Amelioration of *Trichosanthes lobata* in Paracetamol-Induced Hepatic Damage in Rats: A Biochemical and Histopathological Evaluation

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Abstract

The aim of the present work is to investigate the possible therapeutic potential of *Trichosanthes lobata* in paracetamol induced hepatic damage in rats. The hepatotoxicity was induced in rats by administration of paracetamol at higher dose (2 gm/kg) reflected in term of increase lipid peroxidation, impairment in antioxidants [Superoxide Dismutase (SOD), Catalase (CAT) and Reduced Glutathione (GSH)] along with elevation of serum marker enzymes. The possible ameliorative effect of *Trichosanthes lobata* leaf extracts mainly Aqueous (TLAQ) and Alcoholic (TLAL) were administered at various doses for 7 days and found to restored the depleted antioxidants (SOD, GSH, CAT) attenuated lipid peroxidation (MDA formation) and prevented elevated marker enzymes significantly. Further, histopathological alterations were also improved with TLAQ and TLAL in dose dependent manner. Thus, the hepatoprotective activity of TLAQ and TLAL may be correlated with amelioration of oxidative stress by improving endogenous antioxidant defence ability of hepatocytes and histopathological alterations in rats. The ameliorative effect of *Trichosanthes lobata* in paracetamol-induced hepatic damage in rats was studied.

Keywords: Flavonoids, Hepatic Damage, Paracetamol, *Trichosanthes lobata*

1. Introduction

Hepatic damage occurs mainly due to either liver infection or with administration/ingestion of antibiotics in therapeutic ranges. Various chemicals mainly chlorinated hydrocarbons, benzene, alcohol, mercury, arsenic etc are known to induce hepatic damages, leading to overall decline in metabolic functions of liver. Some of these toxicants formed their active metabolites in liver and thus responsible for lipid peroxidation, protein dysfunction and finally oxidative stress. These cellular consequences disturb the vital physiological process which results into cell death and organ dysfunction. The major concerns to noticed the liver diseases includes; firstly almost impossible as liver is extremely regenerative organ in which repairing action of damaging events mostly take place simultaneously; secondly, currently employed allopathic agents does not promise/guarantee for cure and/or prevention of liver diseases. Hence there is urgent need of hepatoprotective agent with least side effect.

*Trichosanthes lobata* Roxb (Family-Cucurbitaceae) known as Patola (Sanskrit) and wild snake gourd (English), is largely found in Maharashtra, India. Traditionally, the plant used as bitter tonic, laxative,
depurative, digestive, cardiotonic, anthelmintic, and in
treatment of jaundice. Documented reports suggest the
presence of various phytoconstituents viz; cucurbita-5,
24-dienol, α, β carotene, lycopene, lutein, vitamin C
and β-sitosterol in *Trichosanthes lobata*. However,
there are no scientific reports unavailable indicating
hepatoprotective effects of *Trichosanthes lobata*. Thus
based on the presence of chemical constituents in
*Trichosanthes lobata*, the present studies was undertaken
to investigate the hepatoprotective effect of
*Trichosanthes lobata* in PCM induced hepatic damage in rats and to
understand the underlying mechanism of action.

2. Materials and Methods

2.1 Plant Material

The plant *Trichosanthes lobata* (Family- Cucurbitaceae)
was collected in the month of June from local region of
Lonavala region of Maharashtra, state, India. The plant
herbarium was taxonomically identified at Botanical
Survey of India (BSI), Pune. A voucher specimen (BSI/
WC/Tech/2008/354-RRW/TL-2.) has been deposited for
future reference.

2.2 Drugs and Chemicals

Paracetamol and silymarin were obtained as gift sample
from Torent Research Centre, Ahmedabad and Cadila
Pharma Ltd. India, respectively. Thiobarbituric Acid
(TBA), reduced glutathione, oxidised glutathione and
nicotinamide adenine dinucleotide (NADPH) were
obtained from Himedia Laboratories, Mumbai, India,
5, 5-dithiobis (2-nitrobenzoic acid)-(DTNB) and
epinephrine were purchased from Sigma chemical Co,
St. Louis, MO, USA. Standard reagents and kits for
determination of AST, ALT, ALP, LDH, Total Proteins and
Total bilirubin were purchased from Span Diagnostics,
Surat, India and Ranbaxy laboratories, Delhi, India. All
other chemicals and reagents were of analytical grade
obtained from local suppliers.

2.3 Preparation of Extracts

The powdered plant material (500 gm) was subjected
to successive solvent extraction with the solvents in the
order of increasing polarity in ratio of (1:2) viz; petroleum
ether (40-60 °C), followed by chloroform and alcohol.

Aqueous extract was prepared by cold maceration
process using chloroform water 1996 as extracting
solvent wherein, chloroform servers as preservative.
The extracts were dried under reduced pressure using
rotary evaporator afforded semi solid extracts. It was
then stored in ambered glass bottle until used.

2.4 Preliminary Phytochemical Analysis

The preliminary phytochemical analyses were carried
out for the presence of various chemical constituents in
alcoholic and aqueous extract of *Trichosanthes lobata*
respectively.

2.5 High Performance Thin Layer
Chromatography (HPTLC) Study

The aqueous and alcoholic extracts of *Trichosanthes
lobata* were dissolved in respective HPTLC grade ethanol
and water which were used for sample application on
precoated silica gel GF 254 aluminium sheet (Made-
Merck, Germany). The samples (5 µL) were spotted
in the form of bands of width 6 mm with a 100 µL
sample using a Hamilton syringe on silica gel which was
precoated on aluminium plate GF-254 plates (20 cm X
10 cm) with the help of Linomat 5 applicator attached
to CAMAG HPTLC system, which was programmed
through WIN CATS software. The linear ascending
development of chromatogram was carried out in a
(20 cm X 10 cm) twin trough glass chamber saturated
with the mobile phase (Ethyl acetate:Formic acid:glacial
acetic acid:Water (99:12:11:27 v/v/v). The developed
plate was dried by hot air to evaporate solvents from the
plate. The plate was sprayed with anisaldehyde sulphuric
acid and 5 % Ferric chloride as spray reagent and dried
at 100 °C in hot air oven for 3 min. The plate was kept in
photo-documentation chamber (CAMAG REPROSTAR
3) and images were taken at 214 nm. captured the images
under UV light at 214 nm, respectively. The Retention
factor (Rf) values and finger print data were recorded
(WIN CATS software). Quercitin was used as reference
standard for comparison.

2.6 Experimental Animals

Wistar rats of either sex (150-200 gm; 10–12weeks
old) were procure from National Toxicology Centre,
Pune. Animals were housed (6/group) in polycarbonate
 cages with standard conditions: humidity (50 ± 5 %),
temperature (25 ± 2 ºC) and light (12 hr light: 12 hr  
dark cycle) in our departmental animal house and were  
fed with a standard diet (Amrut lab Animal feed Pune,  
India) and water ad libitum. Experimental protocols  
was reviewed and approved by the Institutional Animal  
Ethics Committee KLE University’s College of Pharmacy,  
Belagavi, Karnataka. (IAEC Reg. No.: 627/02/a/  
CPCSEA).

2.7 Acute Oral Toxicity Studies  
Acute toxicity studies of aqueous and alcoholic extract  
were carried as per OECD-423 guidelines9. The extracts  
at various doses were administered up to 2000 mg/kg,  
p.o. Animals were observed for toxic effect, behavioural  
changes and mortality, if any for a period of 72 hr. The  
doses of extracts were selected on the basis of findings  
of pilot experiments.

2.8 Dose Selection and Drug Administration  
The aqueous (TLAQ) and alcoholic (TLAL) extracts were  
dissolved/suspended in 1% CMC and administered by  
oral route. The various doses of TLAQ and TLAL were  
administered to PCM induced rats and serum marker  
enzymes were measured for all the groups. The 200, 400  
mg doses were found to be effective and hence used for  
further hepatoprotective activity evaluation.

3. Paracetamol-Induced Hepato-
toxicity in Rats  
Paracetamol (Acetaminophen; Torent Research  
Centre, Ahmedabad) was suspended in 1 % CMC  
and administered per oral (p.o); at a dose of 2 gm /kg.  
Animals, after acclimatization (6-7 days) in the animal  
quarters, were fasted overnight and randomly divided  
into seven groups (n = 6) and treated in the following  
way. Group I served as Normal (vehicle) control and fed  
orally with CMC (10 ml/kg b.w; p.o). Group II vehicle  
control administered with PCM in CMC. Animals of  
Group III were treated with, Silymarin (100 mg.kg b.w;  
p.o). Group IV and V rats were treated with (200 mg  
and 400 mg/kg b.w) of TLAQ and Group VI and VII  
rats were treated with (200 mg and 400 mg /kg b.w)  
of TLAL respectively. The drug treatment and vehicle  
were administered once a day for 7 day to the respective  
group. On the 7th day, paracetamol suspension was given  
by oral route, in a dose of (2 gm/kg) in 1% CMC to all  
rats except the rats in group I10.

3.1 Biochemical Studies  
On 8th day, under light ether anaesthesia blood was  
withdrawn directly from the heart and thereafter  
rats were sacrificed by euthanasia. The liver tissues  
were removed, washed with cold normal saline and  
preserved at -20 ºC. The serum was separated by  
centrifugation at 1200 rpm (Remi, USA) below 30 ºC  
for 15 min and used for the assay of liver marker viz;  
Serum alanine aminotransferase (ALT), Serum aspartate  
aminotransferase (AST)11, alkaline phosphate (ALP)12,  
Lactic Dehydrogenase (LDH)13, Total Bilirubin (TB)14  
and Total Proteins (TP)15 were determined by standard  
methods using enzyme assay kits.

3.2 Antioxidant Assays  
The liver homogenate (10% w/v) was prepared in Triss  
buffer at (pH-7.4) and then centrifuge (Remi, Pvt.  
Ltd.) at 6000 rpm for 15 min and the supernatants  
used for the measurement of antioxidants. The Lipid  
peroxidation was assayed in homogenate by determining  
the formation of MDA16, Catalase (CAT)17, Superoxide  
dismutase (SOD)18, and Reduced Glutathione (GSH)19.

3.3 Histopathological Studies  
The remaining liver tissue preserved in 10 % w/v  
formalin was embedded in paraffin wax and cut into  
3-4 µm thick section on microtome and sections were  
stained using haematoxylin and eosin, and observed  
under microscope for histoarchitecture alterations.

3.4 Statistical Analysis  
The Experimental data was analysed by One way ANOVA  
followed by student ‘t’ test and results were expressed as  
Mean ± Standard Error Mean (SEM). Differences were  
considered statistically significant when p<0.05.

4. Results

4.1 Preliminary Phytochemical Analysis  
Based on preliminary phytochemical analysis of  
TLAQ showed the presence of flavonoids, tannins and
polyphenolic compounds whereas, TLAL showed the presence of flavonoids, tannins, saponins and alkaloids.

4.2 HPTLC Analysis

Optimized HPTLC chromatogram of TLAQ and TLAL extracts showed presence of quercitin as major phytoconstituents at 214 nm which is comparable with standard quercitin and exhibited as blackish (visible) band in the \( R_f \) range of 0.47 to 0.52. Figure 1 showed a good linear relationship \( (r^2 = 0.98 \text{ and } 0.98 \text{ with respective to height and peak area}) \) at the concentration of 5µl/spot. Thus it is possible that standard quercitin and extract showed \( R_f \) value at same wavelength i.e., 214 nm. Hence our extracts might contain quercitin as important flavonoid.

![Image](image_url)

**Fig. 1.** (a) HPTLC peak showing presence of Quercitin at 214 nm in alcoholic extract of *Trichosanthes lobata*. (b) Peak of standard Quercitin at 214 nm. (c) Peak showing presence of Quercitin at 214 nm in aqueous extract of *Trichosanthes lobata*. T2 Quercitin, T1 and T3 replicate spots for TLAL and TLAQ on HPTLC plate 5 μl ml of sample was applied and compared with standard quercitin, at 214 nm.

4.3 Acute Oral Toxicity in Rats

The acute toxicity studies on aqueous and alcoholic extract of *Trichosanthes lobata* were not carried out by oral route at doses 2000 mg/kg, mortality nor were toxic clinical symptoms observed. Furthermore, the pilot experiment was performed for the effective dose for hepatoprotective activity evaluation, based on findings of pilot experiment and oral acute toxicity studies various doses viz. 200 and 400 mg/kg were selected for detailed hepatoprotective activity evaluation.

4.4 Hepatoprotective Activity Screening

Rats administered with paracetamol (2 gm/kg) induced significant \( (p<0.05) \) hepatic damage as evidence from significant elevation of AST, ALT, ALP, LDH and total bilirubin with marked decreased in total protein concentration compared to paracetamol treated group. Pre-treatment with TLAQ and TLAL (200 and 400 mg/kg) and Silymarin (100 mg/kg) prevented the elevation of serum marker enzymes AST, ALT, ALP, LDH and total bilirubin with marked restoration of total proteins in paracetamol treated rats. (Table 1 and 2).
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Table 1: Effect of an aqueous (TLAQ) and alcoholic (TLAL) extracts of *Trichosanthes lobata* on serum marker enzymes in paracetamol-induced liver toxicity in rats

<table>
<thead>
<tr>
<th>Treatment and dose (mg/kg, p.o)</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>LDH (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle treated 10 ml/kg</td>
<td>21.83 ± 2.48</td>
<td>16 ± 3.4</td>
<td>3.1 ± 0.3</td>
<td>221.5 ± 19.58</td>
</tr>
<tr>
<td>PCM 2 gm/kg</td>
<td>127.33 ± 6.59**</td>
<td>72.8 ± 5.1**</td>
<td>54.6 ± 5.5**</td>
<td>861.5 ± 89.5**</td>
</tr>
<tr>
<td>Silymarin 100 mg/kg</td>
<td>43.33 ± 4.13**</td>
<td>37.1 ± 6**</td>
<td>15.6 ± 2.3**</td>
<td>338 ± 26**</td>
</tr>
<tr>
<td>TLAQ 200 mg/kg</td>
<td>113.83 ± 6.5*</td>
<td>83.8 ± 4.9*</td>
<td>47.4 ± 3.2*</td>
<td>766 ± 54.82*</td>
</tr>
<tr>
<td>TLAQ 400 mg/kg</td>
<td>70.6 ± 6.1**</td>
<td>53.8 ± 6**</td>
<td>42.4 ± 3**</td>
<td>575 ± 62.4**</td>
</tr>
<tr>
<td>TLAL 200 mg/kg</td>
<td>113.3 ± 9.8*</td>
<td>64.3 ± 4.5*</td>
<td>47 ± 3.7*</td>
<td>757 ± 47.86*</td>
</tr>
<tr>
<td>TLAL 400 mg/kg</td>
<td>73.5 ± 6.8**</td>
<td>51.5 ± 4**</td>
<td>41 ± 3.5**</td>
<td>639 ± 54.7**</td>
</tr>
</tbody>
</table>

*p < 0.05 and **p<0.01 compared with PCM treated group. # p<0.05 compared with vehicle treated group. PCM: Paracetamol.

Table 2: Alterations in the values of Total Proteins (TL) and Total Bilirubin (TB) with treatment of *Trichosanthes lobata* (TL) leaves extract in paracetamol (PCM) induced liver toxicity in rats

<table>
<thead>
<tr>
<th>Treatment and dose (mg/kg, p.o)</th>
<th>Parameters (Mean ± SEM; n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Proteins (mg/dL)</td>
</tr>
<tr>
<td>Vehicle treated 10 ml/kg</td>
<td>13.3 ± 1</td>
</tr>
<tr>
<td>PCM 2 gm/kg</td>
<td>6.4 ± 0.9**</td>
</tr>
<tr>
<td>Silymarin 100 mg/kg</td>
<td>11.98 ± 1.1**</td>
</tr>
<tr>
<td>TLAQ 200 mg/kg</td>
<td>8.2 ± 0.77*</td>
</tr>
<tr>
<td>TLAQ 400 mg/kg</td>
<td>10.53 ± 0.87**</td>
</tr>
<tr>
<td>TLAL 200 mg/kg</td>
<td>7.96 ± 0.73*</td>
</tr>
<tr>
<td>TLAL 400 mg/kg</td>
<td>9.71 ± 0.88**</td>
</tr>
</tbody>
</table>

*p < 0.05 and **p<0.01 compared with PCM treated group. # p<0.05 compared with vehicle treated group. PCM: Paracetamol

The MDA formation (an index of lipid peroxidation) was increased significantly in paracetamol treated rats and subsequently prevented (p<0.01) the increased MDA formation significantly compared to vehicle control group (Table 3). Depletion of GSH, and the SOD and CAT were restored by pre-treatment by TLAQ and TLAL dose dependently. The effect of TLAQ (200 mg/kg) and TLAL (200 mg/kg) were comparable to that of silymarin 100 mg/kg (Table 3).

Furthermore, in histopathological studies paracetamol treated liver sections showed necrosis, lymphocytes infiltration, congestion and haemorrhage of hepatocytes. However, treatment with TLAQ and TLAL at the dose of (200 and 400 mg/kg) almost prevented the infiltration of lymphocytes and congestion as compared to PCM treated rats (Figure 2(a)-(g)).

Table 3: Effect of an aqueous and alcoholic extracts of *Trichosanthes lobata* on MDA formation, SOD, GSH, CAT in paracetamol induced hepatotoxicity in rats

<table>
<thead>
<tr>
<th>Treatment and dose (mg/kg, p.o)</th>
<th>SOD U/mg of Protein</th>
<th>GSH µg/g of Protein</th>
<th>CAT µmole of H₂O₂/mg of Protein</th>
<th>MDA nmole/gm of Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle treated 10 ml/kg</td>
<td>33.66 ± 3</td>
<td>2.76 ± 0.27</td>
<td>29 ± 2.3</td>
<td>4.2 ± 0.34</td>
</tr>
<tr>
<td>PCM 2 gm/kg</td>
<td>13.5 ± 1.64**</td>
<td>1.33 ± 0.9**</td>
<td>10.15 ± 1.48**</td>
<td>29 ± 3.6**</td>
</tr>
<tr>
<td>Silymarin 100 mg/kg</td>
<td>26.33 ± 2.58**</td>
<td>2.5 ± 0.2**</td>
<td>24.5 ± 2.2**</td>
<td>9.26 ± 1.1**</td>
</tr>
<tr>
<td>TLAQ 200 mg/kg</td>
<td>17.6 ± 1.3*</td>
<td>1.7 ± 0.24*</td>
<td>13.8 ± 1.9*</td>
<td>25.7 ± 2.7*</td>
</tr>
<tr>
<td>TLAQ 400 mg/kg</td>
<td>21.1 ± 3**</td>
<td>2.2 ± 0.2**</td>
<td>20 ± 2**</td>
<td>19.83 ± 1.7**</td>
</tr>
<tr>
<td>TLAL 200 mg/kg</td>
<td>17.3 ± 1.2*</td>
<td>1.7 ± 0.1*</td>
<td>13.6 ± 1*</td>
<td>25.6 ± 2.1*</td>
</tr>
<tr>
<td>TLAL 400 mg/kg</td>
<td>22.5 ± 2.1**</td>
<td>2.25 ± 0.22**</td>
<td>22.5 ± 2.1**</td>
<td>21.8 ± 1.7**</td>
</tr>
</tbody>
</table>

*p < 0.05 and **p<0.01 compared with PCM treated group. # p<0.05 compared with vehicle treated group. PCM: Paracetamol
**Fig. 2A.** Section of liver of normal control rats showed normal hepatic cells with nuclei and cytoplasm.

**Fig. 2B.** Section of paracetamol (PCM) overdose-treated rat liver showed marked necrosis, (yellow arrows), lymphocytes infiltration (black arrows), congestion and hemorrhages.

**Fig. 2C.** Silymarin (100 mg/kg) + paracetamol (2 g/kg mg/kg) treated group showed mild congestion, lymphocytic infiltration and regenerating architecture of hepatocytes with mild necrosis.

**Fig. 2D.** TLAQ (200 mg/kg) + PCM treated group showed vascular dilatation, mild infiltration of lymphocytes.

**Fig. 2E.** TLAQ (400 mg/kg) + PCM treated group, showed regeneration of hepatocytes with prominent nucleus and no signs of necrosis or inflammatory infiltrate and are close to normal.

**Fig. 2F.** TLAL (200 mg/kg) + PCM treated group showed vascular dilatation, mild infiltration of lymphocytes.
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5. Discussion

Paracetamol (Acetaminophen) is widely used clinically as antipyretic and analgesic agent, and found to be safe at therapeutic doses, however, it can induce hepatic damage in human beings, rats and mice at higher doses. Hepatotoxicity of paracetamol has been attributed to the formation of toxic and reactive metabolites N-Acetyl-P-Benzoquinone Imine (NAPQI). NAPQI is detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid. If the rate of NAPQI formation exceeds the rate of detoxification by GSH; it oxidizes tissue macromolecules mainly as lipid or thiol group protein and alters the homeostasis of calcium after depleting GSH. The hepatic damage is largely to reflect in terms of leaking of cellular enzymes into the bloodstream due to disturbances caused in the transport functions of hepatocytes. Therefore determination of enzymes in the serum is a useful biological marker of the extent and nature of hepatocellular damage. In the present experimental findings, the rats treated with paracetamol (2 gm/kg), showed a significant hepatic damage, reflected by elevation of serum marker enzymes (ALT, AST, ALP and LDH) and MDA formation in liver homogenates. Pre-treatment with TLAQ and TLAL extracts at (200 and 400 mg/kg, p.o) significantly prevented the elevation of serum marker enzymes and total proteins. The prevention of elevated serum marker enzymes by TLAQ and TLAL (200 and 400 mg/kg, p.o) might be due to membrane stabilization of hepatocytes which consequently prevents the cystolic released in circulation. These findings are in agreement with the documented fact that, serum transaminase levels return to normal with healing of hepatic parenchyma and regeneration of hepatocytes.

Most of the hepatotoxic chemicals including paracetamol induced damage liver probably by lipid peroxidation directly or indirectly. In higher animals, lipid peroxidation was known to cause destabilization and disintegration of the cell membrane, leading to liver injury, arteriosclerosis and kidney damage. Amongst peroxyl radicals are important agents that mediate lipid peroxidation thereby damaging cell membrane. Administration of TLAQ and TLAL extracts at (200 and 400 mg/kg) significantly attenuated the MDA formation; thereby suggesting that free radicals formation/oxidative reactive species are removed/neutralised by the plant constituents present in the extracts and thus prevented hepatic damage.

Glutathione (GSH) is one of the most abundant tripeptide non-enzymatic intracellular biological antioxidant present in liver. It is involved in removal of free radicals such as H₂O₂, superoxide anions and alkoxy radicals, preserving membrane protein thiols and a substrate for glutathione peroxidase and glutathione reductase. In the present experiments, paracetamol administration exhibited lower GSH content in liver, and subsequently pre-treatment with TLAQ and TLAL extracts at (200 and 400 mg/kg,) able to reverse such effects.

The Reactive Oxygen Species (ROS) are the free radicals that initiate the process of hepatic damage due to high oxidative stress so, formed ROS are scavenged/neutralized by endogenous antioxidant enzymes, thus, there activity get impaired. Since, endogenous defence system activated during such events that include free radical scavengers/Neuratilizers and chain reaction terminators, enzymes such as SOD and CAT. In this study, SOD plays an important role in the elimination of ROS derived from the peroxidative process of xenobiotics in liver tissues. The restoration of SOD due to pre-treatment with TLAQ and TLAL extracts at (200 and 400 mg/kg) have an efficient protective mechanism in response to ROS during hepatic damage.

CAT is a key component of antioxidant defense system. Inhibition of these protective mechanisms results in enhanced sensitivity to free radical-induced cellular...
damage. Excessive production of free radicals may result in alterations in the biological activity of hepatic cellular macromolecules. Pre-treatment with TLAQ and TLAL extracts at (200 and 400 mg/kg) restored CAT activity in paracetamol-induced hepatic damage and thus prevented accumulation of excessive free radicals. The ability of TLAQ and TLAL extracts to protect paracetamol-induced hepatic damage in rats might be attributed to its ability to restore endogenous antioxidants system. Thus, experimental findings suggested that TLAQ and TLAL extracts able to prevent hepatic damage due to paracetamol by their antioxidant property. Since, this model of hepatic damage in the rats simulate many of the features of human liver pathology, our findings suggest that natural antioxidants and scavenging agents in *Trichosanthes lobata* leaves extracts might be involved in hepatoprotection.

This was further supported with histopathological changes. Therefore, it seems that TLAQ and TLAL extracts, due to its antioxidant property, might capable of protecting the hepatic tissues from paracetamol-induced injury and inflammatory changes. The TLAQ and TLAL extracts found to rich in flavonoids. Presence of flavonoids in the extracts was confirmed and agreement with our preliminary phytochemical screening and HPTLC studies. Flavonoids are natural products, which have been shown to possess antioxidant property. As *Trichosanthes lobata* leaves extracts contains large amount of flavonoids it may be possible that the hepatoprotective activity may be due to the presence of flavonoids in the extracts.

6. Conclusion

Based on aforementioned finding, we proposed that hepatoprotective effect of *Trichosanthes lobata* leaves extracts might be due to antioxidant effect (elevation of endogenous antioxidant enzymes and total proteins) and membrane stabilization of hepatocytes (reduce AST, ALT, ALP, LDH and total bilirubin) by scavenging/neutralizing free radicals. The present study thus validate the traditional use of *Trichosanthes lobata* in the treatment of liver diseases and also points out that *Trichosanthes lobata* warrants future detailed investigation as promising hepatoprotective agent.

7. Acknowledgements

Authors are thankful to the Principal, K.L.E University’s College of Pharmacy, Belagavi for providing all necessary facilities to carry out this research work. The authors are also thankful to Mr. Chintan Shah, Research Scientist, Anchrom test lab Pvt. Ltd. Shree Aniket Apt. Navghar road, Mulund East, Mumbai-81 for providing HPTLC instrumentation facility to carry out this research work.

8. References

Amelioration of *Trichosanthes lobata* in Paracetamol-Induced Hepatic Damage in Rats: A Biochemical and Histopathological Evaluation


CERTIFICATE

This is to Certify that the research project
"PHYTOCHEMICAL INVESTIGATION AND HEPATOPROTECTIVE ACTIVITY OF SOME MEDICINAL PLANTS"
Submitted by Mr. Raju R. Wadekar has been approved in the Institutional Animal Ethics Committee meeting held on 19th December 2008, resolution No. JNMC/IAEC/Res-2/10/2008 and was permitted to use 87 Rats/Mice/Rabbits.

You are hereby informed to strictly adhere to the protocol submitted for approval. In case the project needs to be modified later, the modified version of the protocol should be submitted to the Committee, stating valid reasons for such modifications for fresh approval.

You are required to keep the account of animals used for the project in specified proforma, Form-D.

You have to submit the brief report to the Committee after completion of the project along with Form-D

Dr. A. Jagannadha Rao
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J.N. Medical College,
BELGAUM-10.

Dr. P.A. Patil
Member-Secretary,
J.N. Medical College,
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