Protective Effect of *LIV-O-G* a Poly Herbal Formulation on Alcohol-CCL_4 and Paracetamol Induced Hepatotoxicity in Rats

Raghava Naidu S[1], Kumar G.S[2], Sivaji Gawade P[3], Kshama Devi [3]

[1]Department of Pharmacy, Island College of Technology, Balik Pulau, Pulau Penang, 11000, Malaysia.
[2]Department of Pharmacognosy, Sri Krishna Chaithanya College of Pharmacy, Madanapalle-517 325, Andhra Pradesh, India.
[3]Department of Pharmacology, Al-Ameen College of Pharmacy, Bangalore, Karnataka, India.

Summary

Hepatoprotective property of LIV-O-G (Respel pharma) a poly herbal formulation which are known for their hepatoprotective properties in ayurvedic system of medicine. The herbal formulation has been popularly in veterinary medicine. In the present study, the formulation was evaluated for its extent of protective effect against different known hepatotoxic agents viz, alcohol, CCl_4 and paracetamol. Treatment with LIV-O-G was followed up by measuring levels of serum marker enzymes like serum aspartate amino transferase (AST), serum alanine amino transferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH).serum levels of total proteins and bilirubin were also analyzed. The histopathological examination was also carried out to support the above parameters. Silymarin (100 & 200 mg/kg) was used as a

standard hepatoprotective drug. Administration of LIV-O-G (250, 500 and 750 mg/kg, p.o) significantly prevented alcohol-CCL_4 and paracetamol induced elevation levels of GOT, GPT, ALP, LDH and bilirubin. The decreased levels of total proteins (TP) also observed due to hepatic damage induced by alcohol-CCL_4 and paracetamol was found to be increased in LIV-O-G treated rats. Results were comparable to that of standard drug Silymarin. Histopathological studies were also revealed the reduction in degenerative fatty changes, distortion, cell swelling and necrosis of hepatic cells, as compared to CCl_4 treated group. Significant hepatoprotection of LIV-O-G is probably due to combination of all ingredients.

Key words: LIV-O-G, Carbon tetrachloride, alcohol Paracetamol, hepatoprotective activity.

Introduction

Liver, a primary organ of metabolism and excretion, is constantly endowed with the task of detoxification of xenobiotics, environmental pollutants, and chemotherapeutic agents. Therefore disorders associated with these organ are numerous and varied. There is an ever increasing need of an agent which could protect it from such damage. In view of severe undesirable side effects of synthetic drugs, there is growing interest to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines which are claimed to posse's hepatoprotective activity (1, 2).

Various polyherbal formulations like LIV-52, HD-03, Hepatomed, Jingirine and Livex are well known for their hepatoprotective effects. LIV-O-G a poly herbal

formulation combination of 13 herbs. The herbal constituents are mentioned in **Table 1**. These ingredients were earlier reported for their protective activity against different models (3). LIV-O-G has been claimed to improve the sub clinical liver dysfunction in cattle's when administered for six weeks. Liver protective property, as a growth promoter and weight gain following six weeks treatment with LIV-O-G was observed in broiler chicks (4). The present study was aimed to investigate the hepatoprotective effects of LIV-O-G a poly herbal formulation on rats using different hepatotoxic agents such as alcohol-CCL_4 and paracetamol.

Table No: 1

Composition and concentration of LIV-O-G

Botanical name	Family	Concentration
Phylantus niruri	Ephorbiaceae	65 mg
Tephorsia purpurea	Fabaceae	55 mg
Eclipta alba	Asteraceae	65 mg
Picrorrhira kurroa	Scrophulariaceae	65 mg
Tinospora cordifolia	Menispermaceae	60 mg
Cinchorium intybus	Asteraceae	32 mg
Plumbago zeylanica	Plumbaginaceae	16 mg
Andrographis paniculata	Acanthaceae	52 mg
Azadirachta indica	Meliaceae	65 mg
Carum copticum	Apiaceae	16 mg
Coriandrum sativum	Umbelliferae	26 mg
Embelica officinalis	Euphorbiaceae	16 mg
*Ocimum sanctu*m	Lamiaceae	16 mg

Materials and methods

Animals

Male albino rats of Sprague-Dawley strain weighing 150-280 g were used for the study. Approved by the ethics committee Al-Ameen College of pharmacy, Bangalore, India. They are maintained in controlled laboratory conditions of temperature, humidy, light and dark cycles. They were fed on balanced pelleted diet (M/s. Lipton India Ltd, and water *ad libitum)*.

Drug treatment and grouping of animals

Group 1: Control group received normal saline
Group 2: Received standard drug silymarin 50 mg/kg body weight.
Group 3: Received silymarin 100 mg/kg body weight standard drug for 7 days comparison.
Group 4: Received 40% v/v ethanol (2ml/kg) body weight orally for 21 days. On 20th day CCl_4 (1:1 in propylene glycol) served as hepatotoxic control
Group 5: Received test drug LIV-O-G 250 mg/kg + 40% v/v ethanol (2ml/kg) body weight orally daily for 21 days. On 20th day CCl_4 (1:1 in propylene glycol).
Group 6: Received test drug LIV-O-G 500 mg/kg + 40% v/v ethanol (2ml/kg) body weight orally daily for 21 days. On 20th day CCl_4 (1:1 in propylene glycol).
Group 7: Received paracetamol 750 mg/kg for 7 days hepatotoxic drug for comparison.
Group 8: Received LIV-O-G 125 mg/kg + paracetamol 750 mg/kg for 7 days.
Group 9:Received LIV-O-G 250 mg/kg + paracetamol 750 mg/kg for 7 days.
Group 10: Received test drug LIV-O-G 250 mg/kg body weight for 7 days.

The test drug LIV-O-G was suspended in distilled water and administered orally through an intragastric tube once daily.

Collection of serum and tissue samples

At the end of the experimental period, rats were sacrificed by decapitation. Blood was collected by excising the jugular vein. It was allowed to clot and then centrifuged at 3000 rpm for 15 min. The serum samples were collected and stored 0 C until further analysis. Liver was excised and fixed in 10% formalin for histopathological study.

Biochemical estimation

Serum enzymes such as AST, ALT, ALP, and TP were first standardized (5, 6). The protein content of serum was determined according to the procedure described by Lowry et.al. (7). determined by using BM/Hitachi autoanalyser 704/9011. Rallies Research Center, Bangalore, India.

Statistical analysis

The results were analyzed statistically using one-way analysis of variance (ANOVA) followed by Dennett's multiple comparison tests. Results of each group were statistically compared with that of the control group and expressed as mean ± S.E.M.

Results

Rats treated with alcohol-CCl_4 developed significant liver damage and observed with elevated serum levels of hepatic marker enzymes as well as histopathological changes were observed (**Table 2**). Serum AST, ALT, and ALP enzymes were increased in alcohol-CCl_4 and paracetamol treated rats. A decrease in total protein was

Table 2: Effect of LIV-O-G on rat *ser*um enzymes.

Sl.No.	Group	AST (IU/l)	ALT (IU/l)	ALP (IU/l)	TP (g/dl)
1.	Control	88.95± 2.375	43.29 ± 1.488	94.28 ± 1.138	4.728 ± 0.1496
2.	Silymarin (100 mg/kg)	109.6 ± 4.072**	81.53 ± 5.398**	137.70± 5.257**	5.277±0.1104**
3.	Silymarin (200 mg/kg)	102.8±3.234*	74.10±5.393*	129.9±4.866*	5.022±0.1379*
4.	Alcohol + CCl_4	251.8±15.66***	149.2±5.825***	282.7±11.22 ***	6.187±0.2187***
5.	LIV-OG (250 mg/kg) + Alcohol + CCl_4	212.5±16.02***	128.1±4.885***	237.6±8.595***	6.018±0.07939***
6.	LIV-OG (500 mg/kg) + Alcohol + CCl_4	172.4±9.180***	103.3±2.399***	193.3±5.682***	5.608±0.1971***
7.	PCT (750 mg/kg)	268.3±19.45***	143.4±5.285***	268.9±9.913***	6.077±0.1365***
8.	LIV-OG (125 mg/kg) + PCT	239.5±16.06***	128.1±3.199***	234.7±10.65***	5.907±0.1441***
9.	LIV-OG (250 mg/kg) + PCT	199.8±12.59***	113.7±2.023***	197.8±7.961***	5.472±0.1239***
10.	LIV-OG (250 mg/kg)	104.1±4.170 ns	47.20±2.821 ns	99.06±4.003 ns	4.757±0.1439 ns

Values indicate mean ± SEM for 6 animals in each group. *** $P < 0.0001$ significant in drug treated rats
ns: not significant. PCT- Paracetamol, CCl_4- Carbon tetrachloride, LIV-O-G- Poly herbal formulation.

observed in hepatotoxic treated rats. Treatment with test drug LIV-O-G was showed a significant protection against alcohol-CCl_4 and paracetamol induced alterations in serum enzyme levels, and total proteins. The degree of hepatoprotection was observed maximally with the highest dose of poly herbal formulation. (i.e., 500 mg/kg body weight). Histopathological studies (compared to controls) demonstrated congestion of portal vessels, loss of structural integrity, characterized by cell swelling, vacuolar degeneration, fatty degeneration and necrosis induced by CCl_4 and alcohol treated rats and these changes and hepatic architecture is preserved in alcohol-CCl_4 and paracetamol treated rats.(Fig..1-8)

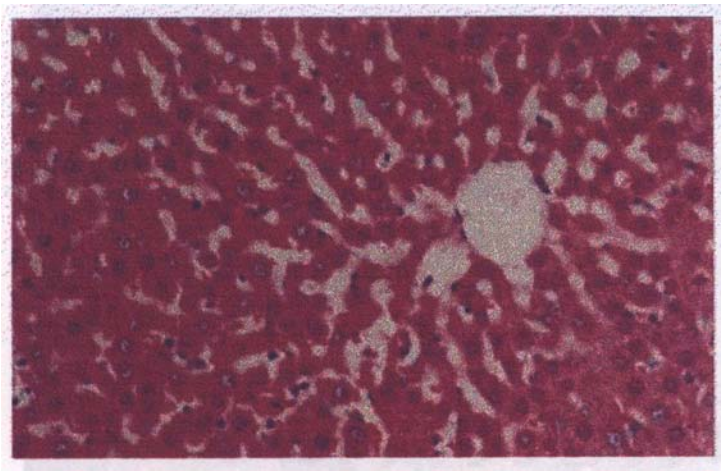

Fig:1 Liver section of control rat showing structural integrity of hepatic architecture (1000 X)

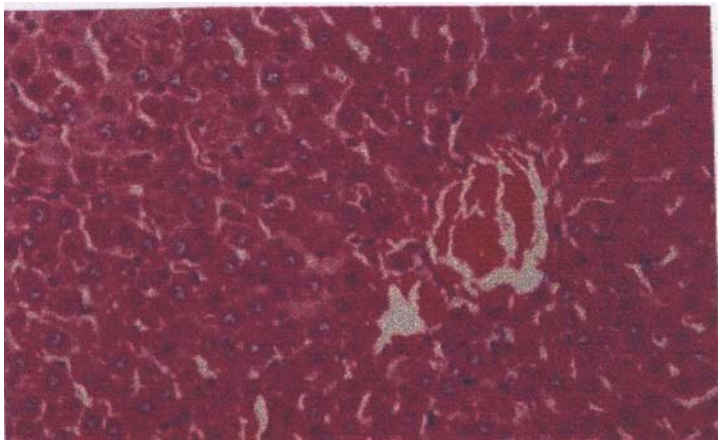

Fig. 2: Hepatocytes were arranged in a cord like structure. Structural integrity was maintained (Silymarin 100 mg/kg)

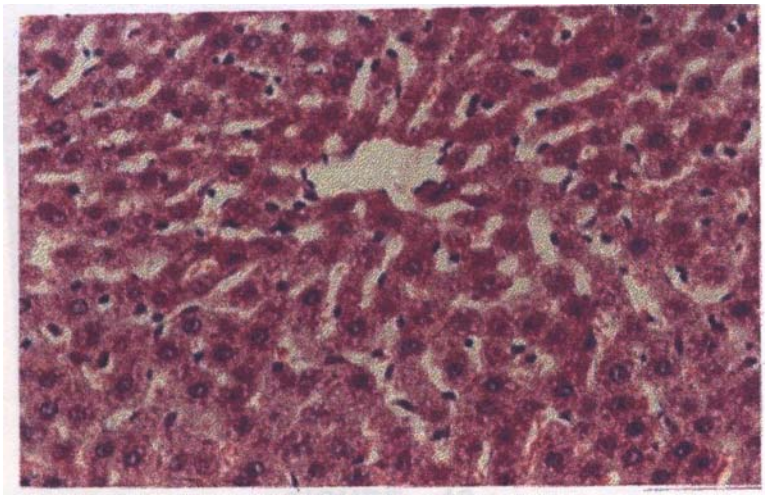

Fig. 3: Liver section showing structural integrity of hepatic architecture (LIV-O-G 500 mg/kg)

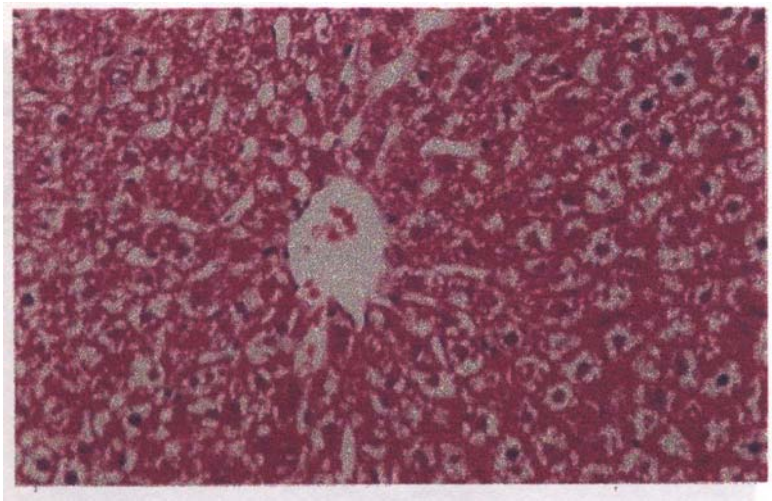

Fig 4. Liver section showing a moderate degree of cell swelling (early degenerative changes) and focal area of necrosis paracetamol (750 mg/kg) (1000 X).

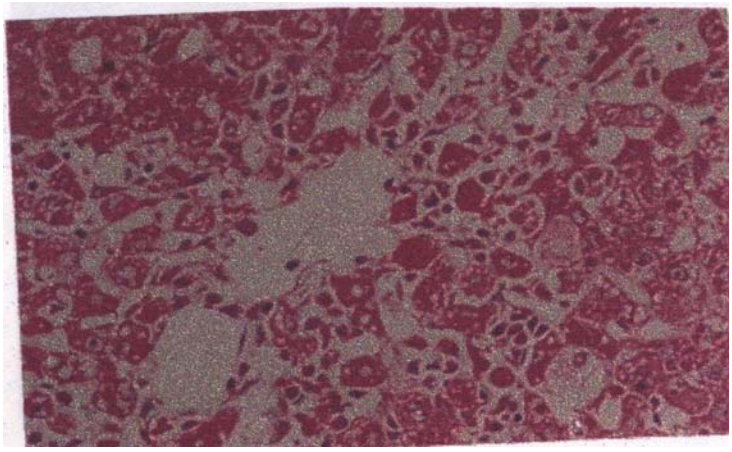

Fig 5. Liver section showing total loss of structural integrity, characterized by cell swelling, vacuolar degeneration, increased mitosis, intracytoplasmic fatty degeneration and necrosis (1000 x) (Alcohol 40% 2ml/kg + CCl_4)

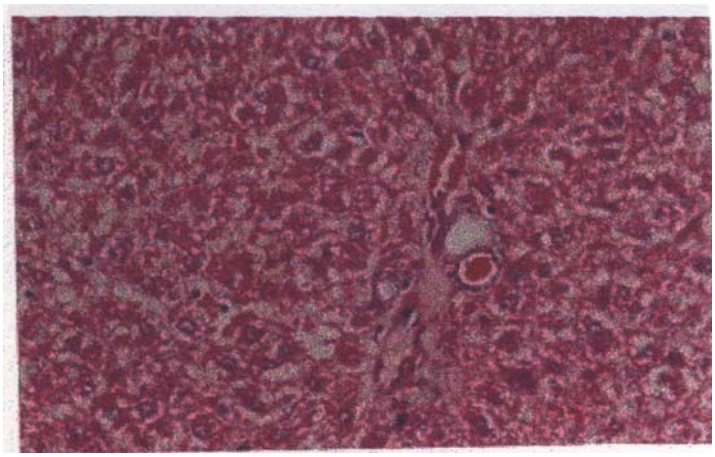

Fig. 6. Liver section showing minimal sinusoidal dilatation, hepatic architecture almost regained (Alcohol-CCl_4 + LIV-OG 250 mg/kg) (1000 x).

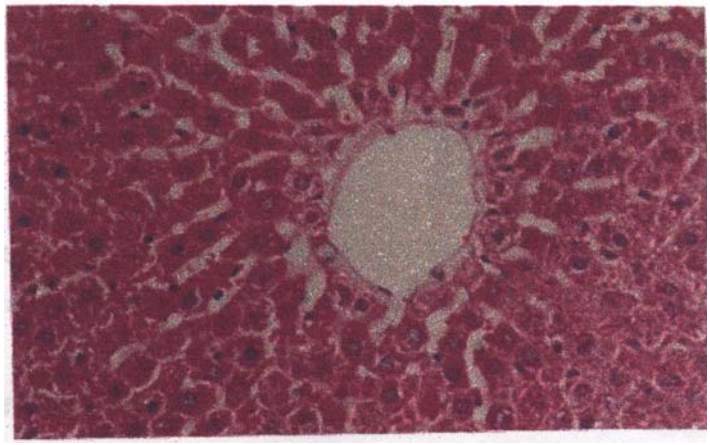

Fig.7. Liver section showing intact hepatocytes and their architecture regenerative hyperplasia can be seen. (PCT 750 mg/kg + LIV-OG 250 mg/kg)
(1000 x)

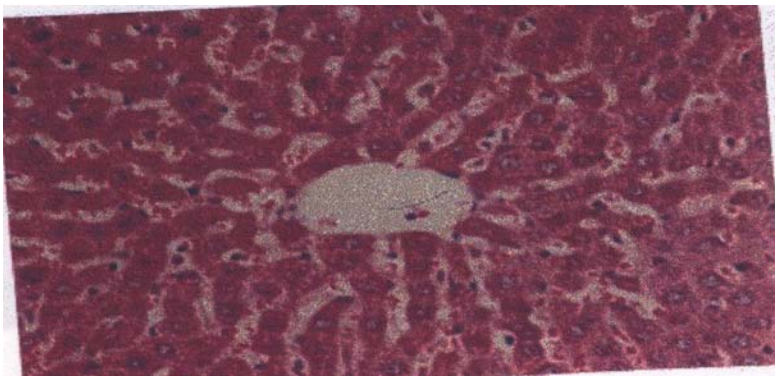

Fig 8. Liver section showing minimal damage with the cell swelling, focal necrosis. Absence of fatty degeneration, mitotic index and hemorrhages are clearly evident. (Alcohol-CCl_4 + LIV-OG)
(1000 x).

Discussion

Liver diseases arise due to liver cell damage and proliferation of fibrous tissue. The damage certainly becomes extensive and normal structure of the liver distorted and its function becomes impaired. This abnormality affects almost every physiologic process including digestion, endocrine, circulation and other metabolic functions. These changes themselves slowly aggravate liver damage.(8). The main aim of the medication is to prevent metabolic abnormalities and regeneration of hepatic cells.

In order to metabolize drugs efficiently, liver secrets drug metabolizing enzymes that help in drug metabolism and are of immense physiological importance (9). Most of these enzymes are mainly located in the hepatic microsomes. Biotransformation of a drug or xenobiotics compound following its exposure can alter its distribution and action leading to its detoxification and excretion or enhance its toxicity due to the activation of the compound or due to the biochemical disruption caused by reactive metabolites arising from biotransformation (10, 11). Biotransformation of xenobiotics usually occurs in two phases. Phase I metabolism (detoxification) involves oxidative, reductive and / or hydrolytic reactions that clone substrate molecule to produce more polar moiety. Phase II reactions (synthetic reaction) involve conjugation of certain endogenous molecules to the products of phase I reaction (12). Cytochrome P 450 and its isoforms are responsible for the metabolic conversion of many drugs to the polar metabolites via phase I and Phase II reaction to earlier excretion. CCl_4 induced hepatotoxicity in rat's represents an adequate established experimental model of liver cirrhosis in man and it's used for the screening of hepatoprotective drugs (13, 14).

The biotransformation of CCl_4 occurs in the endoplasmic reticulum and is mediated by CYP 450 (15). The principle isoform implicated as the catalyst being CYP 2E1 (16). CYP 450 is inhibited suicidally by the reactive metabolities of CCl_4. $CCl_3^{\cdot}$ radical initially formed being relatively unreacting reacts very rapidly with oxygen to yield a highly reactive trichloromethyl peroxy radical (CCl_3 00 $^{\cdot}$) which is the probable initiator of lipid peroxidation (17).

Alcohol-induced liver damage (ALD) is due to increased lipid peroxidation, impaired antioxidant status, the appearance of free radical adducts derived from fatty acid breakdown and CYP2E1-dependent ethanol metabolism to the 1-hydroxyethyl radical have all been shown to correlate with the development of pathology. (18-21).

Paracetamol at subtoxic dose can cause significant liver damage and can be assessed by the level of released cytoplasmic enzymes (ALP, AST and ALT in circulation (22). Both paracetamol and CCl_4 share the common property of being converted into their respective metabolities N-acetyl-p-benzoquinoneimine (NAPQI) and halogenated free radicals (HFR) by hepatic cytochrome P450 s (23). The production of such reactive species is compensated by physiological moieties (glutathione and alpha tocopherol) and the widespread damage to the macromolecules in vital biomembranes, which only occurs when there is depletion of protective moieties due to massive production of reactive species, particularly with paracetamol (24).

Based on these statements we conclude that LIV-O-G a combination of 13 herbs possesses significant hepatoprotective activity against alcohol-CCl_4 and paracetamol induced hepatotoxicity in rats. Histopathological examination also revealed that LIV-O-G preserves the hepatic architecture, reduces cell swelling, and mitosis.

References

1. Handa SS, Sharma A, and Chakraborthi KK. Natural products and plants as liver protecting agents. Fitoterapia. 1986; LVII (5):307-351.
2. Shahani S. Evaluation of hepatoprotective efficacy of APCL-A polyherbal formulation in *Invivo* in rats. Indian drugs. 1999; 36: 628-631.
3. Jindal MN, Patel VK, and Patel NB. Some pharmacological activities of aqueous and alcoholic extract *Cinchorium intibus* Linn. Ind Jour Pharmacol. 1975; 7: 104.
4. Ranade AS, Rajmane BV, Kulkarni PP, Bedse NC. Effect of LIV-O-G an herbal feed supplement on commercial broilers, Department of poultry Science, Bombay Veterinary College, 1998; 1-7.
5. Mohun AF, and Cook IJK. Simple methods for measuring serum levels of the glutamic oxaloacetic and glutamic pyruvic transaminases in routine laboratories. *Jour ClinicPathol*, 1957; 10: 394-399.
6. Kind PRN, and King EJ. Elimination of plasma phosphate by determination of hydrolysed phenol with aminoantipyrine. Jour Clinic Pathol. 1954; 7: 322-326.
7. Lowry OH, Rosebrough NJ, Farr AL, and Randall RJ. Protein measurement with folin-phenol reagent. *Jour Biologl Chem.* 1951;193: 265-275.
8. Fallah Huseini H, Alavian SM, Heshmat R, Heydari MR, and Abolmaali K. The efficacy of Liv-52 on liver cirrhotic patients: A randomized, double-blind, placebo-controlled first approach. Phytomedicine. 2005; 12(9): 619-24.
9. Rao RR. Mechanism of drug induced toxicity. *Ind Jourf Pharmacol.* 1973; 5: 313-318.
10. Athar M, Zakir Hussain S, and Hassan N. Drug metabolizing enzymes in the liver. In: Rana, SVS,

Taketa K. (Eds.), *Liver and Environmental Xenobiotics. Narosa Publishing House*, New Delhi. 1997. p. 189.

11. Plaa GL. Toxic responses of the liver. In: Amdur, M.O., Doull, J., Klassen, C.D. (Eds.), *Casserett and Doull's Toxicology. Pergamon Press,* New York. 1991. p. 876.
12. Remmer HI. The role of liver in drug metabolism. Amr Jour Med. 1970 ;49: 617.
13. Perez-Tamayo R. Is cirrhosis of the liver experimentally produced by CCl_4 an adequate model of human cirrhosis? Hepatology 1983; 3: 112-120.
14. Lopez-Novoa JM, Rengel MA Rodicio JL, and Hernando L. A micropuncture study of salt and water retension in chronic experimental cirrhosis. Amr Jour Physiol. *1977;232: F315-F318.*
15. Castro J, Sasame H, Sussman H, and Bullette P. Diverse effects of SKF 525A and antioxidants on CCl_4-induced changes in liver microsomal P450 content and ethylmorphine metabolism. Life Sciencea. 1968; 7: 129-136.
16. Al-Shabanah OA, Alam K, Nagi MN, Al-Rikabi AC, and Al-Bekairi AM. Protective effect of aminoguanidine, a nitric oxide synthase inhibitor against CCl4-induced hepatotoxicity in mice. Life Sciences. 2000;66: 265-270.
17. Bhat VB, Madyastha KM. C-phycocyanin: a potent peroxyl radical scavenger *In Vivo* and *Invitro*. BiochemBiophy Res Comm. 2000;275: 20-25.
18. Albano E. In: Sherman, C. D. I. N., Preedy, V. R., Watson, R. R., Eds. Ethanol and the liver. *London: Taylor & Francis* 2002. p. 153-159.
19. Paradis V, Kollinger M, Fabre M, Holstege A, Poynard T, and Bedossa P. In situ detection of lipid peroxidation of lipid peroxidation by-products in

chronic liver diseases. Hepatology. 1997;26: 135-142.
20. Knecht KT, Bradford BU, Mason RP, and Thurman RG. *Invivo* formation of a free radical metabolite of ethanol. *Mol. Pharmacol*. 1990;38: 26-30.
21. Albano E, Clot P, Morimoto M, Tomasi A, Ingelman-Sundberg M, and French SW. Role of cytochrome P4502E1-dependent formation of hydroxyethyl free radical in the development of liver damage in rats intragastrically fed with ethanol. Hepatology. 1996; 23:155-163.
22. Chenoweth MB. and Hake CL. The smaller halogenated aliphatic hydrocarbons. Ann Rev Pharmacol. 1962; 2: 363-398.
23. Lee WM. Review article: drug-induced hepatotoxicity. Alim Pharmacol Therap. 1993;7:241-250.
24. Miller MG, Beyer J, Hall GL, Grafenried LA, and Adams PE. Predictive value of liver slices for metabolism and toxicity *in vivo*: use of acetaminophen as model hepatotoxicant. *Toxicol App Pharmacol*. 1993; 122: 108-116.