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RESEARCH ARTICLE

Anti-Hepatoprotective Effect of *Clerodendrum phlomidis* (L) Against CCL_4 Induced Hepatotoxicity in Rats

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ABSTRACT

In the present investigation, studies on Anti-Hepatoprotective Effect of *Clerodendrum phlomidis* (L) Against CCL_4 Induced Hepatotoxicity in albino Rat model. In this study the ethanolic extract of *Clerodendrum phlomidis* was given CCL_4 (intramuscularly) intoxicated rats in which the Hepatotoxicity induced by the administration of Carbon tetra chloride for 7 days. Biochemical parameters were assessed the protective effect of *Clerodendrum phlomidis* extract to observe the results when compared with normal rats. The results showed increased activity of SGOT, SGPT and ALP in CCL_4 induced rats compared with control rats. Ethanolic extract of *Clerodendrum phlomidis* significantly decreased the level of SGOT, SGPT and ALP in rats. The present study the decreased content of GSH in CCL_4 intoxicated rat compared to control rats. After administration of ethanolic extract of *Clerodendrum phlomidis* significantly increased in the level of GSH in CCL_4 intoxicated rats.

KEYWORDS

Clerodendrum phlomidis, SGOT, SGPT, ALP, GSH, CCL_4, Anti-Hepatoprotective activity

INTRODUCTION

Plants have been used as a source of medicine throughout history and continue to serve as the basis for many pharmaceuticals used today. The liver plays a central in transforming and clearing chemicals and is susceptible to the toxicity from these agents. More than 900 drugs have been implicated in causing liver injury. (Friedmen et al., 2003). Drugs continue to be taken off the market due to late discovery of hepatotoxicity. Due to its unique metabolism and close relationship with the gastrointestinal tract, the liver is susceptible to injury from drugs and other substances.75% of blood coming to the liver arrives directly from gastrointestinal organs and then spleen via portal veins which bring drugs and Xenobiotics in concentrated form. Several mechanisms are responsible for either inducing hepatic injury or worsening the damage process. Many chemicals damage Mitochantria, an intracellular organelle that produces energy. Its dysfunction releases excessive amount of Oxidants which in turn injuries hepatic cells.

Clerodendrum phlomidis(L) belongs to family Verbinaceae, is an important indigenous endemic medicinal herb. It's a large bush or a small tree growing throughout India. Indian system of medicines particularly Ayurvedha and Siddha uses *Clerodendrum phlomidis*(L) as a single drug or in combination with other drugs. Roots are values as tonic diuretic febrifuge, anti-diabetic, anti-inflammatory and anti-tussive (Nadkarni, 1982).

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MATERIALS AND METHOD

Plant Materials

Fresh Leaves of *Clerodendrum phlomidis* (L) were collected from Tanjore. The leaves of *C .phlomidis* were dried in shade for 4-6 days. After 6 days, the leaves with lost moisture content were made into fine powder using mixer grinder.

Preparation of Leaf Extract

The powdered form of *C. phlomidis* was obtained after grinding (100g) was defatted with Distilled water using Soxhlet apparatus till exhaustion for about 32hrs (Bose *et al.*,2007).

Procurement of Animals

Healthy male albino rats (100-150g) were used for the present studies. They were housed in groups of three in standard cages at room temperature with light cycles. They were allowed free access to standard pellet diet and water ad-libitum.

Experimental Design

The rats were divided into four groups, containing 4 rats in each group. Initial body weight each was recorded. Group I Served as a control received standard. Group II was CCL_4 induced into normal diet for 7 days. Group III was received aqueous extract of *C. phlomidis* after induction with CCL_4. Group IV was received Dimercaptosuccinic acid (DMSA) of after with CCL_4. DMSA has been recommended for removal of Cadmium from blood.

Collection of Blood

The blood was collected by sino-orbitol puncture and allowed to clot and then centrifuged at 3000rpm for 5mts. The clear serum was collected and used for estimation of biochemical parameters, such as activity of SGOT, SGPT, ALP, GSH, MDA and TBL.

Biochemical Parameters

Estimation of SGOT (AST)

The serum SGOT was estimated by the method Reitman and Frankel (1957).

0.1 ml of serum was mixed with 0.5 ml of substrate reagent and incubated for 60mts at 37^0C. 0.5ML of colour reagent was added and further incubation for 20mts at 37^0C.After the incubation added 3ml of alkaline reagent stopped the reaction and the colour intensity was read at 505nm.

The SGOT activity was expressed as IU/L.

Assessment of SGPT (ALT)

The serum SGPT was estimated by the method of Reitman and Frankel (1957).

0.1ml of serum was mixed with 0.5ml of substrate reagent and incubated for 60mts at 37^0C. 0.5ml of colour reagent was added and further incubation for 20min at 37^oC. After the incubation, added 3.0ml of alkaline reagent. Stopped to reaction and the colour intensity was read at 505nm.

The SGPT activity was expressed as IU/L.

Activity of Alkaline Phosphatase (ALP)

Alkaline phosphatase activity was determined by Kind (1954).

0.5ml of buffered substrate (buffered substrate, pH 10.0) was added to control, blank and test and 1.5ml of distilled water was added to all the tubes. Mix well and incubate for 3 mts at 37^oC. 0.05ml of serum was only added to the test. Mix and incubate for 15mts at 37^oC. 1ml of colour reagent (Chromogen reagent) was added to all the tubes 0.05ml of serum was added to control tube. Mix well after the addition of each reagent and measure the optical density of Blank (B), Standard(S), Control(C) and Test (T) against purified water using a green filter (510nm).

Activity of Reduced Glutathione (GSH)

Reduced glutathione was estimated by method (Moron *et al.*, 1979).

0.5ml of serum sample was precipitated with 1ml of 10%TCA and the precipitate was removed by centrifugation. To 0.5 ml of the supernatant 1ml of DTNB was added. (0.6nm) DTNB in 0.2m Sodium phosphate and the total volume was made up to 3ml of phosphate buffer

(0.2ml phosphate buffer) at pH (8.0) the absorbance was read at 412nm.

Activity of Malondialdehyde (MDA)

Malondialdehyde was estimated by the Thiobarbituric acid assay method (Begue and Aust, 1978).

The serum sample was combined with 2.0ml of TCA-TBA-HCl reagent (Trichloro acetic acid 15%, thiobarbituric acid: 0.375% HCl acid-0.25N, mix the reagent in the ratio of 1:1:1) and mixed thoroughly. The solution was heated for 15mts in a boiling water bath. The centrifuge at 1000rpm for 10mts. The absorbance of the sample was read at 535nm against a blank without sample.

Activity of Total Bilirubin (TBL)

The total bilirubin activity was determined by Aniya *et al.*, (2005).

1ml of Diazo A, 0.1ml of Diazo B, 1ml of activator was diluted with 2.5 ml distilled water. To this 0.2ml of serum was added mixed and read at yellow green filter after 5mts in room temperature. The control without activator was read also. The value was expressed as mg/dl.

Statistical Analysis

The results obtained in the present investigation were subject to statistical analysis like Mean(x-) and SD by Zar (1984).

$$\text{Mean}(x) = \frac{\sum x}{N}$$

The SD was calculated by the following formula.

$$SD\ (\delta) = \sqrt{\frac{\Sigma(x - x)^2}{N - 1}}$$

Where, Add together all the values of X and $\sum x$

N-Total number of observation

RESULTS AND DISCUSSION

In this study Carbon tetra chloride (CCl_4) hepatotoxicity were analyzed and treated with *Clerodendrum phlomidis* plant extract using male albino rats. In this study ethanolic extract of *Clerodendrum phlomidis* were given Carbon tetra chloride (intramuscularly) intoxicated rats in which the hepatotoxicity induced by the administration of Carbon tetra chloride for 7 days. Biochemical parameters were assessed the protective effect of *Clerodendrum phlomidis* also examined. The observations were compared with normal rats.

In our current investigation it was observed hepatoprotective effect of *Clerodendrum phlomidis* elevated against carbon tetra chloride (CCL_4) toxicity induced rats. In our present study increased activity of SGOT, SGPT and ALP in Carbon tetra chloride induced rats compared with control rats. Ethanolic extract of *Clerodendrum phlomidis* significantly decreased the level of SGOT, SGPT and ALP in rats.

The present study the decreased content of GSH in Carbon tetra chloride intoxicated rat compared to control rats. Administration of ethanolic extract of *Clerodendrum phlomidis* significantly increased in the level of GSH in Carbon tetra chloride intoxicated rats.

Estimating the activity of serum markers enzyme like SGOT, SGPT and ALP can make the assessment of the liver function. When the liver cells plasma is damaged, a variety of enzymes normally located in cytosol and released in to the blood stream. The estimation in serum is useful quantitative marker of the intent type of hepatocellular damage (Mithra *et al.*, 1998).

Melondialdehyde (MDA) the major aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acids. MDA, a secondary product of lipid peroxidation is used as an indicator of tissue damage by series chain reaction. In the context in carbon tetra chloride induced rats when compared to control rats. Administrative ethanolic extract of *Clerodendrum phlomidis* significantly decreased the level of MDA in Carbon tetra chloride induced rats.

In the present study the level of total bilirubin increased in Carbon tetra chloride induced rats compared to control rats. The increased level of

bilirubin indicated the abnormal liver function administration of ethanolic extract of *Clerodendrum phlomidis* significantly restored in the level of bilirubin carbon tetra chloride induced rats.

CONCLUSION

From the result it was concluded that the hepatoprotective study indicated that *Clerodendrum phlomidis* has higher hepatoprotective activity. The plants therefore may be used as *medicine* as described in *Ayurvedic* literature. Finally, it has been suggested that further comparative characterization of chemical constituents of each species is essential to reveal the potent Hepatoprotective components along with their proportionate combination.

Table 1: The level of SGOT and SGPT in normal, Induced and Treated rats

SN	Experimental Model	SGOT IU/dl	SGPT IU/dl
1	Group-I (Normal)	52.44±0.87	70.51±.86
2	Group-II (Induced)	175.24±1.3	195.1±0.62
3	Group-III (Treated herbal)	92.35±1.78	113.1±0.25
4	Group-IV (Standard drug)	68.17±0.19	78.16±0.27

Table-1 indicates the level of SGOT and SGPT in normal, Induced and Treated (Herbal and Standard drugs) rats SGOT and SGPT was extensively increased in group-II intoxicated rats compare with Group-I normal rats. Group – III Treated rats distinctly decreased in the level of SGOT and SGPT compared with Group II. The standard drug treated rat SGOT and SGPT level also decreased compared than Group-III.

Table-2 reveals the level of ALP in normal, Induced and treated rats ALP was significantly increased in group-II intoxicated rats compare with Group-I normal rats. Group-III treated rats markedly decreased in the level of ALP compared with Group-II.

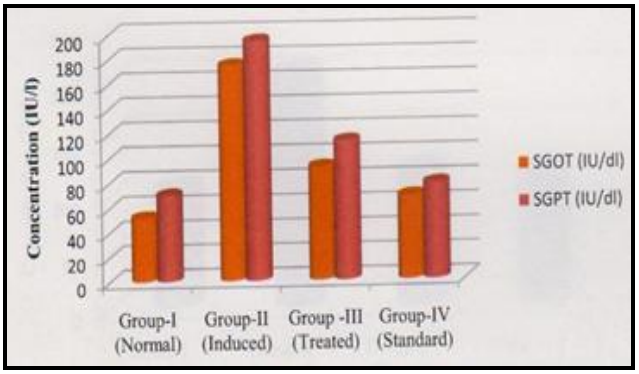

Figure 1: The Level of SGOT and SGPT in Normal, Induced and Treated Rats

Table 2: The level of ALP in normal, Induced and Treated rats

SN	Experimental Model	ALP IU/dl
1	Group-I (Normal)	104.92±0.61
2	Group-II (Induced)	207.12±0.42
3	Group-III (Treated herbal)	119.25±0.92
4	Group-IV (Standard drug)	114.75±0.24

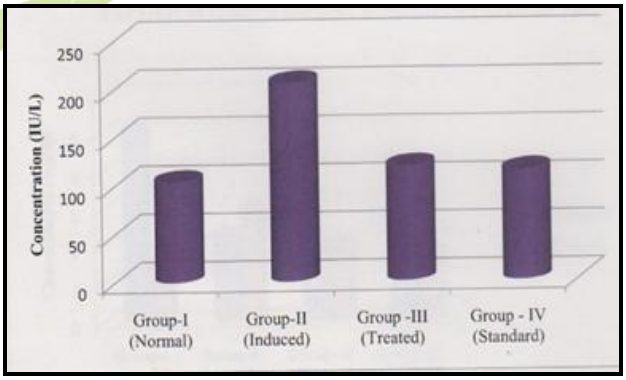

Figure 2: The Level of ALP in Normal, Induced and Treated Rats

Table-3 Depicts the level of GSH and MDA in normal, Induced and Treated rats GSH was significantly decreased and MDA was slightly increased in group-II intoxicated rats compare with Group-I normal rats. Group-III treated rats markedly decreased in the level of GSH and MDA compared with Group-II.

Table 3: the level of GSH and MDA in normal, Induced and Treated rats

SN	Experimental Model	GSH mg/dl	MDA mg/ml
1	Group-I (Normal)	4.08±0.07	1.57±0.09
2	Group-II (Induced)	2.25±0.09	3.02±0.15
3	Group-III (Treated herbal)	1.96±0.02	2.04±0.17
4	Group-IV (Standard drug)	1.850±.06	1.84±0.49

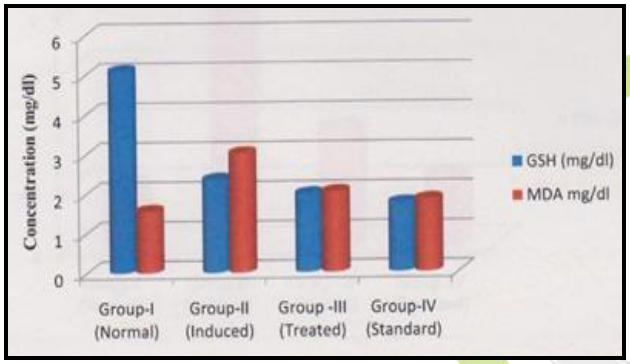

Figure 3: The Level of GSH in Normal, Induced and Treated Rats

Table 4: The level of TBL in normal, Induced and Treated rats

SN	Experimental Model	TBL mg/dl
1	Group-I (Normal)	0.52±0.05
2	Group-II (Induced)	2.47±0.14
3	Group-III (Treated herbal)	0.99±0.19
4	Group-IV (Standard drug)	0.59±0.07

Table-4 shows the level of TBL in normal, Induced and Treated rats TBL was drastically increased in group-II intoxicated rats compare with Group-I normal rats. Group-III treated rats prominently decreased in the level of TBL compared with Group-II. Standard drug treated rat TBL level also decreased.

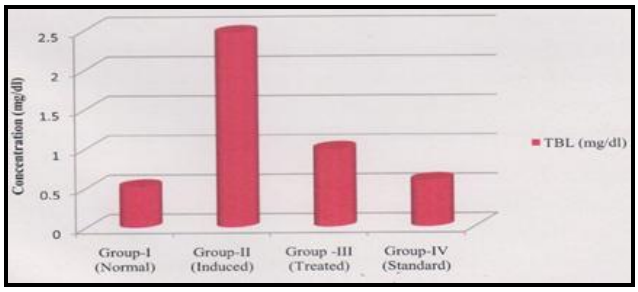

Figure 4: The Level of TBL in Normal, Induced and Treated Rats

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