includes botanical, animal, mineral and metallic preparations.

In Siddha system of medicine, the drug sources are mainly obtained from plants, animal products, minerals and metals. Coral reefs are one of the oldest and largest living ecosystem on earth, similar marine communities have existed for hundreds of thousands of years. Coral reefs are storehouses of genetic resources with vast medicinal potential. The rocky frame work of coral reef is formed by polyps, which secrete calcium carbonate deposited mainly by calcareous algae and the stony corals. Precious coral or red coral are called *Corallium rubrum,* (Coralliidae) a word that is derived from Latin word related to Greek word *Koralliom*. Red coral grows on cave walls, vertical cliffs and overhangs at 10 to 200 m depth. [6] These writings, preserved in codified poems and oral transmission, are a KPC accumulation of knowledge about the pharmacology and ecology of herbal, animal and mineral medicines. The few studies available depict red coral as a long-lived species (decades) with a slow growth rate. [7, 8] Hence, in this present study, an attempt was made systematically to study the toxicity profile of the Siddha drug.

MATERIALS & METHODS

Animals

Male wistar rats weighing about 200-220gms were selected and kept under standard laboratory conditions. The animals were allowed free access to standard pellet diet and water *ad libitum*. The blood samples were drawn after application of topical lignocaine anaesthesia to minimize pain to the animals. The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for KPC lemmatization to the laboratory conditions. This study protocol was approved by the Institutional Animal Ethics Committee (IAEC).

Acute toxicity studies

The Acute toxicity study of KPC was evaluated in rats as per the OECD guide line 423. [9] It is the principle of the test that based on stepwise procedure with the use of the minimum number of animal per step. Three animals were used for each step. The dose level of 300 to 4000 mg/kg body weight was administered stepwise. Observations were made and recorded systematically and continuously observed as per the guideline after substance administration. The number of survivors was noted after 24 hours and these animals were then maintained for a further 14 days and observations made daily. Since there is inadequate information on the test substance, hence for animal welfare reasons the starting dose of 300mg/kg body weight was selected. The time interval between treatment groups is determined by the onset, duration, and severity of toxic signs.

Justification for Dose Selection

As stated in results of acute toxicity studies in wistar rats indicated that *KPC* was nontoxic up to the maximum dose level of 4000 mg/kg body weight but toxic symptoms was observed after 48 hours of oral drug treatment at the dose level of 2000 mg/kg. On the basis of these results, the doses selected for the study was 100 mg/kg, 200 mg/kg and 400 mg/kg body weight. The oral route was selected for use because oral route is considered to be a proposed therapeutic route.

Preparation and administration of dose

KPC was suspended in 2% CMC in distilled water to obtain concentrations of 200 mg/ml. It was administered to animals at the dose levels of 100 mg/kg, 200 mg/kg and 400 mg/kg in the dose volume of 10 mL/kg. The test substance suspensions were freshly prepared every two days once for 28 days. The control animals were administered vehicle only. Administration was by oral (gavage), once daily for 28 consecutive days.

Repeated dose oral toxicity study

Repeated dose oral toxicity studies were conducted as per OECD guideline 407 on four groups of rats. Each group was containing 6 rats. Of these were 3 males and 3 females. The test substance suspensions were freshly prepared every 2 days once for 28 days. The control animals were administered vehicle only. *KPC* suspended in 2% CMC with vigorous mixing and was administered to the groups of wistar rats in a single oral dose by gavage using a feeding needle. Animals were fasted prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously observed as per the guideline after substance administration. The visual observations included skin changes, mobility, aggressiveness, sensitivity to sound and pain, as well as respiratory movements. They were deprived of food, but not water 16–18 h prior to the administration of the test suspension.

Finally, the number of survivors was noted after 24 h and these animals were then maintained for a further 28 days and observations made daily. The toxicological effect was assessed on the basis of mortality. Administration was by oral gavage once daily for 28 consecutive days. Experimental animals were kept under observations throughout the course of study for the following: Clinical signs and mortality, body weight, food and water consumption; Hematological parameters were determined using Hematology analyzer; Bio chemical parameters were determined using auto analyzer; Gross necropsy: All the animals were sacrificed on day 29. Necropsy of all animals was carried out and the weights of the organs including Liver, Spleen, Kidney, Brain, Lung, Pancreas, Heart, Stomach, Testis were recorded; Histopathology: Liver, Spleen, Kidney, Brain, Lung, Pancreas, Heart, Stomach, Testis were fixed in 10% formalin for routine histopathological examination. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50 ^{0}C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with haematoxylin and eosin.

Statistical analysis

All the values are expressed as mean ± S.E.M. The data were statistically analyzed by one-way ANOVA followed by Dunnet-t test. P values < 0.05 were considered significant.

RESULTS

Acute toxicity studies

At the dose level of 2000 mg/ kg and 4000 mg/kg 2-2 animals were died. And behavioral changes observed at every dose level and bodyweight was increased (Table 1).

Repeated dose oral toxicity study

Body weight, Food and water consumption

Body weight was gain (Table 2) and Food (Table 3) and water consumption (Table 4) was found to be normal throughout the dosing period of 28 days when compared the treatment groups with control.

Table 1: Dose finding experiment and its behavioral Signs of Toxicity

No	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	300	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	2000	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	+	+
3	4000	+	-	-	+	+	+	-	+	-	-	-	-	-	+	-	+	+	-	+	+

1. Alertness 2. Aggressiveness 3. Piloerection 4. Grooming 5. Gripping 6. Touch Response 7. Decreased Motor Activity 8. Tremors 9. Convulsions 10. Muscle Spasm 11. Catatonia 12. Muscle relaxant 13. Hypnosis 14. Analgesia 15.Lacrimation 16. Exophthalmos 17. Diarrhoea 18. Writhing 19. Respiration 20. Mortality

Table 2: Body wt (g) of albino rats exposed to *KPC* for 28days.

Dose (mg/kg/day)	Days				
	1	7	14	21	28
Control	112.13±5.26	115.87±5.18	118.10±6.25	119.14±6.10	120.17±8.74
100	114.10±6.12	121.32±8.24	123.15±7.36	125.41±10.15	127.12±11.00
200	112.42±5.18	114.52±10.51	116±10.04	115.10±8.56	118.05±8.44
400	116.21±8.21	118.22±9.24	121.45±10.69	120.08±7.88	122.00±6.82

Values are MEAN ± S.E.M. (Dunnett test).where N=6 and ^{ns}P>0.05 Vs control group.

Table 3: Food (g/day) intake of albino rats exposed to *KPC* for 28days.

Dose (mg/kg/day)	Days(gm/rats)				
	1	7	14	21	28
Control	41.15±2.77	40.20±2.56	38.85±3.18	42.10±2.59	39.15±4.18
100	38.75±3.54	39.14±2.12	39.30±2.32	41.40±2.64	40.37±2.45
200	40.19±3.10	38.25±2.36	39.10±2.48	44.98±2.88	42.98±2.61
400	43.41±4.55	40.19±2.78	40.00±2.48	40.72±4.26	41.61±2.87

Values are MEAN ± S.E.M. (Dunnet 't' test). Where N=6; ^{ns}P>0.05 Vs control group.

Table 4: Water (ml/day) intake of male and female albino rats exposed to *KPC* for 28days.

Dose (mg/kg/day)	Days (ml/rat)				
	1	7	14	21	28
Control	44.19±3.41	46.41±2.45	45.78±2.59	42.41±2.24	42.08±3.27
100	38.28±3.20	42.56±3.27	45.17±2.57	44.20±3.15	46.46±3.54
200	40.19±2.17	42.46±3.88	42.30±3.09	44.98±2.08	44.28±3.31
400	42.40±2.74	45.66±2.32	41.18±3.46	45.24±2.98	42.14±2.88

Values are MEAN ± S.E.M. (Dunnett's test). Where N=6; ^{ns}P>0.05 Vs control group Hematological investigations

Table 5:Hematological parameters after 28days treatment with *KPC* in rats.

Parameter	Control	100 mg/kg	200 mg/kg	400 mg/kg
Red blood cell (mm^3)	7.40±0.16	9.04 ± 0.30	8.095 ± 0.41	8.81 ± 0.17
HB (%)	15.20±0.41	15.00±0.36	15.10±0.68	15.9±0.68
Leukocyte ($x10^6$/mL)	8139±126.53	7550 ± 2.192	8730 ±2.493	7422 ± 1.689
Platelets ($10^5/mm^3$)	3.014 ± 0.288	3.022 ± 0.132	2.988 ± 0.146	2.868 ± 0.191
MCV (gl)	59.77±4.46	55.42±3.74	54.28±2.12	54.20±2.90
N	55.56 ± 1.234	57.11 ± 0.69	57.95 ± 1.362	60.51 ± 2.3
L	39.72 ± 0.70	37.37 ± 0.97	37.90 ± 0.14	38.87 ± 0.21
M	6.0±0.48	5.2±0.40	4.37±1.10	2.46±0.42
E	0.49 ± 0.028	0.508 ± 0.023	0.576 ± 0.035	0.47 ± 0.022
B	0	0	0	0
ESR(mm)	1±00	1±00	1±00	1±00
PCV	47.11±1.80	46.13 ± 4.38	48.1 ± 1.42	47.47 ± 2.39
MCH pg	17.28±0.40	18.40±1.42	19.60±0.38	19.60±0.34
MCHC g/dl	32.54±0.90	33.02±0.46	32.24±1.20	31.54±0.47

Values are MEAN ± S.E.M. (Dunnett's test). N=6; $^{ns}P>0.05$ Vs control group.

The results of haematological investigations (Table 5) conducted on day 29, revealed following significant changes in the values of different parameters investigated when compared with those of respective controls. However, the increase or decrease in the values obtained was within normal biological and laboratory limits or the effect was not dose dependent. No major changes in the values of MCHC were observed for animals in all the dose groups. Similarly slight decrease in total WBC count and MCV values were obtained for animals in the dose group of 100 and 400 mg/kg. Increase in MCH values were obtained for animals in dose groups administered 200 and 400mg/kg group sacrificed on day 29.

Table 6: Effect of *KPC* treatment on biochemical parameters.

Dose (mg/kg)	Control	100 mg/kg	200 mg/kg	400 mg/kg
Total Bilirubin (mg/dL)	0.220±0.04	0.216±0.05	0.210±0.04	0.217±0.04
Bilirubin direct (mg/dL)	0.1±0.04	0.1±0.05	0.1±0.04	0.1±0.04
Bilirubin indirect(mg/dL)	0.1±00	0.1±00	0.1±00	0.1±00
ALP (U/L)	386.14±8.12	366.10±10.28	322.62±10.02	364.01±10.05
SGOT (U/L)	162.99±6.17	155.19±6.16	150.12±5.96	151.08±6.77
SGPT(U/L)	51.22 ± 1.38	44.35 ± 1.18	46.19 ± 0.40	44.25 ± 0.85
Total Protein(g/dl)	8.24 ± 0.10	7.15 ± 0.23	7.98 ± 0.20	8.11 ± 0.22
Albumin(g/dl)	4.10±0.24	3.98±0.21	3.85±0.25	3.74±0.22
Globulin(g/dl)	4.88±0.19	5.0±0.12	4.80±0.28	5.04±0.28
A/G Ratio(g/dl)	0.60±0.05	0.68±0.10	0.64±0.16	0.72±0.12
GGT(U/L)	6.1±0	6.2±0	6.3±0	6.2±0

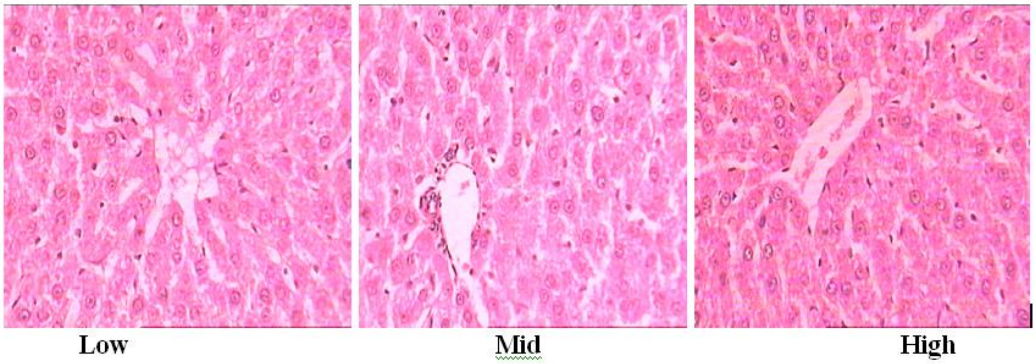
Values are MEAN ± S.E.M. (Dunnet's 't' test). N=6; $^{ns}P>0.05$ Vs control group.

Biochemical Investigations

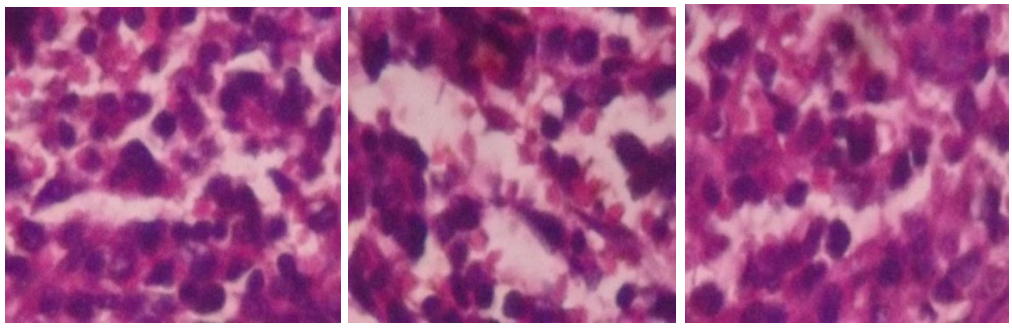
Results of Biochemical investigations conducted on days 29 and recorded in Table 6 revealed the following significant changes in the values of different parameters studied when compared with those of respective controls; however, the values obtained were within normal biological and laboratory limits. In other words, the effect was not dose-dependent. Decreased ALP levels showed in animals in 200 mg/kg dose group, Protein levels decreased in animals of 200 and 400 mg/kg group. All other parameters were found to be near normal.

Histopathological examination (Figure 1 A-I) for histopathology there were no changes to be observed.

Liver

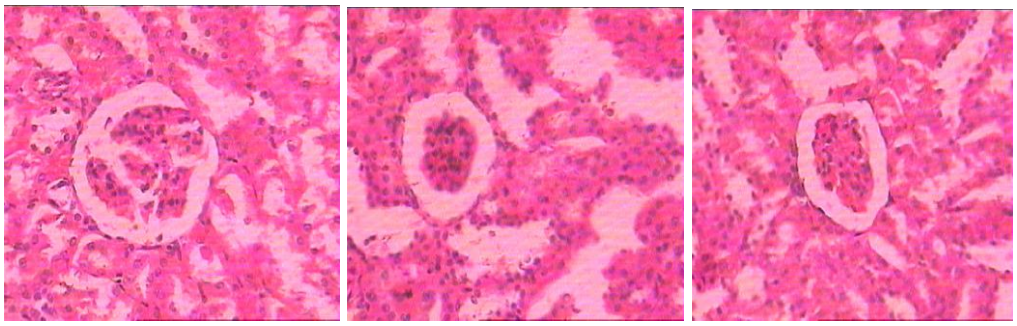


Spleen



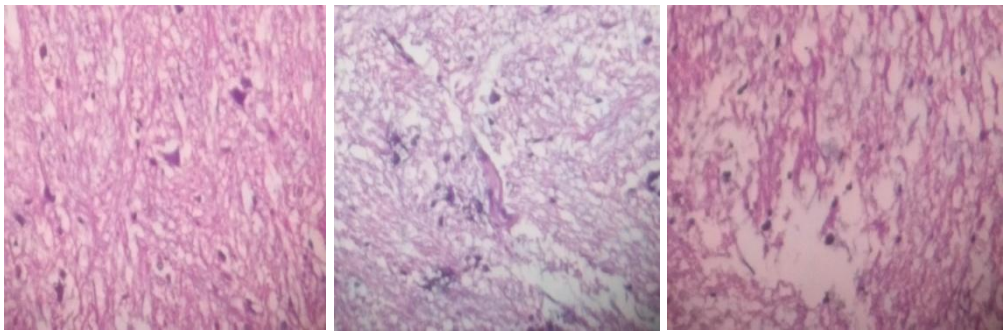
Low **Mid** **High**

Kidney



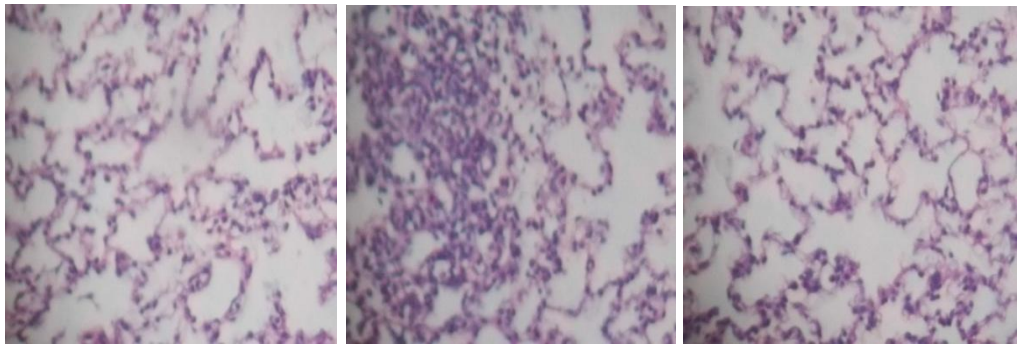
Low **Mid** **High**

Brain



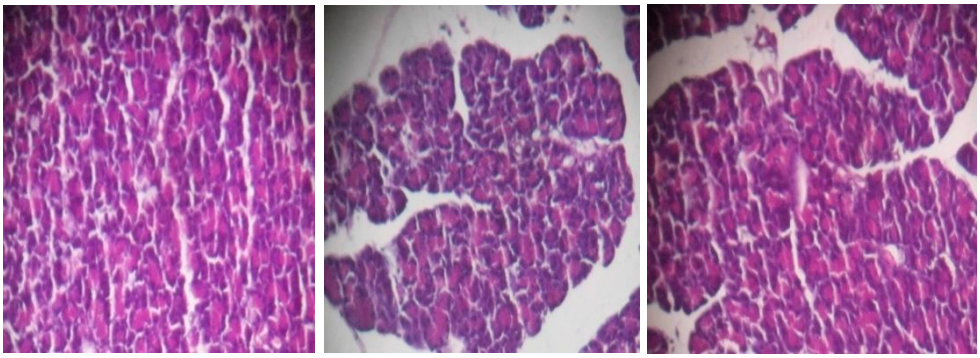
Low **Mid** **High**

Lung



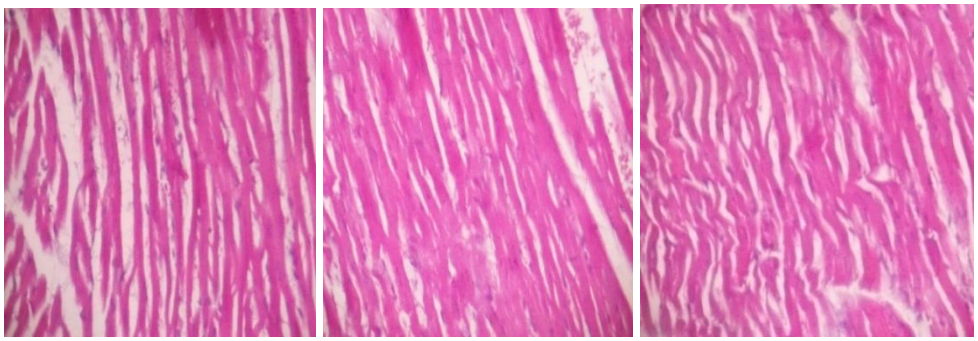
Low **Mid** **High**

Pancrea



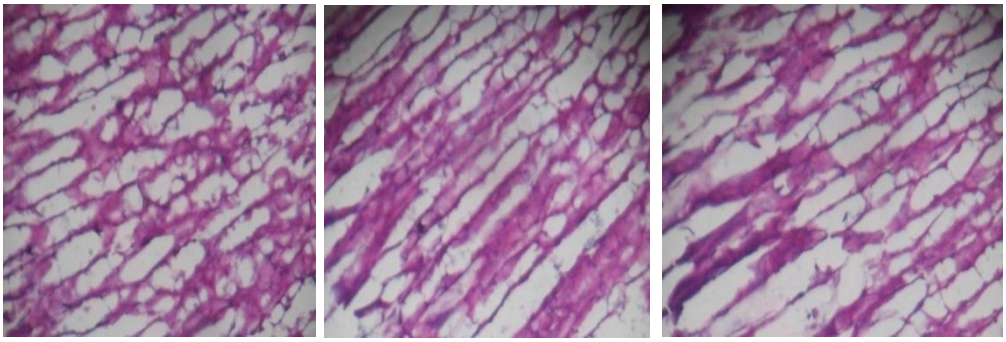
Low **Mid** **High**

Heart



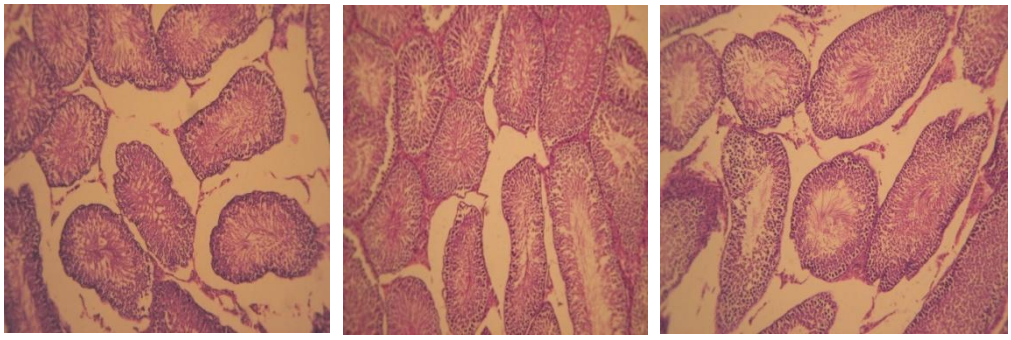
Low **Mid** **High**

Stomach



Low **Mid** **High**

Testis



Low **Mid** **High**

Figure 1(A-I): Histopathological Analysis of *KPC* (Liver, Spleen, Kidney, Brain, Lung, Pancreas, Heart, Stomach and Testis)

DISCUSSION

A world health organization survey indicates that about 70-80% of the world's populations rely on non-conventional medicine mainly of herbal source in their primary healthcare. [10] Natural products from including plants, animals and minerals have been used for basic treatment of human diseases. [11] All the animals from control and all the treated dose groups up to 400 mg/kg survived throughout the dosing period of 28 days. Except mild diarrhoea no signs of major or significant intoxication were observed in animals from lower and middle dose groups during the dosing period of 28 days. Animals from all the treated dose groups exhibited comparable body weight gain with that of controls throughout the dosing period of 28 days. Food consumption of control and treated animals was found to be comparable throughout the dosing period of 28 days. Hematological analysis conducted at the end of the dosing period on day 29, revealed no abnormalities attributable to the treatment. Biochemical analysis conducted at the end of the dosing period on day 29, revealed no abnormalities attributable to the treatment. Histopathological examination did not reveal any abnormality.

CONCLUSION

Based on these findings, no toxic effect was observed up to 400 mg/kg of *KPC* treated via oral route over a period of 28 days. So, it can be concluded that the *KPC* can be prescribed for therapeutic use in human with the dosage recommendations of up to 400 mg/kg/p.o.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Ishari. K. Ganesh, Chancellor, Vels University for providing the facilities necessary to carry out this research.

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