Int J Pharm Bio Sci 2013 Jan; 4(1): (P) 829- 839

Research Article **Toxicology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF *KODI PAVALA CHUNNAM* IN CARBON TETRACHLORIDE INDUCED LIVER DAMAGE IN RATS

VELPANDIAN[*1], J. ANBU[2] AND S. PREMA[3]

[1]Department of Pharmacology and Toxicology, Govt. Siddha Medical College, Arumbakkam, Chennai
[2]Departmentof Pharmacology, School of Pharmaceutical Sciences, Vels University, (VISTAS), Chennai-600117
[3]Department of Siddha Medicine, Tamil University, Tanjore-613010

ABSTRACT

The *Kodi Pavala Chunnam* (KPC) is traditionally used in Siddha system. The present investigation was evaluated the hepatoprotective activity KPC against carbon tetrachloride (CCl_4) induced liver damage in wistar rats. The KPC was used 100 mg/kg and 200 mg/kg administered orally to the animals. The Silymarin (100 mg/kg) was given as reference standard. The KPC was effective in protecting the liver against the injury as there was significant produced action in wet liver weight, Liver volume, Direct and total bilirubin, SGPT, SGOT, ALP, Total protein, Cholesterol, Triglyceride, CAT, SOD, LPO.

KEY WORDS: ***Kodi Pavala Chunnam*, Hepatoprotective, Coral, Carbon tetrachloride, bilirubin, Silymarin**

VELPANDIAN
Department of Pharmacology and Toxicology, Govt. Siddha Medical College, Arumbakkam, Chennai

INTRODUCTION

The traditional system of medicine became significantly more popular all over the globe because of the curative property, less toxic and has no side effects.[1] Siddha system of medicine (SSM) is one such ancient traditional system of India. This interest has led to the discovery of almost lines 8,500 marine natural products to date and many of the compounds have shown very promising biological activity. [2, 3] A great numbers of biologically active diterpene derivatives have been isolated from the extracts of several soft corals. [4, 5, 6] Recently, interest of natural product research has actively moved to marine organisms. As a result, almost 50% of reported natural cytotoxic compounds were isolated from marine organisms such as soft corals and sponges. [7, 8] Coral reefs are one of the oldest and largest living ecosystems 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. Drugs or their metabolites can cause toxic effect on the liver. Many of the intermediate metabolites have a short half-life, some estimated to be less than a minute, which makes detecting them a challenging task. [9] The liver is the central organ in the metabolism and detoxification of drugs and toxins. [10] Liver is a major metabolic organ affected by various chemicals and toxins daily and identification of a successful hepatoprotective agent will provide a useful tool for the treatment of hepatic diseases. Carbon tetrachloride induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs. It is a potent hepatotoxic producing centrilobular necrosis which causes liver injury. CCl_4 is bio transformed by the cytochrome P450 system to produce the trichloromethyl free radicals, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation.[11] Carbon tetrachloride is a potent hepatotoxin, and a single exposure to it can rapidly lead to an increase in the level of several enzymes, severe centrizonal necrosis and steatosis. Depending on the type of cell and the membrane involved, lipoperoxidation due to CCl_4 results in hemolysis, which increases the serum bilirubin level. The hepatotoxic effects of CCl_4 are largely due to its active metabolite, trichloromethyl radical. These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxidase, which in turn gives products like malondialdehyde (MDA) that cause damage to the membrane. [12, 13]

MATERIALS AND METHODS

Animals

Male wistar rats weighing about 200-220 gm 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 anesthesia 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. [14] 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 5, 50, 300 and 2000 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. One tenth and one twenty dose was considered as for hepatoprotective activity.

Preparation and administration of dose

KPC was suspended in 2% CMC in distilled water to obtain concentrations of 200 mg/ml. 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.

Experimental design for hepatoprotective activity

The model was described and employed with some modifications. [15] Animals were divided into five groups, each group containing six animals. Group I (normal control) received Saline (10 ml/kg) for 7 days. Group II (Toxicant control) received CCl_4 1.25 ml/kg, i. p. Group III (Standard) received Silymarin (100mg/kg, p.o.) for 7 days and CCl_4 induction on 5^{th} day. Groups IV-V, received KPC (100mg/kg and 200mg/kg p.o) for 7th days and CCl_4 induction on 5th day. On the 8th day, the animals were sacrificed under ether anesthesia, blood and liver samples were collected. The blood was used for biochemical estimations. The liver was quickly removed and use for wet liver weight and volume.

Assessment of hepatoprotective activity

The following parameters were considered for hepatoprotective activity like Wet liver weight, Liver volume, Direct and total bilirubin, SGPT, SGOT, ALP, Total protein, Cholesterol, Triglyceride, CAT, SOD, LPO. [16]

Statistical analysis

The results were represented as Mean ± Standard Error Mean. The statistical significance was computed using One Way ANOVA followed by Tukey-Kramer's test and compared with toxicant control group with Standard, KPC-1 and KPC-2 where the n=6 animals in each one group were used.

RESULT AND DISCUSSION

One of the most commonly used chemical agents for liver damage in hepatoprotective study is CCl_4.[17] The active radical of this compound is CCl_4 which bind to the macromolecules and induce peroxidative degradation of membrane lipids of Endoplasmic reticulum. This result in the formation of lipid peroxides whose product malondialdehyde (MDA) causes severe membrane damage. [18, 19] The hepatoprotective potential of a drug depends upon its ability in reducing the harmful effects caused by a hepatotoxin. [20] The medicinal property of a plant is due the presence of its chemical constituents. In hepatoprotective study, these phytoconstituents play a vital role in inducing microsomal enzymes thereby accelerating the excretion of CCl_4, or inhibiting the lipid peroxidation induced by CCl_4. [21, 22] Most experiments involving the induction of liver injury by CCl_4 is usually accompanied by the elevation in the levels of liver enzyme markers (AST, ALT and ALP). The elevated levels of these biochemical parameters are direct reflection of alterations in the hepatic structural integrity. [23] Liver injuries by toxicants cause cellular leakage and loss of functional integrity. [24] The liver regulates many important metabolic functions. Hepatic injury is associated with distortion of these metabolic functions. [25] Therefore, many folk remedies from plant origin are evaluated for its possible antioxidant and hepatoprotective effects against different chemical induced liver damage in experimental animals. CCl_4 induced hepatotoxicity model is frequently used for the investigation of hepatoprotective effects of drugs and plant extracts. [26, 27] The present studies were performed to assess the hepatoprotective activity in rats against Carbon tetrachloride as hepatotoxin. Clinical signs in rats that received CCl_4 (Group B) included dullness and loss of appetite. The all result significantly found when all groups were compared with the toxicant

control groups of CCl_4 induced hepatotoxicity action on rat model. From the Table 1 it was evident that KPC was able to reduce or normalized the wet liver weight (gm/100gm) and wet liver volumes (gm/100gm) when compared with others groups like toxicant control, standard group. The values are represent the significant reduced in the liver weight and liver volume where standard group (Silymarin 100 mg/kg), KPC-1 (100 mg/kg) and KPC-2 (200 mg/kg), the results was found significantly as $P<0.001$ and $P<0.01$ respectively.

Table 1

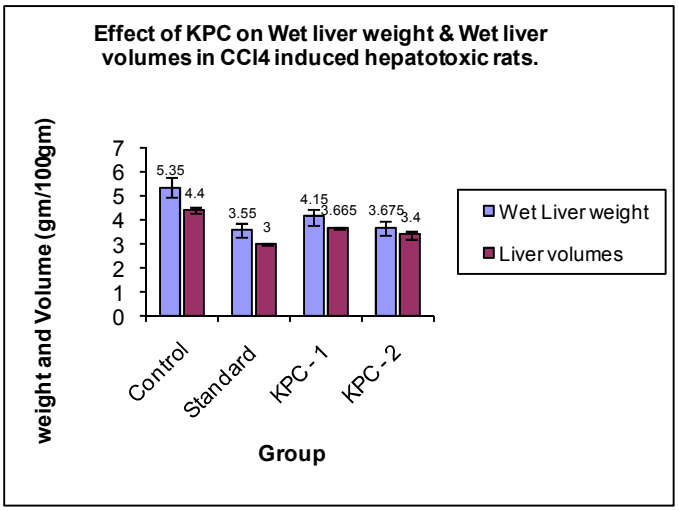
Effect of KPC on Wet liver weight & Wet liver volumes in CCl_4 induced hepatotoxic rats.

Group	Treatment	Dose (p.o.)	Wet Liver weight (gm/100gm)	Liver volumes (ml/100gm)
A	Normal control	Saline (10ml/kg)	2.875 ± 0.375	2.75 ±0.10
B	Toxicant Control	CCl_4-1.25 ml/kg	5.35 ± 0.150	4.4 ±0.15
C	Standard (Silymarin)	100mg/kg+CCl_4	3.55 ± 0.05***	3.0 ±0.05***
D	KPC - 1	100mg/kg+CCl_4	4.15 ± 0.10**	3.665 ±0.015***
E	KPC - 2	200mg/kg+CCl_4	3.675 ± 0.125***	3.40 ±0.150***

Values are MEAN ± SEM (n=6) one way ANOVA followed by Tukey-Kramer's test. Where the values are *** P<0.001; ** P<0.01

Graph1

Effect of KPC on Wet liver weight & Wet liver volumes in CCl_4 induced hepatotoxic rats



From the Table 2 it was evident that KPC was able to reduce or normalized the direct bilirubin (mg/dl) and total bilirubin (mg/dl) when compared with others groups like toxicant control, standard group. The values represent the reduced in the direct bilirubin and total bilirubin where standard group (Silymarin 100 mg/kg), KPC-1 (100 mg/kg) and KPC-2 (200 mg/kg), the results was found significantly as $P<0.001$ respectively.

Table 2

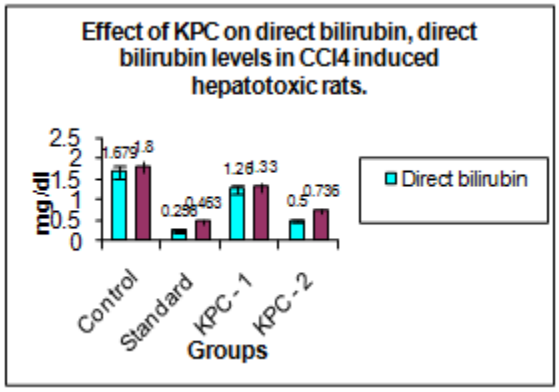
Effect of KPC on direct bilirubin, direct bilirubin levels in CCl_4 induced hepatotoxic rats.

Group	Treatment	Dose (p.o.)	Direct bilirubin (mg/dl)	Total bilirubin (mg/dl)
A	Normal control	Saline (10ml/kg)	0.173 ±0.0120	0.293±0.029
B	Toxicant Control	CCl_4-1.25 ml/kg	1.679 ±0.099	1.8±0.005
C	Standard (Silymarin)	100mg/kg+CCl_4	0.256 ±0.012***	0.463±0.049***
D	KPC - 1	100mg/kg+CCl_4	1.26 ±0.032***	1.33±0.059***
E	KPC - 2	200mg/kg+CCl_4	0.50 ±0.040***	0.736±0.1105***

Values are MEAN ± SEM (n=6) one way ANOVA followed by Tukey-Kramer's test. Where the values are *** P<0.001

Graph 2

Effect of KPC on direct bilirubin, direct bilirubin levels in CCl_4 induced hepatotoxic rats.



From the Table 3 it was evident that KPC was able to reduce or normalized the SGPT (u/l), SGOT (u/l) ALP (mg/dl) when compared with other groups like toxicant control, standard group. The values represent the reduced in the SGPT, SGOT, ALP level where standard group (Silymarin 100 mg/kg), KPC-1 (100 mg/kg) and KPC-2 (200 mg/kg), the results was found significantly as P<0.001 respectively.

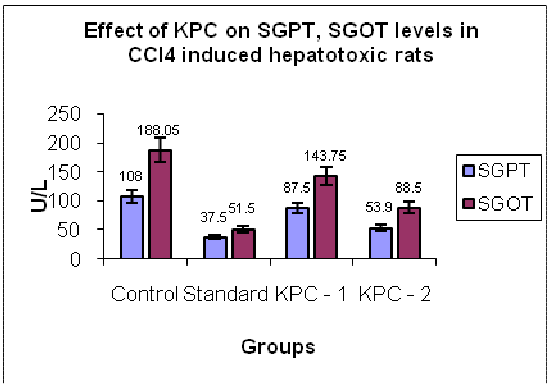
Table 3

Effect of KPC on SGPT, SGOT, ALP levels in CCl_4 induced hepatotoxic rats.

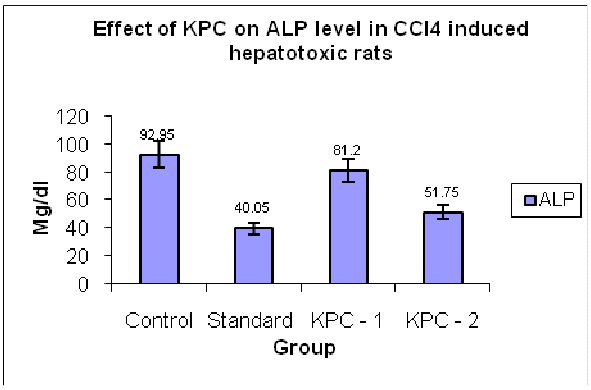
Group	Treatment	Dose (p.o)	SGPT (U/L)	SGOT (U/L)	ALP (mg/dl)
A	Normal control	Saline (10ml/kg)	28.175±0.325	34.05±4.5	33.0±0.50
B	Toxicant Control	CCl_4-1.25 ml/kg	108±2.50	188.05 ±2.50	92.95±0.550
C	Standard (Silymarin)	100mg/kg+CCl_4	37.5±1.0***	51.5±1.0***	40.05±0.55***
D	KPC - 1	100mg/kg+CCl_4	87.5±2.0***	143.75±8.750***	81.2±0.30***
E	KPC - 2	200mg/kg+CCl_4	53.9±1.30***	88.5±3.0***	51.75±0.250***

Values are MEAN ± SEM (n=6) one way ANOVA followed by Tukey-Kramer's test. Where the values are *** P<0.001

Graph 3. ***Effect of KPC on SGPT, SGOT levels***



Graph 4. ***Effect of KPC on ALP levels***



From the Table 4 it was evident that KPC was able to normalize the onset of sleep (sec) and duration of sleep (min) when compared with other groups like toxicant control, standard group. The values represent the normalized sleep time (onset and duration), where standard group (Silymarin 100 mg/kg), KPC-1 (100 mg/kg) and KPC-2 (200 mg/kg), the results was found significantly as P<0.001 and P<0.01 respectively.

Table 4

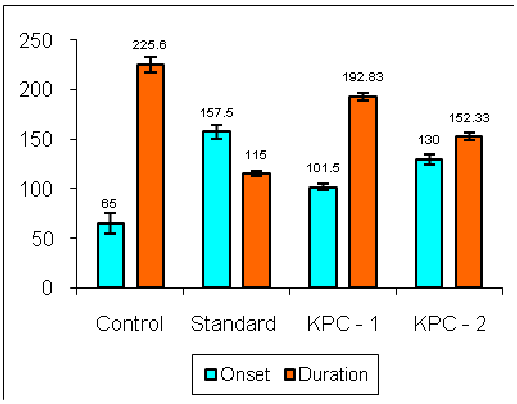
Effect of KPC on Onset of sleep & Duration of sleep in CCl_4 induced hepatotoxic rats.

Group	Treatment	Dose (p.o.)	Onset of time (sec)	Duration of sleep (min)
A	Normal control	Saline (10ml/kg)	177.5±2.5	96.75±3.775
B	Toxicant Control	CCl_4-1.25 ml/kg,	65±10.0	225.6±7.68
C	Standard (Silymarin)	100mg/kg+CCl_4	157.5±7.5***	115±2.74***
D	KPC – 1	100mg/kg+CCl_4	101.5±3.5**	192.83±3.712***
E	KPC – 2	200mg/kg+CCl_4	130±5.0***	152.33±3.84***

Values are MEAN ± SEM (n=6) one way ANOVA followed by Tukey-Kramer's test. Where the values are *** P<0.001; ** P<0.01

Graph 5

Effect of KPC on Onset of sleep & Duration of sleep in CCl_4 induced hepatotoxic rats.



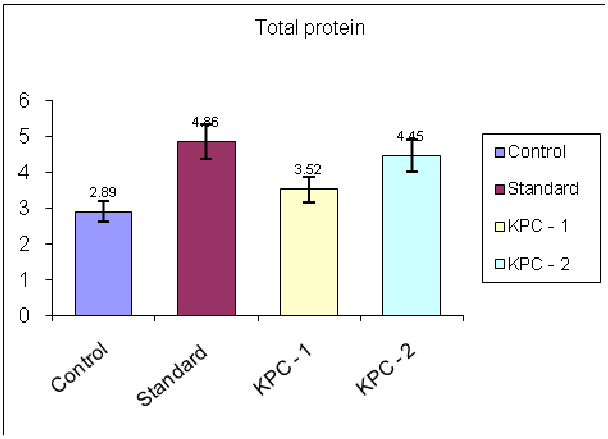
From the Table 5 it was evident that KPC was able to normalize the total protein (gm/dl), total cholesterol (mg/dl) and triglyceride (mg/dl) levels when compared with other groups like toxicant control, standard group. The values represent the total protein, total cholesterol and triglyceride levels where standard group (Silymarin 100 mg/kg), KPC-1 (100 mg/kg) and KPC-2 (200 mg/kg), the results was found significantly as P<0.001; P<0.01 and P<0.05 respectively.

Table 5
Effect of KPC on Serum Total protein, Total cholesterol & Triglyceride levels in CCl_4 induced hepatotoxic rats.

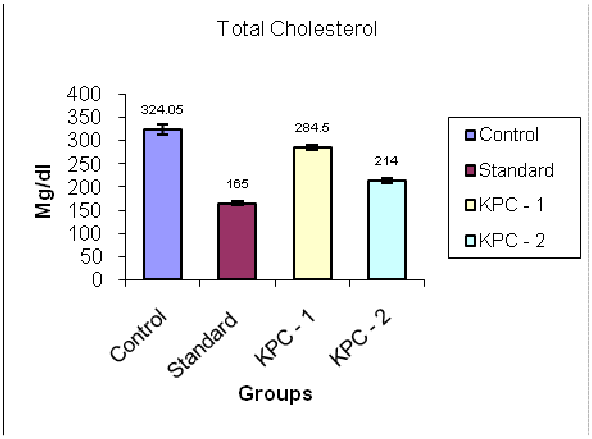
Group	Treatment	Dose (p.o.)	Total protein (gm/dl)	Total Cholesterol (mg/dl)	Triglyceride levels (mg/dl)
A	Normal control	Saline (10ml/kg)	5.45±0.07	135.6±4.59	0.515±0.0123.6
B	Toxicant Control	CCl_4-1.25 ml/kg	2.70±0.25	324.05±10.5	2.38±0.0879
C	Standard (Silymarin)	100mg/kg+ CCl_4	4.86±0.06**	165±3.46***	0.890±0.0382***
D	KPC - 1	100mg/kg+ CCl_4	4.33±0.78*	284.5±4.53**	2.015±0.0182***
E	KPC - 2	200mg/kg+ CCl_4	4.45±0.12*	214±5.50***	1.08±0.0694***

Values are MEAN ± SEM (n=6) one way ANOVA followed by Tukey-Kramer's test. Where the values are *** P<0.001; ** P<0.01; * P<0.05

Graph 6. ***Effect of KPC on Total protein***

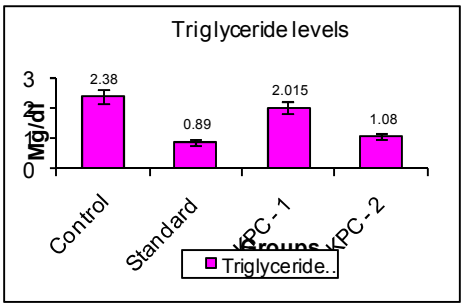


Graph 7. ***Effect of KPC on Total Cholesterol***



Graph 8

Effect of KPC on Serum Triglyceride levels in CCl_4 induced hepatotoxic rats.



From the Table 6 it was evident that KPC was able to normalize the CAT, SOD and LPO levels when compared with other groups like toxicant control, standard group. The values represent the CAT, SOD and LPO levels where standard group (Silymarin 100 mg/kg), KPC-1 (100 mg/kg) and KPC-2 (200 mg/kg), the results was found significantly as P<0.001 respectively.

Table 6

Effect of KPC on Catalase, Super oxide dismutase and Lipid peroxidation in CCl_4 induced hepatotoxic rats.

Group	Treatment	Dose (p.o)	CAT	SOD	LPO
A	Normal control	Saline (10ml/kg)	91.8±3.412	14.5±0.5774	3.83±0.60
B	Toxicant Control	CCl_4-1.25 ml/kg	22.3±0.8819	3.23±0.088	89.26±0.1856
C	Standard (Silymarin)	100mg/kg+CCl_4	83.05±0.622***	11.0±0.352***	6.53±0.202***
D	KPC-1	100mg/kg+CCl_4	36.4±0.585***	5.8±0.152***	7.86±0.03***
E	KPC-2	200mg/kg+CCl_4	51.03±1.093***	7.7±0.2517***	7.26±0.0667***

Values are MEAN ± SEM (n=6) One Way ANOVA followed by Tukey-Kramer's test. Where the values are *** P<0.001

Graph 9

Effect of KPC on Catalase, Super oxide dismutase and Lipid peroxidation in CCl_4 induced hepatotoxic rats.

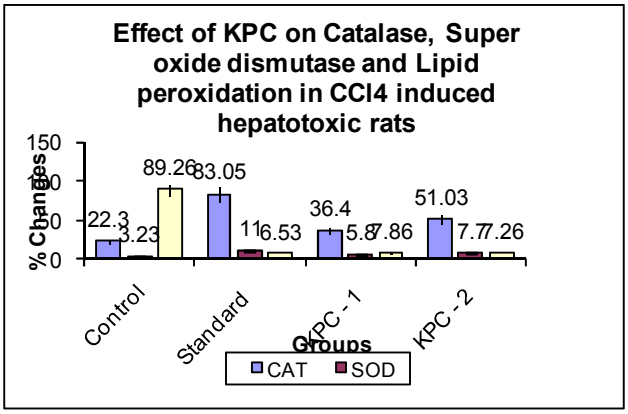
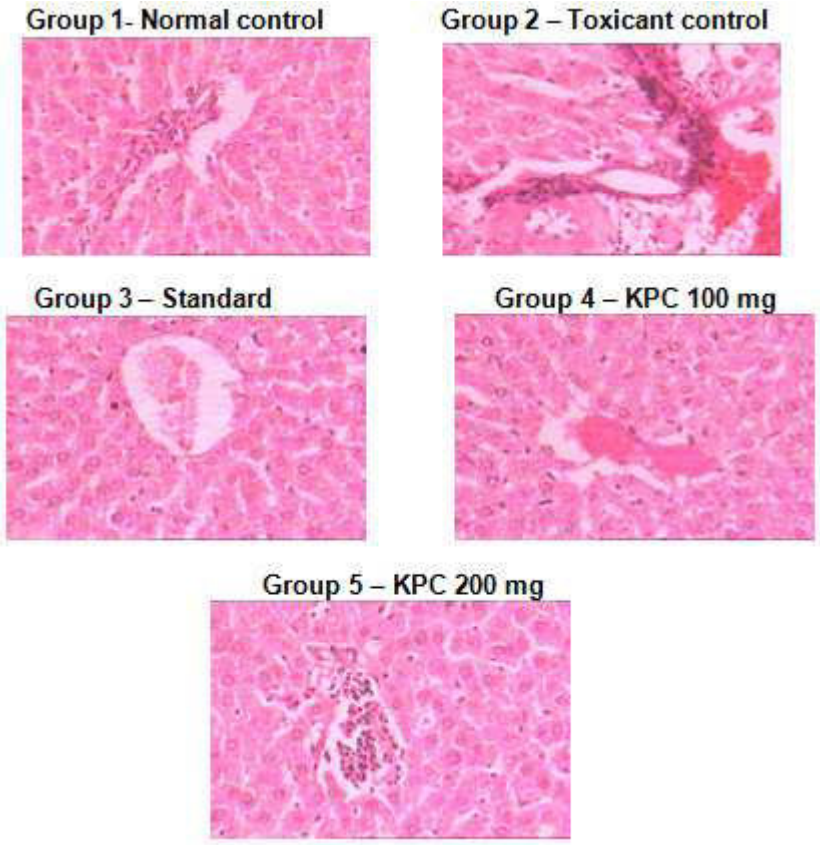


Figure 1
HISTOPATHOLOGY OF LIVER



CONCLUSION

It is concluded that *Kodi Pavala Chunnam* from coral have shown the hepatoprotective effect in the CCl_4 induced hepatotoxicity in rodents and produced the significant action on enzymes and proteins for protection of liver like Wet liver weight, Liver volume, Direct and total bilirubin, SGPT, SGOT, ALP, Total protein, Cholesterol, Triglyceride, CAT, SOD, LPO. This effect might explain the apparent usage of this drug in Siddha system of therapy.

ACKNOWLEDGEMENTS

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

REFERENCES

1. Meenadevi VN, Nagendraprasad P, Kalirajan K. Infrared spectral studies on Siddha drugs *Pavalaparpam*. Int J Pharma Bio Sci 2010; 1(4): 474-483.
2. Ram A, Joseph DA, Balachandar S, Singh VP. Medicinal plants from Siddha system of medicine useful for treating respiratory

diseases. Int J Phar Anal 2009; 1 (2): 20-30.
3. Abdel-Wahhab MA, El-Nekeety AA, Hassan NS, El-Hefnawy MS, Kotb MM, El-Mekkawy SA, Khalil NA, Hanna AG. Hepatoprotective effect of Sarcophine isolated from soft coral (*Sarcophyton glaucum*) in rats. Global Vet 2012; 8 (3): 244-253.
4. Dong H, Gou YL, Kini RM, Xu HX, Chen SX, Teo SL, But PP. A new cytotoxic polyhydroxysterol from soft coral *Sarcophyton trocheliophorum*. Chem Pharm Bull (Tokyo) 2000; 48: 1087-1089.
5. Sheu JH, Chang KC, Duch CY. A cytotoxic 5α, 8α-Epidioxysterol from a soft coral *Sinularia species*. J Nat Prod 2000; 63: 149-151.
6. Grote D, Soliman HSM, Shaker KH, Hamza M, Seifert K. Cembranoid diterpenes and a briarane diterpene from corals. Nat Prod Res 2005.
7. Kim J, Park EJ. Cytotoxic anticancer candidates from natural resources. Curr Med Chem Anti Cancer Agent 2002; 2: 485-537.
8. Ahmed HH, Mannaa F, Estefan SF. Modulatory effect of the red sea soft coral extracts on hepatotoxicity induced by carcinogenic agents in rats model. J Egypt Soc Toxicol 2006; 35: 97-107.
9. Park BK, Kitteringham NR, Maggs JL, Pirmohamed M, Williams DP. The role of metabolic activation in drug-induced hepatotoxicity. Annu Rev Pharmacol Toxicol. 2005; 45: 177-202.
10. Adebayo AH, Abolaji AO, Kela R. Hepatoprotective activity of *Chrysophyllum albidum* against carbon tetrachloride induced hepatic damage in rats. Canadian J Pure app sci. 2011; 2 (5): 1597- 1602.
11. Recknagel, RO, Glende EA, Dolak JA, Waller RLC. Mechanism of carbon tetrachloride toxicity. Pharmacol Ther. 1989; 43: 139-154.
12. De-Leve LD, Kaplowitz N. Mechanisms of drug-induced liver disease. Gastroenterol Clin N Am. 1995; 24: 787-810.
13. Farrel GC. Liver disease caused by drugs, anesthetics and toxins. Sleisenger & Fordtran's Gasrointestinal and Liver Disease: Pathophysiology Diagnosis Management. 2nd ed. Philadelphia: WB Saunders Co: 1998; 1221-1253.
14. OECD Test Guideline 423, OECD Guideline for Testing of Chemicals. Available: [http://www.oecd.org/document/html], (2001).
15. Chakraborti KK, Handa SS. Antihepatotoxic investigations on *Boerhaavia repanda* Wild. Indian Drugs, 1989; 2: 19-24.
16. Chaudhari BP, Chaware VJ, Joshi YR, Biyani KR, Hepatoprotective activity of Hydroalcoholic extract of *Momordica charantia* Linn. Leaves against Carbon tetra chloride induced Hepatopathy in Rats. Int J Chem Tech Res Coden (USA): 2009; 1 (2): 355-358.
17. Johnston DE, Kroening C. Mechanism of early carbon tetrachloride toxicity in cultured rat hepatocytes. Pharmacol Toxicol. 1998; 83: 231-239.
18. Cotran RS, Kumar V, Robbins SL. Cell injury and cellular death. Robbin's Pathologic Basis of Disease, 5th ed. Prism Book Pvt Ltd; 1994: 379-430.
19. Kaplowitz N, Aw TY, Simon FR, Stolz A. Drug-induced hepatotoxicity. Ann Int Med 1986; 104: 826-839.
20. Manjunatha BK, Mankani KL, Vidya SM, Krishna V, Manohara YN. Hepatoprotective activity of *Butea superba* against carbon tetrachloride induced hepatic damage in rodents. Phcog Mag 2008; 4 (15): 41-45.
21. Mehta RS, Shankar MB, Geetha M, Saluja AK. Hepatoprotective activity of *Trianthema portulacastrum.* Indian drugs 1999; 36: 241-243.
22. Singh S, Metha A, P Metha. Hepatoprotective activity of *Cajanus* against carbon tetrachloride induced liver damage. Int J Pharm Pharmaco Sci 2011; 3 (2): 146-147.

23. Patrick-Iwuanyanwu KC, Wegwu MO, Okiyi JK. Hepatoprotective effects of African locust bean (*Xylopia aethiopica*) in CCl4 induced liver damaged Wistar Albino rats. Int J Pharmacol 2010; 6: 744-749.
24. Sallie R, Tredger JM, Williams R. Drugs and the liver part 1: Testing liver function. Biopharm Drug Dispos 1991; 12: 251-259.
25. Wolf PL. Biochemical diagnosis of liver diseases. Indian J Clin Biochem 1999; 14: 59–90.
26. Rubinstein D, Epinephrine release and liver glycogen levels after carbon tetrachloride administration. Am J Physiol 1962; 203: 1033–1037.
27. Suja SR, Latha PG, Pushpangadan P, Rajasekharan S. Aphrodisiac property of *Helminthostachys zeylanica* in mice. J Trop Med Plants 2002; 3: 191–195.