ABSTRACT

There is an increasing interest in herbal remedies because of their effectiveness, less side effects in clinical experiments and relatively low cost. Buchanania lanzan Spreng. (B. lanzan) belongs to the Family Anacardiaceae, is commonly known as ‘Chaar’ in India. In ayurvedic medicine various part this plant used as astringent, depurative, constipating, brain tonic, cardio tonic and for glandular swelling. This plant is used in treatment and prevention of cancer by traditional healers and herbalists of the Chhattisgarh state of India. The phytoconstituents reported in bark of this plant are flavonoids, sterols, tannins, quercetin, glycosides, Triterpenoid, Saponins, carbohydrates, phenolic compounds. The present study was conducted to evaluate the hepatoprotective activity of methanolic and aqueous extract of bark of B. lanzan against paracetamol induced liver damage in rats. The methanolic and aqueous extract of B. lanzan was administered orally to the animals with hepatotoxicity induced by Paracetamol. Silymarin was used as reference standard. The plant aqueous extract was effective in protecting the liver against the injury induced by Paracetamol in rats. Silymarin, methanolic extract of bark of B. lanzan (MEBBL) and aqueous extract of bark of B. lanzan (AQEBBL) treated groups when compared to paracetamol (PCM) induced hepatotoxic rats the increased ALT, AST, ALP, BILD, BILT, CHO and TG levels were significantly reduced.
and ALB and PRO levels were significantly increased. The histopathological changes i.e. fatty changes (steatosis), necrosis etc. were partly or fully prevented. It was concluded from the result that the AQEBBL possesses hepatoprotective activity against PCM induced hepatotoxicity in rats and it is less when compared to Silymarin as evidenced by the significant difference in biochemical parameters.

**KEYWORDS:** Buchanania lanzan Spreng. Bark, Aqueous extract, Methanolic extract, PCM, Hepatoprotective, and Silymarin.

**INTRODUCTION**
Liver is one of the largest organs in the human body and chief site for intense metabolism and excretion. Liver diseases are one of the major health problems in the world. These are caused by toxic chemicals, autoimmune disorders, infections and excess consumption of alcohol. The hepatotoxic chemicals can induce lipid peroxidation and oxidative damages. It is involved in almost all the biochemical pathways to growth, fight against the disease, nutrient supply, energy provision and reproduction.\(^1,2\)

*Buchanania lanzan* Spreng (locally called as Chironji), a member of family Anacardiaceae is a commercially useful tree species found in several areas of India. The plant has well-known traditional uses and its seeds are used as expectorant and tonic. The oil extracted from kernels is applied on skin diseases and also to remove spots and blemishes from the face. The root is used as expectorant, in biliousness and also for curing blood diseases. The juice of the leaves is digestive, expectorant, aphrodisiac and purgative. The rhizome of *B. lanzan* finds an important place in indigenous medicine as an expectorant, diuretic and carminative. It is also found to have anticancer, antihypertensive, larvicidal and anti-diabetic activities.\(^3-6\) It is a commercially useful tropical plant. Chironji tree is a medium evergreen deciduous tree, growing 50 ft tall. It bears fruits each containing a single seed, which is a popular edible nut, known as chironji. It is common in India mostly in eroded lands. It has tickly leathery leaves which are broadly oblong, with blunt tip and rounded base.\(^7,8\)

**MATERIALS AND METHODS**
**Plant Material**
Fresh bark of *Buchanania lanzan* Spreng. were collected from forest of Basana, Chhattisgarh and authenticated from BSI Attapura, Hyderabad then Shade dried in Ranga reddy district of Telangana, India. A voucher specimen of *B. lanzan* has been deposited at the Dept. of
Pharmacognosy, (Voucher specimen No.-BSI/DRC/2015-16/Tech./552), KVK College of Pharmacy, Surmaiguda, Hyderabad (TS).

**Preparation of Extracts**
Bark of *Buchanania lanzan* Spreng. was coarsely powdered and extracted with double distilled water by maceration for 24 hours. For the methanolic extract bark powder packed in Soxhlet apparatus was extracted with 95% methanol for 18 h and appearance of colorless solvent in the siphon tube was taken as the termination of extraction. The extract was concentrated using rotary evaporator *invacuo* until the extract acquired semisolid consistency. The extracts were finally dried under vacuum in desiccator over phosphorous pentoxide.

**Phytochemical Analysis**
The extract was subjected to preliminary phytochemical screening for phytochemical constituents such as Flavonoids, tannins, alkaloids, glycosides, Triterpenoid, Saponins, sterols, carbohydrates, phenolic compounds, gum and mucilage[10] (Table 2).

**Acute Oral Toxicity**
The acute toxicity of total alcoholic and aqueous extracts and fractions *B. lanzan* fruits were determined as per OECD guideline no. 423 (OECD, 2001). Based on the cut-off Value of the median lethal dose (LD$_{50}$), the therapeutically effective dose was derived.

**Evaluation of Hepatoprotective Activity**
Sanzyme Limited is a CPCSEA registered body with a registration number of 546/02/a/CPSCEA The animals were divided into nine groups of six animals each. Group A was served as normal control which was given with vehicle only. Group B with Silymarin (100 mg/Kg p.o.) that serves as standard. Group C with Paracetamol (2 gm/Kg p.o.). Animals in groups D, E and F were treated with three different doses (low, medium and high) of MEBBL and groups G, H and I with AQEBSBL Groups B, C, D, E, F, G, H and I were intoxicated with Paracetamol (2 gm/Kg p.o.) for 3 days and from 4th-10th day with different doses of MEBBL & AQEBSBL in related groups respectively. They were anaesthetized with ether. Blood was collected through retro orbital puncture, later sacrificed by overdose of ether. Livers removed were washed with saline, weighed and stored in 10% Formaldehyde for histological studies.
Biochemical Analysis
Blood samples are allowed to coagulate for 30 min at 37 °C. Then it was centrifuged at 1500rpm (Micro centrifuge) for 15min to separate the serum. The clear serum was subjected to biochemical investigation viz., Serum alanine aminotransferase (ALT), Serum aspartate aminotransferase (AST), Serum alkaline phosphatase (ALP), Serum direct bilirubin (BILD), Serum total bilirubin (BILT), Serum cholesterol (CHO), Serum triglycerides (TG), Serum albumin (ALB) and Serum total proteins (TP).

Statistical Analysis
All the recorded results expressed as mean ± SEM from 6 animals. Statistical difference in mean analyzed by using one-way ANOVA (analysis of variance) followed by ‘t’ test. P< 0.05*, 0.01** and 0.001*** considered as statistically significant. (Used graph pad prism software).

RESULTS
A. Effect of PCM on biochemical parameters in rats
Biochemical parameters like ALT (145.62±2.50 U/L), AST (258.87±2.46 U/L), ALP (263.54±2.75 U/L), BILD (2.01±0.22 mg/dL), BILT (2.37±0.13 mg/dL), CHO (237.23±5.28 mg/dL) and TG (216.7±2.91 mg/dL) are significantly increased whereas ALB (3.45±0.26 g/dL) and TP (13.06±0.38g/dL) are significantly decreased.

B. Effect of Silymarin on biochemical parameters in PCM induced hepatotoxic rats
Biochemical parameters like ALT (62.45±3.37 U/L), AST (141±2.21 U/L), ALP (127.67±2.13 U/L), BILD (0.39±0.04 mg/dL), BILT (0.5±0.02 mg/dL), CHO (155.47±1.22 mg/dL) and TG (105.22±3.25 mg/dL). Whereas ALB (5.66±0.15 g/dL) and PRO (21.22±0.72 g/dL), are significantly increased.

C. Effect of MEBBL on biochemical parameters in PCM induced hepatotoxic rats
Biochemical parameters like ALT, AST, ALP (U/L), BILD, BILT, CHO and TG (mg/dL) ) levels are significantly reduced with med and high dose and similarly ALB and PRO (g/dL) levels are significantly increased.

D. Effect of AQEBBL on biochemical parameters in PCM induced hepatotoxic rat
Biochemical parameters like ALT, AST, ALP (U/L), BILD, BILT, CHO and TG (mg/dL) levels are significantly reduced with med and high dose and similarly ALB and PRO (g/dL) levels are significantly increased.
Table No.1 Effect of MEBBL and AQEBBL on biochemical parameters in paracetamol induced hepatotoxicity rats:

<table>
<thead>
<tr>
<th>Biochemi</th>
<th>Normal</th>
<th>Standard</th>
<th>Toxicant</th>
<th>MEBBL 100mg/kg</th>
<th>MEBBL 200mg/kg</th>
<th>MEBBL 400mg/kg</th>
<th>AQEBBL 100mg/kg</th>
<th>AQEBBL 200mg/kg</th>
<th>AQEBBL 400mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT U/L</td>
<td>53.25±2.06</td>
<td>62.45±3.37 ***</td>
<td>145.62±2.50 ***</td>
<td>142.37±2.79 ns</td>
<td>130.81±1.64 ***</td>
<td>90.92±2.55 ***</td>
<td>134.85±1.93 *</td>
<td>91.5±1.78 ***</td>
<td>70.08±2.26 ***</td>
</tr>
<tr>
<td>AST U/L</td>
<td>121.35±3.20</td>
<td>141±2.21 ***</td>
<td>258.87±2.46 ***</td>
<td>249.3±2.20 ns</td>
<td>197.65±2.17 ***</td>
<td>160.23±2.81 ***</td>
<td>241.28±10.59 *</td>
<td>180.77±3.26 ***</td>
<td>150.4±2.50 ***</td>
</tr>
<tr>
<td>ALP U/L</td>
<td>120.6±2.36</td>
<td>127.67±2.13 ***</td>
<td>263.54±2.75 ***</td>
<td>240.95±2.80 **</td>
<td>213±5.38 ***</td>
<td>169.52±3.23 ***</td>
<td>248.72±8.06</td>
<td>152.08±1.94 ***</td>
<td>130.75±2.83 ***</td>
</tr>
<tr>
<td>BILD mg/dL</td>
<td>0.24±0.04</td>
<td>0.39±0.04 ***</td>
<td>2.01±0.22 **</td>
<td>1.64±0.24 ns</td>
<td>0.96±0.15 ***</td>
<td>0.53±0.01 ***</td>
<td>1.60±0.24 ns</td>
<td>0.9±0.02 ***</td>
<td>0.48±0.02 ***</td>
</tr>
<tr>
<td>BILT mg/dL</td>
<td>0.38±0.06</td>
<td>0.5±0.02 ***</td>
<td>2.37±0.13 ***</td>
<td>2.05±0.26 ns</td>
<td>1.27±0.14 ***</td>
<td>0.98±0.06 ***</td>
<td>1.97±0.14 ns</td>
<td>0.83±0.03 ***</td>
<td>0.75±0.03 ***</td>
</tr>
<tr>
<td>ALB g/dL</td>
<td>5.86±0.07</td>
<td>5.66±0.15 ***</td>
<td>3.45±0.26 ***</td>
<td>3.75±0.07 ns</td>
<td>4.16±0.07 **</td>
<td>4.75±0.07 ***</td>
<td>3.88±0.06 ns</td>
<td>4.54±0.10 ***</td>
<td>5.24±0.13 ***</td>
</tr>
<tr>
<td>TP g/dL</td>
<td>25.16±0.90</td>
<td>21.2±0.72 ***</td>
<td>13.06±0.38 ***</td>
<td>12.22±0.59 ns</td>
<td>18.27±2.37 ***</td>
<td>20.6±1.69 ***</td>
<td>14.52±1.34 ns</td>
<td>17.79±0.58 *</td>
<td>22±0.33 ***</td>
</tr>
<tr>
<td>CHO mg/dL</td>
<td>142.15±1.68</td>
<td>155.47±1.22 ***</td>
<td>237.23±5.28 ***</td>
<td>225.37±4.58 ns</td>
<td>191.82±2.68 ***</td>
<td>178.27±5.04 ***</td>
<td>209.69±15.40ns</td>
<td>188.36±5.65 ***</td>
<td>161.11±10.09 ***</td>
</tr>
<tr>
<td>TG mg/dL</td>
<td>91.27±2.60</td>
<td>105.22±3.25 ***</td>
<td>216.7±2.91 ***</td>
<td>191.6±18.17 ns</td>
<td>170.3±4.0 ***</td>
<td>143.92±1.15 ***</td>
<td>183.75±1.05 **</td>
<td>159.27±2.97 ***</td>
<td>109.52±3.90 **</td>
</tr>
</tbody>
</table>

n = 6, Significant at P < 0.05*, 0.01**, and 0.001***, ns = not significant, MEBBL-Methanolic extract of bark of *B.lanzan*, AQEBBL- Aqueous extract of bark of *B.lanzan*. 
Table No.2 Preliminary phytochemical evaluation of MEBBL and AQEBBL.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Test</th>
<th>MEBBL</th>
<th>AQEBBL</th>
<th>S.N.</th>
<th>Test</th>
<th>Bark powder of BBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Flavonoids</td>
<td>(+) ve</td>
<td>(+) ve</td>
<td>IV</td>
<td>Glycosides</td>
<td>(-) ve</td>
</tr>
<tr>
<td></td>
<td>FeCl3 test</td>
<td>(+) ve</td>
<td>(+) ve</td>
<td></td>
<td>Borntrager’s test</td>
<td>(+) ve</td>
</tr>
<tr>
<td></td>
<td>NAOH Solution</td>
<td>(+) ve</td>
<td>(+) ve</td>
<td></td>
<td>Modified Borntrager’s test</td>
<td>(+) ve</td>
</tr>
<tr>
<td></td>
<td>Lead acetate</td>
<td>(+) ve</td>
<td>(+) ve</td>
<td></td>
<td>Keller kiliani test</td>
<td>(+) ve</td>
</tr>
<tr>
<td></td>
<td>Mineral acid</td>
<td>(+) ve</td>
<td>(+) ve</td>
<td></td>
<td>Baljit test</td>
<td>(+) ve</td>
</tr>
<tr>
<td></td>
<td>Alkali-acid</td>
<td>(+) ve</td>
<td>(+) ve</td>
<td></td>
<td><strong>Gums and Mucilage</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bark powder</td>
<td>Ppt with 90% alcohol</td>
<td>(-) ve</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Tannins</td>
<td>(+) ve</td>
<td>(+) ve</td>
<td>VI</td>
<td><strong>Triterpenoid and Saponins</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FeCl3 test</td>
<td>(+) ve</td>
<td>(+) ve</td>
<td></td>
<td>Foam test</td>
<td>(+) ve</td>
</tr>
<tr>
<td></td>
<td>Dil FeCl3 test</td>
<td>(+) ve</td>
<td>(+) ve</td>
<td></td>
<td>Haemolysis test</td>
<td>(-) ve</td>
</tr>
<tr>
<td></td>
<td>Gelatine test</td>
<td>(+) ve</td>
<td>(+) ve</td>
<td></td>
<td><strong>Sterol</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Match stick test</td>
<td>(-) ve</td>
<td>(-) ve</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chlorogenic acid test</td>
<td>(-) ve</td>
<td>(-) ve</td>
<td></td>
<td>Lieberman burchard reaction</td>
<td>(+) ve</td>
</tr>
<tr>
<td>III</td>
<td>Alkaloids</td>
<td>(-) ve</td>
<td>(-) ve</td>
<td>VIII</td>
<td><strong>Carbohydrates</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mayer’s reagent</td>
<td>(-) ve</td>
<td>(-) ve</td>
<td></td>
<td>Molischs test</td>
<td>(+) ve</td>
</tr>
<tr>
<td></td>
<td>Wagner’s reagent</td>
<td>(-) ve</td>
<td>(-) ve</td>
<td></td>
<td>Fehling’s test</td>
<td>(+) ve</td>
</tr>
<tr>
<td></td>
<td>Hager’s reagent</td>
<td>(-) ve</td>
<td>(-) ve</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Effect of MEBBL & AQEBBL on serum AST level**

![Graph showing the effect of MEBBL and AQEBBL on serum AST level](image)

**Effect of MEBBL & AQEBBL on serum ALT level**

![Graph showing the effect of MEBBL and AQEBBL on serum ALT level](image)
Effect of MEBBL & AQBBL on serum ALP level

ALP (U/L)

Control
Standard
Toxicant
MEBBL 100 mg/kg
MEBBL 200 mg/kg
MEBBL 400 mg/kg
AQBBL 100 mg/kg
AQBBL 200 mg/kg
AQBBL 400 mg/kg

Effect of MEBBL & AQBBL on serum BILD level

BILD (mg/dl)

Control
Standard
Toxicant
MEBBL 100 mg/kg
MEBBL 200 mg/kg
MEBBL 400 mg/kg
AQBBL 100 mg/kg
AQBBL 200 mg/kg
AQBBL 400 mg/kg

Effect of MEBBL & AQBBL on serum BILT level

BILT (mg/dl)

Control
Standard
Toxicant
MEBBL 100 mg/kg
MEBBL 200 mg/kg
MEBBL 400 mg/kg
AQBBL 100 mg/kg
AQBBL 200 mg/kg
AQBBL 400 mg/kg
Effect of MEBBL & AQBBL on serum ALB level

- Control
- Standard
- Toxicant
- MEBBL 100 mg/kg
- MEBBL 200 mg/kg
- MEBBL 400 mg/kg
- AQBBL 100 mg/kg
- AQBBL 200 mg/kg
- AQBBL 400 mg/kg

Effect of MEBBL & AQBBL on serum TP level

- Control
- Standard
- Toxicant
- MEBBL 100 mg/kg
- MEBBL 200 mg/kg
- MEBBL 400 mg/kg
- AQBBL 100 mg/kg
- AQBBL 200 mg/kg
- AQBBL 400 mg/kg

Effect of MEBBL & AQBBL on serum CHO level

- Control
- Standard
- Toxicant
- MEBBL 100 mg/kg
- MEBBL 200 mg/kg
- MEBBL 400 mg/kg
- AQBBL 100 mg/kg
- AQBBL 200 mg/kg
- AQBBL 400 mg/kg
Effect of MEBBL & AQBBBL on serum TG level

Figure 1: Histology of normal hepatic tissue

Figure 2: PCM induced damage in hepatic tissue

Figure 3: Effect of Silymarin on PCM induced hepatic damage

Figure 4: Effect of MEBBL (High) dose on PCM induced hepatic damage

Figure 5: Effect of AQEBBL (High) dose on PCM induced hepatic damage
DISCUSSION

The liver is a vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification from the exogenous and endogenous challenges, like xenobiotics, drugs, viral infections and chronic alcoholism. If during all such exposures to the above mentioned challenges the natural protective mechanisms of the liver are overpowered, the result is hepatic injury. Liver damage is always associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like AST, ALT, triglycerides, cholesterol, bilirubin, alkaline phosphatase are elevated. Paracetamol is normally eliminated mainly as sulfate and glucuronide. Only 5% of the paracetamol is converted into N-acetyl-p-benzoquinoneimine (NAPQI). Higher dose of paracetamol and NAPQI can alkylate and oxidize intracellular GSH and protein thiol group, which results in the depletion of liver GSH pool subsequently, leads to increased lipid peroxidation and liver damage.\(^{[15]}\)

Aspartate and alanine aminotransferases are normally localized within the cells of the liver, heart, gill, kidney, muscles and other organs. The enzymes are of major importance in assessing and monitoring liver cytolysis. Their presence in the serum may give information on organ dysfunction.\(^{[16]}\)

ALT is more specific cytosolic enzyme for liver, whereas AST is localized in cytosol and mitochondria that are released into circulation in the early phase of injury. Prolonged destruction in hepatic cells results in more hepatic releases to exacerbate hepatic dysfunction and causes an elevation of ALP, LDH and bilirubin in serum.\(^{[17]}\)

The results obtained reveal that Chronic administration of (Paracetamol) PCM to rats increased the levels of marker enzymes like ALT, AST and ALP as these are stored in the liver cells and increase in the levels of these marker enzymes in serum indicate damage to the liver cells. Pretreatment with AQEBBL decreased the levels of ALT, AST, ALP, BILD, BITD, CHO, TG levels and increased PRO and ALB levels, an indication for the hepatoprotective activity of the extract against drug induced hepatotoxicity. Intoxication with drugs cause increase in cholesterol and triglyceride levels. MEBBL and AQEBBL prevented elevated cholesterol and triglyceride levels due to hepatic lipid peroxidation occurred after drug intoxication.
CONCLUSION

The preliminary phytochemical analysis of the MEBBL and AQEBBL revealed the presence of flavonoids, sterols, tannins, quercetin, glycosides, Triterpenoid, Saponins, carbohydrates, phenolic compounds. The medium and higher doses of MEBBL and AQEBBL (200 and 400 mg/kg) treated groups showed better hepatoprotective activity when compared to standard drug silymarin (100 mg/Kg p.o.) treated group. Though both extracts contained similar phytoconstituents, the different in quantity of phytoconstituents present may responsible for the difference in hepatoprotective activity.

REFERENCES


