Hepatoprotective Potential of **Tecomella undulata** Bark on Paracetamol and CCL₄ Induced Hepatotoxicity in Rats: Invitro Analysis

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**Authors’ contributions**

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

**Article Information**

DOI: 10.9734/JPRI/2021/v33i42A32409

Editor(s):
(1) Dr. Rafik Karaman, Al-Quds University, Palestine.
Reviewers:
(1) Shiara Martins de Souza, Universidade Federal de Ouro Preto, Brazil.
(2) Alexander Lykov, Research Institute of Tuberculosis, Russia.
Complete Peer review History: https://www.sdiarticle4.com/review-history/73542

Received 22 June 2021
Accepted 27 August 2021
Published 28 August 2021

**ABSTRACT**

In this study the T. undulata bark was tested for its hepatoprotection against paracetamol (PCM) induced hepatic damage and Carbon tetra chloride (CCl₄) induced hepatotoxicity. The Invitro study was performed on HepG2 cell line .The Levels of serum marker enzymes i.e. AST, ALT (aminotransferases), ALP (alkaline phosphatase), GSH (reduced glutathione) and MDA (Malondialdehyde) in 70% Ethanol treated rats were monitored, respectively. The 70% Ethanol extract gave promising results as studied in detail. The Present study showed that the 70 % ethanolic extract of bark of **T.undulata** apparently revive the physiological integrity of hepatocytes. Thus the present study demonstrated the Hepatoprotective property of Tecomella undulate Bark.

**Keywords:** Hepatoprotective; Tecomella undulate; HepG₂; AST; ALT; ALP; GSH; MDA.
1. INTRODUCTION

Plant products play a crucial role in the hepatoprotection through its antioxidants property. Therefore, search for modern medicine of plant origin with this property has become a central focus on hepatoprotection today [1]. The occurrence of liver diseases is most common in present era. The management of such diseases is most challenging in present medicinal system. The present researches have now focused towards the ethnobotanical sources. Tacomella undulata belonging to family bignoneaceae has shown Hepatoprotective activity [2-3]. It is an evergreen tree found in almost all parts of India. It is known by different names in the country desert teak in English, rugtrora in Hindi, rohira in Punjabi, lohira in Sindhi, rakhtroda in Marathi and rohita in Sanskrit. The tree has been mentioned in almost all Ayurvedic texts [4]. The Bark of the tree is included in n number of ayurvedic preparations like Rohitakarista, Rohitakaghrta, Rohitakadyachoorna, Rohitakaloha etc. Tacomella undulata has also shown its significance in the treatment of syphilis, painful swellings and cancer traditionally [5]. The plant is also known for its antibacterial, hepatoprotective, immunomodulatory, anti-inflammatory activities etc thus the aim of the present study is to determine the Hepatoprotective Activity potential of Tecomella undulate Bark.

2. MATERIALS AND METHODS

Plant Material: The plant Tecomella undulata was collected from Rajasthan district, Jaipur in the August Month and authenticated in Department of Botany Ch.Charan Singh University, Meerut and the voucher specimen was deposited for future reference.

Phytochemical Screening: Preliminary Phytochemical Screening of 70% ethanolic and ethyl acetate extract was carried out by using standard procedure [6] shows the presence of various Phytoconstituent like Carbohydrates, fixed oil, alkaloids, Saponins, flavanoids, tannins, phenol compounds in the extract which are shown in the table

2.1 Determination of Total Phenolic and Flavonoids Content

Reagents and Chemicals: Folin-Ciocalteu reagent, gallic acid, and quercetin, aluminum chloride hexahydrate, methanol, and sodium carbonate.

1. Total Phenolic contents determination assay: The total polyphenol content (μg/mg extract) was analyzed using the Folin-Ciocalteu reagent method [7].

2. Total Flavonoid contents determination assay: The total flavonoid content (μg/mg extract) was analyzed using the quercetin reagent method [8].

3. DPPH radical scavenging activity of Tecomella Undulata

The radical scavenging activity was done by already predetermined methods via, DPPH radical scavenging assay. The results were expressed as % radical scavenging activity.DPPH assay of 70% ethanol stem bark extract was estimated by using ascorbic acid solution as standard. The absorbance data were recorded against the selected concentration (10 –100 μg/ml). The % inhibition curves for ascorbic acid and that for 70% ethanol stem bark extract was plotted, from which, IC50 value (concentration of extracts that inhibits the formation of DPPH radicals by 50 %.) of DPPH by ascorbic acid and 70% ethanol stem bark extract was calculated using calculated by regression equation [9].

2.2 Pharmacological Activity

Chemicals: Paracetamol, Carbon tetra chloride and Country made liquor

Extract Preparation: The Bark were kept for air shaded dry 1.5 kg of bark powder was macerated to remove the impurities like fatty substances and further extracted with 70% ethanol for 5 days by cold maceration method, filter the extract Centrifuge at 10000 rpm/min, concentrate on Buchi rotary evaporator and further dried in lophilizer freeze drier under reduce pressure, this yielded 98.00 gm of solid residue (6.5% w/w).

Experimental Animals: All experiment were performed on healthy adult male wistar albino rats weighing 200-250 gram. All the animal were procured from the animal house, I.T.S College of Pharmacy, Ghaziabad, India (1044/PO/Re/S/0 7/CPCSEA,27th Frb.2007).

Five Group of rats, six animal in each group has been used to study the effect of 70 % ethanolic
extract of T. undulata in three model for the treatment hepatotoxicity (Table 1).

2.3 Hepatoprotective Assay

2.3.1 Paracetamol induced hepatotoxicity

Paracetamol induced hepatotoxicity model was adopt for the study [10]. The rats were divided into 5 groups of 6 animals each. Group I served as a control and received normal saline, 5 mL/kg body weight, daily for 7 days. Group II constituted the hepatotoxic group and were treated with 2 gm/kg paracetamol. Group III received the standard drug Silymarin (100 mg/kg) daily, Group IV and Group V received 70 ethanolic extract (100 and 400 mg/kg body weight per day, respectively) suspended in 0.5% sodium carboxymethylcellulose for 14 days. On the 7th day, paracetamol suspension was given orally, 2 g/kg body weight, to all the rats except those in Group I. At the end of the experimental period, the rats were fasted overnight and sacrificed by ether. Blood and liver samples were collected for biochemical analysis [11].

2.3.2 CCl4 induced hepatotoxicity

Carbon tetra chloride (CCl4) induced hepatotoxicity model was adopt for the study [12]. The rats were divided into 5 groups of 6 animals each. Group I served as a control and received normal Saline 10 ml/kg, i.p once in a day for 7 days. Group II constituted the hepatotoxic group and were treated with 0.5 ml/kg, i.p. Group III received the standard drug Silymarin (100 mg/kg) daily, Group IV and Group V received 70 ethanolic extract (100 and 400 mg/kg body weight per day, respectively) suspended in 0.5% sodium carboxymethylcellulose for 14 days. On the 7th day, CCl4 suspension was given orally, 0.5 ml/kg body weight, to all the rats except those in Group I. At the end of the experimental period, the rats were fasted overnight and sacrificed by ether. Blood and liver samples were collected for biochemical and histological studies.

2.3.3 Body weight

Body wt. of individual animal was taken for each group and record was maintained. Body wt. was taken daily from the starting day of the study till the last dosing was done also before sacrificing the animal. If death of any animal occurs in between the study time, its weight was also to be taken. Any change in the body wt. of the animal was record.

2.3.4 Measurement of ALT, AST, ALP

Serum ALT, AST and ALP was assess as per standard kit methods using UV spectrophotometer and the standard kit methods was obtain in detail from the leaflets provide in the commercially kits [13].

2.3.5 Estimation of glutathione level

GSH a key antioxidant biomarker is a superoxide radical scavenger where it protects thiol group required for maintaining the cell integrity against oxidation. Glutathione was estimated [14].

2.3.6 Estimation of MDA level

MDA forms a 1:2 adduct with thiobarbituric acid which can be measured by fluorometry or spectrophotometry [14].

2.4 Acute Toxicity Study

The acute toxicity was performed according to OECD guidelines (OECD 423, 2001). The selected Male Wistar rats were used for toxicity studies. The animals were divided into three groups of three in each. The animals were fasted overnight prior to the experimental procedure. The acute toxicity study was performed for deciding safe doses for further pharmacological studies along with this any behavioral or physiological changes due to extract administration was also observed. Extracts were given orally to rats at the graded dose of 1000, 2000, 4000 mg/kg body wt. Immediately, after dosing, the animals were observed continuously for first four hours for behavioral changes and for mortality at the end of 24 h and daily up to 14 days for any behavioral change or mortality. Since No mortality was reported even after 14 days. This indicated that the extracts are safe up to a single dose of 4000 mg/kg body weight. Hence the selected doses for the administration in experimental animals were considered 1/10th and 1/5th of maximum safe dose [15].

3. RESULTS

3.1 Total Phenolic content assay of Tecomella undulate

The absorbance of gallic acid at different concentrations (10-100 μg/ml) was determined (Fig. 1; Tabs 3,4). Standard curve of gallic acid is shown in figure. The total Phenolic content of
Tecomella undulata bark ethyl acetate extract was found to contain 106.89±0.294 μg/mg of Galic acid. The total Phenolic content of Tecomella undulata bark 70% ethanolic extract was found to contain 172.77 μg/mg of Galic acid.

3.2 Total Flavonoid Content Assay of Tecomella undulate

Standard curve of Quercetin is shown in figure. The absorbance of quercetin at different concentrations (10-100 μg/ml) was determined. The total Flavonoid content of Tecomella undulata bark 70% ethanol extract was found to contain 110.33 ± 0.964 μg/mg of Quercetin. The total Flavonoid content of Tecomella undulata bark ethyl acetate extract was found to contain 36.66 ± 0.19μg/mg of Quercetin(Tabs 5-7: Fig. 2).

3.3 DPPH Radicals Scavenging Activity of Tecomella undulate

The DPPH radical scavenging activity of Tecomella undulata for 70% Ethanolic Extract and Ethyl Acetate extract was determined by using ascorbic acid solution as standard. The absorbance data recorded against the selected concentration (10 –100 μg/ml). The IC 50 (μg/ml) for 70% ethanolic extract of Tecomella undulata was found to be 56.31% and 86.64% Ethyl Acetate extract of Tecomella undulata in comparison to the 37.09 % for the standard Ascorbic acid respectively (Fig. 3 Tab 8). The study revealed the antioxidant property of Tecomella undulata bark. The 70% ethanol extract of Tecomella undulata shows the higher amount of Phenols and flavonoids content. These phytochemicals are known to possess good antioxidant property which could further help in protection against hepatotoxicity. This provides supportive evidence for the rationale behind selecting the following extract for further animal activities.

3.4 Paracetamol Induced Hepatotoxicity

Body weight: The body weight of the animal was decreased in toxic control. The treatment of animal with the extract showed increase in the body weight. There was no significant decrease in the body weight in comparison to the normal control. On administration of Silymarin the body weight was found to be near normal. In group 4 and 5 the effect was found to be in dose dependent manner (Fig. 4). At higher dose of extract the promising effect was seen. The ethanolic extract showed significant activity.

3.5 Effect on Biochemical Markers

Under the influence of Paracetamol there in the level of biochemical markers i.e. ALT, AST and ALP. The administration of extract to the animals showed a dose depend change in the level of ALT, AST and ALP (Figs 5-7). At higher dose i.e. 400 mg/kg the results were near to the normal. The level of GSH and SOD (Figs 7-10 ) were decreased in toxic control whereas on administration of extract the levels were revived near to the normal. The level of MSH was increased in toxic control which was significantly altered under the influence of extract.

### Table 1. Experimental animals

<table>
<thead>
<tr>
<th>S.N</th>
<th>Groups</th>
<th>Paracetamol Model</th>
<th>CCl4 Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group 1(GP1) (Control) Normal Saline</td>
<td>Normal Saline 5 ml/kg po</td>
<td>Normal Saline 10 ml/kg , i.p.</td>
</tr>
<tr>
<td>2</td>
<td>Group 2 (GP2) (Negative Control)</td>
<td>2gm/kg (07 Days) po</td>
<td>0.5 ml/kg, i.p. (07 Days)</td>
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<tr>
<td>3</td>
<td>Group 3 (GP 3) (Standard) Silymarin</td>
<td>100mg/kg (14 Days) po</td>
<td>100mg/kg (14 Days) po</td>
</tr>
<tr>
<td>4</td>
<td>Group 4 (GP 4) (Extract)</td>
<td>100 mg/kg (14Days) po</td>
<td>100 mg/kg (14Days) po</td>
</tr>
<tr>
<td>5</td>
<td>Group 5 (GP 5) (Extract)</td>
<td>400 mg/kg (14 Days) po</td>
<td>400 mg/kg (14Days) po</td>
</tr>
</tbody>
</table>

### Table 2. Acute toxicity study

<table>
<thead>
<tr>
<th>Groups</th>
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<th>Treatment</th>
<th>Route</th>
<th>Dosage</th>
<th>Duration</th>
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</thead>
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<td>A1</td>
<td>3</td>
<td>70% Ethanol extract of Tecomella undulata</td>
<td>Oral</td>
<td>1000 mg/kg bodyweight</td>
<td>14 Day</td>
</tr>
<tr>
<td>A2</td>
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<td>Oral</td>
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<td>14 Day</td>
</tr>
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<td>A3</td>
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<td>Tecomella undulata</td>
<td>Oral</td>
<td>4000 mg/kg bodyweight</td>
<td>14 Day</td>
</tr>
</tbody>
</table>
Fig. 1. Absorbance of Galic Acid

Fig. 2. Absorbance of Quercetin

Fig. 3. Antioxidant activity of *T. undulata* extract
Table 3. Phenolic content of *Tecomella undulata* For 70% Ethanol

<table>
<thead>
<tr>
<th>Sample Solution µg/ml</th>
<th>Wt of dry extract gram/ml</th>
<th>Absorbance</th>
<th>Gallic acid Concentration µg/ml</th>
<th>Gallic acid Concentration mg/ml</th>
<th>Total phenol content as gallic acid mg/gm</th>
<th>Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>0.001</td>
<td>0.525</td>
<td>172.66</td>
<td>0.17266</td>
<td>172.660</td>
<td>172.77±0.113</td>
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<tr>
<td>1000</td>
<td>0.001</td>
<td>0.525</td>
<td>172.66</td>
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<td>173</td>
<td>Mean 0.196</td>
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Table 4. Phenolic content of *Tecomella undulata* for Ethyl acetate

<table>
<thead>
<tr>
<th>Sample Solution µg/ml</th>
<th>Wt of dry extract gram/ml</th>
<th>Absorbance</th>
<th>Gallic acid Concentration µg/ml</th>
<th>Gallic acid Concentration mg/ml</th>
<th>Total phenol content as gallic acid mg/gm</th>
<th>Mean±SEM</th>
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<td>0.001</td>
<td>0.328</td>
<td>107</td>
<td>0.107</td>
<td>107.00</td>
<td>106.89±0.294</td>
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<tr>
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<td>0.001</td>
<td>0.328</td>
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<td>Mean 106.33</td>
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<td>106.33</td>
<td>Mean 0.510</td>
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Table 5. Flavonoids content of *Tecomella undulata* for 70% Ethanol

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<tr>
<th>Sample Solution µg/ml</th>
<th>Wt of dry extract gram/ml</th>
<th>Absorbance</th>
<th>Quercetin Concentration µg/ml</th>
<th>Quercetin Concentration mg/ml</th>
<th>Total phenol content as Quercetin mg/gm</th>
<th>Mean ± SEM</th>
</tr>
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<td>37</td>
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<td>36.66±0.19</td>
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<tr>
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<td>36.33</td>
<td>0.03633</td>
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<tr>
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Table 6. Flavonoids content of *Tecomella undulata* for 70% Ethanol

<table>
<thead>
<tr>
<th>Sample Solution µg/ml</th>
<th>Wt of dry extract gram/ml</th>
<th>Absorbance</th>
<th>Quercetin Concentration mg/ml</th>
<th>Total phenol content as Quercetin mg/gm</th>
<th>Mean ± SEM</th>
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<tr>
<td>1000</td>
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<td>0.251</td>
<td>0.037</td>
<td>37.00</td>
<td>36.66±0.19</td>
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<td>0.03633</td>
<td>36.33</td>
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<td>0.03666</td>
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<td>0.193</td>
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</table>
Fig. 4. Effect on body weight due to Paracetamol induced Hepatotoxicity
Values are expressed as mean± SEM. (n=6). Statistical analysis was carried out by one-way ANOVA ** (p< 0.01) Vs Toxic group

Fig. 5. Effect of ALT due to Paracetamol induced Hepatotoxicity
Values are expressed as mean± SEM. (n=6). Statistical analysis was carried out by one-way ANOVA ** (p< 0.01) Vs Toxic group
Fig. 6. Effect of AST due to Paracetamol induced Hepatotoxicity
Values are expressed as mean± SEM. (n=6). Statistical analysis was carried out by one-way ANOVA ** (p< 0.01)
Vs Toxic group

Table 7. Total Phenol and Flavonoids content in both extract

<table>
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<tr>
<th>S.No</th>
<th>Plant extract</th>
<th>70% Ethanol</th>
<th>Ethylacetate</th>
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<tr>
<td></td>
<td>Total Phenol</td>
<td>172.77±0.113</td>
<td>106.89±0.294</td>
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<tr>
<td></td>
<td>Total Flavonoid</td>
<td>110.33±0.964</td>
<td>36.66±0.19</td>
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</table>
Table 8. DPPH radicals scavenging activity of *Tecomella undulate*

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>% Inhibition of DPPH Radical</th>
<th>Ascorbic acid</th>
<th>70% Ethanolic Extract</th>
<th>Ethyl Acetate</th>
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<tr>
<td>10</td>
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<tr>
<td>IC 50 (µg/ml)</td>
<td>37.09</td>
<td>56.31</td>
<td>86.64</td>
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</table>

Fig. 8. Effect of ALP due to Paracetamol induced Hepatotoxicity
Values are expressed as mean± SEM. (n=6). Statistical analysis was carried out by one-way ANOVA ** (p< 0.01) Vs Toxic group

Estimation of GSH level:

Fig. 9. Effect of GSH due to Paracetamol induced Hepatotoxicity
Values are expressed as mean± SEM. (n=6). Statistical analysis was carried out by one-way ANOVA ** (p< 0.01) Vs Toxic group
3.6 Carbon Tetrachloride Induced Hepatotoxicity

**Body weight:** The body weight of the animal was decreased in toxic control. The treatment of animal with the extract showed increase in the body weight. No change in the body weight normal control was seen. On administration of Silymarin the body weight was found to be near normal. On administration of extract the body weight was found near to the normal. At higher dose of extract the promising effect was seen (Fig. 11).

![Graph showing SOD levels](image1)

**Fig. 10. Effect of SOD due to Paracetamol induced Hepatotoxicity**
*Values are expressed as mean± SEM. (n=6). Statistical analysis was carried out by one-way ANOVA ** (p< 0.01) Vs Toxic group*

**Body weight:**

![Graph showing weight changes](image2)

**Fig. 11. Effect on body weight due to Paracetamol induced Hepatotoxicity**
*Values are expressed as mean± SEM. (n=6). Statistical analysis was carried out by one-way ANOVA ** (p< 0.01) Vs Toxic group*
3.7 Effect on Biochemical Markers

Under the influence of CCL₄, the level of biochemical markers i.e. ALT, AST and ALP was increased. The administration of extract to the animals showed a dose depend change in the level of ALT, AST and ALP (Figs 12-14). At higher dose i.e. 400 mg/kg the results were near to the normal. The level of GSH and SOD were decreased in toxic control whereas on administration of extract the levels were revived near to the normal (Fig 15). The level of MSH was increased in toxic control which was significantly altered under the influence of extract (Fig 16).

![Graph showing effect on biochemical markers](image1)

**Fig. 12. Effect on body weight due to Paracetamol induced Hepatotoxicity**
Values are expressed as mean± SEM. (n=6). Statistical analysis was carried out by one-way ANOVA ** (p< 0.01) Vs Toxic group

Estimation of AST level:

![Graph showing effect on AST level](image2)

**Fig. 13. Effect on body weight due to Paracetamol induced Hepatotoxicity**
Values are expressed as mean± SEM. (n=6). Statistical analysis was carried out by one-way ANOVA ** (p< 0.01) Vs Toxic group
Estimation of ALP level:

**Fig. 14. Effect on body weight due to Paracetamol induced Hepatotoxicity**
Values are expressed as mean± SEM. (n=6). Statistical analysis was carried out by one-way ANOVA **(p< 0.01)** Vs Toxic group

**Fig. 15. Effect of MDA due to CCL₄ induced Hepatotoxicity**
Values are expressed as mean± SEM. (n=6). Statistical analysis was carried out by one-way ANOVA **(p< 0.01)** Vs Toxic group
Fig. 16. Effect on SOD due to CCL\textsubscript{4} induced Hepatotoxicity

Values are expressed as mean± SEM. (n=6). Statistical analysis was carried out by one-way ANOVA ** (p< 0.01) Vs Toxic group

4. DISCUSSION AND CONCLUSION

In the present study, 70% ethanolic and ethyl acetate extracts of \textit{Tecomella undulata} bark were evaluated for its hepatoprotective activity using Paracetamol and CCL\textsubscript{4} induced . The hepatoprotective effect of fractions of ethanolic extract of \textit{Tecomella undulata} bark was compared with Silymarin. The damage to the Liver was determined by biochemical markers (AST, ALT, ALP, SOD,GSH and MDA level). Further the body weight was also determined. Paracetamol is the most commonly used toxic control for the study of hepatoprotective effects of the medicinal plants extracts and drugs [16].

Paracetamol is known for its widely used NSAIDs and its long term use causes hepatic injury in man and experimental animals by depletion of glutathione and binding of toxic metabolite to vital proteins and enzymes. The enzyme Cytocromes P450E1 (CYP2E1) and 3A4 (CYP3A4) causes the conversion of paracetamol toN-acetyl-p-benzoquinone imine (NAPQI) a highly reactive intermediary metabolite [17]. In normal course this metabolite , NAPQI is detoxified in conjugation with glutathione. Due to paracetamol toxicity or CCL\textsubscript{4}, the sulfate and glucuronide pathways become saturated, and more paracetamol is shunted to the cytochrome P450 system to produce NAPQI [18]. This hinders the hepatocellular supplies of glutathione and NAPQI is free for the reaction with cellular membrane molecules. This results in hepatocytes damage and death, i.e. acute hepatic necrosis [19-20]. In this regard, the reduced level of AST and ALT towards the normal under the influence of extract indicates the plasma membrane of stabilization. Further this shows the rejuvenated hepatic tissue damage caused by paracetamol. The results of biochemical parameters showed the hepatoprotective activity of ethanolic extracts of bark in dose dependent manner. The photochemical screening of the extracts has shown the presence of flavonoids which has further shown its antioxidant activities. Thus, it can be apparently said that that possible mechanism of hepatoprotective activity of \textit{Tecomella undulata} bark may be due to its free radical-scavenging and antioxidant activity. Thus the present shows significant hepatoprotective action of \textit{Tecomella undulata} (Sm.) bark extract against experimentally induced liver damage in the rats, this also supports its traditional folk medicine use.

ETHICAL APPROVAL

All animal procedure was approved by the ethical committee of I.T.S College of Pharmacy, Muradnagar, Ghaziabad.
CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/73542