

Screening of hepatoprotective effect of a herbal mixture against CCl₄ induced hepatotoxicity in Swiss albino mice

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Abstract: The hepatoprotective potential of a herbal mixture was evaluated against CCl₄ induced liver injury in Swiss albino mice. Liv 52, a commercially available polyherbal hepatoprotective drug was evaluated for comparison. The potential toxicity of the above herbal hepatoprotective agents was also compared. It was observed that there was a reduction in the enzyme biomarkers (Aspartate and Alanine Transaminase) of liver injury in the herbal mixture treated groups, which was similar to the reduction initiated by Liv 52. An increase in glutathione was observed in the herbal mixture treated groups and it was assumed that the herbal mixture protects the liver by virtue of its antioxidant nature along with high regeneration initiation potential. From the study it is also concluded that the herbal mixture is safer than Liv 52.

Key words: *Asparagus racemosus*, *Boerhaavia diffusa*, *Glycyrrhiza glabra*, *Hemidesmus indicus*, *Phyllanthus amarus*, *Phyllanthus emblica*, *Picrorhiza scrophulariiflora*, *Ricinus communis*, *Tinospora cordifolia*, CCl₄ hepatotoxicity, Liv 52, Liver

Introduction

Liver, the largest organ of the body comprising 2-3% of the total adult body weight, is primarily concerned with the metabolic activity of organisms (Sheila and Dooley, 1993). It is also the central site for the biotransformation of xenobiotic chemicals and therefore is involved in the detoxifying mechanism of the body. Liver is responsible for detoxifying the chemical substances in the blood and in this process it is exposed to high concentrations of toxicants and toxic metabolites making it susceptible to injury (Glaister, 1986). The liver damage caused by pathogens as well as chemical agents is of similar nature and a proper treatment regime or plan is absent for both. The fact that reliable liver protective drugs are explicitly inadequate in allopathic medicine (Neha and Rawal, 2000), exhorted the scientists to explore herbal remedies.

In the traditional medical practices, followed throughout the world, herbs play a major role in the management of various liver disorders. Poly-herbal formulations are preferred by the traditional healer than a single herb. The phytotherapists always prefer to prescribe chemically complex remedies and also administer them as complex formulations because the basic fundamental of phytotherapy is that life is chemically complex and so is our food, and therefore the medicines should also be chemically complex (Mills and Bone, 2000). The different plants in the herbal mixture will have different modes of action for curing the disease and in the combined form may sometimes exhibit synergistic activity (enhanced activity than that of the individual herbs). Components of the plants, which are not active themselves, can act to improve the stability,

solubility and bioavailability or half life of the active components. Hence a particular active principle in the pure form may have only a fraction of the pharmacological activity that it has in its plant matrix, which again highlights the importance in using the plant as a whole or a mixture of plants for treating a disease (Mills and Bone, 2000).

Individual and polyherbal preparations are used for the treatment of various diseases by the local medical practitioners all over India. The constituent plants in the herbal mixture or the different combinations of the same are used in folklore remedies. In the rural areas of India, people usually use combinations of these plants to prepare medicines, for the treatment of various disease including liver disorders, right at home (Sairam, 1998). Some of the constituent plants of the herbal mixture namely *Glycyrrhiza glabra* Linn., (Yamamura *et al.*, 1997), *Hemidesmus indicus* (Prabakan *et al.*, 2000), *Phyllanthus amarus* (syn. *Phyllanthus niruri*) (Rajeshkumar and Kuttan, 2000; Liu *et al.*, 2001; Xin-Hua *et al.*, 2001), *Phyllanthus emblica* (Xia *et al.*, 1997), *Picrorhiza scrophulariiflora* Pennel (Dwivedi *et al.*, 1990; Saraswat *et al.*, 1993; Vaidya *et al.*, 1996) *Ricinus communis* Linn., (Chemexil, 1993) and *Tinospora cordifolia* Willd., (Nagarkatti *et al.*, 1994) are traditionally used and scientifically proven for the treatment of liver disorders. The constituent plants can be used individually but are usually used in the form of various combinations and the pertaining information was obtained from a book on preparation of traditional herbal medicines Ayurveda

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Oushadha nimanam, Sidhandavum prayogangalum (preparation of Ayurvedic medicines – principles and uses) (Prince and Rajani, 1997) in a local South Indian language (Malayalam).

Chemically induced hepatotoxicity in animals has been widely used for the screening of hepatoprotective herbal remedies (Liu *et al.*, 1994). Chemical induced damage of animal liver, especially rodents, mimic both pathogen induced as well as chemical induced liver injury in man. In the present study the hepatoprotective effect of the herbal mixture was evaluated against CCl_4 induced hepatotoxicity in mice. Studies have already shown that CCl_4 induced cirrhosis of the liver is an adequate model of alcoholic cirrhosis in humans and the histological and biochemical changes that develop in the CCl_4 treated animals were found to mimic human cirrhosis observed in several etiological types (Perez-Tomayo, 1983). Acute administration of carbon tetrachloride to rodents especially rats and mice induce centrilobular necrosis and steatosis and the chronic administration leads to fibrosis and cirrhosis of the liver (McLean *et al.*, 1969). CCl_4 induces liver damage by the free radical mechanism and consists of two stages. The initial phase of injury to the liver is caused by the free radical of CCl_4 namely trichloromethyl radical ($\text{CCl}_3\cdot$). The second phase of injury is by the cascade of events in the metabolism of the trichloromethyl radical ($\text{CCl}_3\cdot$) which leads to lipid peroxidation (Timbrell, 1991). Since the mode of hepatic injury by CCl_4 is biphasic, it can be used for predicting the mode of action of the hepatoprotective agent. Many studies have been carried out using CCl_4 induced liver injury in rodents for the screening of herbal remedies. Commercial formulations like Picroliv (Dwivedi *et al.*, 1990) and Liv 52 (Kataria and Singh, 1997) and extracts from plants like *Ginkgo biloba* (Ashok Shenoy *et al.*, 2001) and *Acanthus ilicifolius* (Babu *et al.*, 2001) have been tested and found effective against CCl_4 induced liver injury, *in vivo*.

Herbs and herbal preparations are generally viewed as safe by the general public. But along with the beneficial components they may contain many bioactive compounds which could impose potentially deleterious effects. The prime objective of the experiment was to confirm the hepatoprotective activity of the herbal mixture against CCl_4 induced hepatotoxicity in Swiss albino mice. The activity of the herbal mixture was compared with that of a known hepatoprotective herbal drug, Liv 52. The toxicity of the herbal mixture was also evaluated as a part of the experiment.

Materials and Methods

Herbal mixture and Liv 52: The herbal mixture, evaluated for hepatoprotective effect comprised the extracts of 9 plant species namely *Asparagus racemosus* Willd. (root), *Boerhaavia diffusa* Linn. (whole plant), *Glycyrrhiza glabra* Linn. (root), *Hemidesmus indicus* R.Br. (root), *Phyllanthus amarus* Linn. (whole plant), *Phyllanthus emblica* Linn. (fruit), *Picrorhiza scrophulariiflora* Pennel (root), *Ricinus communis* Linn. (fruit) and *Tinospora*

cordifolia Willd (stem) mixed in equal proportion. The herbal mixture was obtained in the form of tablets on gratis from Cybele Herbal Laboratories (P) Ltd., Cochin, India. The herbal mixture was administered by oral intubation at a dose of 1000 mg/ kg body weight.

Liv 52 (Himalaya Drugs, India), a commonly available hepatoprotective drug (purchased from a local medical shop) was administered by oral intubation at a dose of 1000 mg/ kg body weight. The composition of Liv 52 is *Capparis spinosa*, *Cichorium intybus*, *Solanum nigrum*, *Cassia occidentalis*, *Terminalia arjuna*, *Achillea millefolium*, *Tamarix gallica*, *Eclipta alba*, *Phyllanthus niruri*, *Berberis aristata*, *Taphanus sativus*, *Phyllanthus emblica*, *Plumbago zeylanica*, *Boerhaavia diffusa*, *Tinospora cordifolia*, *Embelia ribes*, *Terminalia chebula* and *Fumaria officianlis* (Singh *et al.*, 1983).

Prior to dosing, the tablets of herbal mixture Liv 52 were powdered and made into a fine suspension in distilled water using mortar and pestle.

Carbon tetrachloride: The chemical used for inducing hepatic injury namely carbon tetrachloride [CCl_4] (Ranbaxy Fine Chemicals Ltd., Punjab) was diluted in liquid paraffin at 1:2 ratio and administered intraperitoneally at a dose of 2 ml/ kg body weight.

Animals: Swiss albino mice (*Mus musculus*), 5 to 6 weeks old, 20±2 g. were procured from the animal house of international institute of biotechnology and toxicology (IIBAT) and acclimated to laboratory conditions (temperature $21 \pm 3^\circ\text{C}$, humidity 30-70% and 12 hr light and 12 hr dark rhythm) for a week prior to the start of the experiment.

Allocation of groups and experimental procedure: The animals (24 males + 24 females), were randomly divided into 6 groups (4 males + 4 females per group). Group 1 was maintained as the untreated control. Groups 3, 5 and 6 received 5 administrations (once every 48 hr) of CCl_4 intraperitoneally. Oral administration of herbal mixture to groups 2 and 3 and Liv 52 to groups 4 and 5 was carried out once daily for 9 days. The treatment was fixed to nine days based on the results of an earlier study in rats. The dose regime of CCl_4 followed in the present study is sufficient to induce hepatotoxicity as evidenced by the increase in plasma levels of the liver function enzymes. The objective of the study was to investigate the hepatoprotective of the herbal mixture when administered simultaneously with induction of hepatic injury. The terminal blood collection did prove that the CCl_4 alone treated group showed an elevation in the liver function enzymes while the herbal mixture and Liv 52 treated groups showed a lowering of these enzymes. The period of nine days was solely based on the time period required by CCl_4 to induce full-fledged hepatotoxicity.

Blood collection was carried out in anaesthetized animals, 24 hr after the last administration of CCl_4 , from the retro-orbital

sinus using heparinised haematocrit capillaries (TOP Syringe Mfg. Co., Bombay). A portion of blood was collected in heparinised vials for biochemistry and the other portion in EDTA-ed vials for hematology. A blood smear was also prepared. After the blood collection, the animals were euthanised by intraperitoneal injection of thiopental sodium (100 mg/kg body wt.). Gross pathological examinations of the animals were carried out and the weight of liver was taken. Homogenated liver was prepared in phosphate buffer (pH 7.4) for biochemical estimation.

Hematological and biochemical estimations: Red blood corpuscles (RBC), white blood corpuscles (WBC), hemoglobin (Hb) and packed cell volume (PCV) were read on an Erma particle counter (Model PC 607 Erma Inc., Japan). In addition to above, differential count of cells in the blood smears were also carried out. The biochemical parameters in the plasma and liver homogenate were estimated using Erba test reagent kits (Transasia Biomedicals Ltd., Bombay, India) on an Erba smartlab auto-analyser (Transasia Biomedicals Ltd., Bombay, India). The biochemical parameters estimated in blood include alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose (GLU), blood urea nitrogen (BUN) and creatinine (CRE). In the liver homogenate alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH) and glutathione (GSH) were estimated.

Statistical analysis: Data were subjected to Barlett's test for homogeneity, followed by analysis of variance (ANOVA). For post hoc comparison Student Newman Keul's test was employed.

Results and Discussion

Hematological investigations did not reveal any significant changes among the groups while a sex wise variation was noticed in the case of biochemical parameters both in the blood as well as in the liver homogenate.

Biochemical estimations in blood: In male mice, ALT was similar in control and the groups treated with either herbal mixture (G2) or Liv 52 (G4) alone. Although the ALT in group 3 (Herbal mixture + CCl₄) and group 5 (Liv 52 + CCl₄) were higher than the control, they showed a significant decrease when compared with the CCl₄ alone treated group (G6). But in female mice, the ALT levels were significantly higher in all the CCl₄ treated groups (G3, G5 and G6) when compared to untreated control (G1), herbal mixture alone treated (G2) and Liv 52 alone treated (G4) groups. In male mice, the AST values in groups 2, 3, 4 and 5 were similar to that of untreated control and a significant increase was noticed only in the CCl₄ alone treated group (G6), but in female mice a significant increase of AST was observed in all the CCl₄ treated groups (G3, G5 and G6). The glucose values in all the groups were similar to control in both male and female mice but a significant increase of glucose was observed in males of Liv 52 alone treated group, when compared with the males of CCl₄ alone treated group. In both males and females, the BUN values in the herbal mixture treated groups (G2 and G3) were similar to the untreated control (G1) while it was significantly higher in the Liv 52 treated groups (G4 and G5) and CCl₄ alone treated group (G6). Creatinine did not show any sex wise variation and was similar to control in all the groups (Table 1).

Biochemical estimations in liver homogenate: In the liver homogenate enzymes like GGT and ALP did not show any variation among the groups. In the case of male mice, the LDH values in the herbal mixture treated groups (G2 and G3) were similar to that of the untreated control (G1), but it was significantly higher in the Liv 52 treated groups (G4 and G5) and in CCl₄ alone treated group while in female mice an increase of LDH was observed only in group 5 (Liv 52 + CCl₄) and group 6 (CCl₄ alone). A significant increase in glutathione levels was observed in the herbal mixture treated groups (G2 and G3) in the case of

Table - 1: Effect of herbal mixture and Liv 52 on the biochemical parameters in the plasma of mice subjected to CCl₄ toxicity

Group	ALT (U/l)	AST (U/l)	GLU (mg/dl)	BUN (mg/dl)	CRE (mg/dl)
Male					
G1 (Untreated control)	42.43 ^a ± 14.74	100.99 ^a ± 27.06	134.95 ^{ab} ± 14.04	22.75 ^a ± 2.29	1.18 ^a ± 0.23
G2 (HM 1000 mg/kg)	64.59 ^a ± 15.19	113.08 ^a ± 20.83	142.90 ^{ab} ± 13.78	24.28 ^a ± 1.07	1.66 ^a ± 0.12
G3 (CCl ₄ + HM 1000 mg/kg)	1488.30 ^b ± 940.50	554.50 ^a ± 119.70	130.17 ^{ab} ± 8.18	32.61 ^{ab} ± 4.00	1.82 ^a ± 0.09
G4 (Liv 52 1000 mg/kg)	214.55 ^a ± 52.09	323.76 ^a ± 154.79	169.37 ^b ± 16.88	47.26 ^b ± 8.13	1.58 ^a ± 0.15
G5 (CCl ₄ + Liv 52 1000 mg/kg)	713.63 ^{ab} ± 168.88	450.04 ^a ± 171.05	138.78 ^{ab} ± 10.21	47.19 ^b ± 5.25	1.59 ^a ± 0.44
G6 (CCl ₄ alone)	2047.65 ^c ± 327.98	2778.87 ^b ± 531.56	101.46 ^a ± 16.57	50.74 ^b ± 8.69	0.85 ^a ± 0.15
Female					
G1 (Untreated control)	43.94 ^a ± 2.52	105.70 ^a ± 9.01	137.90 ^a ± 18.70	24.46 ^a ± 1.13	1.07 ^a ± 0.20
G2 (HM 1000 mg/kg)	45.27 ^a ± 6.09	130.51 ^a ± 41.22	158.20 ^a ± 3.40	20.68 ^a ± 2.34	1.43 ^a ± 0.10
G3 (CCl ₄ + HM 1000 mg/kg)	2702.15 ^c ± 227.15	2049.85 ^b ± 629.75	126.63 ^a ± 7.24	29.69 ^{ab} ± 1.37	1.14 ^a ± 0.14
G4 (Liv 52 1000 mg/kg)	121.86 ^a ± 22.95	162.86 ^a ± 31.22	142.03 ^a ± 6.47	41.05 ^{bc} ± 3.93	0.91 ^a ± 0.134
G5 (CCl ₄ + Liv 52 1000 mg/kg)	1207.11 ^b ± 470.21	1880.63 ^b ± 630.71	177.30 ^a ± 39.32	40.40 ^{bc} ± 4.44	1.11 ^a ± 0.31
G6 (CCl ₄ alone)	1696.19 ^b ± 331.99	2175.80 ^b ± 481.99	145.25 ^a ± 10.66	39.81 ^c ± 3.55	1.27 ^a ± 0.19

Values are presented as mean ± standard error

Values having similar superscripts are not statistically significant (p>0.05)

HM – Herbal mixture



Table - 2: Effect of herbal mixture and Liv 52 on the biochemical parameters in the liver homogenate of mice subjected to CCl₄ toxicity

Group	ALP (U/g)	GGT (U/g)	LDH (U/g)	GSH (mg/g)
Male				
G1 (Untreated control)	2.08 ^a ± 0.27	0.47 ^a ± 0.02	5.44 ^a ± 0.02	0.64 ^a ± 0.07
G2 (HM 1000 mg/kg)	1.77 ^a ± 0.14	0.38 ^a ± 0.13	12.54 ^a ± 1.48	2.42 ^{ab} ± 0.24
G3 (CCl ₄ + HM 1000 mg/kg)	1.68 ^a ± 0.16	0.52 ^a ± 0.08	8.11 ^a ± 3.38	3.33 ^b ± 1.47
G4 (Liv 52 1000 mg/kg)	1.93 ^a ± 0.19	0.53 ^a ± 0.15	29.71 ^b ± 6.15	0.38 ^a ± 0.04
G5 (CCl ₄ + Liv 52 1000 mg/kg)	1.84 ^a ± 0.18	0.43 ^a ± 0.13	31.67 ^b ± 2.87	0.97 ^a ± 0.08
G6 (CCl ₄ alone)	1.86 ^a ± 0.15	0.44 ^a ± 0.02	36.22 ^b ± 4.85	0.66 ^a ± 0.04
Female				
G1 (Untreated control)	1.83 ^a ± 0.27	0.41 ^a ± 0.08	6.78 ^a ± 1.50	0.91 ^a ± 0.23
G2 (HM 1000 mg/kg)	2.11 ^a ± 0.34	0.34 ^a ± 0.15	7.64 ^a ± 0.64	0.45 ^a ± 0.23
G3 (CCl ₄ + HM 1000 mg/kg)	2.25 ^a ± 0.41	0.53 ^a ± 0.06	13.28 ^a ± 0.92	3.73 ^b ± 1.65
G4 (Liv 52 1000 mg/kg)	2.85 ^a ± 0.25	0.49 ^a ± 0.17	12.53 ^a ± 1.35	0.55 ^a ± 0.11
G5 (CCl ₄ + Liv 52 1000 mg/kg)	2.40 ^a ± 0.09	0.31 ^a ± 0.11	39.03 ^b ± 7.72	0.99 ^a ± 0.24
G6 (CCl ₄ alone)	2.17 ^a ± 0.17	0.50 ^a ± 0.04	60.95 ^c ± 9.90	0.54 ^a ± 0.07

Values are presented as mean ± standard error

Values having similar superscripts are not statistically significant (p>0.05)

HM – Herbal mixture

males and in group 3 (Herbal mixture + CCl₄) alone in the case of females, when compared to the other groups (Table 2).

Gross pathology observations did not reveal any significant pathological manifestations among the groups. In male mice a significant increase in liver weight was observed in Groups 3, 4 and 5, when compared to the untreated control (G1) but no difference could be observed among the groups in the case of female mice (Table 3).

Liv 52 when tested against rats chronically treated with antitubercular drug was found to be hepatoprotective (Vijaya Padma *et al.*, 1998). The hepatoprotective effect of Liv 52 was also observed in mice and it protected the liver from aldrin induced hepatotoxicity (Rathore *et al.*, 1994).

In the present study, the hepatoprotective effect of the herbal mixture could be deciphered from the decrease in ALT and AST levels in the herbal mixture + CCl₄ and Liv 52 + CCl₄ (G3 and G5) treated groups (male mice) when compared with the CCl₄ alone treated group (G6). In an earlier study, the administration of isosafrole reduced the levels of ALT and AST in male mice treated with CCl₄ (similar to the decrease in ALT and AST in the present study) confirming the hepatoprotective effect of isosafrole (Zhao and O'Brien, 1996). But in the present study, the change in the levels of ALT and AST was easily noticeable, as a decreasing trend was not observed in female mice of the same groups (G3 and G5). The levels of ALT and AST indicated the protection offered by both herbal mixture and Liv 52 against CCl₄ induced injury in male mice while offering little or no protection to female mice. Earlier studies in Sprague Dawley rats have shown the sex-wise variation in ALT and AST following an acute CCl₄ exposure. In female rats, hepatic damage peaked at 24 hr following the treatment and was approximately 2.5-fold (AST 2.7-fold, ALT 2.3 fold) greater than the damage observed in male

Table - 3: Effect of herbal mixture and Liv 52 on relative liver weight of mice subjected to CCl₄ toxicity

Group	Liver weight to body weight ratio (%)	
	Male	Female
G1 (Untreated control)	3.98 ± 0.17 ^a	6.11 ± 0.29 ^a
G2 (HM 1000 mg/kg)	4.91 ± 0.32 ^{ab}	5.41 ± 0.32 ^a
G3 (CCl ₄ + HM 1000 mg/kg)	6.36 ± 0.58 ^b	6.27 ± 0.14 ^a
G4 (Liv 52 1000 mg/kg)	5.70 ± 0.20 ^b	5.50 ± 0.33 ^a
G5 (CCl ₄ + Liv 52 1000 mg/kg)	5.76 ± 0.38 ^b	5.37 ± 0.25 ^a
G6 (CCl ₄ alone)	5.02 ± 0.29 ^{ab}	5.62 ± 0.44 ^a

Values are presented as mean ± standard error

Values having similar superscripts are not statistically significant (p>0.05)

HM – Herbal mixture

rats the hepatic damage in male rats appearing to peak around 3 hr post exposure (Moghaddam *et al.*, 1998). In the present experiment the animals were sacrificed 24 hr after the last dose of CCl₄. The high enzyme levels (ALT and AST) in the herbal mixture + CCl₄ and CCl₄ treated female mice could be because the peak damage due to CCl₄ occurs 24 hr following its administration. In male mice the peak damage would have occurred around 3 hours after CCl₄ dosing and the drugs (herbal mixture and Liv 52) would have enhanced the recovery rate of liver thus lowering the enzyme levels while it still remained high in the CCl₄ alone treated group (G6). In female mice the expected time for peak injury due to CCl₄ could be around 24 hr after CCl₄ dosing and the high enzyme levels of ALT and AST in the herbal mixture/ Liv 52 + CCl₄ treated groups (G3 and G5) could be due to the lack of prevention of CCl₄ induced damage by the drugs. So it could be postulated that both the drugs rather than preventing CCl₄ induced hepatic injury, enhance the rate of recovery of the liver after damage. But in the female mice of group (G3) (herbal mixture + CCl₄) the ALT levels were significantly higher than even the CCl₄ alone treated group. It is known that agents that induce drug metabolizing enzyme systems

tend to potentiate hepatic injury produced by compounds such as chloroform, carbon tetrachloride and halothane (Gopinath and Ford, 1975). Similarly, the herbal mixture would have potentiated CCl_4 induced hepatic injury to a certain extent (which lead to the rise of ALT in female mice of G3) and the same reason could be attributed for the lack of prevention of hepatic injury although enhancing the recovery of liver. The changes (both increase as well as decrease) in the levels of glucose can be due to liver injury or liver disease (Ellefson and Caraway, 1976). In the present experiment there were slight changes in glucose levels in male mice (increase of glucose in Liv 52 alone treated group compared to CCl_4 alone treated group) but since it was similar to untreated control the findings did not carry any biologically relevant information. No change was noticed in females with regards to levels of glucose in the plasma.

It could be assumed from the biochemical analysis of blood that both the drugs (herbal mixture and Liv 52) did not have any hepatotoxicity as the levels of ALT, AST and glucose in the drug alone treated groups (G2 and G4) showed similar values as that of untreated control (G1). Renal injury is known to increase BUN (Miura *et al.*, 1987) and an increase of BUN was observed in the Liv 52 treated groups (G4 and G5) and CCl_4 alone treated group (G6) when compared to untreated control, in both male and female mice. Carbon tetrachloride is known to cause kidney damage (Zimmerman *et al.*, 1983). So the increase in BUN in the CCl_4 alone treated group (G6) and Liv 52 + CCl_4 treated group (G5) was predictable but the increase of BUN in Liv 52 alone treated group indicated towards the toxicity of the drug. But the levels of BUN in the herbal mixture treated groups (G2 and G3) were similar to that of untreated control revealing not only the lack of nephrotoxicity of the herbal mixture but also the nephroprotective effect of the herbal mixture in mice. However, creatinine, another biomarker of kidney damage [usually shoots up only after extensive kidney damage] (Faulkner and King, 1976) did not show any changes among the groups.

The biochemical analysis of the liver can provide some valuable information on the status or condition of the liver. Alkaline phosphatase (ALP) and GGT, which are biomarkers of cholestatic liver injury (Batsakis, 1974; Zimmerman, 1984), did not show any increase confirming that CCl_4 induced liver injury was not of the cholestatic type but is of the necrogenic type (Rouiller, 1964). Carbon tetrachloride is known to cause an increase in the LDH levels (Korsrud and Grice, 1972) and an increase of LDH in liver homogenate was observed in the CCl_4 alone treated group and Liv 52 + CCl_4 treated group in both male and female mice when compared to untreated control, while the LDH in the herbal mixture treated groups were similar to that of control indicating a better hepatoprotective effect of the herbal mixture than Liv 52. In male mice, the level of LDH was high in the Liv 52 alone treated group, while it was normal in female mice of the same group. It could be due to sex-wise variation in the activity of the enzyme (LDH) or in the metabolism of Liv 52.

Glutathione is an intercellular thiol compound involved in the protection of cell from reactive oxygen species and free radicals (Meister and Anderson, 1983; Sen, 1997). Earlier studies in rats have shown that the levels of glutathione are reduced during CCl_4 induced hepatotoxicity and the administration of S-adenosylmethionine restores the levels of glutathione in liver reducing or minimizing hepatic damage (Gassao *et al.*, 1996). The increase of GSH in the herbal mixture treated groups (G2 and G3) provided a hint on the mode of protection of the herbal mixture. The enrichment of glutathione in the liver leads to reduced free radical formation (the metabolite of CCl_4 degradation trichloromethyl and trichloromethyl peroxy radicals) which leads to reduced lipidperoxidation and therefore it could be assumed that it is the antioxidant and free radical scavenging properties of the herbal mixture which protects the liver from CCl_4 induced liver injury. An earlier study reports that treatment of rats with glutathione before or after CCl_4 administration offered partial protection from CCl_4 induced hepatic necrosis and the suggested mode of action was that GSH prevents liver necrosis by changing the cellular response rather than by changing early steps in the process (Gorla *et al.*, 1983). Of the nine plants of the herbal mixture, *Asparagus* sp. (Kamat *et al.*, 2000), *Glycyrrhiza* sp. (Vaya *et al.*, 1997), *Phyllanthus* sp. (Bandyopadhyay *et al.*, 2000) and *Tinospora* sp. (Mathew and Kuttan, 1997) have proven antioxidant properties. Therefore it can be assumed that although the herbal mixture does not protect the liver from the initial onslaught of CCl_4 , the antioxidant nature of the herbal mixture prevents the damages due to lipid peroxidation, the major route of CCl_4 induced hepatic damage or may be by altering the cellular response to CCl_4 as mentioned in the earlier reports by Gorla *et al.* (1983).

Studies in mice with silymarin against CCl_4 induced hepatotoxicity have shown that silymarin prevents carbon tetrachloride-induced lipid peroxidation and hepatotoxicity, firstly, by decreasing the metabolic activation of CCl_4 and secondly, by acting as a chain breaking antioxidant (Lettaeron *et al.*, 1990). As postulated in the previous paragraphs, the herbal mixture seems to act as a chain breaking antioxidant (breaking the chain of events in lipid peroxidation) rather than a metabolic suppressant of CCl_4 . If the herbal mixture could decrease metabolic activation of CCl_4 it would have reduced the initial damage and that would have offered protection to the female mice also.

The gross pathology findings showed an increase of liver weight in the drug + CCl_4 treated groups (G3 and G5) and Liv 52 alone treated group in male mice when compared to the untreated control and CCl_4 alone treated group. The increase of liver weight in male mice of G3 and G5 is of less concern (can be considered healthy) as no enzyme parameters indicated otherwise. But the increase of liver weight in male mice of the Liv 52 alone treated group cannot be ignored because LDH, a biomarker of liver injury was high in this group. So it could be assumed that Liv 52 at the dose of 1g/kg body weight did exhibit a certain amount of stress on the liver especially in male mice. Female mice did not show any variation in liver weight among the groups.



It can be concluded that the herbal mixture has hepatoprotective effect against CCl_4 induced hepatotoxicity (necrosis) in male Swiss albino mice. It was found that the herbal mixture exhibited similar hepatoprotective effect as that of Liv 52 while having fewer side effects. Based on the findings the herbal mixture can be considered for development as a herbal hepatoprotective agent after carrying out further pre clinical efficacy and long term toxicity studies.

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