Hepatoprotective Activity Of Terminalia Chebula Retz. Against paracetamol Induced Hepatotoxicity

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ABSTRACT

Terminalia chebula Retz. (TC) is used as herbal plants in many countries such as India, West Africa, China, Egypt, Nigeria; for ulcer, gastric acidity, wound healing. The aim of present study is to carried out and evaluates the hepatoprotective effect of aqueous leaves extract of TC against paracetamol (PCM) induced hepatotoxicity in rats. The phytochemical screening was carried out using standard laboratory methods. Hence the leaves of TC was successively extracted with distilled water against PCM (2mg/kg) induced hepatotoxicity using standard drug silymarin (10mg/kg). There was a significant change in the biochemical parameters as increase in (Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphate (ALP), Total bilirubin) and decrease in total proteins. In silymarin treated rats, which was restored towards normalization in TC (200mg/kg) treated rats when compared with control and normal base treated groups. Thus the detected phytochemical constituents includes alkaloids, flavanoids, tannins, glycosides, phenol, protein and tri-terpenoids confirms that the leaf extract of T. chebula possesses significant hepatoprotective activity.

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INTRODUCTION:
Liver is the largest organ of human body it plays a major role in detoxification and excretion of endogenous and exogeneous components of body and also helps in repairing any injury or improper functioning which leads to complications to health. (Handa & Kapoor et al., 2002). Liver disease is still a worldwide health problem in human beings. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate. (Pang et al., 1992). Unfortunately, conventional or synthetic drugs used in the treatment of liver disease are inadequate and sometimes can have serious effects.(C. Hari Kumar et al.,2014) In the absence of modern drug therapy of liver protecting drug there are no of herbal medicinal preparation in Ayurveda used for the treatment of hepatotoxicity.(Chatterjee TK et al.,2000).

The herbal medicines can be identified and isolated as potential for treatment of disease. Herbal medicines are prepared from variety of biological active ingredients from various parts of plant as leaves, roots, stems, bark etc (Meshram et al, 2011). Terminalia chebula Retz. (TC) is a flowering evergreen tree belonging to family Combretaceae. It has several synonyms as black myrobalan, haritaki, harad etc. It is also known as “King of Medicine”. (Aneja KR et al., 2009).

TC contains several phytoconstituents as tannins, flavanoids, resin, sterols, fixed oil, amino acid, glucose and sorbitol (Gupta et al., 2012). The tannins of myrobalan are of pyrogallol type (hydrolysable tannins), which on further hydrolysis gives chebulic acid and d-galloyl glucose. Chebulinic, chebulagic, ellagic and gallic acids all are the contents of myrobalan.(Kokate CK.,2008).Triterpenoids & glycosides are isolated from stem bark of TC (Kundu AP et al.,1993). Presently it has been studied that TC contains more phenolics than any other plant (Saleem A et al., 2002).

TC is used for various therapeutic purposes as astringent, laxative, cardiotonic, stomachins etc. It is also an anthelminic and fruit pulp is used in bleeding (Ashwini et al., 2011). It is an active ingredient of ayurvedic preparation “Triphala”. TC is also used in treatment of piles and external ulcers (Kokate CK., 2008). TC is also reported to be used as antimicrobial (Manoj kumar et al., 2009), antibacterial (kannan P. et al., 2009), anti-hyperglycemic (Murali et al., 2004), immunomodulators (Vaibhav et al 2011), hepatoprotective (Vuyyala Balakrishna et al.,2019), wound healing (Chaudhary G.P et al.,2011).

MATERIALS AND METHODS

Drugs and chemicals
Paracetamol was used to cause hepatotoxicity in the animals and silymarin was used as standard drug for comparison with test drug. Aqueous extract of leaves of TC was used as test drug.

Sources of plant material and preparation of plant extracts
The leaves of TC were collected and the specimen was certified by Dr. G.P. Sinha scientist, Botanical Survey of India Central Regional Center, 10 Chatham Lines, Prayagraj, 212002. Specimen with Voucher number SIP/2018/556 has been deposited at the Botanical Survey of India.

The leaves of TC were shade dried and then powdered by mechanical grinder. The coarse powder was passed through a 40-mesh sieve. The successive solvent cold extraction method used to obtain various extracts including petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extract. The solvent were removed from the extracts under reduced pressure by using a rotary vacuum evaporator. The % of yield of extract was noted. The brownish extract was obtained and is dissolved in their respective solvents for pharmacological studies.

Qualitative phytochemical screening
The preliminary qualitative phytochemical screening of TC was conducted for the presence or absence of alkaloids, glycosides, flavonoids, tannins, anthraquinones, saponins, volatile oils, cyanogenic glycosides, triterpenes using standard laboratory procedure.

Determination of total phenolic content
The total phenolic content of TC was determined by the Folin-Ciocalteu reagent assay. (Akthar Nayeem et al., 2013). Concentration of phenolic content in the extract was expressed as gallic
acid and was measured according to the methods described by Singleton and Rossi. (Singleton et al., 1965)

ANIMAL STUDIES

Experimental animals

Male Albino wistar rats weighing 150-200gm were procured from Indian Veterinary Research Institute, Izatnagar, Bareilly, India. The animals were placed in the propylene cages with paddy husk bedding at the temperature of 24±3 °C and relative humidity 30-70%, maintained under standard condition 12 h light; and 12 h dark cycle. The experimental study protocol was authenticated by Institutional Animal Ethical Committee (IAEC) with Registration no. SIP-IAEC/005/09/18.

Acute oral Toxicity

Acute oral toxicity testing was carried out as per the guidelines of OECD (423), revised from CPCSEA, Ministry of social justice and empowerment, Government of India (OECD, 2005). The animals were randomly selected and marked for individual identification and keep them in cage for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The test substance is administered in single dose by using oral gavage or incubation canula (Litchfield et al., 1949)

Animal Grouping and Treatments (Krishna KL et al., 2010)

The rats were divided into five (5) groups of six (6) rats each (n=6) for evaluation of hepatoprotective activity aq. leaves extract of T.chebula as given below.

Group 1: Served as an untreated control for 4 days.
Group 2: Hepatotoxic control rats have been given 1% Tween 80 for 4 days followed by Paracetamol (2mg/kg) on 3rd day.
Group 3: Served as standard control rats have been given Silymarin (100mg/kg) for 4 days followed by PCM on 3rd day.
Group 4: Test rats have been given aqueous extract of leaves of T.chebula (200mg/kg) followed by PCM on 3rd day.
Group 5: Test rats have been given aqueous extract of leaves of T.chebula (300mg/kg) followed by PCM on 3rd day.

Estimation of liver marker enzymes

At the end of 5th day animals were sacrificed by cervical dislocation with chloroform and blood sample were obtained by heart puncture and allowed to clot for 45min at room temperature. The serum was separated by centrifugation for the estimation of various biochemical parameters like SGOT, SGPT, ALP, Total bilirubin, Total protein.

Histopathological examination

Liver was isolated from rats of all groups and kept in 10% formalin solution for histopathological study. The liver tissues was placed in plastic container and immersed in neutral buffered formaline for 24hrs. The fixed tissues was embedded in paraffin, cut it into 4µm thick and stained with hematoxylin and eosin. The extent of PCM induced hepatic damage was evaluated by assessing the morphological changes in the liver section. (Akther Nayeem et al., 2013).

STATISTICAL ANALYSIS

Data were represented as mean ±SEM. Result were analysed by one-way ANOVA followed by Dunnet’s t-test using Graph pad instat 3.1 software. P<0.01 values was as considered to be statistically significant.

RESULTS

Phytochemical screening

The preliminary qualitative screening of TC revealed the presence of protein, alkaloids, flavanoids, tannins, glycosides, phenols and terpenes (Table 1) (Khandelwal KR., 2000). The total phenolic content in the aqueous extract of TC was found as 92.6 µg/mg of extract.

Effect of aqueous extract of T.chebula on marker enzymes in serum

In vivo hepatoprotective affect of aqueous extract of TC (200 and 300 mg/kg) was studied against PCM (2mg/kg) body weight causes hepatic toxicity in
wistar rats. The biochemical parameters (SGOT, SGPT, ALP, bilirubin and total protein) of all groups animals are given in Table 2. The oral administration of PCM caused chronic liver damage as showed by significant increase in serum marker enzymes SGOT, SGPT, ALP (p<0.001) compared to control group. The rats treated with TC (200 and 300 mg/kg) with PCM showed significant healing against PCM induced toxicity by restoring the levels of SGOT, SGPT, ALP. Significant increase in total bilirubin was observed after PCM administration. The effect of aq. extract of TC on total bilirubin was seen decrease in levels. The standard group (silymarin) also showed significant protection against PCM caused toxicity.

Effect of aqueous extract of T.chebula on total protein

The significant increase in protein levels in toxic group treated with PCM only. On treatment with aqueous extract of T.chebula at both doses (200 and 300 mg/kg) causes decrease in elevation of protein concentration in liver tissues. Silymarin treated group also showed a significant decrease in of protein level as compared to only PCM treated toxic group.

Table 1: Preliminary phytochemical study of leaves extract

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Name of Test</th>
<th>Constituents</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Fehling solution</td>
<td>Reducing Sugar</td>
<td>ND</td>
</tr>
<tr>
<td>2.</td>
<td>Xantho-protein</td>
<td>Proteins</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>5% FeCl₃ solution</td>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Mayer’s test</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Salwoski test</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Lead Acetate test</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Killer Killani test</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Liber Burchard test</td>
<td>Tri Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Ferric chloride test</td>
<td>Tannins</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Detected
ND = Not detected

Table 2: Activity on serum marker enzymes in paracetamol induced hepatotoxicity

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Treatment</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
<th>Total Bilirubin</th>
<th>Total Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal</td>
<td>42.66±1.14</td>
<td>42.5±2.45</td>
<td>118.5±6.5</td>
<td>42.66±1.14</td>
<td>5.66±0.6</td>
</tr>
<tr>
<td>2.</td>
<td>PCM 2g/kg</td>
<td>58.5±3.08</td>
<td>57.1±2.3</td>
<td>182.3±4.9</td>
<td>1.63±0.13</td>
<td>14.8±1.3</td>
</tr>
<tr>
<td>3.</td>
<td>Silymarin 100mg/kg</td>
<td>+</td>
<td>43.3±0.8</td>
<td>48±0.93</td>
<td>122.5±6.48</td>
<td>0.46±0.06</td>
</tr>
<tr>
<td>4.</td>
<td>Aqueous extract of TC 200mg/kg+ PCM</td>
<td>48.66±1.33</td>
<td>52.5±1.05</td>
<td>131.16±9.7</td>
<td>0.51±0.05</td>
<td>7.1±0.87</td>
</tr>
<tr>
<td>5.</td>
<td>Aqueous extract of TC 300mg/kg+PCM</td>
<td>52.66±1.69</td>
<td>52.16±2.1</td>
<td>140.1±7.1</td>
<td>0.66±0.06</td>
<td>7.78±0.5</td>
</tr>
</tbody>
</table>

Value are expressed as mean ± SEM for (n=6) rats in each group. Data are compared with toxic group. One way analysis of variance (ANOVA). p<0.01
**Histopathological observation**

The hepatoprotective effect of leaves aqueous extract of TC was confirmed after histopathological analysis of liver tissue of both control and toxic group treated rats. Histopathological studies provide supportive evidence for the biochemical analysis. The histological architecture of liver section of healthy rats showed normal cellular architecture with distinct hepatic cells, portal track, central vein and sinusoids (Fig 1). In the liver section of rats intoxicated with PCM (Fig 2) shows loss of normal liver architecture there was disarrangement and several necrosis of normal hepatic cells, fibrosis, sinusoidal space and inflammatory cells. Rats treated with silymarin and intoxicated with PCM are normal in cell architecture and arrangement no fibrosis, central vein are normal (Fig 3). The rats treated with low dose of aqueous extract of TC (200mg/kg) shows hepatocytes regeneration, mild fibrosis, sparse inflammatory component and central vein are normal. (Fig 4) Where as drug extract (300mg/kg) shows mild regeneration of hepatocytes and minimal fibrosis (Fig 5).
DISCUSSION

Hepatotoxicity, it is an injury to liver function associated with the impaired free radicals derived from oxygen or lipid peroxidation induced by drugs & chemicals exposure (Olusola Bolaji Adewale et al., 2005).

PCM is a very common antipyretic-analgescic drug potentially safe in therapeutic doses. Many investigational studies shows that the induction of hepatic cell necrosis on high dose of PCM in animals (Ramadan.A et al., 1984). High dose of PCM causes toxicity to liver it is extensively metabolized into N-acetyl-p-benzoquinone imine (NAPIQI) is formed by cytochrome P450 enzymes in the liver which depletes glutathione (GSH) and leads to hepatotoxicity (Biswas.K et al., 2010) (Setty SR et al., 2007). PCM directly inhibit the cellular proliferation, induces oxidative stress, lipid peroxidation, depletion of ATP levels also alter the Ca++ homeostasis (K.C. Gini et al., 2010) (H. Rabiul et al., 2011).

In the present study was undertaken to establish the hepatoprotective effect aqueous extract of TC against PCM induced liver injury. Hepatotoxicity due to overdose of PCM develops significant liver damage, which was observed by elevation in the concentration of serum marker enzymes (SGOT, SGPT, ALP) and total bilirubin with significant reduction in total protein.

Transaminase (SGOT, SGPT) catalyse the conversion of alanine to pyruvate and glutamate and is release in similar manner. Transaminase is more specific to liver and hence better parameter for detecting liver injury. On the other hand serum level of ALP is due to elevation in synthesis in presence of increased biliary pressure (Kozer E et al, 2003). On administration of PCM significantly increase the level of SGOT, SGPT, ALP, Total bilirubin and decrease the level of protein in serum which is ascribe to damage of liver as these enzymes are present in the cytoplasm on increase level causes leaked to blood therefore causes cell damage indicating the formation of hepatotoxicity on comparing with normal (Gutierrezl RMP et al, 2009). Treatment of aqueous extract of TC (200 and 300 mg/kg) caused restoration of these serum markers. Same observation was reported on treatment with silymarin (100mg/kg). The changed in elevated level of serum markers in PCM induced liver toxicity by aqueous extract of TC may
be due to stabilization of membrane by preventing the leakage of intracellular enzymes and regeneration of hepatocytes.

Hyperbilirubinemia was due to excessive hem-destruction and block of bile duct within the liver due to which severity of necrosis and its accumulation is a measure of binding, conjugation & excretory capacity of hepatocytes. Thus on treatment with aqueous extract of T.chebula (200mg/kg & 300mg/kg) demonstrated the decrease in serum bilirubin indicates the effectiveness of the extract in normal functioning status of liver.

Decrease in total protein is also an indicator of liver damage thus on significant fall in protein synthesis hypoproteinemia was observed due to PCM administration. On pretreatment with aqueous extract of TC (200mg/kg & 300mg/kg) turns towards normal indicate the enhancement of protein synthesis.

Hence silymarin and TC leave extract showed similar hepatoprotective effect. In these study histopathological studies also shows normal, toxic, standard (higher & lower dose) treated hepatocytes of liver.

CONCLUSION

Our investigational report on aqueous leaves extract of T.chebula exhibited accelerated hepatoprotective activity due to its free radical scavenging activity & antioxidant effect. These findings justify the medicinal uses of T.chebula for hepatotoxic management. Further studies are required to isolate, purify, characterize and elucidate the hepatoprotective activity of bioactive components of this plant.

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