

## Hepatoprotective Effect of *Crotalaria longipes* Wight & Arn, Ethanol Extract in CCl<sub>4</sub> Induced Hepatotoxicity in Wistar Rats

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### ABSTRACT

The aim of the present study is to investigate the hepatoprotective activity of ethanol extract of aerial part of *Crotalaria longipes* in CCl<sub>4</sub> induced hepatotoxic rats. Administration of hepatotoxins (CCl<sub>4</sub>) showed significant elevation of serum GOT, GPT, ALP, total bilirubin, conjugated, unconjugated and lipid peroxidation. Treatment with *Crotalaria longipes* (100, 200 and 400 mg/kg) significantly reduced the above mentioned parameters. Regarding antioxidant activity, ethanol extract of *Crotalaria longipes* exhibited a significant effect showing increasing levels of SOD, CAT, GP<sub>x</sub>, GSH, and GRD by reducing malondialdehyde (MDA) levels. The ethanol extract of *C. longipes* have significant effect on the CCl<sub>4</sub> induced hepatotoxic animal models.

**Key words** Bilirubin, CCl<sub>4</sub>, Hepatotoxicity, MDA.

### INTRODUCTION

Liver is one of the most complex internal organs in the body. It plays an important role in the maintenance of the internal environment through its multiple and diverse functions. It is involved in the intermediary metabolism of proteins, fats, carbohydrates and foreign bodies and is responsible for the synthesis of a number of plasma proteins. It also plays an important role in the production of various enzymes and formation and excretion of bile. It acts as a store house of proteins, glycogen, various vitamins and minerals. Hence, any injury to it or impairment of its function has grave influence on the health of the affected person. Liver disease is still a worldwide health problem. Although viral infection is one of the main reasons of hepatic injury, xenobiotics, excessive drug therapy, environmental pollutants and chronic alcohol ingestion can also cause severe liver injury. Hepatotoxicity is a common undesirable finding following overdosing with non-steroidal anti-inflammatory drugs (NSAIDs)<sup>1</sup>. Treatment options for common liver diseases such as cirrhosis, fatty liver and chronic hepatitis are problematic. The effectiveness of treatment such as interferon colchicines, penicillamine and corticosteroid are inconsistent at best and incidence of side effect is profound though the treatment is worse than the disease<sup>2</sup>. Drugs from natural sources are showing remarkable benefit with negligible side effects against different pathological conditions. Hence people are looking at the traditional system of medicine for remedies to treat hepatic disorders<sup>3</sup>. The genus *Crotalaria* has 300 species worldwide and about 18 species are reported in India. The genus produces mainly pyrrolizidine alkaloids but some flavonoid, glycosides have also been reported<sup>4</sup>. *C. longipes* are woody shrub

growing upto 4 m tall with bright yellow flowers endemic to Nilgiris and Kolli hills. However, perusal of literature survey reveals that hepatoprotective activity of *C. longipes* is totally lacking and hence the present investigation was undertaken. The present study focuses on evaluating the hepatoprotective activity of aerial part of *C. longipes*.

### MATERIALS AND METHODS

#### *Collection of Plant Sample*

The aerial parts of *Crotalaria longipes* was collected from Kothagiri, Nilgiris Biosphere Reserve, Tamil Nadu. With the help of flora, voucher specimen were identified and preserved in the Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin, Tamil Nadu for further references.

#### *Preparation of plant extract for phytochemical screening and hepatoprotective studies*

The aerial part of the plant was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder, which was then subjected to extraction in a Soxhlet apparatus using ethanol. The extract was subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures<sup>5,7</sup>. The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract were used for hepatoprotective studies.

#### *Animals*

Normal healthy male Wistar albino rats (180-240g) were used for the present investigation. Animals were housed under standard environmental conditions at room temperature (25±2°C) and light and dark (12:12h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum*.

Table 1: Effect of ethanol extract of *Crotalaria longipes* aerial part on the body weight of in the normal, liver damaged and drug treated rats.

Treatment	Initial Body weight (Gm)	Final Body weight (Gm)	Mean weight Gain (G↑) / loss(L↓) (Gm)	% of Difference
Group-I	218.15±4.84	226.36±3.94	8.21↑	3.76
Group-II	196.33±3.94	191.16±2.83**	5.17↓	2.63
Group- III	192.46±2.84	206.36±3.16ns	13.90↑	7.22
Group- IV	196.15±2.16	214.18±2.16a	18.03↑	9.19
Group- V	206.11±1.93	219.38±3.18aa	13.27↑	6.43
Groups VI	214.24±1.86	220.18±3.84aaa	5.94↑	2.77

Values are mean ± SD of 6 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. \*\* $p < 0.01$  as compared with Normal Control to liver damaged control: a  $p < 0.05$ ; aa  $p < 0.01$  aaa  $p < 0.001$  as compared with liver damaged control to drug treated animal, ns- not significant.

Table 2: Effect of ethanol extract of *Crotalaria longipes* aerial part on the serum protein, albumin, globulin concentration and serum GOT, GPT and ALP enzyme activity in the normal, liver damaged and drug treated rats.

Groups	Parameters						
	T.Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G Ratio	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
Group-I	8.34±0.34	4.86±0.65	3.48±0.16	1.3:1	18.13±1.34	20.36±1.75	168.31±3.07
Group-II	7.16±0.18*	3.85±0.74*	3.31±0.22	1.16:1	63.91±1.58**	54.18±1.22**	198.39±3.18*
Group- III	7.96±0.13ns	4.89±0.27a	3.07±0.15	1.59:1	26.34±1.36 a	34.84±1.68*	181.68±2.16*
Group- IV	8.26±0.19ns	4.58±0.56	3.68±0.18	1.24:1	21.63±1.18aa	29.16±1.84a	153.29±1.84a
Group- V	8.30±0.27a	4.68±0.39	3.62±0.21	1.29:1	17.96±1.54aaa	24.39±1.28aa	136.84±1.95a
Groups VI	8.43±0.18a	4.93±0.28a	3.50±0.13	1.40:1	19.36±1.48aaa	21.16±1.94aa a	149.39±1.27aa a

Values are mean ± SD of 6 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. \*\* $p < 0.01$  as compared with Normal Control to liver damaged control : a  $p < 0.05$ ; aa  $p < 0.01$  aaa  $p < 0.001$  as compared with liver damaged control to drug treated animal, ns- not significant.

#### Acute Toxicity Studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study<sup>8</sup>. The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 100 upto 2000 mg/kg body weight.

#### Experimental Design

In this investigation, a total of 30 rats (25 CCl<sub>4</sub> hepatic toxicity induced rats and 5 normal rats) were taken and divided into six groups of 5 rats each.

Group I: Rats received normal saline was served as a normal control.

Group II: CCl<sub>4</sub> hepatic toxicity induced control: Rats received 2.5ml/kg body weight of CCl<sub>4</sub> for 14 days.

Group III: Liver injured rats received ethanol extract of aerial part of *C. longipes* at the dose of 100mg/kg body weight for 14 days.

Group IV: Liver injured rats received ethanol extract of aerial part of *C. longipes* at the dose of 200mg/kg body weight for 14 days.

Group V: Liver injured rats received ethanol extract of aerial part of *C. longipes* at the dose of 400mg/kg body weight for 14 days.

Group VI: Liver injured rats received standard drug silymarin at the dose of 100mg/kg body weight for 14 days.

#### Biochemical Analysis

The animals were sacrificed at the end of experimental period of 7 days by decapitation. Blood was collected, sera separated by centrifugation at 3000 g for 10 minutes. Serum protein<sup>9</sup> and serum albumins was determined quantitatively by colorimetric method using bromocresol green. The total protein minus the albumin gives the globulin. Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) was measured spectrophotometrically by using the method of Reitman and Frankel<sup>10</sup>. Serum alkaline phosphatase (ALP) and serum acid phosphatase (ACP) was measured by the method of King and Armstrong<sup>11</sup>. Lactate dehydrogenase (LDH) was determined by the method of Mercer<sup>12</sup>. Total bilirubin and conjugated bilirubin were determined as described by Balistrei and Shaw<sup>13</sup>. The unconjugated bilirubin concentrations were calculated as the difference between total and conjugated

Table 3: Effect of ethanol extract of *Crotalaria longipes* aerial part on the serum Total, conjugated, unconjugated bilirubin and GGTP levels in the normal control, liver injured and drug treated rats.

Groups	Parameters			
	Total Bilirubin (Mg/dl)	Conjugated (Mg/dl)	Unconjugated (Mg/dl)	GGTP (U/L)
Group-I	0.68±0.06	0.20±.05	0.48±0.02	10.31±0.13
Group-II	2.96±0.05***	2.18±0.07***	0.78±0.03*	28.13±0.28**
Group- III	1.84±0.04*	1.24±0.08**	0.60±0.07*	16.31±0.19ns
Group- IV	1.24±0.03a	0.95±0.01*	0.29±0.06a	11.84±0.11a
Group- V	1.04±0.01a	0.54±0.02ns a	0.50±0.02a	7.84±0.18aa
Groups VI	0.91±0.06aa	0.62±0.05ns aa	0.29±0.04a	7.13±0.15aaa

Values are mean ± SD of 6 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test.\*\* $p < 0.01$  as compared with Normal Control to liver damaged control: <sup>a</sup>  $p < 0.05$  ; <sup>aa</sup>  $p < 0.01$  <sup>aaa</sup>  $p < 0.001$  as compared with liver damaged control to drug treated animal ns: not significant.

bilirubin concentrations. Quantitative estimation of MDA formation was done by determining the concentration of thiobarbituric acid reactive substances (TBARS) in plasma by the method of Satoh<sup>14</sup>. Enzymatic antioxidants, superoxide dismutase (SOD)<sup>15</sup> Catalase (CAT)<sup>16</sup> glutathione reductase (GRD)<sup>17</sup> reduced and glutathione (GSH)<sup>18</sup> and glutathione peroxidase (GPx)<sup>19</sup> were also assayed in erythrocytes. Seven  $\mu$ m thick paraffin sections of buffers formalin fixed liver samples were stained with haematoxylin- eosin for photomicroscopic observations of the liver histological architecture of the control and treated rats.

## RESULT

### Preliminary phytochemical screening

The preliminary phytochemical screening of the aerial part ethanol extract showed the presence of alkaloid, coumarin, catechin, flavonoid, steroid, saponin, glycosides, phenol, tannin, terpenoid and sugar.

### Acute toxicity

Oral administration of the ethanol extract of *C.longipes* aerial part did not cause any acute toxicity in experimental rats at all the tested dosage, confirming that it has potential safe for consumption.

### Effect of aerial part extract of *Crotalaria longipes* on body weight

The effect of ethanol extract of *C.longipes* aerial part on body weight of the normal control, CCl<sub>4</sub> intoxicated control and drug treated rat groups are shown in Table-1. An increase in body weight was noticed in all the groups except the rats in Group II, the liver damaged control (CCl<sub>4</sub> intoxicated control), when compared to normal control. The rats treated with aerial part extract of *C.longipes* (100, 200, and 400mg/kg bodyweight) also gained body weight during the experimental period.

### Effect of aerial part extract of *Crotalaria longipes* on biochemical parameters

The results of serum biochemical parameters are presented in Table-2. In CCl<sub>4</sub> control group, significantly decreased levels of total protein ( $p < 0.05$ ) albumin ( $p < 0.05$ ) and globulin were observed. But the group when received the ethanol extract of aerial part of *C.longipes* at the dose of 400mg/kg body weight showed an increase in the levels of total protein, albumin and globulin. There was significant ( $p < 0.01$ ;  $p < 0.05$ ) increase in serum GOT,

GPT and ALP levels in CCl<sub>4</sub> intoxicated group (Group II) when compared to the normal control group (Group I). Aerial part ethanol extract of *C.longipes* at a dose of 400mg/kg body weight helped to decrease the elevated serum marker enzymes significantly. Treatment with the standard drug silymarin reversed the altered protein, albumin and liver marker enzymes to almost normal level.

### Effect of aerial part extracts of *Crotalaria longipes* on total bilirubin, conjugated, un conjugated, and r-glutamyltranspeptidase (GGTP)

The effect of ethanol extract of *Crotalaria longipes* aerial part on total, conjugated and unconjugated bilirubin and r-glutamyltransferase are depicted in Table 3. There was a significant elevation of conjugated, unconjugated bilirubin ( $p < 0.001$  and  $p < 0.05$ ) and r-glutamyltransferase or r-glutamyltranspeptidase (GGTP) ( $P < 0.01$ ) in the serum of CCl<sub>4</sub> intoxicated control rats (Group II) as compared to normal control (Group I). The aerial part ethanol extract of *Crotalaria longipes*, at the dose of 400mg/kg body weight, reduced the elevated levels of total conjugated and unconjugated bilirubin in plant extract treated rats. Decrease in the concentration of total bilirubin, conjugated bilirubin, unconjugated bilirubin and r-glutamyltransferase was found to be greater in silymarin, standard drug treated rats (Group VI) followed by Group V and Group VI and Group III rats treated with the plant extract.

### Effect of aerial part extract of *Crotalaria longipes* on antioxidant enzymes

The effect of *Crotalaria longipes* aerial part extract on LPO, GP<sub>x</sub>, GRD, SOD, CAT, and GSH activities are shown in Table-4. When compared to the normal control rats (Group I), the level of lipid peroxidation (LPO) had increased significantly ( $p < 0.01$ ) and levels of glutathione peroxidase (GP<sub>x</sub>) glutathione (GRD), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) had decreased significantly ( $p < 0.01$  and  $p < 0.05$ ) in CCl<sub>4</sub> intoxicated control rats (Group II). Treatment with the aerial part ethanol extract of *C.longipes* decreased the elevated lipid peroxidation level significantly and restored the altered glutathione level towards normal in a dose dependent manner. The results were well comparable with that of silymarin treated rats. Liver sections from control rats pre-treated with normal

Table 4: Effect of ethanol extract of *Crotalaria longipes* aerial part on serum LPO, GP<sub>x</sub>, GRD, SOD, CAT and GSH activity in the normal control, liver injured and drug treated rats.

Groups	Parameters					
	LPO (n mole of MDA/mg protien)	GP <sub>x</sub> (u/mgProtien)	GRD (u/mg)	SOD (u/mg)	CAT (u/mg)	GSH (u/mg)
Group-I	2.094±0.054	2.918±0.061	0.381±0.011	0.318±.0113	6.311±0.154	39.46±1.16
Group-II	5.134±0.37**	1.631±0.054*	0.198±0.016**	0.209±0.0114**	2.84±0.039**	20.84±1.54**
Group- III	4.113±0.058ns	1.804±0.027ns	0.241±0.024*	0.284±0.036ns	3.85±0.041*	28.55±1.34*
Group- IV	2.863±0.018ns	2.112±0.013a	0.248±0.011*	0.298±0.018a	4.11±0.036ns	32.16±0.64ns a
Group- V	2.113±0.051aa	3.016±0.027aa	0.261±0.018*a	0.308±0.081a	4.26±0.054a	37.16±0.96aa
Groups VI	2.108±0.018aaa	3.113±0.041a	0.364±0.014a	0.324±0.036a	6.816±0.036a	32.67±0.54aa a

Values are mean ± SD of 6 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. \*\* $p < 0.01$  as compared with Normal Control to liver damaged control :<sup>a</sup>  $p < 0.05$  ;<sup>aa</sup>  $p < 0.01$  <sup>aaa</sup>  $p < 0.001$  as compared with liver damaged control to drug treated animal ns: not significant.

saline showing the normal lobular architecture and normal hepatic cells with a well preserved cytoplasm and well-defined nucleus and nucleoli are shown plate-I Fig A. In the liver damaged group (plate-I Fig B) the liver showed hepatocytic necrosis and inflammation in the centrilobular region with portal triaditis. Co-administration of *C. longipes* aerial part showed minimal inflammation with moderate portal triaditis and their lobular architecture was normal. Plate-I (Fig C, D and F). On the other hand, in comparison, the liver tissue of rats pretreated with reference hepatoprotective agent, (Fig G - 100 mg/kg ) silymarin also demonstrated normal liver histology . These above findings indicated the hepatoprotective effect of *C. longipes* aerial part extracts act on a dose dependent manner.

## DISCUSSION

Liver is one of the largest organs in human body and the chief site for internal metabolism and excretion; which play an important role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction<sup>20</sup>. The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus to maintain a healthy liver is a crucial factor for overall health and wellbeing. Liver is continuously and variedly exposed to environmental toxins and abused by poor drug habits and alcohol which can eventually lead to various liver ailment like hepatitis, cirrhosis and alcoholic liver disease<sup>21</sup>. There is no rational therapy available for treating liver disorders and management of liver diseases is still a challenge to the modern medicine<sup>22</sup>. In the absence of reliable live protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders. The use of natural remedies for the treatment of various hepatic diseases has a long history

and medicinal plants and their derivative are still used all over the world<sup>23</sup>. CCl<sub>4</sub> has been one of the most intensively studied hepatotoxicants to date and provides a relevant model for other halogenated hydrocarbon that are used widely<sup>24</sup>. A single exposure to CCl<sub>4</sub> can lead to severe centizonal necrosis and steatosis<sup>25</sup>. The changes associated with CCl<sub>4</sub>- induced liver damage are similar to that of acute viral hepatitis<sup>26</sup>. CCl<sub>4</sub> is transformed by cytochrome P<sub>450</sub> system to produce trichloromethyl free radicals. These free radicals may again react with oxygen to form trichloromethylperoxyl radicals which may attack lipids on the membrane of endoplasmic reticulum to elicit lipid peroxidation, finally resulting in cell necrosis and consequent cell death<sup>25</sup>. Marked increases in release of hepatic enzymes into the blood stream is often associated with massive necrosis of the liver. CCl<sub>4</sub> is known to cause marked elevation of serum enzymes.

In the present investigation, CCl<sub>4</sub> administration results in elevated activities of SGOT, SGPT and ALP in serum against normal control rats. Similarly bilirubin level and GGTP were also found to be increased significantly ( $p < 0.01$ ) as a result of CCl<sub>4</sub> toxicity. On the other hand, total serum protein level was lowered in response to CCl<sub>4</sub> administration when compared with control. Abnormally higher activities of serum SGOT, SGPT and ALP after CCl<sub>4</sub> administration are an indication of the development of hepatic injury, which is responsible for leakage of cellular enzymes into the blood, when liver plasma membrane gets damaged, a variety of enzymes normally located in the cytosol are released into the circulation<sup>27,28</sup>. Oral administration of various doses of ethanol extract of *C. longipes* aerial part (100, 200 and 400mg/kg body weight) to CCl<sub>4</sub> intoxicated rats resulted in gradual normalization of the activities of SGOT, SGPT and ALP. This evidently suggests the protective effect of the extract in improving the functional integrity of liver cells. Alkaline phosphatase, a hydrolase enzyme is responsible for removing phosphate groups from many types of

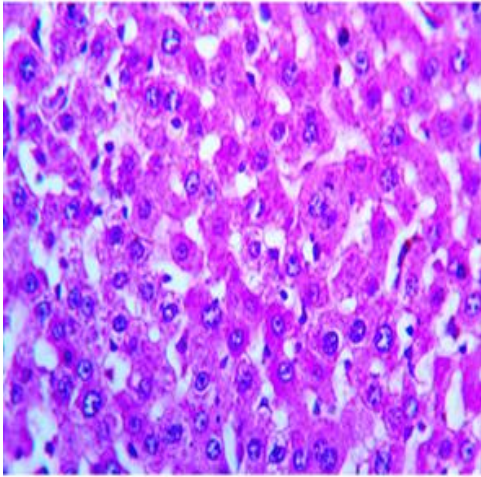


Figure A: Histoarchitecture of the normal control rat liver showing normal hepatic cells arrangement.

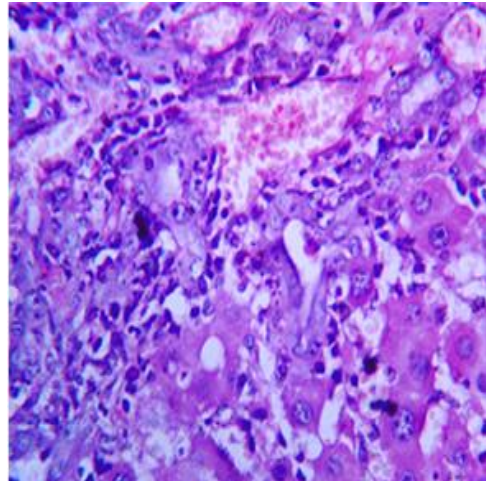


Figure B: Histoarchitecture of the liver damaged control rat liver showing steatosis, centrilobular necrosis and vacuolization was seen.

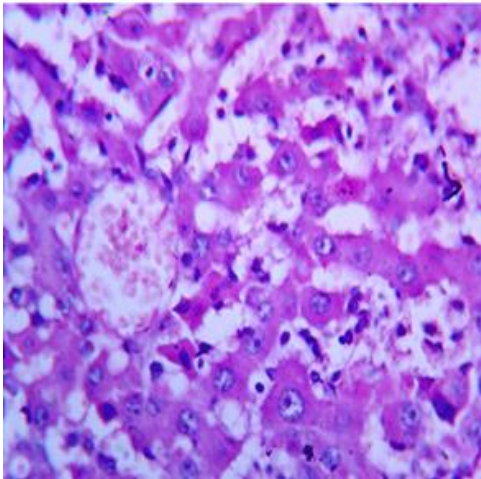


Figure C: Histoarchitecture of the liver damaged rat liver treated with *C. longipes* (100mg/Kg) (Low dose) showing the minimal recovery of the hepatocytes from necrosis.

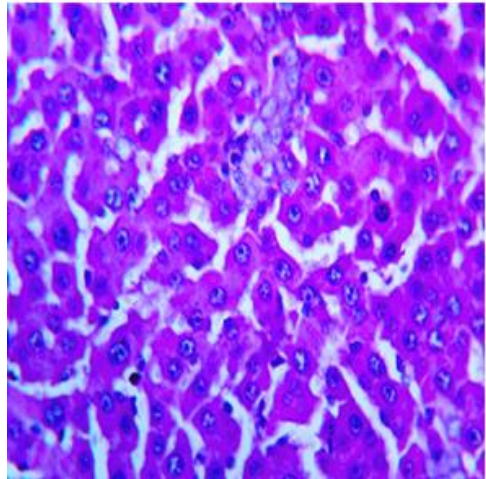


Figure D: Histoarchitecture of the liver damaged rat liver treated with *C. longipes*(200mg) extract (moderate dose) showing almost 60% of recovery

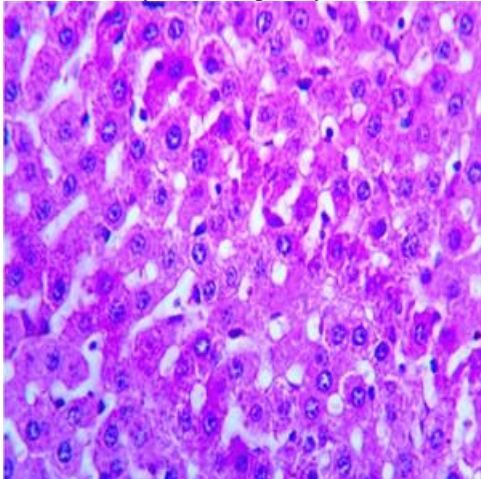


Figure E: Histoarchitecture of the liver damaged rat liver treated with *C. longipes* (400mg/kg)extract (high dose)showing high recovery of the hepatocytes from necrosis.

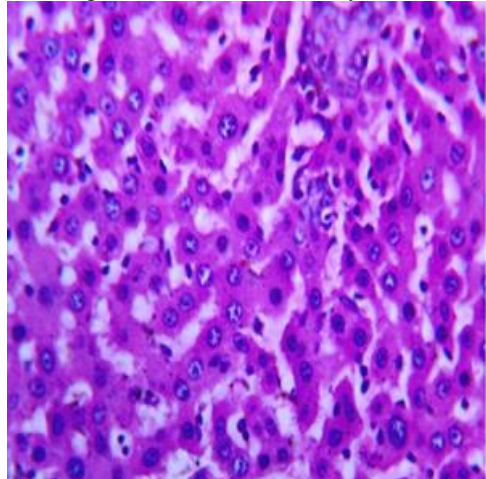


Figure F: Histoarchitecture of the liver damaged rat liver treated with known (silymarin) showing normal hepatic cell arrangement

molecule, including nucleotides, proteins and alkaloids. The alkaline phosphatase is the prototype of these

enzymes that reflects the pathological alteration in binary flow<sup>29</sup>. Serum bilirubin is considered as an index for the

assessment of hepatic function and any abnormal increase indicates hepatobiliary disease and severe disturbances of hepatocellular architecture<sup>30</sup>. CCl<sub>4</sub> administration resulted in increased serum bilirubin level, thereby suggesting severe hepatic injury and confirming the hepatotoxic nature of CCl<sub>4</sub>. Treatment with ethanol extract of *C. longipes* aerial part significantly ( $p < 0.05$ ) decreased the elevated level of total bilirubin in serum towards normalcy indicating its hepatoprotective efficacy. Hepatotoxins impair the capacity of liver to synthesize albumin. Decreased total serum protein level in CCl<sub>4</sub> treated rats may be attributed to impaired protein synthesis by damaging liver tissue. Subsequent treatment of CCl<sub>4</sub> intoxicated rats with ethanol extract of *C. longipes* aerial part increased the total serum protein level. This further signifies the curative nature of extract against CCl<sub>4</sub> toxicity. Increased lipid peroxidation associated with CCl<sub>4</sub> administration has often been used as an indicator of oxidative stress in both animal models and human clinical trials<sup>31</sup>. CCl<sub>4</sub> intoxicated rats showed increased liver tissue levels of lipid peroxidation marker such as TBARS. The increased peroxidation can result in changes in cellular metabolism of the hepatic and extra hepatic tissues. Increased accumulation of lipid peroxidation products in cells can result in cellular dehydration, whole cell deformity and cell death<sup>32</sup>. A massive decrease in lipid peroxidation in liver tissue of plant extract treated groups indicates that *C. longipes* aerial part administration suggest a decreased impact of reactive oxygen species (ROS) on lipid membranes, and therefore increased protection against -CCl<sub>4</sub> induces liver injury. Free radical scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GP<sub>x</sub>) are the first line of defence against oxidative injury. The second line of defence consists of the non-enzymatic scavengers such as GSH, ascorbic acid (vitamin C) and  $\alpha$ -tocopherol (vitamin E), which scavenge residual free radicals escaping decomposition by the antioxidant enzymes. Moreover, enzymatic antioxidants are inactivated by the excessive levels of free radicals and hence the presence of non-enzymatic antioxidants is presumably essential for the removal of these radicals<sup>33,34</sup>. Decrease in enzyme activity of SOD is a sensitive index in hepatocellular damage and is the most sensible enzymatic index in liver injury. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by radicals. Decrease in SOD production can be attributed to an enhanced superoxide generation and the utilization of this enzyme during reactive metabolites detoxification. High amounts of superoxide inhibit catalase, which is another important antioxidant enzyme<sup>35</sup>. CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals<sup>36</sup>. Therefore reduction in the activity of CAT may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide. GP<sub>x</sub> works in tandem with CAT to scavenge excess H<sub>2</sub>O<sub>2</sub> as well as other free radicals in response to oxidative stress. The equilibrium between these enzymes is

important for the effective removal of oxidative stress in intracellular organelles. The sulfhydryl group is invariably affecting the activity of the enzyme. This antioxidant defence system is significantly altered by CCl<sub>4</sub> administration<sup>37</sup>. CCl<sub>4</sub> intoxicated rats showed the decreased activities of these enzymes; resulted in the accumulation of highly reactive free radicals, leading to deleterious effects such as loss of cell membrane function<sup>38</sup>. Treatment with ethanol extracts of *C. longipes* aerial part significantly ( $p < 0.01$ ;  $p < 0.05$ ) increased the hepatic SOD, CAT and GP<sub>x</sub> activity and thus reactive free radical induced oxidative damage to liver. Glutathione is one of the most abundant tripeptide non-enzymatic biological antioxidant present in the liver. It removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thiols. Also it is a substrate for glutathione peroxidase<sup>39</sup>. decreased level of GSH is associated with an enhanced lipid peroxidation in CCl<sub>4</sub> intoxicated rats significantly increased the levels of GSH. The ability of *C. longipes* aerial part extract might be potentially useful in countering free radical mediated injuries involved in the development of liver damage caused by CCl<sub>4</sub>. Comparative histopathological study of the liver from different groups of rats corroborated the hepatoprotective efficacy of ethanol extract of *C. longipes* (fig. A to F). Various pathological changes like steatosis, centrilobular necrosis and vacuolization seen in group II (toxicant rats) is due to oxidative damage by free radical generation. These pathological changes were prevented to moderate extent in both the test drug groups and known drug groups. This might be due to presence of flavonoids and ascorbic acid. Antioxidant property is claimed to be one of the mechanism of hepatoprotective drugs. Further flavonoids and ascorbic acid have been suggested to act as antioxidants by free radical scavenging. Thus the hepatoprotective activity of *C. longipes* aerial part may be attributed to the presence of flavonoids and ascorbic acid. Preliminary phytochemical screening carried out in this study indicated that ethanol extract of *C. longipes* contain alkaloids, flavonoids, tannins and saponin. These phytochemicals are known to perform several general and specific functions in plants and may also exhibit different biochemical and pharmacological actions in different species of animals when ingested. These actions range from cell toxicity to cell protective effects<sup>40</sup>. The phytochemical report of this study suggested that the hepatocellular function-enhancing effect of the extract may result from the action of the various phytochemical contents, especially the presence of flavonoids which have been reported to have antioxidative effects<sup>41</sup>. Furthermore, saponins also present in the extract are known to have hypocholesterolemic activities<sup>42</sup>, which may aid in lessening the metabolic burden on the liver.

## CONCLUSION

It is concluded from the data, that the ethanol extract of *C. longipes* aerial part possesses significant hepatoprotective activity and may prove to be effective for the treatment of liver disorders. However, longer

duration studies on chronic models are necessary to elucidate the exact mechanism of action so as to develop it as a potent hepatoprotective drug.

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