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## Study of Hepatoprotective Activity of *Solanum nigrum* and *Cichorium intybus*

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**Abstract:** The study was conducted to evaluate the hepatoprotective activity of *Solanum nigrum* and *Cichorium intybus*. Hepatotoxicity was induced in albino rats with 1:1(v/v) mixture of CCL<sub>4</sub> in olive oil, administered at the dose of 1.5 mg kg<sup>-1</sup> i.p on 7th day. The extract of *Solanum nigrum* and *Cichorium intybus* were administered to the experimental rats. The hepatoprotective effect of these extracts was evaluated by the assay of serum protein, Serum bilirubin and serum enzymes like Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Markers for oxidative stress like Glutathione (GSH), Superoxide Dismutase (SOD), Lipid Peroxidase (LPO) and histopathological studies of liver. In the groups where hepatic injury induced by CCL<sub>4</sub> and extract were given simultaneously, the toxic effect of CCL<sub>4</sub> was controlled significantly (<0.05) by maintenance of structural integrity of hepatocyte cell membrane and normalisation of functional status of liver. Histology of liver sections from *Solanum nigrum* and *Cichorium intybus* + CCL<sub>4</sub> treated rats revealed moderate centrilobular hepatocytes degeneration, few areas of congestion with mild fatty changes. The extract of *Solanum nigrum* and *Cichorium intybus* possess significant hepatoprotective activity in comparison to standard drug Silymarin.

**Key words:** CCL<sub>4</sub>, silymarin, hepatoprotective, oxidative stress

### INTRODUCTION

Liver disease has become a global concern worldwide. The principal causative factors are increasing alcohol consumption, infection, malnutrition, anaemia and availability of hepatotoxic drugs over the counter (Yu *et al.*, 2010).

Modern medicine has very few choices in terms of treatment for liver diseases. The conventional drugs used in the treatment of liver diseases viz., corticosteroids, antiviral drugs and immunosuppressant agents are sometimes inadequate and may lead to serious adverse effects. Paradoxically, these may themselves cause hepatic damage e.g., cholestatic jaundice with Azathioprine and elevation of serum transaminases by interferons (Hayden, 2006). More over these drugs are very expensive. It is therefore imperative to search alternative drugs for the treatment of liver disease to replace the currently used drugs of doubtful efficacy and safety (Nadeem *et al.*, 1997).

In India, numerous medicinal plants and their formulations are used for liver disorders in traditional systems of medicine. Some of these plants viz. *Silybum marinum*, *Picrorrhiza kurroa*, *Andrographis paniculata*, *Phyllanthus niruri* and *Eclipta alba* are evaluated for their hepatoprotective actions against hepatotoxins

(Bisset and Wichtl, 1994). However, the readily available hepatoprotective herbal drugs are not sufficiently active to effectively combat severe liver disorders. In view of lack of synthetic agents for the treatment of hepatic disorder, there is a growing focus to evaluate traditional herbal medicines for hepatoprotective activity. Therefore, there is a need to develop satisfactory hepatoprotective drugs. Also scientific evaluation for safety and efficacy is needed for the drugs of alternative medicine, because of incidence of hepatotoxicity amongst herbal medicines (Dienstag, 2008).

*Solanum nigrum* and *Cichorium intybus* are well known medicinal plants which grows wild in India. These two plants are commonly known as Kakamachi (Sanskrit) or Black nightshade (English), Kashi (Sanskrit) or Chicory (English) respectively. Different ethnic groups traditionally use both plant products as antidiarrhoeal, diuretic, cathartic, antispasmodic and disorders of menstruation. There is a dire necessity of reliable hepatoprotective drugs in modern medicine. Plants and natural products are providing to be hepatoprotectants as evident from voluminous published work on their hepatoprotective potential (Wagner, 1981). *Cichorium intybus* have been listed among nineteen commonly used herbs against diabetes (Karim *et al.*, 2011). Though the plants *Solanum nigrum* and *Cichorium intybus* are

traditionally used for different disease conditions in India, but they hepatoprotective potential has not been evaluated.

In view of this, the present study has been undertaken on the following experimental models:

- To study the preventive activity of *Solanum nigrum*, *Cichorium intybus* in liver injury induced by CCL<sub>4</sub>
- To compare the hepatoprotective activity of *Solanum nigrum*, *Cichorium intybus* to a standard drug Silymarin in liver injury induced by CCL<sub>4</sub>

## MATERIALS AND METHODS

**Animals:** Wistar albino rats (156-182 g) were procured from Centre for Toxicology and Developmental Research, Sri Ramachandra University. The animals had free access to standard rat pellet with water supplied *ad libitum* under strict hygienic conditions. Animals were habituated to laboratory conditions (i.e. room temperature of 25±1°C; relative humidity 45-55% and a 12:12 light/dark cycle) for 48 h prior to experimental protocol to minimize non-specific stress if any. The approval of the Institutional Animal Ethical Committee (IAEC) of Sri Ramachandra University (IAEC/XII/SRU/75/2008) was taken prior to the experiments. All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) from 28-6-2008 to 5-9-2008.

**Plant materials:** The hydroalcoholic extracts of Fruits of *Solanum nigrum* and Roots of *Cichorium intybus* were used in the study (Obtained from pharmacognocny Department, Sri Ramachandra University, Chennai).

**Silymarin suspension:** The stock solution was prepared by dissolving 100 mg of Silymarin (Micro labs, Bangalore) in 5 mL aqueous suspension of Carboxymethyl cellulose.

**Induction of hepatic injury:** CCL<sub>4</sub> (E.Merck, Mumbai) was used as hepatotoxine. Liver damage was induced in these rats with 1:1(v/v) mixture of CCL<sub>4</sub> in olive oil, administered at the dose of 1.5 mL kg<sup>-1</sup> i.p on 7th day.

The parameters assessed are:

- Estimation of serum enzymes like AST, ALT
- Markers for oxidative stress like GSH, SOD and LPO
- Serum bilirubin
- Total protein

- Histological examination of liver

**Experimental design:** The experiment was carried out for a period of 14 days with 30 healthy albino rats. Before starting the experiment, the animals were allowed to acclimatize to the laboratory environment for 1 week. Then animals were randomly divided into 5 groups of 6 animals each.

**Group 1:** Carboxymethyl Cellulose 1 mL kg<sup>-1</sup>/day p.o. for 7 days (CMC)

**Group 2:** Control + CCL<sub>4</sub> 1.5 mL kg<sup>-1</sup> i.p. on 7th day (C+CCL<sub>4</sub>)

**Group 3:** Silymarin (100 mg/kg/day, p.o.) for 7 days+CCL<sub>4</sub> 1.5 mL kg<sup>-1</sup> i.p. on 7th day (SLM+CCL<sub>4</sub>)

**Group 4:** *Solanum nigrum* fruit extract 250 mg/kg/day for 7 days + CCL<sub>4</sub> 1.5 mL kg<sup>-1</sup> i.p. on 7th day (SNF+CCL<sub>4</sub>)

**Group 5:** *Cichorium intybus* root extract 75 mg/kg/day p.o for 7 days + CCL<sub>4</sub> 1.5 mL kg<sup>-1</sup> i.p. on 7th day (CIR+CCL<sub>4</sub>)

Animals were treated as mention above, food was withdrawn 12 h before carbon tetrachloride administration to enhance the acute liver damage in animals of Groups 2, 3, 4 and 5 and received a single dose of CCL<sub>4</sub> (1.5 mL kg<sup>-1</sup>, i.p.) diluted with olive oil (1:1) on 7th day (1 h after treatment).

**Assessment of hepatoprotective activity:** Blood was withdrawn directly from the heart on 8th and 15th day under light ether anaesthesia. Then, the blood was kept for 30 min without disturbing. The clot was dispersed with a glass rod and then centrifuged for 15 and 20 min at 2000 rpm to separate the serum. Biochemical investigations were carried out to assess liver function viz., total protein, albumin globulin ratio (Kingsley and Frankel, 1939), Bilirubin level, serum alkaline phosphatase (Gowenlock *et al.*, 1981) and serum transaminases (Reitman and Frankel, 1957). Liver homogenate was collected for estimation of markers of oxidative stress viz. GSH, SOD and LPO.

**Histopathology:** After collection of blood, liver samples were excised and washed with normal saline. A careful record of each liver was made, regarding the size and shape, colour and presence or absence of any nodule. Then, the liver were taken and fixed immediately in 10% formalin solution. Paraffin section were taken at 5 mm thickness processed in alcohol-xylene series and were stained with haematoxylin and eosin (Culling, 1974). The sections were examined microscopically for histopathological changes.

**Statistical analysis:** Mean, standard error of mean were calculated for each parameters in each group. One way ANOVA was used for multiple group comparisons followed by Post- Hoc Tukey HSD (Honestly Significant Difference) test for inter group comparisons of all the parameters. p-values less than <0.05 was taken to be significant.

**RESULTS**

**Solanum nigrum and CCL<sub>4</sub> group:** Levels of ALT, AST and S. Bilirubin were significantly less in *Solanum nigrum* group as compared with group treated with CCL<sub>4</sub> alone, Mean difference of -92.92 U L<sup>-1</sup> (p<0.0001), -133.37 U L<sup>-1</sup> (p<0.0001), -0.71 mg dL<sup>-1</sup> (p<0.0001), respectively. Levels of total protein were significantly high in *Solanum nigrum* group as compared to group treated with CCL<sub>4</sub> alone Mean difference +0.60 mg dL<sup>-1</sup> (p<0001) (Table 1).

Levels of SOD and GSH were significantly more in *Solanum nigrum* group as compared with group treated with CCL<sub>4</sub> alone, Mean difference of +2.23 U mg<sup>-1</sup> protein (p<0.0001) and 3.35 μmol mg<sup>-1</sup> protein (p<0.004), respectively. Levels of LPO were significantly low in *Solanum nigrum* group as compared to group treated with CCL<sub>4</sub> alone, Mean difference of -0.168 μmol mg<sup>-1</sup> protein (p<0.0001) (Table 1).

**Cichorium intybus and CCL<sub>4</sub> group:** Levels of ALT, AST and S.Bilirubin were significantly less in *Cichorium intybus* group as compared with group treated with CCL<sub>4</sub> alone. Mean difference of -114.44 U L<sup>-1</sup> (p<0.01), -163.38 U L<sup>-1</sup> (p<0.0001), -0.91 mg dL<sup>-1</sup> (p<0.0001) respectively. Levels of Total protein were significantly high in *Cichorium intybus* group as compared to group treated with CCL<sub>4</sub> alone. Mean difference of +0.99 mg dL<sup>-1</sup> (p<0.0001) (Table 1).

Levels of SOD and GSH were significantly more in *Cichorium intybus* group as compared with group treated with CCL<sub>4</sub> alone. Mean difference of +3.54 U mg<sup>-1</sup> protein (p<0.0001) and +5.82 μmol mg<sup>-1</sup> protein (p<0.0001), respectively. Levels of LPO were significantly low in *Cichorium intybus* group as compared to group treated

with CCL<sub>4</sub> alone. Mean difference -0.251 μmol mg<sup>-1</sup> protein (p<0.0001) (Table 1).

**Solanum nigrum and Silymarin group:** Levels of ALT, AST and S. Bilirubin were significantly less in silymarin group as compared with group treated with *Solanum nigrum* alone, Mean difference of ALT, AST, S.Bilirubin and Total protein 44.73 U L<sup>-1</sup>, 62.19 U L<sup>-1</sup>, 0.4166 mg dL<sup>-1</sup> and -0.768 mg dL<sup>-1</sup> respectively. Compared to Silymarin, *Solanum nigrum* has got significantly less antioxidant activity. Mean difference of SOD, GSH and LPO was -2.59 U mg<sup>-1</sup> protein, -5.53 μmol mg<sup>-1</sup> protein and +0.161 μmol mg<sup>-1</sup> protein, respectively.

**Cichorium intybus and Silymarin group:** Levels of ALT, AST and S.Bilirubin were significantly less in silymarin group as compared with group treated with *Cichorium intybus* alone, Mean difference of ALT, AST, S. Bilirubin and Total protein +23.21 U L<sup>-1</sup>, +32.18 U L<sup>-1</sup>, +0.22 mg dL<sup>-1</sup> and -0.37 mg dL<sup>-1</sup> respectively. Compared to Silymarin, *Cichorium intybus* has got significantly less antioxidant activity. Mean difference of SOD, GSH and LPO was -1.28 U mg<sup>-1</sup> protein, -3.06 μmol mg<sup>-1</sup> protein and +0.078 μmol mg<sup>-1</sup> protein respectively.

**Cichorium intybus and Solanum nigrum group:** Levels of ALT, AST and S.Bilirubin were significantly less in *Cichorium intybus* group as compared with group treated with *Solanum nigrum*, Mean difference of -21.52 U L<sup>-1</sup> (p<0.02), -30.01 U L<sup>-1</sup> (p<0.008), -0.191 mg dL<sup>-1</sup> (p<0.044) respectively. Levels of Total protein were significantly high in *Cichorium intybus* group as compared to group treated with *Solanum nigrum*. Mean difference +0.396 mg dL<sup>-1</sup> (p<05) (Table 1).

Levels of SOD and GSH were significantly more in *Cichorium intybus* group as compared with group treated with *Solanum nigrum*, Mean difference of -1.30 U mg<sup>-1</sup> protein (p<0.039) and -2.46 μmol mg<sup>-1</sup> protein (p<0.048), respectively). Levels of LPO were significantly less in *Cichorium intybus* group as compared to group treated with *Solanum nigrum*, Mean difference -0.0833 (p<0.031) (Table 1).

Table 1: Results of liver function test in-group 1 to 5

Groups	Serum ALT (I U L <sup>-1</sup> )*	Serum AST (I U L <sup>-1</sup> )*	Serum bilirubin (mg dL <sup>-1</sup> )*	Plasma total protein (mg dL <sup>-1</sup> )*	SOD (Units mg <sup>-1</sup> of protein)*	GSH (μmoles mg <sup>-1</sup> of protein)*	LPO (μmoles mg <sup>-1</sup> of protein)*
CMC	45.90±0.96	81.27±3.42	0.286±0.018	8.21±0.12	12.84±0.41	22.40±0.60	0.736±0.02
C+CCl <sub>4</sub>	217.70±6.92	327.37±8.30	1.616±0.079	6.05±0.11	5.33±0.28	9.51±0.42	1.158±0.01
SLM+CCl <sub>4</sub>	80.04±0.83	131.80±2.17	0.4817±0.047	7.42±0.05	10.16±0.37	18.40±0.57	0.828±0.01
** SNF+CCl <sub>4</sub>	124.77±2.99	194.00±8.19	0.898±0.018	6.65±0.10	7.57±0.18	12.86±0.63	0.990±0.06
** CIR+CCl <sub>4</sub>	103.25±1.59	163.99±2.66	0.706±0.032	7.05±0.08	8.87±0.15	15.33±0.67	0.906±0.03

\*Results are expressed as Mean±SEM (n = 6). \*\*p values of<0.05 (statistically significant). ALT: Alanine transaminase, AST: Aspartate aminotransferase, CCL<sub>4</sub>: Carbon tetrachloride, CMC: Carboxymethyl Cellulose GSH: Glutathione, SOD: Superoxide Dismutase, LPO: Lipid peroxidase

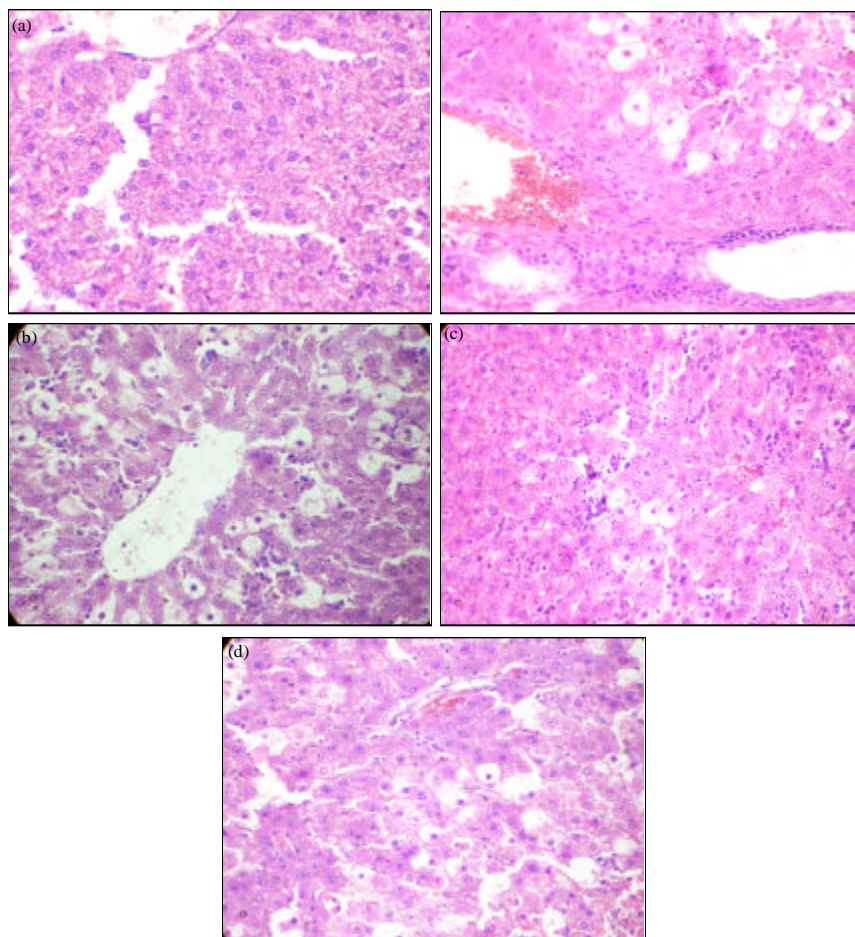


Fig. 1(a-d): Photomicrographs of rat liver (HPE). (a) Normal control (CMC) Positive control ( $\text{CCl}_4$ ) (Normal arrangements of hepatocytes with nuclei) (Intense centrilobular necrosis) (b) Silymarin ( $100 \text{ mg kg}^{-1}$ , p.o.) (Scanty degeneration of hepatocytes without any necrotic changes) (c) *Solanum nigrum* ( $250 \text{ mg/kg/day}$ , p.o.) and (d) *Cichorium intybus* ( $75 \text{ mg/kg/day}$ , p.o.) (Moderate centrilobular hepatocytes degeneration) (Mild centrilobular hepatocytes degeneration)

#### Histopathological study

**Control group:** Histology of liver from control group showed portal triad, rows of hepatocytes and normal arrangements of hepatocytes with nuclei.

**$\text{CCl}_4$  group:** Histology of  $\text{CCl}_4$  treated group rat liver showed intense centrilobular necrosis, bridging type of necrosis, congestion of sinusoids with small lipid globules and hydropic vacuolation Fig. 1a.

**Silymarin+ $\text{CCl}_4$  group:** Histology of liver section from Silymarin+ $\text{CCl}_4$  treated rats showed marked decrease in inflammation, very scanty degeneration of hepatocytes without any necrotic changes and mild fatty degeneration Fig. 1b.

***Solanum nigrum*+ $\text{CCl}_4$  group:** Histology of liver sections from *Solanum nigrum*+ $\text{CCl}_4$  treated rats revealed moderate centrilobular hepatocytes degeneration, few areas of congestion with mild fatty changes Fig. 1c.

***Cichorium intybus*+ $\text{CCl}_4$  group:** Histology of liver sections from *Cichorium intybus*+ $\text{CCl}_4$  treated rats revealed mild centrilobular hepatocytes degeneration, few areas of congestion with mild fatty changes Fig. 1d.

#### DISCUSSION

$\text{CCl}_4$  mediated acute hepatotoxicity was taken here as the experimental model for liver injury. It has been established that  $\text{CCl}_4$  is accumulated in hepatic

parenchymal cells and metabolically activated by cytochrome p-450 dependent monooxygenases to form a trichloromethyl free radical ( $\text{CCl}_3^{\cdot}$ ) which alkylates cellular proteins and other macromolecules with simultaneous attack on polyunsaturated fatty acids in the presence of oxygen to produce lipid peroxidases leading to liver damage (Recknagel *et al.*, 1989).

The Mean serum AST, ALT and Bilirubin levels in control group rats were  $45.90 \text{ U L}^{-1}$ ,  $81.27 \text{ U L}^{-1}$  and  $0.29 \text{ mg dL}^{-1}$  respectively, whereas in  $\text{CCl}_4$  treated rats, the levels significantly increased to  $217.70 \text{ U L}^{-1}$ ,  $327.37 \text{ U L}^{-1}$  and  $1.62 \text{ mg dL}^{-1}$  respectively, similar observations of increased AST, ALT and Bilirubin levels are seen in ethanol induced hepatotoxicity (Baranisrinivasan *et al.*, 2009). *Solanum nigrum* and *Cichorium intybus* significantly reduced the serum AST, ALT and Bilirubin levels to  $124.77 \text{ U L}^{-1}$  and  $103.25 \text{ U L}^{-1}$ ,  $194.00 \text{ U L}^{-1}$  and  $163.99 \text{ U L}^{-1}$  and  $0.89 \text{ mg dL}^{-1}$  and  $0.70 \text{ mg dL}^{-1}$  respectively. Silymarin significantly reduced the serum AST, ALT and Bilirubin levels to  $80.04 \text{ U L}^{-1}$ ,  $131.80 \text{ U L}^{-1}$  and  $0.48 \text{ mg dL}^{-1}$  respectively. There was significant decrease in serum protein levels after  $\text{CCl}_4$ , similar observations were made by others (Samudram *et al.*, 2008). In the present study serum protein levels was reversed by *Solanum nigrum*, *Cichorium intybus* and Silymarin with statistical significance.

The Mean levels of SOD and GSH content of liver in control group rats were  $12.84 \text{ U mg}^{-1}$  protein and  $22.40 \text{ } \mu\text{mol mg}^{-1}$  protein respectively, whereas in  $\text{CCl}_4$  treated rats, the levels significantly decreased to  $5.33 \text{ U mg}^{-1}$  protein and  $9.51 \text{ } \mu\text{mol mg}^{-1}$  protein, respectively similar parameter are used to assess antioxidant properties against paracetamol induced hepatotoxicity (Malathi and Gomez, 2007). *Solanum nigrum* and *Cichorium intybus* significantly increased the SOD and GSH levels to  $7.57 \text{ U mg}^{-1}$  protein and  $12.86 \text{ U mg}^{-1}$  protein and  $8.87 \text{ } \mu\text{mol mg}^{-1}$  protein and  $15.33 \text{ } \mu\text{mol mg}^{-1}$  protein respectively. Silymarin significantly increased the SOD and GSH content of liver to  $10.16 \text{ U mg}^{-1}$  protein and  $18.40 \text{ } \mu\text{mol mg}^{-1}$  protein, respectively. There was significant increase in LPO levels after  $\text{CCl}_4$  which was reversed by *Solanum nigrum*, *Cichorium intybus* and Silymarin. Similar studies against  $\text{CCl}_4$ -induced rat liver injury through potentiating antioxidant capacity of liver cells and prevention of oxidative stress that accompanied with  $\text{CCl}_4$  hepatotoxicity was established (Khadr *et al.*, 2007).

Histology of control group rat liver showed normal architecture of hepatic lobule. Histology of  $\text{CCl}_4$  treated group rat liver showed intense centrilobular necrosis, bridging type of necrosis, congestion of sinusoids with small lipid globules and hydropic vacuolation which

proves successful development of liver injury model. Similar histopathological observations were made by other researchers (Prakash *et al.*, 2008; Turel *et al.*, 2009; Chavda *et al.*, 2010). Histology of *Solanum nigrum* and  $\text{CCl}_4$  treated group rats showed moderate centrilobular degeneration of hepatocytes and mild congestion. Histology of *Cichorium intybus* and  $\text{CCl}_4$  treated group rats showed mild centrilobular degeneration of hepatocytes and mild congestion. Histology of Silymarin and  $\text{CCl}_4$  treated group rats showed almost normal lobular architecture with mild congestion.

The extract of *Solanum nigrum* and *Cichorium intybus* confers significant protection against  $\text{CCl}_4$  induced liver injury and probable mechanism is by maintenance of structural integrity of hepatocyte cell membrane and may be due to their ability to suppress the oxidative degradation of DNA (Sultana *et al.*, 1995). Histological examination of the liver also confirms these findings by showing moderate centrilobular hepatocytes degeneration, few areas of congestion with mild fatty changes.

## CONCLUSION

The results indicate that the extract of *Cichorium intybus* and *Solanum nigrum* has significant hepatoprotective activity in albino rats with  $\text{CCl}_4$  liver injury. *Cichorium intybus* has got significantly better hepatoprotective activity as compared to *Solanum nigrum*. Both are commonly used traditional herbs which are safe, cost effective and grow widely in India and used by different ethnic groups for various diseases. Further studies are necessary to evaluate the molecular and biochemical basis of its pharmacological action as a hepatoprotective agent.

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