Asian Journal of Chemistry Vol. 21, No. 6 (2009), 4559-4563

Comparative Hepatoprotective Efficacy of Livfit and Livomyn Against Carbon Tetrachloride Induced Hepatic Damage in Rats

S.K. MASTAN*, K.E. KUMAR†, N. SREEKANTH‡ and D.S. PILLAI
Department of Pharmacology, Roland Institute of Pharmaceutical Sciences, P.O. Khodasingi, Ambapua, Berhampur-760 010, India
E-mail: shkmastan@gmail.com

Livfit and livomyn are polyherbal hepatoprotective formulations used in Indian system of medicine. Livfit and livomyn were evaluated for their hepatoprotective efficacy against carbon tetrachloride induced hepatic injury in rats. Biochemical parameters like total bilirubin, serum glutamic oxaloacetic transaminases (SGOT), serum glutamic pyruvate transaminases (SGPT) and alkaline phosphatase (ALP) and total proteins were estimated to assess liver function. Silymarin used as reference standard also exhibited hepatoprotective activity against carbon tetrachloride. The biochemical observations were supplemented with histopathological examination of rat liver sections. It was concluded from the study that livfit has shown more significant hepatoprotective activity against carbon tetrachloride induced hepatic damage in rats.

Key Words: Hepatoprotective activity, Livfit, Livomyn.

INTRODUCTION

Liver is the major organ for metabolism and excretion. About 20,000 deaths found every year due to liver disorders. In India, about 40 polyherbal commercial formulations reputed to have hepatoprotective action are being used. It has been reported that 160 phytoconstituents from 101 plants have hepatoprotective activity[1]. There are different marketed formulations available for treating liver diseases, *viz*., livfit, livomyn, liv-52, liver cure, livol, jigrine and livogen *etc*. Livfit and livomyn are popular herbal formulations indicated for liver diseases[2]. Hepatoprotective efficacy of some of the individual herbal ingredients of both the formulations was reported in literature[3-6]. The aim of the present study was to compare the hepatoprotective efficacy of two marketed formulations, livfit and livomyn against carbon tetrachloride induced hepatic damage in rats.

¶Present address: Trident Life Sciences Ltd., Pharmacokinetics Unit, Clinical Pharmacology Department, Survey No. 66-67 Part, Miyapur Village, Serilingampally Mandal, Hyderabad-500 050, India.
†Department of Pharmacology, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530 003, India.
‡Siddharth Institute of Phamacy, Kantepudi(V), Sattenapally (M), Guntur-522 438, India.

EXPERIMENTAL

Male wistar albino rats (150-200 g) were procured from Mahaveer Enterprises, Hyderabad and maintained under standard laboratory conditions at an ambient temperature of 25 ± 2 ºC and 50 ± 15 % relative humidity with a 12 h light/12 h dark cycle. Rats were fed with commercial pellet diet (Rayans Biotechnologies Pvt Ltd., Hyderabad) and water *ad libitum*. The study was performed in accordance with our institutional animal ethics committee.

Livfit (Dabur Pharmaceuticals, India) and livomyn (Charak Pharma Ltd., India) were used to evaluate the hepatoprotective activity. Silymarin (Micro labs, Tamil Nadu, India) was used as a standard. Carbon tetrachloride (E-Merck, Mumbai, India) was used to induce hepatic toxicity. All the biochemicals and chemicals used were of analytical grade.

Evaluation of hepatoprotective activity: The animals were divided into 5 groups of 6 rats each. The animals in group I served as control and received distilled water p.o for 14 d. All the animals of group II to V received CCl_4 (0.1 mL/Kg/day; i.p) for 10 d[7]. Group III, IV and V animals received the standard drug silymarin (100 mg/Kg/day; p.o), livfit (2.85 mL/Kg/day; p.o) and livomyn (2.85 mL/Kg/day; p.o) for 14 d, respectively. Livfit and livomyn doses were calculated based on human dose from the label. The CCl_4, silymarin, livfit and livomyn were administered concomitantly to the respective group of animals.

Assessment of hepatoprotective activity: All the animals were sacrificed on day 14 under light ether anesthesia. The blood samples were collected separately by carotid bleeding into sterilized dry centrifuge tubes and allowed to coagulate for 0.5 h at 37 ºC. The clear serum was separated at 2500 rpm for 10 min and biochemical investigations were carried out to assess liver function *viz.*, total bilirubin[8], total protein[9], serum transaminases[10] and serum alkaline phosphatase[11].

The results are expressed as mean ± SEM of 6 animals from each group. The data were evaluated by one-way ANOVA followed by Turkey's multiple comparison test. p values ≤ 0.01 were considered statistically significant.

Histopathology: The liver from all the animals was isolated and washed with the normal saline, blotted with filter paper and weighed[12]. Liver was sliced and pieces were fixed in 10 % buffered neutral formalin for 48 h and processed for paraffin embedding. The sections were taken at 5 µ thickness using microtome, processed in alcohol-xylene series and were stained with alum-haemotoxylin and eosin[13]. The sections were examined microscopically for the evaluation of histological changes.

RESULTS AND DISCUSSION

The results of carbon tetrachloride induced hepatotoxicity are shown in Table-1. At the end of 14 d treatment, blood samples of CCl_4 treated animals showed significant increase in the levels of total bilirubin, serum glutamic oxaloacetic transaminases (SGOT), serum glutamic pyruvate transaminases (SGPT) and alkaline phosphatase

TABLE-1
EFFECT OF LIVFIT AND LIVOMYN ON CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN RATS

Design of treatment	Dose	Liver (wt/100 g b.wt)	SGPT (U/L)	SGOT (U/L)	ALP (U/L)	Total bilirubin (mg %)	Total protein (g %)
Control	–	3.6 ± 0.12	133.5 ± 1.96	46.2 ± 0.80	174.3 ± 1.23	0.49 ± 0.03	9.25 ± 0.12
CCl_4	0.1 mL/kg	6.7 ± 0.30*	219.2 ± 4.20*	342.5 ± 2.60*	443.1 ± 2.06*	2.22 ± 0.13*	5.09 ± 0.32*
CCl_4 + Silymarin	100 mg/kg	3.8 ± 0.28**	140.0 ± 2.16**	83.2 ± 4.20**	184.6 ± 1.20**	0.50 ± 0.01**	8.72 ± 0.01**
CCl_4 + Livfit	2.85 mL/kg	4.3 ± 0.07**	160.0 ± 1.16**	91.5 ± 6.12**	204.4 ± 2.20**	0.65 ± 0.04**	8.17 ± 0.03**
CCl_4 + Livomyn	2.85 mL/kg	4.6 ± 0.08**	168.0 ± 1.20**	100.2 ± 7.10**	238.9 ± 2.30**	0.67 ± 0.05**	8.12 ± 0.05**

All values are expressed as mean ± SEM, *$p < 0.01$ compared to control group, **$p < 0.01$ compared to CCl_4 treated group.

(ALP) compared to normal control groups but the total protein level decreased reflecting the liver injury caused by CCl_4. Where as blood samples from the animals treated with livfit and livomyn showed significant decrease in the levels of serum markers and significant increase in total protein to the near normal which are comparable to the values registered in the standard drug treated group of animals, indicating the protection of hepatic cells. It was observed that the size of the liver was enlarged in CCl_4 treated rats but it was normal in drug-treated groups. A significant reduction in liver weight supports this finding.

Histological profile of the control animals showed normal hepatocytes (Fig. 1). The section of liver of the group II animals showed severe intense centrilobular necrosis, vacuolization and macro vesicular fatty changes (Fig. 2). The liver sections of silymarin-treated animals showed normal hepatic architecture (Fig. 3). Significant liver protection was observed in the liver sections of livfit treated animals as evident by the presence of normal hepatic cords, absence of necrosis with few fatty lobules and regenerative activity of hepatocytes (Fig. 4). However, the liver sections of the animals treated with livomyn exhibited moderate accumulation of fatty lobules (Fig. 5).

Carbon tetrachloride is one of the most commonly used hepatotoxins in experimental study of liver disease[14]. The lipid peroxidative degradation of biomembrane is one of the principle causes of hepatotoxicity of CCl_4[15]. The hepatotoxic effect of CCl_4 are largely due to its active metabolite trichloromethyl radical[16], which binds to the macromolecule and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxide which in turn gives toxic aldehyde that causes damage to liver. This is evident from an elevation in the serum marker analysis (SGOT, SGPT, ALP and total bilirubin). This is indication of cellular leakage and loss of the functional integrity of the cell membrane in liver[17].

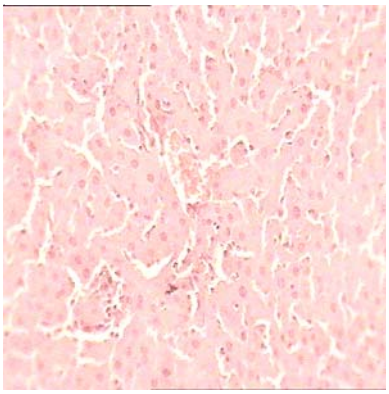

Fig. 1. Histopathological examination of liver section of control group

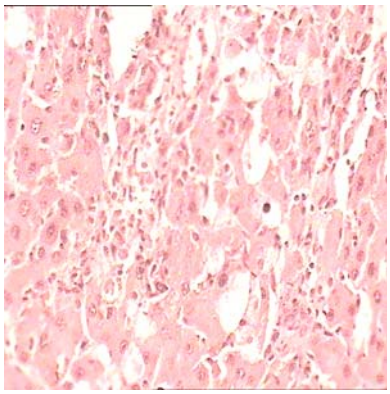

Fig. 2. Histopathological examination of liver section of CCl_4 treated group

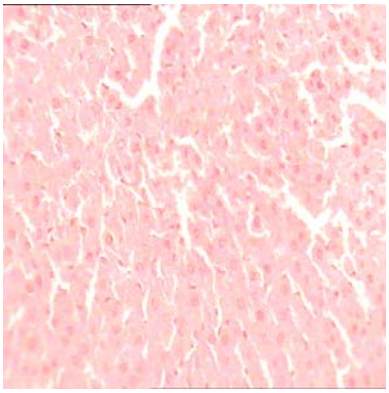

Fig. 3. Histopathological examination of liver section of silymarin treated group

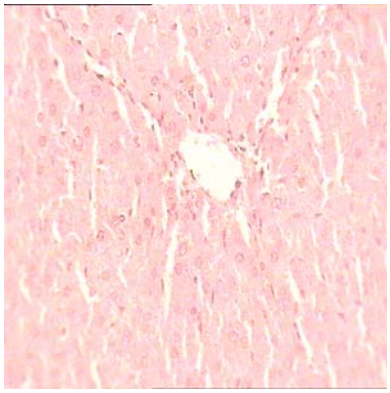

Fig. 4. Histopathological examination of liver section of livfit treated group

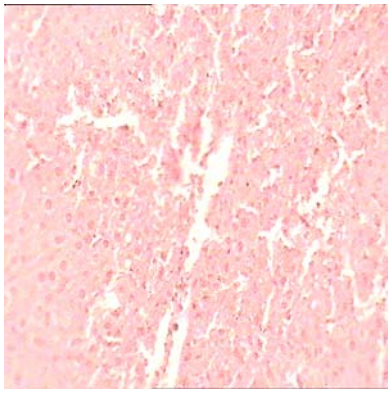

Fig. 5. Histopathological examination of liver section of livomyn treated group

The biochemical studies in wistar albino rats revealed that CCl_4 induced hepatic injury was significantly inhibited by livfit and livomyn. All these results were comparable with the standard drug silymarin.

The comparative histopathological study of the liver from different groups of rats corroborated the hepatoprotective efficacy of polyherbal formulations. Various pathological changes such as steatosis, centrilobular necrosis and vacuolization observed in CCl_4 treated rats were prevented to a moderate extent in group III, IV and V. All the effects of livfit and livomyn were comparable with silymarin as a positive control. These results showed that livfit has shown significant hepatoprotective activity in comparison to livomyn against carbon tetrachloride induced hepatic damage in rats.

Conclusion

Overall, the present study indicates that livfit and livomyn demonstrated a significant hepatoprotective activity against carbon tetrachloride induced hepatic damage in rats. Moreover livfit has shown significant hepatoprotective activity in comparison to livomyn.

ACKNOWLEDGEMENTS

The authors are thankful to Prof. M.E. Bhanoji Rao, Principal, Roland Institute of Pharmaceutical Sciences and Prof. S. Satyanarayana, HOD, University College of Pharmaceutical Sciences, Andhra University for their kind support and encouragement.

REFERENCES

1. S.S. Handa, A. Sharma and K.K. Chakraborti, *Fitoterapia*, **57**, 307 (1986).
2. P.B. Reddy, C.K. Reddy, D. Rambhau, V. Venkateshvarala and V.N. Murthy, *Indian J. Pharm. Sci.*, **55**, 137 (1993).
3. S.M. Karandikar, G.V. Jnglckar, G.K. Chitale and J.H. Balwani, *Acta Pharmacol. Toxicol.*, **20**, 274 (1963).
4. M.N. Jindal, V.K. Patel and N.B. Patel, *Indian J. Pharmacol.*, **7**, 104 (1975).
5. A.H. Gilani and K.H. Janbaz, *Phytomedicine*, **1**, 193 (1994).
6. S. Sultana, P. Shahid, I. Mohammad and A. Mohammad, *J. Ethnopharmacol.*, **45**, 189 (1995).
7. B. Jaiprakash, R. Aland, R.V. Karadi, R.V. Savadi and V.I. Hukkeri, *Indian Drugs*, **40**, 296 (2003).
8. H.T. Mallory and E.A. Evelyn, *J. Biol. Chem.*, **119**, 481 (1937).
9. S.R. Kingsley and S.J. Frankel, *J. Biol. Chem.*, **128**, 131 (1939).
10. S. Reitman and S.A. Frankel, *Am. J. Clin. Pathol.*, **28**, 56 (1957).
11. O.A. Bessey, D.H. Lowery and M.J. Brock, *J. Biol. Chem.*, **164**, 321 (1964).
12. H. Matsuda, K. Samukawa and M. Kubo, *Planta Med.*, **57**, 523 (1991).
13. A.E. Galigher and E.N. Kayloff, Essentials of Practical Microtechniques, Philadelphia: Lea and Febiger, p. 77 (1971).
14. E. Johnson and C. Krocning, *Pharmacol. Toxicol.*, **83**, 231 (1998).
15. S. Cotran, V. Kumar and S.L. Robbins, Cell Injury and Cellular Death, In: Robbins Pathologic Basic of Disease, Prism Book Pvt. Ltd., edn. 5, pp. 379-430 (1994).
16. N. Kaplowitz, T.Y. Aw, F.R. Simon and A. Stolz, *Ann. Int. Med.*, **104**, 826 (1986).
17. R.B. Drotman and G.T. Lawhorn, *Drug Chem. Toxicol.*, **1**, 163 (1978).

(*Received*: 3 July 2008; *Accepted*: 20 March 2009) AJC-7364