Chapter Six

DISCUSSION
The liver holds a position of singular importance in the overall functions of the human body. Besides its secretary and excretory functions, it effectively controls numerous vital metabolic functions. The normal functioning of the liver can be disturbed by various infections, infiltrations or toxic agents such as alcohol, drugs and environmental factors (Price 1978).

Acetaminophen (Paracetamol) is a widely used antipyretic and analgesic which seems safe when taken in therapeutic doses but larger amounts may cause fatal hepatic necrosis (Prescott et.al, 1971; Proud foot and wright 1970). This necrosis is primarily centrilobular but may also extend through midzonal area towards the periportal areas (Hinson 1980; Boyd and Berezcky, 1966). Paracetamol hepatotoxicity occurs not only in persons who ingest massive amounts of the drug with suicidal intent, but also in some individuals who ingest quantities within the therapeutic range (Zakim, 1971). So paracetamol was used as acute hepatotoxic model for inducing liver damage for one week & for Prophylactic studies.

Metabolism in the liver protects the tissues in higher organisms from potentially harmful blood-borne environmental chemicals. Ironically the metabolic products of detoxification reactions that protect other tissues from effects of the primary toxicant can be destructive to the liver when in excess or chronically present. Carbon tetrachloride (CCl₄) intoxication is a widely used model of both acute and chronic liver injury. CCl₄ causes hepatocyte injury that is characterized by centrilobular necrosis followed by hepatic fibrosis. Its metabolism occurs predominantly in the pericentral zone (zone 3) of the liver where its products cause hepatocyte injury which can be of
both short and long-term consequences to the liver (Gumcio, 1988; Jungerman, 1982; Thurman. 1986). So, in the present study CCl₄ was used as a chronic model to study the curative effect of 50% ethanolic extracts of Taraxacum officinale and Cichorium intybus.

Selection of the rats and mice has been done due to their high resistance to infection, smaller body weight, less dose requirement, cheapness and convenience in handling a large number of animals and easy availability.

Albino rats and mice (Wistar strain) were most of the times purchased from Central Animal House of Regional Research Laboratory (RRL), Jammu and ASCOMS, Sidhra Jammu, so that animals of uniform strain, robust health and free from any infectious disease could be obtained and who have not been previously tested for any other activity.

Mice and hamsters are very sensitive to paracetamol induced liver cell damage (Jollow, 1974). Therefore, mice were used for one week study and prophylactic study as paracetamol induced liver damage is reproducible in mice. The animals (rats / mice) were randomly distributed according to age, sex, weight and were housed in clean polypropylene cages under identical conditions of food, water, temperature and degree of nursing care.

Taraxacum officinale and Cichorium intybus whole plant were collected from Kashmir University campus and were also purchased from a recognized shop and were identified microscopically by a botanist. They were freed from any unwanted material before being subjected to the process of maceration.
50% ethanolic extracts of *Taraxacum officinale* and *Cichorium intybus* were administered in the form of suspensions prepared in 1% gum acacia, as these extracts were insoluble in water.

Paracetamol and 50% ethanolic extracts of the plants were administered by the oral route as it is the most common route employed in therapeutics. Also oral administration of paracetamol results in more consistent liver lesion (Walker and Racz, 1980) and more closely approximates the situation of over dosage in human beings.

Paracetamol has been used in a single oral dose of 500mg/kg body weight by Pandey and Shrivastava (1990) for producing degenerative changes and necrosis in mice. In our studies also, paracetamol was administered in a single dose of 500mg/kg, 48 hrs before the administration of drug suspensions and in prophylactic studies, 24 hrs. after the administration of the extracts of plants.

Paracetamol powder was made in the form of solution by dissolving in warm distilled water and given by forced oral administration. Controls received an equal volume (0.4 to 0.6 ml) of 1% gum acacia suspension as vehicle.

Carbon tetrachloride (CCl₄), given subcutaneously, at the dose of 2.0ml/kg body weight, twice a week for 14 days, has been reported to produce centrlobular necrosis and fatty changes in the liver (Chundong 2002). So, in our studies as a chronic toxic model, CCl₄ was administered at the dose of 2.0 ml/kg b.w (along with liquid paraffin 1:1) twice a week for 14 days.
Food was withheld after the administration of the last dose of the drug i.e. 16 hrs before biochemical estimations were performed as it was found that drug treated animals eat different amount of food as compared with controls and this introduces a variable.

The assessment of liver function was done by performing various biochemical estimations and by histopathological studies of liver sections. Transaminases exist in the tissues of many organs. Necrotic activity in these organs causes a release of abnormal quantities of enzymes into blood (Karmen and Wroblewski, 1955). In liver diseases the values of both AST and ALT are increased but the increase in ALT is much more than that of AST (Nath, 1970). Heart tissue is rich in AST and myocardial infection results in high AST activity, while high levels of SAP are indication of involvement of biliary tract (Nath, 1970).

Acetaminophen hepatotoxicity leads to prominent increase in the serum AST and ALT levels (often into several thousands) and lesser increase in the serum alkaline phosphatase (Black, 1980). In CCl₄ poisoning, the transaminase levels may go upto 27,000 units (Boyd, 1970). So, in the present study, AST, ALT and SAP estimations were performed.

In our experimental studies, 50% ethanolic extract of *Taraxacum officinale* and *Cichorium intybus* whole plant were studied for their prophylactic and curative effect against paracetamol and CCI₄ induced liver damage in albino rats and mice.

The high dose (100mg/100g/day) of *Taraxacum officinale* was administered in case of paracetamol induced liver damage so that if activity...
was observed at higher dose experiment could be performed with smaller
doses of the drug.

Albino rats (Wistar strain), in the weight range of 135-180g, were
used. Male and female rats were kept in separate cages so that there was no
interference in dosing later on.

The temperature of the room in which the rats were kept ranged
between 16-19° C and humidity was in the range of 71-76%.

In the first experiment, paracetamol given in a single dose of
500mg/kg b.w produced a significant rise in ALT, SAP and serum
cholesterol levels reflecting liver cell injury (Table 1,2,3,4 ). Taraxacum
officinale extract (100mg/100g/day) given daily for 5 days after
administration of single dose of paracetamol (500mg/kg) reduced the AST,
SAP and Cholesterol levels while producing highly significant fall
(***p<0.01) in ALT levels. The average body weight and liver weight of
the rats was reduced after administration of the extract.

The attributivity of the observed alterations of serum enzyme levels to
hepatic damage of health was confirmed by histopathological studies of
liver in paracetamol treated group which showed severe fatty degeneration
of cells around the portal tract (Fig 2a,2b) while Taraxacum officinale
(100mg/100g/day) treated group revealed only mild fatty changes and
regenerative activity in 63% of rats. These observations point towards
antihapatotoxic role of 50% ethanolic extract of Taraxacum officinale in this
model.
In the next experiment, the aim was to study the prophylactic activity of the extracts of *Taraxacum officinale* and *Cichorium intybus* against paracetamol induced hepatotoxicity. The extracts were given at three dose levels, 50, 100 and 200 mg/100g/day, by forced oral administration to mice daily in two divided doses for five days. 24hrs. after the administration of last dose of the extracts, single dose of paracetamol was administered to find out whether the extracts were effective and the optimum dose at which protection was afforded.

Results suggested a dose dependent fall in the ALT levels of the mice that had received *Taraxacum officinale* extract prophylactically for 5 days, the fall being highly significant (*p<0.01) at the doses 100 and 200mg/100g/day of 50% ethanolic extract of *Taraxacum officinale* (Table 7; Fig g). The biochemical results were supported by histopathological studies which revealed 33% livers normal at the dose of 50mg/100g/day while regenerative activity and anisonucleosis were observed in 47 % livers at the dose of 100mg/100g/day while 60% livers showed normal hepatocytes and regenerative activity at the dose of 200mg/100g/day (Fig 6a,6b; 7a,7b; 8a, 8b). Both these biochemical and histopathological studies point towards a protective role of 50% ethanolic extract of *Taraxacum officinale*, at the doses of 50,100 and 200mg/g/day, in the prophylactic study against paracetamol induced hepatotoxicity with maximum protection being afforded at the dose of 200mg/100g/day. There was no significant effect on the average liver weight of mice at the doses of 50, 100 mg/100g /day of the extract while 200mg dose increased it significantly.

50% ethanolic extract of *Cichorium intybus* whole plant reduced the ALT levels significantly fall (*p<0.05) and (**p<0.001) respectively, when given at the doses of 100 and 200mg/100g/day prophylactically, daily for
five days, before the administration of paracetamol while the fall was not so significant at the dose of 50mg/100g/day (Table 9). Histopathological studies revealed 17% livers normal at the dose of 50mg/100g/day, 80% at the dose of 100mg/100g/day and 84% at the dose of 200 mg/100g/day (Fig 9a, 9b; 10a, 10b; 11a,11b). Both the biochemical and histopathological studies suggest that 50% ethanolic extract of *Cichorium intybus* when given prophylactically, at the doses of 50, 100 and 200mg/100g /day, for five days, affords protection against paracetamol induced hepatocellular damage in rats.

After the preliminary screening of 50% ethanolic extracts of *Taraxacum officinale* and *Cichorium intybus* whole plant against the acute hepatotoxic model paracetamol were assessed for their curative activity against chronic liver cell damage. Administration of Carbon tetrachloride (CCl₄) to rodents is a widely used model to study mechanisms of acute and chronic hepatic injury (Chundong, 2002). Metabolism of CCl₄ occurs predominantly in the pericentral zone (zone 3) of the liver where its products cause hepatocyte injury which can be of both short and long term consequences to the liver (Jungerman 1982).

So, in the next experiment for inducing chronic toxicity in rats, Carbon tetrachloride was administered at the dose of 2.0ml/kg body weight (along with liquid paraffin 1:1), twice a week for 14 days (Chundong 2002). 50% ethanolic extract of *Taraxacum officinale* was administered at two dose levels 50 and 100mg/100g/day, twice a day daily for 14 days along with CCl₄. The results of this experiment reveal that extract of whole plant of *Taraxacum officinale* has a definite antihepatotoxic effect against the deleterious effect of CCl₄ upon the structure and function of liver as estimated by various parameters. The biochemical studies seem to support...
the beneficial effect of *Taraxacum officinale* in improving the metabolic function of the liver. The AST, ALT and SAP levels which were raised by CCl₄ were effectively antagonized by concurrent administration of *Taraxacum officinale* (Table 11, 12, 13) which confirms the protective ability of *Taraxacum officinale* against the hepatotoxic effect of CCl₄, the effect being more pronounced at the dose of 100mg/100g/day than at the dose of 50mg/100g/day.

Histopathological studies also confirm the beneficial role of *Taraxacum officinale* in antagonizing the deleterious effect of CCl₄ on the histology of liver. While CCl₄ treated rats showed extensive histological changes, the animals treated with CCl₄ and *Taraxacum officinale* concurrently at the doses of 100 and 50mg/100g/day showed only mild to moderate histopathological changes (Fig 13, 14). The dose of 100mg/100g/day of *Taraxacum officinale* was found to be more effective than the dose of 50mg/100g/day in protecting the liver against the hepatocellular injury caused by CCl₄.

In our study, *Cichorium intybus* extract seemed to offer protection and maintain the structural integrity of hepatic cells against CCl₄ induced damage. The protective effects were more significant and much better when the rats were treated with 50mg/100 g dose than 25mg/100g dose. This was evident from a fall (*p<0.05) in ALT levels at the dose of 25mg/100g /day while a significant fall in both AST and ALT levels (*p<0.05) was observed at the dose of 50mg/100g/day when given concurrently with CCl₄ (Table 16, 17).

Biochemical results were confirmed by the histopathological studies which revealed less damage of the hepatic architecture in the rats given CCl₄.
and *Cichorium intybus* concurrently when compared to the rats that had received CCl₄ alone. Histopathology also revealed that the dose of 50mg/100g/day of *Cichorium intybus* extract afforded more protection as fatty changes were observed in 42% livers at the dose of 25 mg/100/day and only in 20% livers at the dose of 50mg/100g/day of *Cichorium intybus*. Also marked regenerative activity and anisonucleosis was observed in the livers at the dose of 50mg/100g/day than 25mg/100g/day of *Cichorium intybus*.

The above results are in corroboration with those of an earlier study by Gadgoli & Mishra (1995) who have reported that total aqueous extracts of *Cichorium intybus* seeds reduced the levels of AST & ALT in Paracetamol-Carbon tetrachloride induced hepatotoxicity & (Kalantari, 1997). Zafar & Mujahid 1998 have reported that root callus extracts of *Cichorium intybus* showed better antihepatotoxic activity as compared to natural root extracts against carbon tetrachloride induced (5 days) hepatic damage.

Our studies indicate that 50% ethanolic extract of *Cichorium intybus* afforded better antihepatotoxic activity when given prophylactically at the dose of 50, 100 and 200 mg/kg against paracetamol induced acute hepatic damage in Mice while optimum antihepatotoxic effect was observed at the dose of 50mg/kg of *Cichorium intybus* against Carbon tetrachloride induced (administered for 14 days) chronic hepatic damage in rats thus confirming the beneficial role of *Cichorium intybus* in antagonizing the harmful effects of CCl₄ on liver of rats.