Evidences of hepatoprotective and antioxidant effect of *Citrullus colocynthis* fruits in paracetamol induced hepatotoxicity

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**Abstract:** The objective of present study was to explore the hepatoprotective and antioxidant profile of *Citrullus colocynthis* fruits. Hepatoprotective profile of methanolic extract of *Citrullus colocynthis* fruits (MECCF) was investigated on rats, which were made hepatotoxic using paracetamol. The antioxidant profile of MECCF was evaluated by conducting Catalase, Superoxide Dismutase, Lipid Peroxidation and Diphenyl Picryl Hydrazyl tests. During hepatoprotective investigation, the Paracetamol treated group II showed significant increase in total bilirubin (TB), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) level. The results so obtained showed that pretreatment of rats with MECCF 300mg/kg p.o. decreases the elevated TB, SGOT, SGPT and ALP serum levels. Also, MECCF inhibitory profile was found comparable to toxicant group (Paracetamol 2g/kg, p.o). The present study concludes that MECCF fruit possess significant hepatoprotective and antioxidant activity.

**Keywords:** Hepatoprotective, antioxidant, MECCF, Paracetamol.

**INTRODUCTION**

In human body liver is an imperative organ (Ahmad and Sharafatullah, 2008). The liver is considered imperative because the nutritional level of a person does not depends only on what eatables consumed, but also on what liver processed. Regrettably it is not possible to diagnose early symptoms of imbalance in liver metabolism. A person could be a chronic patient of some liver disorder without known to it. Apart from treatment of liver disorders, daily care of liver can be a keystone for good health of a person (Kiran et al., 2011).

Liver is associated with vital functions such as it maintains and regulates the homeostasis in body. It plays an astonishing role in human body as it mediates various biochemical pathways like pathways for body defense against diseases, for body growth, for energy production and for supply of nutrition. Liver acts as a centre for metabolism of proteins, lipids, carbohydrates, and it also removes unwanted metabolites. Liver secretes a biochemical called bile, which plays an important role in digestion (Naskar et al., 2011).

Universal health problem is nothing but the liver disease. It is fact that for treatment for liver disorders the conventional or synthetic drugs are inadequate and are generally associated with various side effects. Although, plant sources provides many folk remedies with potential antioxidant and hepatoprotective activities. In India there exist three different systems for traditional medicines namely Ayurveda, Siddha and Unani that makes use of plant materials. The three features of herbal drugs such as safety, cost effectiveness and efficacy, made herbal drugs to gain high popularity and importance (Das et al., 2012).

From a long time in India herbal-based therapeutics for liver disorders has been in use and has been famous world over by leading pharmaceuticals. As per literature, numerous plants and formulations are reported to possess hepatoprotective profile. It is an estimate that from more than 100 plants about 160 phytoconstituents are known to have hepatoprotective action. Moreover, in India, about 87 plants are reported for their use in different commercial herbal multi component preparations (Saleem et al., 2010).

*Citrullus colocynthis* (L.) Schrad belonging to the melon family of cucurbitaceae (Ramanathan et al., 2011). Plant of *Citrullus colocynthis* is most commonly distributed in East Asian continent. Bitter apple or bitter cucumber is another name of *Citrullus colocynthis*. Generally, the fruit of *Citrullus colocynthis* plant is traditionally used for treatment of urinary disorders, jaundice and diabetes as well. The pulp of *Citrullus colocynthis* fruit contains a strong laxative phytoconstituent called colocynthis (Shasthree et al., 2010).

**MATERIALS AND METHODS**

**Plant material**

The *Citrullus colocynthis* fruit used for the present studies was collected from the province of Akola district of Maharashtra, India. Plant was further authenticated by...
Preparation of the extract
The powdered drug was dried and packed well in Soxhlet apparatus and then was successively extracted with benzene, chloroform and methanol (Agarwal et al., 2012).

Qualitative phytochemical screening
The three obtained herbal extracts (benzene, chloroform and methanolic) of Citrullus colocynthis were further subjected to different standardized tests to detect presence of different phytoconstituents such as carbohydrates, alkaloids, triterpenoids, glycosides, phenolics, tannins, saponins, flavones, flavonoids and phytosterols (Agarwal et al., 2012, Ramachandra et al., 2011).

Animals
In present study Male albino Wistar rats (weight between 150 to 200g) and albino mice (weight between 20 to 25g) of either sex were used. The animals were procured from Anuradha College of pharmacy, Chikhli, Dist-Buldhana (MS) India and were kept in animal house at a room temperature of 25±1°C. The animals were exposed to 12 h light and 12 h dark cycle; and were fed with standard pellets and tap water. The relative humidity of animal house was maintained up to 60%. Experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) after careful evaluation of research project during 2013 meeting (Proposal No. 751/03/abc/CPCSEA and Ref. No. IAEC/ 2012-2013).

Acute oral toxicity studies
The acute oral toxicity studies were performed on mice, using guidelines of OECD-423. The delivery dose was chosen from one of the four levels such as 5, 50, 500 and 2000 mg/kg. To determine mortality and behavioral response, the mice were kept under strict observation for 48 h, and thereafter once daily up to fourteen days.

Experimental design
A total of 24 rats were procured and equally divided into 4 groups. Each group contained six rats. The Normal control administered group was named as Group I, and was a recipient of daily oral dose of 2 ml/kg of saline up to seven days. Toxicant control administered group was named as Group II, and was a recipient of daily oral dose of 2 ml/kg of saline up to seven days. The methanolic extract of Citrullus colocynthis fruit (MECCF) administered group was named as Group III, and was a recipient of daily oral dose of 300 mg/kg up to seven days. The reference control (Standard) administered group was named as Group IV and was a recipient of daily oral dose of 1ml/kg of marketed preparation of Liv 52 up to seven days.

Moreover, on day seven, the rats of group II, III, and IV were orally administered with 2 g/kg of Paracetamol, that too after 30 min of the last dose (Rajesh et al., 2009). Nextly, after 36h of oral administration of 2g/kg of Paracetamol all groups of rats were anesthetized using ether and blood sample from respective groups of rats were withdrawn and collected in sterile tube for clotting. Later, the sterile tubes were subjected to centrifugation for 15 min at 2500 rpm to separate the serum. The serum’s so obtained were subjected to several biochemical assays. On the other hand, after last blood sample withdrawn all groups of rats were sacrificed and liver were isolated for histopathological studies from all groups of rats respectively.

Physical parameters (Qureshi et al., 2010)
Determination of wet liver weight
The respective livers of sacrificed animal group were isolated and were subjected to saline washing. Next, the weights of livers were determined using an electronic balance. The liver weights were expressed with respect to its body weight i.e. g/100g.

Determination of wet liver volume
After recording the weight, all the livers were dropped individually in a measuring cylinder containing a fixed volume of distilled water or saline and the volume displaced was recorded.

Biochemical parameters
The various groups of animals were assayed (according to standard methods) for different bio components such as total bilirubin (TB) (Ghosh et al., 2007), serum glutamate oxaloacetate transaminase (SGOT) (Khan et al., 2008), serum glutamate pyruvate transaminase (SGPT) (Balaraman and Bafna, 2013), and alkaline phosphatase (ALP) (Rathi et al., 2010).

Antioxidant parameters
All the four groups were estimated for In-vivo antioxidant parameters Catalase (CAT), Super oxide Dismutase (SOD) and Lipid Peroxidation (LPO) (Pietrzycka et al., 2007), serum glutamate (SGOT) and serum glutamate pyruvate transaminase (SGPT) (Pietrzycka et al., 2010).

Histopathological studies
Animals from all groups after treatment were sacrificed and small portions of each liver were fixed for 48 h in 10% formalin solution. Further, processing of livers was done by using paraffin embedding method. Nextly, the liver sectioning (cutting up to 5µm thickness) was performed. Later, the liver sections were subjected to staining with eosin and hematoxylin. Lastly, the stained liver sections were kept under microscope for histopathological studies.
STATISTICAL ANALYSIS

After following the experimental methodology the resultant data so obtained were subjected to statistical investigation. Resultant values were expressed in Mean ± SEM. The statistical significance of resultant data was assessed by ANOVA test. The resultant values with P<0.05 were considered significant.

RESULTS

Phytochemical constituents present in Citrullus colocynthis fruits

After extraction of Citrullus colocynthis fruits, the extracts were subjected to different standard qualitative tests for phytochemical identification. The data so obtained is represented in table 1.

According to table 1, the identified phytochemical constituents in Citrullus colocynthis fruits in three different extracts were found to be carbohydrates, alkaloids, glycosides, triterpenoids, phenolics and tannins, saponins, flavones & flavonoids, and phytosterols

In, preliminary phytochemical studies of extracts of Citrullus colocynthis confirmed the strong presence of desired phytochemicals in methanolic extracts when compared to benzene and chloroform extracts. Hence, for the further investigational studies, the MECCF was selected.

Physical parameters

Wet liver weight and volume

Treatment of rats with Paracetamol caused liver enlargement which was evident by increased in the wet liver weight and volume. The groups treated with Paracetamol displayed a significant increase in the wet liver weight and wet liver volume, in comparison to group I. Administration of MECCF (300mg/kg) and Liv 52 syrup (1ml/kg) in rats caused significant reduction in the wet liver weight and wet liver volume in comparison to group II. The results and data on Wet liver weight and Wet liver volume are shown in table 2.

Biochemical parameters

Effect of MECCF on biochemical parameters in Paracetamol induced hepatotoxic rats.

Total bilirubin level

Elevation of total bilirubin levels after administration of Paracetamol indicates its hepatotoxicity. Pretreatment with Liv 52, MECCF significantly reduced level of total bilirubin when compared to toxic control group, which indicates Hepatoprotective effect of MECCF. Resultant data of bilirubin levels obtained during this study are shown table 3.

SGOT, SGPT and ALP level

The group II, Paracetamol treated rats suffered from significant hepatotoxicity. This was confirmed by observing high serum levels of several bio chemicals such as SGOT, SGPT and ALP in comparison to normal control. Pretreatment with Liv 52, MECCF showed significant reduction in SGOT, SGPT and ALP serum level against paracetamol induced toxicity in liver. Resultant data of SGOT, SGPT and ALP serum level are shown in table 4.

In- vivo antioxidant parameters

From the results it was found that rats treated with Paracetamol showed a marked decrease in activities of catalase and super oxide dismutase in comparison to group I. The rats pretreated with syrup of Liv 52 and MECCF caused a marked increase in activities of catalase and super oxide dismutase when compared to toxicant group. During In vivo lipid peroxidation study Paracetamol treated group displayed a marked elevation in Malondialdehyde (MDA) level in comparison to group I. From present study it was revealed that MECCF and Liv 52 were able to significantly prevent the rise in MDA level, which is evident from table 5.

In-vitro antioxidant activity of MECCF

DPPH radical scavenging activity

After commencement of DPPH radical scavenging experiment, it was found that scavenging of free radicals by MECCF was concentration dependent. The MECCF extract at concentration of 800µg/mL showed 76% inhibition of DPPH radicals (table 6). Whereas standard ascorbic acid drug at a concentration of 200µg/mL displayed 90% inhibition of the DPPH radicals.

Histopathological studies of liver in paracetamol induced hepatotoxicity

The histopathological evaluation of Paracetamol toxicity in all the groups was examined and showed in fig. 1. According to fig. 1, Section 1 of rat liver treated with vehicle control group showed liver parenchyma with intact architecture, which is the normal appearance. Section 2 of liver in toxicant control group showed partially effaced architecture. Where Section 2 showed extensive vacuolar changes with congestion indicating severe degree of hepatotoxicity. It also shows round cell infiltration. Center lobular vein also shows hyperplasia of bile duct. Section 3 of rat liver in test drug treated groups (300mg/kg) showed intact architecture, at few places granular changes. Hemorrhages at few places seen. Whereas Section 4 of liver treated with standard (Liv 52 syrup) group showed mild vacuolar tubules in focal areas indicating incomplete restoration of parenchyma with intact architecture. No hyper plastic changes of the periphery of central tubular vein suggesting changes of restoration as compare to MECCF.
DISCUSSION

The preliminary phytochemical investigations showed presence of various secondary metabolites. The qualitative phytochemical investigation on different extracts of *Citrullus colocynthis* showed positive test for carbohydrate, triterpenes, steroids, tannins and flavonoids. The methanolic extract showed significant hepatoprotective activity. This might be due to higher content of the triterpenes, tannins and flavonoids.

Table 1: List of tests for determination of various phyto constituents in *Citrullus colocynthis* fruits extracts

<table>
<thead>
<tr>
<th>S. No</th>
<th>Phytochemical constituents</th>
<th>Test</th>
<th>Benzene Extract</th>
<th>Chloroform Extract</th>
<th>Methanolic Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbohydrates</td>
<td>Molish's test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td>Dragendorf’s test</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Glycosides</td>
<td>Bornträger's test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Triterpenoids</td>
<td>Tin + thionyl chloride</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Phenolics and tannins</td>
<td>Ferric chloride test</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Saponins</td>
<td>Foam test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Flavones and Flavonoids</td>
<td>Shinoda test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Phytosterols</td>
<td>Libermann-Burchards test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+, Presence of component -, Absence of component

Table 2: Effect of MECCF on Wet liver weight and Wet liver volume in Paracetamol induced hepatotoxic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>Wet Liver weight (gm/100gm)</th>
<th>Liver volumes (ml/100gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal Control (saline solution)</td>
<td>2 ml/kg p. o.</td>
<td>2.50±0.03</td>
<td>2.57±0.01</td>
</tr>
<tr>
<td>II</td>
<td>Toxicant Control (Paracetamol)</td>
<td>2 g/kg, p. o.</td>
<td>4.81±0.09</td>
<td>4.55±0.024</td>
</tr>
<tr>
<td>III</td>
<td>MECCF + Paracetamol</td>
<td>300 mg/Kg p.o. + 2 g/kg, p. o.</td>
<td>2.75±0.02**</td>
<td>2.798±0.01**</td>
</tr>
<tr>
<td>IV</td>
<td>Standard (Liv 52) + Paracetamol</td>
<td>1ml /kg p. o. + 2 g/kg, p. o.</td>
<td>2.64±0.02***</td>
<td>2.62±0.02***</td>
</tr>
</tbody>
</table>

Table 3: Effect of MECCF on bilirubin levels in Paracetamol induced hepatotoxic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>Total bilirubin levels (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal Control (saline solution)</td>
<td>2 ml/kg, p. o.</td>
<td>1.158±0.02</td>
</tr>
<tr>
<td>II</td>
<td>Toxicant Control (Paracetamol)</td>
<td>2 g/kg, p. o.</td>
<td>2.96±0.02</td>
</tr>
<tr>
<td>III</td>
<td>MECCF + Paracetamol</td>
<td>300 mg/Kg p. o. + 2 g/kg, p. o.</td>
<td>1.343±0.02*</td>
</tr>
<tr>
<td>IV</td>
<td>Standard (Liv 52) + Paracetamol</td>
<td>1ml /kg p. o. + 2 g/kg, p. o.</td>
<td>1.475±0.02***</td>
</tr>
</tbody>
</table>

Table 4: Effect of MECCF on SGOT, SGPT & ALP levels in Paracetamol induced hepatotoxic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>SGOT levels ( U/L )</th>
<th>SGPT levels ( U/L )</th>
<th>ALP levels ( U/L )</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal Control (saline solution)</td>
<td>2 ml/kg, p. o.</td>
<td>97.07±1.31</td>
<td>114.6±1.62</td>
<td>163.8±1.99</td>
</tr>
<tr>
<td>II</td>
<td>Toxicant Control (Paracetamol)</td>
<td>2 g/kg, p. o.</td>
<td>275.8±2.04</td>
<td>294.9±4.38</td>
<td>322.4±1.78</td>
</tr>
<tr>
<td>III</td>
<td>MECCF + Paracetamol</td>
<td>300 mg/Kg p. o. + 2 g/kg, p. o.</td>
<td>209.8±3.47*</td>
<td>219.3±6.46**</td>
<td>251.3±1.09*</td>
</tr>
<tr>
<td>IV</td>
<td>Standard (Liv 52) + Paracetamol</td>
<td>1ml /kg p. o. + 2 g/kg, p. o.</td>
<td>171.6±2.09***</td>
<td>156.4±4.15**</td>
<td>201.0±5.23**</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for six rats each. Values are stastically significant *at p<0.05, ** represents highly significant at p< 0.01, and *** represents very significant at p<0.001 Toxicant rats were compared with normal control rats. MECCF treated rats were compared with Toxicant rats, Liv 52 treated rats were compared with Toxicant.
Table 5: Effect of MECCF on Catalase, SOD and Lipid peroxidation in Paracetamol induced hepatotoxic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>SOD (units/mg protein)</th>
<th>CAT (units/mg protein)</th>
<th>LPO (nM of MDA/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal Control (saline solution)</td>
<td>2 ml/kg, p. o.</td>
<td>12.48±0.20</td>
<td>95.22±1.59</td>
<td>4.360±0.16</td>
</tr>
<tr>
<td>II</td>
<td>Toxicant Control (Paracetamol)</td>
<td>2 g/kg, p. o.</td>
<td>3.95±0.32</td>
<td>25.22±0.99</td>
<td>10.04±0.89</td>
</tr>
<tr>
<td>III</td>
<td>MECCF + Paracetamol</td>
<td>300 mg/Kg p. o. + 2 g/kg, p. o.</td>
<td>5.588±0.16*</td>
<td>35.54±1.46*</td>
<td>7.28±0.48*</td>
</tr>
<tr>
<td>IV</td>
<td>Standard (Liv 52) + Paracetamol</td>
<td>1ml /kg p. o. + 2 g/kg, p. o.</td>
<td>8.53±0.14***</td>
<td>82.76±1.01***</td>
<td>6.65±0.28***</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for six rats each. Values are stastically significant *at p<0.05, ** represents highly significant at p< 0.01, and *** represents very significant at p<0.001. Toxicant rats were compared with normal control rats. MECCF treated rats were compared with Toxicant rats, Liv 52 treated rats were compared with Toxicant.

Fig. 1: Histopathology of the liver in Paracetamol induced hepatotoxicity studies
In toxic liver, level of bilirubin gets elevated to cause hyperbilirubinemia, which can be attributed to impaired hepatic uptake of unconjugated bilirubin. This condition commonly occurs in general injury of hepatic cell. In present investigation MECCF treatment to rats significantly decreased bilirubin serum level. Such observation further supports to hepatoprotective profile of MECCF.

**Table 6: DPPH scavenging activity of MECCF**

<table>
<thead>
<tr>
<th>MECCF (µg/ml)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>8.52±0.09</td>
</tr>
<tr>
<td>50</td>
<td>39.44±0.05</td>
</tr>
<tr>
<td>100</td>
<td>48.92±0.04</td>
</tr>
<tr>
<td>200</td>
<td>56.80±0.03</td>
</tr>
<tr>
<td>400</td>
<td>63.37±0.04</td>
</tr>
<tr>
<td>800</td>
<td>76.35±0.23</td>
</tr>
<tr>
<td>Ascorbic acid (200)</td>
<td>90.32±0.24</td>
</tr>
</tbody>
</table>

Values represents number of readings in each group = 3mean ± SEM

Liver toxicity elevates the SGOT and SGPT levels. Alcoholic liver damage and cirrhosis can also be associated with mild to moderate elevation of transaminases. In the present investigation the MECCF treatment to rats caused a remarkable reduction in SGOT and SGPT serum levels. This indicates and confirms the hepatoprotective profile of MECCF.

Furthermore, in hepatotoxicity, the level of alkaline phosphatase was very high. This must be due to defect in hepatic excretion or must be due to higher production of ALP by duct cells or hepatic parenchymal cells. The present study revealed that MECCF treatment to rats reduced ALP serum level, which supports to hepatoprotective profile of MECCF.

The present investigation further supports that chronic exposure of paracetamol reduced the reactive oxygen species (ROS) scavenging activities of CAT and SOD. The present study reveals that MECCF have the ability to restore the activity of both CAT and SOD. So, MECCF may reduce hepato cellular damage and free radicals generation.

Paracetamol induced hepatotoxicity is also associated with high lipid peroxidation. The present study supports that MECCF administration reduced lipid peroxidation. This indicates that MECCF possesses antioxidant activity and reduces paracetamol induced membrane lipid peroxidation.

The plant extract MECCF showed maximum percentage inhibition of DPPH radicals. Free radicals of DPPH are comparatively stable. These DPPH radicals when comes in contact with antioxidants undergoes quenching and their absorbance is reduced. The result suggested that in comparison with ascorbic acid DPPH radicals showed scavenging activity of the MECCF.

Histopathological liver sections also revealed that liver's microscopic structure was disrupted in Paracetamol treated group. On the other hand the hepatic section of the rats treated with the MECCF and toxicants, retained normal cellular architecture as compared with the toxicant group. Hence study confirms the significant hepatoprotective effect of 300mg/kg of orally administered MECCF.

**CONCLUSION**

Based on the results of serum marker enzyme levels, physical, antioxidant, and histopathological studies the present study concludes that *Citrullus colocynthis* fruits possesses hepatoprotective activity and thus supports the traditional use of same for treatment of liver disorder.

**REFERENCES**


