Research Article

Hepatoprotective Activity of *Inula cuspidata* Flower, Stem and Whole Plant Extract against Carbon tetrachloride Induced Toxicity in Rats

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ABSTRACT

The liver serves a vital function in human system. It has the primary metabolic function of regulating the blood concentration of most metabolites, particularly glucose and amino acids. Any injury to it or impairment of its function may lead to many implications on one's health. Management of liver diseases is still a challenge to modern medicine. The allopathic medicine has little to offer for the alleviation of hepatic ailments whereas the most important representatives are of phytoconstituents. In the present study, the aqueous extract of flower, stem and whole plant of *Inula cuspidata* at the dose of 200mg/kg and 400mg/kg (orally administered) was studied for the hepatoprotective effect using Carbon tetrachloride induced liver damage in wistar albino rats. Various determinants of liver injury, such as serum glutamate oxaloacetate transferase (SGOT), serum glutamic pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total bilirubin (TB), direct bilirubin (DB) were evaluated. It was observed that SGOT, SGPT, TB and DB were found to be elevated in CCl_4 group as CCl_4 causes severe liver injury leading to elevated enzyme level and increased bilirubin levels. High dose (400mg/kg) of flower extract of *Inula cuspidata* produced the maximum decrease in the elevated SGPT level followed by the high dose of Whole plant extract. The minimum SGOT level was observed with high dose flower extract followed by high dose of whole plant extract. Similar results were observed in the case of alkaline phosphatase, total bilirubin and direct bilirubin. The stem extract was found to have the least activity. The overall experimental results suggests that the biologically active phytoconstituents such as flavonoids, glycosides, sesquiterpene lactones present in the aqueous extract of plant *Inula cuspidata* might be responsible for the significant ($p<0.001$) hepatoprotective activity and the results justify the use of *Inula cuspidata* as a hepatoprotective agent.

Keywords: Aqueous extract, Carbon tetrachloride, Hepatoprotective activity, *Inula cuspidata*.

INTRODUCTION

Liver is one of the largest organs in the human body and the main site for intense metabolism and excretion. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision, and reproduction.[1] Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity.

The genus Inula (family-Asteracea) is most widely distributed genera and is comprised of 20 species in India and several of these are reported to possess medicinal properties and used in folk medicines, as tonic, stomachic, diuretic, diaphoretic, anti-inflammatory, bactericidal, hepatoprotective, carminative and anti-tumour. Among these Inula species, *Inula cuspidata* C.B.Clark (Asteracea) known as jhuri[2] is a small or medium sized deciduous or sub deciduous shrub of India and is found in the western Himalayas from Kashmir to Uttrakhand. It has been found growing on the steep, rocky or precipitous ground.[3] Isoquercetin, geranyl linalool, Incaspitolides A, B, C, D, hydroxygermacrene, two isomeric acetylenic sulfoxides, β-sitosterol and its βD glucoside, and squalene have been isolated from the aerial parts of the plant.[4,5] The essential oil present in leaves possess good anti fungal activity against plant and human pathogenic fungi.[6] Thymyl isobutyrate, thymol, thymyl isovalerate, 8α hydroxyl presilphiperfolene and intermedeol have been isolated from the steam volatile extract of *Inula cuspidate.*[7]

MATERIALS AND METHODS

Plant material

The plant *Inula cuspidata* was collected from Nanital in the month of December-2012. The plant was identified and authenticated by Dr.G.C.Joshi, Research officer incharge, Regional research institute of Himalayan flora, CCRAS, Thapala (Ranikhet), India.

Preparation of extract

The coarsely powdered flower, stem and whole plant (root, stem, flower, leaf) of *Inula cuspidata* was decocted in purified boiling water in the ratio of 1:9 for 30 mins. Decoction was kept overnight and filtered as per procedure adopted by M. Anusha et.al.[8] The aqueous

extract was filtered through a cotton plug, followed by whatman filter paper (no.1) and then concentrated by using a rotary evaporator at a low temperature (40-60°C) and reduced pressure.

Preliminary phytochemical analysis

The aqueous extracts (of flower, stem and whole plant) were then subjected to preliminary phytochemical[9] analysis to assess the presence of various phytoconstituents; it revealed the presence of flavonoids, sesquiterpene lactones, carbohydrates, phenolic compounds and glycosides. Preliminary Thin layer chromatography studies also confirmed these constituents.[10]

Animals

Wistar albino rats weighing 180-250g of either sex maintained under standard husbandry conditions (temp 23±2°C, relative humidity 55±10% and 12 hours light dark cycle) were used for the screening. Animals were fed with standard laboratory food and ad libitum during the study period. The experiments were performed after the experimental protocols approved by the institutional animal ethics committee, India 2009.

Toxicity studies

Acute oral toxicity study was performed as per OECD-423 guidelines.[11] The drugs was administered orally in doses of 5, 50, 300, 2000 mg/kg bodyweight to groups of rats (*n* = 3) and the percentage mortality was recorded over a period of 24 h. During the first 1 h of drug administration, rats were observed for gross behavioural changes as described by Irwin et; al.[12]. If mortality was observed in two animals out of three animals, then the dose administered was assigned as toxic dose. If the mortality was observed in one animal, then the same dose was repeated to confirm the toxic dose. As mortality was not observed, the procedure was repeated for further higher doses such 50, 200 & 2000mg/kg body weight. The animals were observed for toxic symptoms for 72 h as per procedure adopted by Rekha rajendran.[13]

Carbon tetrachloride induced hepatotoxicity

The animals were divided into nine groups of six animals each and labelled (Groups F1 to F9).

Group I (F1) served as normal control and received sterile olive oil (vehicle) 1ml/kg (p.o).

Group II (F2) animals constituted the hepatotoxic group, and received CCl4 suspended in sterile olive oil (1:1 v/v, 2 ml/kg, i.p.) every 72 h for 10 days.

Group III (F3) received standard drug silymarin 100 mg/kg (p.o.) for 10 days and CCl4 suspended in sterile olive oil (1:1 v/v, 2 ml/kg, ip).

Groups IV - IX (F4 – F9) received aqueous extract (200 and 400 mg/kg/day) of flower, stem and complete plant suspended in 0.5 % sodium carboxy methyl cellulose for 10 days and CCl4 suspended in sterile olive oil (1:1 v/v, 2 ml/kg, ip).

At the end of the experimental period, the rats were fasted overnight and sacrificed by ether anaesthesia. Blood and liver samples were collected for biochemical and histological studies.[14]

Assessment of liver function

Blood was collected from all the groups by puncturing the retro-orbital plexus and was allowed to clot at room temperature and serum was separated by centrifugation at 2500 rpm for 10 min. The serum was used for estimation of biochemical parameters to determine the functional state of the liver.[13,14] Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphate (ALP), Total bilirubin (TB), Direct bilirubin (DL) were estimated [15] by Arba kit (Trans Asia bio medical ltd., Mumbai) and Autopak kit (Siemens health care diagnostic company, Baroda).

Histopathological studies

The animals were sacrificed and the abdomen was cut open to remove the liver. Paraffin sections (7 µm thick) of buffered formalin–fixed liver samples were stained with hematoxylin–eosin (which stains the nuclei blue and the cytoplasm pink) to study the liver histological structure of the control and treated rats. The sections were then observed under microscope for histopathological changes in liver architecture and their photomicrographs were taken.[13, 14]

Statistical analysis

For determination of significant inter-group differences, each parameter was analyzed separately by‘t’ test and one way analysis of variance. Dunnet’s test was used for individual comparisons. The $p < 0.05$ was considered as statistical significant.

RESULTS

Acute toxicity studies

Aqueous extracts of *Inula cuspidata* did not produce any toxic symptoms or mortality up to the dose level of 2000mg/kg body weight in rats, and hence the extract was considered to be safe and non-toxic for further pharmacological screening.

Hepatoprotective activity

Hepatoprotective effects of flower, whole plant and stem extracts at high dose and low dose were studied. Various determinants of liver injury, such as Serum glutamate oxaloacetate transferase (SGOT), Serum glutamic pyruvate transaminase (SGPT), Alkaline phosphatase (ALP), Total bilirubin (TB), Direct bilirubin (DB) were evaluated. It was observed that SGOT, SGPT, TB. and DB were found to be elevated in CCl_4 group as CCl_4 causes severe liver injury leading to elevated enzyme level and increased bilirubin levels. High dose of flower extract of

Inula cuspidata produced the maximum decrease (287.78) in the elevated SGPT level followed by the high dose of Whole plant extract, which decreased the level up to 298.53. The minimum SGOT level was observed with high dose flower extract (266.84) followed by high dose of whole plant extract (282.41). Similar results were observed in the case of alkaline phosphatase, Total bilirubin and direct bilirubin. The stem extract was found to have the least activity (Table 1).

Table 1: Effect of CCl_4, Silymarin, Flower, Whole Plant and Stem Extracts on liver function tests X

Parameters → Groups ↓	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	T. Bil. (mg/dl)	D. Bil. (mg/dl)
Normal Control	39.44±0.90	48.28±1.32	137.80±2.62	0.26±0.019	0.15±0.009
CCl_4	648.06±8.39 ###	666.81±7.48 ###	733.89±5.72###	1.89±0.078 ###	0.85±0.036###
Silymarin	246.21±5.05***	243.87±3.99***	269.17±3.06***	0.60±0.004***	0.36±0.015***
Low Dose (Flower)	328.00±6.87 ***	305.86±5.30***	350.54±4.25***	1.33±0.023***	0.70±0.015***
High Dose (Flower)	287.78±5.89 ***	266.84±2.81***	287.39±4.72***	0.72±0.003***	0.42±0.011***
Low Dose (Whole Plant)	334.48±5.00***	329.22±4.33 ***	326.84±4.27***	1.24±0.022***	0.67±0.017***
High Dose (Whole Plant)	298.53±4.33***	282.41±5.09***	312.58±3.74***	0.74±0.006***	0.52±0.016***
Low Dose (Stem)	417.69±5.39***	385.28±3.13 ***	471.93±4.21***	1.55±0.02***	0.77±0.01 ***
High Dose (Stem)	392.81±4.04***	355.59±3.72***	420.59±4.06***	1.41±0.01 *	0.73±0.01***

###p<0.01, when compared CCl_4 group with normal control group using't' test; ***p<0.001 & *p<0.05; when compared the groups with CCl_4 group, using one way ANOVA followed by Dunnett´s test; (Each value represents Mean ± SEM of N=6/group).

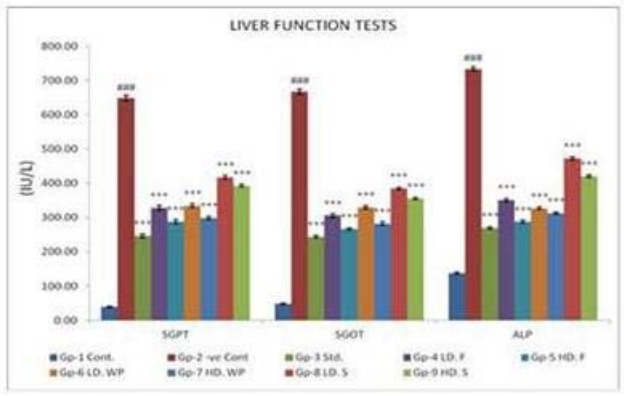

Figure 1: Graph of liver function test

###p<0.01, when compared CCl_4 group with normal control group using't' test; ***p<0.001 & *p<0.05; when compared other groups with CCl_4 group, using one way ANOVA followed by Dunnett´s test; (Each value represents Mean ± SEM of N=6/group.

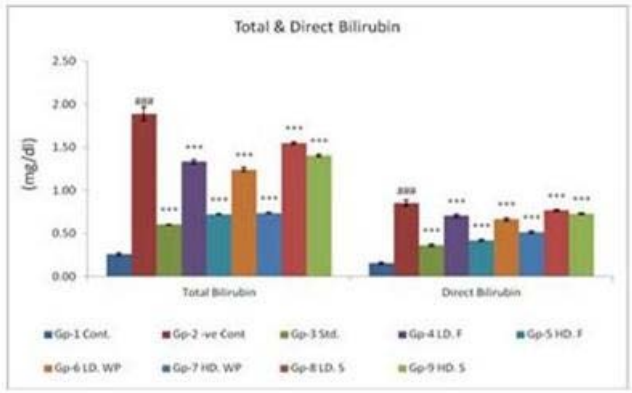

Figure 2: Graph of Total and Direct bilirubin

###p<0.01, when compared CCl_4 group with normal control group using't' test; ***p<0.001 & *p<0.05; when compared other groups with CCl_4 group, using one way ANOVA followed by Dunnett´s test; (Each value represents Mean ± SEM of N=6/group.

Effect of Silymarin and Extracts Pre-treatment on Pathological Histology of Rat Livers after CCl_4 Induced Acute Toxicity

Histopathological studies of the liver section of rats were carried out to confirm the serum analysis results and hepatoprotective effect of the treatments. Histological observations supported the results obtained from serum enzyme assays. The CCl_4 induced histopathological changes in liver were confirmed. Silymarin and extracts pre-treatment reversed the effect of CCl_4.

Figures show that CCl_4 induced liver injury caused significant fatty degeneration of hepatic cells; vacuole formation in the central vein, hepatocyte ballooning and inflammation, after CCl_4 treatment. Treatment with silymarin and extracts reduced the injury level, vacuole formation and inflammation and showed a preventive effect against CCl_4 induced acute hepatotoxicity.

The histopathological findings of rat livers pre-treated with silymarin and extracts were as follows:

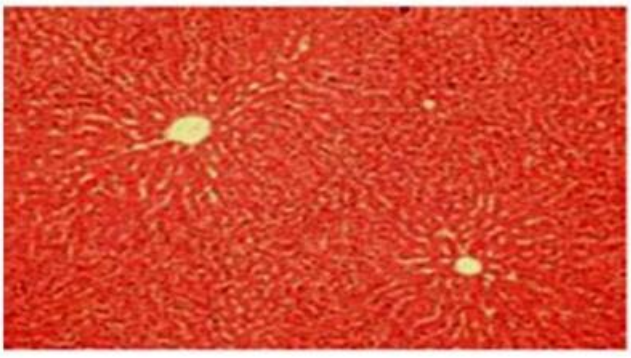

Figure 3: Normal control group

In case of Normal Control group (figure 3) hepatic globular structure; central veins, portal tracts,

hepatocytes and sinusoids appeared normal; the lobular unit was also well identified.

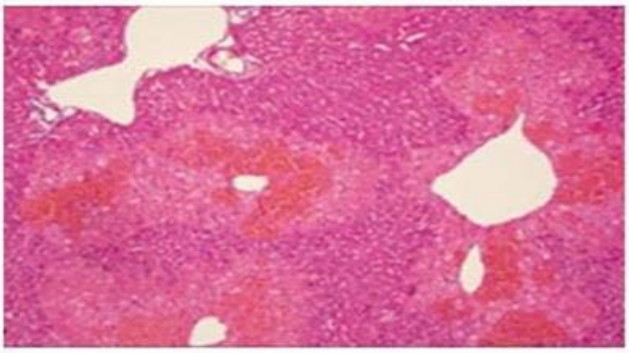

Figure 4: CCl_4 treated group

In case of CCl_4 treated group (figure 4); liver section showed the damage of the liver cells, with ballooning of hepatocytes, patchy parenchymal necrosis along with marked fatty & proteineous degeneration and lobular inflammation; sinusoidal spaces were flooded with inflammatory cells.

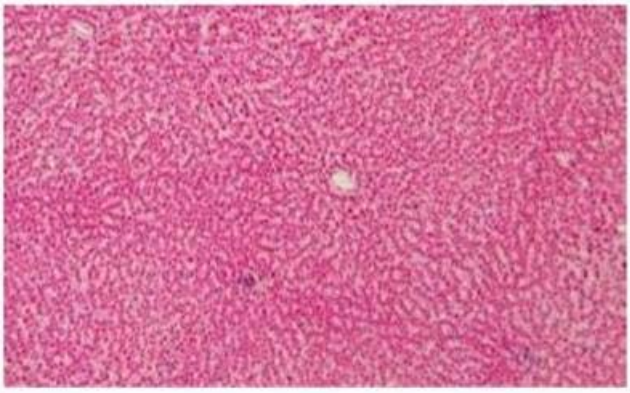

Figure 5: Silymarin treated group

Liver section of Silymarin treated group (figure 5) showed normal architecture with very mild degree of proteineous degeneration; slight periportal mono-nuclear infiltration with patchy necrosis of hepatocyte.

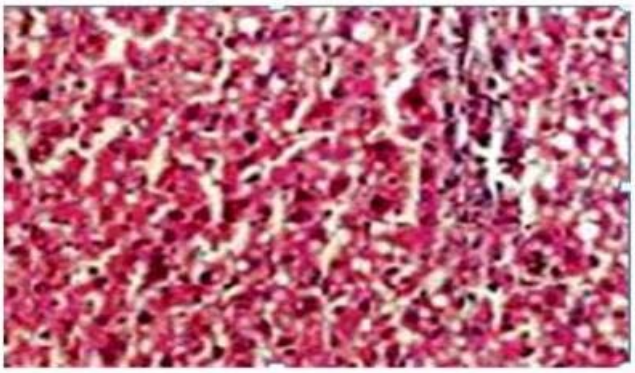

Figure 6: Flower extract treated group

In Low dose (Flower) extract treated group (figure 6); section of liver showed normal architecture of hepatocytes and moderate accumulation of fatty vacuoles with proteineous degeneration.

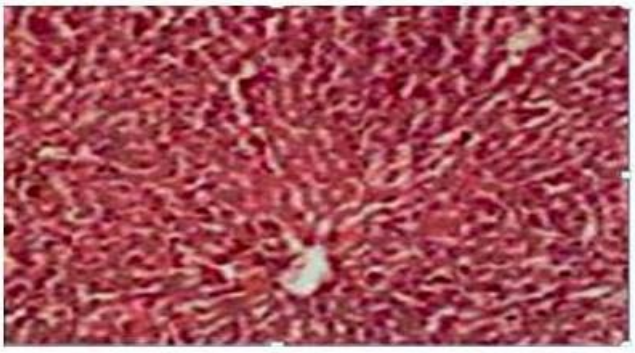

Figure 7: High dose flower treated group

In case of High dose (Flower) treated group (figure 7); section of liver showed normal architecture of hepatocyte, with very mild inflammation in portal tract and no necrosis was seen. Some of the hepatocytes showed regenerative activity.

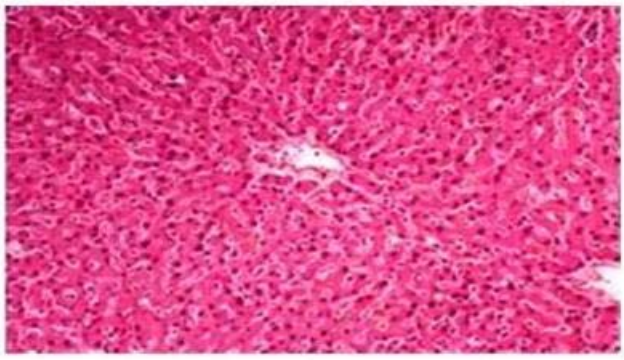

Figure 8: Low dose whole plat treated group

In Low dose (Whole Plant) treated group (figure 8); liver section showed normal architecture with no degenerative changes and no necrosis of hepatocytes. There was slight periportal inflammation along with fatty degeneration.

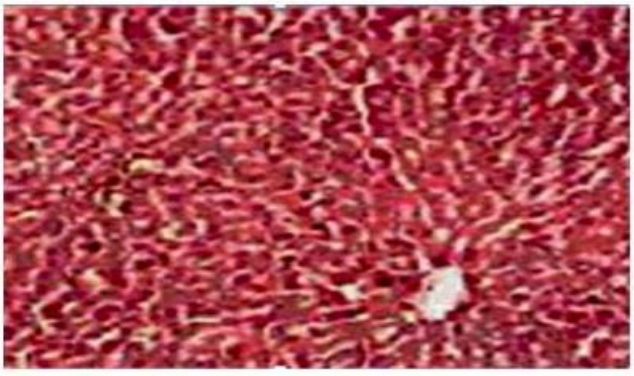

Figure 9: High dose whole plant treated group

Liver section of High dose (Whole Plant) treated group (figure 9) showed normal architecture and reversal of toxic effects in the liver cells. No necrosis was seen; central vein and portal triads appear normal. Some of the hepatocytes showed regenerative activity.

RESULTS AND DISCUSSION

The present study was performed to assess the hepatoprotective activity in rats, against carbon tetrachloride as hepatotoxin to prove its claim in folklore practice against liver disorder. Carbon tetrachloride is a widely used experimental hepatotoxicant, which is biotransformed by the cytochrome P-450 system to produce the trichloromethyl free radical, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation, disturb Ca 2+ haemostasis and finally result in cell death.[16]

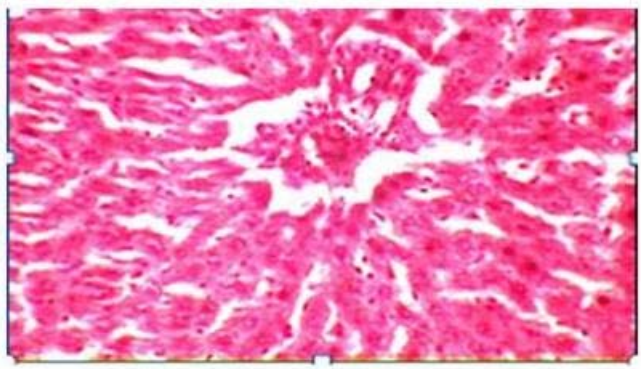

Figure 10: Liver section of low dose treated group

Liver section of Low dose (Stem) treated group (figure 10) showed normal architecture of hepatocytes; central vein and portal triads appear normal along with marked fatty degeneration.

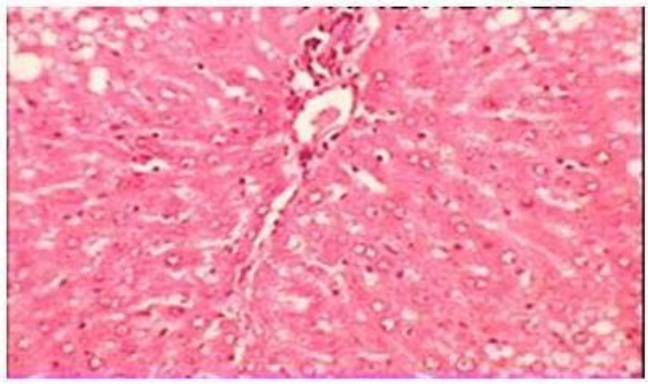

Figure 11: High dose stem treated group

In High dose (Stem) treated group (figure 11); liver section showed normal architecture with no degenerative changes and patchy necrosis of hepatocytes. There was slight periportal inflammation along with fatty degeneration.

Liver function tests are done to diagnose liver diseases. It includes the measurement of different liver enzymes, proteins, bilirubin and other chemicals produced by liver. Liver function tests (LFT) are done to detect any infection, inflammation or damage to the liver cells. Increased levels of LFT indicate abnormality of the liver. Liver function tests are done to measure the level of enzymes which are raised in some liver diseases such as SGOT, SGPT, and ALP. Liver function tests also help to evaluate if the liver is infected by viruses such as hepatitis. Liver function tests help to determine the prognosis of a disease such as alcoholic hepatitis, its further management and treatment. Liver function tests are also done to check the side effects of recently started medication. Increased level of particular enzymes indicates a specific disorder. Thus LFT also helps to evaluate specific diagnosis of the disease and its treatment.

Estimating the activities of serum marker enzymes, like SGPT, SGOT and ALP can the assessment of liver function when liver cell plasma membrane is damaged, a variety of enzyme normally located in the cytosol are released into the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage.[17] The tendency of these enzymes to return to near normally in extract administered group is a clear manifestation of anti-hepatotoxic effects of the extract. Reduction in the levels of SGPT and SGOT towards the normal value is an indication of regeneration process. Reduction in ALP levels with concurrent depletion of raised bilirubin levels suggests the stability of the biliary function during injury with Carbon tetrachloride. High dose of flower extract of *Inula cuspidata* produced the maximum decrease in the elevated SGPT level followed by the high dose of Whole plant extract. The minimum SGOT level was observed with high dose flower extract followed by high dose of whole plant extract. Similar results were observed in the case of alkaline phosphatase, Total bilirubin and direct bilirubin. The stem extract was found to have the least activity.

Histopathological liver sections also revealed that the normal liver architecture was disturbed by hepatotoxin in carbon tetrachloride group, whereas in the liver sections of the rat treated with the aqueous extract and intoxicated with carbon tetrachloride the normal cellular architecture was retained and it in comparable with the standard Silymarin group. Hence confirming the significant hepato protective effect of flower extract of *Inula cuspidata* at the dose of 400mg/kg body weight followed by whole plant extract and least hepatoprotective effect was observed with stem extract.

These findings suggested that *Inula cuspidata* extract significantly neutralized the toxic effects of carbon tetrachloride and helped in regeneration of hepatocytes.

In accordance with these results, the presence of phytoconstituents such as flavonoids, sesquiterpene lactones, carbohydrates, phenolic compounds and glycosides in the aqueous extract could be considered as, responsible for the significant hepatoprotective activity. In conclusion, it can be said that the aqueous extract of flower (400mg/kg body weight) of *Inula cuspidata* exhibited the maximum hepatoprotective effect followed by the high dose of whole plant extract. The stem extract was found to have least activity as compared with flower and whole plant extract against carbon tetrachloride induced hepatotoxicity.

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