CHAPTER 6

RESULTS OF INVIVO STUDY

6.1 SAFETY EVALUATION STUDY

Rats when fed with ethanol extract of MA and BF up to 2000 mg/ kg body weight p.o. exhibited no mortality or any sign of gross behavioral changes when observed initially for 72 h, and finally up to 15 days.

DISCUSSION

The safe evaluation study of ethanol extract of MA and BF showed that no mortality of rats occurred up to a limit dose of 2000 mg /kg body weight given p.o. This is an indication that the extract has low acute toxicity when administered p.o.

According to Clarke and Clarke (1977), substances with LD$_{50}$ of 1000 mg/kg body weight/oral route are regarded as being safe or of low toxicity. The high LD$_{50}$ obtained is an indication that the extract could be administered with a high degree of safety where the absorption might be incomplete due to inherent factors impeding absorption along the gastrointestinal tract (Dennis, 1984).

6.2 ACETAMINOPHEN (APAP) INDUCED HEPATOTOXIC STUDIES IN ALBINO RATS

The serum activities of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and bilirubin were used as biochemical markers to assess early acute hepatic damage.
Many people all over the world are suffering from hepatic damage induced by alcohol and drug abuse. The liver plays a major role in the digestion, metabolism and storage of nutrients. Today the increasing impact of liver disease, especially liver injury due to pharmacological treatment is being recognized. In recent years new insights have been gained into the pathological mechanisms of liver injury. In certain cases this forms the basis for novel therapeutic strategies (Gerbes et al., 2006).

The liver is the first organ to metabolize all foreign compounds and hence it is susceptible to different diseases. Some are rare but there are a few, including hepatitis, cirrhosis, alcohol related disorders and liver cancer which are fatal. A major cause of these disorders is the exposure to different environmental pollutants and xenobiotics e.g., Paracetamol (APAP), carbon tetrachloride (CCl₄), thioacetamide, alcohol, H₂O₂ etc. These toxicants mainly damage the liver by producing reactive oxygen species (ROS).

Acetaminophen (N-acetyl-para-aminophenol; Paracetamol, APAP) is one of the most widely and commonly used over-the-counter drugs for the relief of fever and headaches due to its antipyretic and analgesic properties, and is a major ingredient in cold and flu remedies. Though APAP is generally considered safe for human use at recommended doses, potentially fatal liver damages occurred in rare cases when an acute over-dose or even a normal dose was taken. Accordingly, APAP overdose is one the most common causes of drug poisoning world-wide. Excessive use of APAP can cause multiple organ damages, especially of the liver and kidney (Bertolini 2006; Ypar, et al., 2007). Other tissues have been shown to be affected by acetaminophen, for instance eye (Zhao et al., 1997), lung (Hart et al., 1998), testes (Boyd 1970), heart (Prescott, 1980) and lymphoid tissues (Cohen et al., 1997).
Table 6: Effect of MA on serum enzymes (ALT, AST and ALP), total bilirubin and total protein on APAP induced hepatotoxicity in rats

<table>
<thead>
<tr>
<th>Groups and treatment</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
<th>ALP IU/L)</th>
<th>Total Bilirubin (mg/dl)</th>
<th>Total Protein (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 Control</td>
<td>44.50± 1.47</td>
<td>48.26± 2.73</td>
<td>14.03± 0.45</td>
<td>1.65± 0.05</td>
<td>7.47± 0.18</td>
</tr>
<tr>
<td>Group 2 APAP 750mg /kg B/w</td>
<td>97.25± 4.28 a,**</td>
<td>98.25±2.75 a,**</td>
<td>28.71±1.23 a,**</td>
<td>6.44± 0.22 a,**</td>
<td>5.09± 0.19 a,**</td>
</tr>
<tr>
<td>Group 3 MA (250mg/kg bw) + APAP</td>
<td>87.83± 2.00 b,*</td>
<td>88.91± 2.38 b,*</td>
<td>24.52± 0.55 b,*</td>
<td>5.55± 0.07 b,*</td>
<td>5.21± 0.15 b,*</td>
</tr>
<tr>
<td>Group 4 MA (500mg/kg) + APAP</td>
<td>79.06±1.66 b,**</td>
<td>71.65±2.47 b,**</td>
<td>18.36±0.29 b,**</td>
<td>3.41± 0.31 b,**</td>
<td>5.43± 0.09 b,**</td>
</tr>
<tr>
<td>Group 5 MA (500mg/kg)</td>
<td>60.23± 2.59 b,**</td>
<td>54.98±1.10 b,**</td>
<td>16.01±0.43 b,**</td>
<td>1.90± 0.05 b,**</td>
<td>6.49±0.13 b,*</td>
</tr>
</tbody>
</table>

Values are mean ± s.D. (N = 6). A as compared with control, b as compared with apap, ** represents p<0.001, * represents p<0.01
The serum activities of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and bilirubin were used as biochemical markers to assess hepatic damage

The activity levels of serum ALT, AST, ALP and bilirubin were observed in control, APAP induced. In group II animals which were treated with APAP the levels of biochemical marker enzymes like ALT, AST and bilirubin were significantly elevated (P<0.001) as shown in table 6 and in figure 13.

Figure 13: Effect of ethanolic extract of MA on serum levels of ALT, AST and ALP

Values are mean ± s.D. (N = 6). A as compared with control, b as compared with APAP, ** represents p<0.001, * represents p<0.01.

The activity level of ALP however, was significantly (P<0.001) elevated when compared to level in normal rats (Table: 6, Figure 13).
Bilirubin, an endogenous organic anion, binds reversibly to albumin and is transported to the liver, where it is conjugated to glucuronic acid and excreted in the bile. It is derived primarily from catabolism of red blood cells, heme and to a lesser extent from degradation of myoglobin, cytochrome, catalase and peroxidase.

Figure 14: Effect of ethanolic extract of MA on serum levels of total Bilirubin and total protein on APAP induced hepatotoxicity

Values are mean ± s.D. (N = 6) a as compared with control, b as compared with APAP, ** represents p<0.001, * represents p<0.01

Table 6; Figure 14, depict serum level of total bilirubin and total protein in control and experimental groups of rats. Rats intoxicated with acetaminophen (Group II) showed a significant elevation (P<0.001) in total bilirubin
and total protein. Pretreatment with MA extract (Group 3 & 4) resulted in reversal of the above changes to near normal.

DISCUSSION

The liver is the largest organ in the vertebrate body and is the major site of xenobiotic metabolism and excretion. Liver injury can be caused by toxic chemicals, drugs and virus infiltration from ingestion or infection. The toxins absorbed from the intestinal tract gain access first to the liver resulting in a variety of liver ailments. Thus liver diseases remain one of the serious health problems (Karan et al., 1999).

Acetaminophen is known to cause hepatotoxicity in experimental animals and humans at high doses (Mitchell, 1988; Eriksson et al., 1992; Thompsen et al., 1995). The laboratory features of hepatotoxicity induced by APAP resemble other kinds of acute inflammatory liver diseases with prominent increase of ALT, AST and ALP levels (Davidson and Eastham, 1966).

The serum level of hepatic enzymes ALT, AST, ALP and total bilirubin levels were increased and reflected the hepatocellular damage in the APAP-induced hepatotoxicity animal model. This is indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotman et al., 1978).

6.3 NEPHROPROTECTIVE EFFECT OF MA AND BF EXTRACT ON APAP INDUCED NEPHROTOXICITY IN ALBINO RATS

Acetaminophen (APAP), discovered in 1889, has been a widely used analgesic and antipyretic agent alternative to aspirin, when administered within the therapeutic range. This drug belongs to the para-aminophenol class of the non-
steroidal anti-inflammatory drugs (NSAIDs) (Jackson-Robert II and Morrow, 2001). Cause of hepatic and renal failure is due to the overdose of APAP (Hamid et al., 2012). These serious disorders are required to be treated to overcome the APAP overdose.

Daily high doses greater than 4 g in adults, commonly cause severe and devastating renal damage (Ostapowicz et al., 2002). It has been observed that the numbers of emergency visits and deaths have been in raise during the previous years either due to intentional or unintentional overdoses (Nourjah and Wiley, 2002). Acetaminophen-induced renal damage is reported to be mediated through an increased lipoperoxidation in renal tissues and urine volume, in glutathione status, creatinine clearance (Schnellman, 2001; Bessems and Vermeulen, 2001).

Overdose of Acetaminophen is normally associated with several metabolic disorders including serum electrolyte, urea and creatinine rearrangements. To investigate drug induced nephrotoxicity in animals and human (Rai et al., 2006), increased concentration of serum urea and creatinine are considered. CYP-mediated conversion of acetaminophen to a highly reactive quinone imine, \( N\)-acetyl-\( p\)-benzoquinoneimine (NAPQI) is the cause of acetaminophen toxicology. The primary role of NAPQI in the toxicity of acetaminophen has been reported by several papers in literature such as (Corcoran et al., 1980, Dahlin et al., 1982, Holme et al., 1984, Dahlin et al., 1984, Lowry et al., 1951, Streeter et al., 1984).

Blood urea nitrogen is found in the liver protein get usually excreted in the urine. Source of blood urea nitrogen is may be through diet or tissues. Renal disease occurs due to the serum urea accumulation, which is owing to the rate of
serum urea production exceeding the rate of clearance (Mayne, 1984). Increase in urea and creatinine levels in the serum has been considered as the index of nephrotoxicity (Ali et al., 2001, Anwar et al., 1999, Bennette et al., 1982). Creatinine is often obtained from endogenous sources by tissue creatinine breakdown (Mayne, 1984). And hence serum urea concentration is mostly treated as a more reliable renal function predictor than serum creatinine.

Most widely used method for monitoring renal function is creatinine concentration. This creatinine level in blood is used to compute creatinine clearance (CrCl), which reflects the glomerular filtration rate (GFR). A simple blood test will reveal the presence of nephrotoxicity. Renal failure is diagnosed by a decreased creatinine clearance, whose normal level should be in the range of 80 - 120 μmol/L.

Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing lifestyle related disorders of nephrotoxicity and relatively very little knowledge is available about their mode of action. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits.

6.3.1 Effect of MA and BF extract on serum urea, uric acid and creatinine concentrations

Statistical analysis:

Administration of APAP to rats resulted in nephrotoxicity and development of oxidative stress damage in renal tissues. Biochemical parameters
such as urea, uric acid and creatinine levels obtained through this study is tabulated in Table 7. In this study, APAP induced nephrotoxicity demonstrates a significant (p<0.01) increase in the serum urea and creatinine concentrations in Group II (APAP induced) rats when compared to the normal group (Group I). In addition, oral administration of ethanol extract of Melia Azadirachtah has significantly (p<0.01) decreased the concentration of urea and creatinine in Group III, IV and V respectively, when compared with the p values of Group II. However the level of uric acid is significantly decreased (P<0.01) in the Group II rats when compared to Group I. Oral administration of plant extract significantly (P<0.01) increases the uric acid level in Group III, IV and V respectively, when compared to the APAP induced rats of Group II. The above discussion can also be seen from the pictorial representations of the three phytochemical parameters, Urea, Uric acid and Creatinine in figures 15 and 16.

Rats treated with plant extract alone (group V) did not show any significant effect on serum urea, uric acid and creatinine levels when compared to control. The activities of serum Urea and creatinine were found to be significantly increased (P<0.01) in APAP intoxicated rats (Group II) and were brought back to near normal significantly (P<0.01 and P<0.001; Groups VI and VII) upon BF pretreatment.
Table 7: Effect of treatment with ethanol extract of MA & BF on the serum Urea (mM/L), uric acid (µM/L) and creatinine levels (µM/L) in rats with APAP-induced nephrotoxicity

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 Control (NaCl 0.9% w/v)</th>
<th>Group 2 APAP (750 mg/kg)</th>
<th>Group 3 MA (250 mg/kg) + APAP (750 mg/kg)</th>
<th>Group 4 MA (500 mg/kg) + APAP + 750 mg/kg</th>
<th>Group 5 MA only (500 mg/kg)</th>
<th>Group 6 BF (250 mg/kg) + APAP (750 mg/kg)</th>
<th>Group 7 BF (500 mg/kg) + APAP (750 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>6.62±0.44</td>
<td>12.32±0.32a,**</td>
<td>10.28±0.22b,*</td>
<td>8.34±0.22b,**</td>
<td>7.43±0.14</td>
<td>10.32±0.51 b,**</td>
<td>8.07±0.61 b,**</td>
</tr>
<tr>
<td>Uric acid</td>
<td>124.64±4.22</td>
<td>113.43±6.54b,**</td>
<td>109.74±8.02b,*</td>
<td>123.16±8.12b,**</td>
<td>125.62±8.22</td>
<td>114.22±6.64b,**</td>
<td>121.68±7.24b,**</td>
</tr>
<tr>
<td>Creatinine</td>
<td>64.52±2.12</td>
<td>94.88±6.14a,**</td>
<td>82.51±6.12b,*</td>
<td>80.63±2.52b,**</td>
<td>65.22±2.72</td>
<td>80.42±6.04b,**</td>
<td>75.65±4.62b,**</td>
</tr>
</tbody>
</table>

Values are expressed mean ± S.D for six rats in each group. a As compared with control, b As compared with APAP, ** represents P<0.001, * represents P<0.01
The levels of uric acid (UA) decreased significantly when compared with control, in APAP treated animals. However on treatment with BF extract at dose level of 250 mg and 500 mg, the level of uric acid increased significantly P<0.001 in Group VI and VII respectively.

Figure 15: Effect of treatment with ethanol extract of MA & BF on the, blood Urea (UR; mM/L) level, in rats with APAP-induced nephrotoxicity

Data are expressed as mean ± S.D., (n = 6). a As compared with control, b As compared with APAP, **represents P<0.001 , *represents P<0.01.
Figure 16: Effect of treatment with MA & BF on the serum uric acid (µM/L) and creatinine levels (µM/L) in rats with APAP-induced nephrotoxicity

Data are expressed as mean ± S.D., (n = 6). a As compared with control, b As compared with APAP, **represents P<0.001, *represents P<0.01.

6.4 EFFECT OF THE MA AND BF EXTRACT ON KIDNEY - ANTIOXIDANT STATUS

The oxidative stress in the renal tissue was assessed by measuring the levels of thiobarbituric acid reactive substances (TBARS), lipid hydro peroxides and antioxidant defense enzymes viz., Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx) and Glutathione-S- transferase (GST) in APAP administrated as well as plant extract treated groups.
Due to the improved lipid peroxidation or inactivation of the antioxidative enzymes because of overdose of APAP, it has been observed that activities of SOD, CAT, and GSH have been drastically reduced when compared with normal control rats. During kidney injury, superoxide radicals are generated at the site of damage and modulate SOD and CAT, resulting in a loss of activity and accumulation of superoxide radical, which damages the kidney. SOD and CAT are the most important enzymes involved in ameliorating the effects of oxygen metabolism (Pande and Flora 2002; Linares et al., 2006).

When the rat was treated with the ethanol extract of MA and BF, the reduction of SOD, CAT, and GSH activity was increased significantly when compared with the induced group \((p < 0.01)\) (group III). Our observations have suggested that intracellular GSH plays an essential role in detoxification of APAP and prevention of APAP-induced toxicity in the liver and kidney (Nelson 1990; Richie et al., 1992; Newton et al., 1996). However, APAP was found to increase the microsomal superoxide and hydrogen peroxide production in mice. The generation of the reactive oxygen species appears as an early event which precedes intracellular GSH depletion and cell damage in APAP nephrotoxicity (Manov et al., 2003). APAP administration also caused a significant decrease in GSH content. Administration of ethanol extract of MA and BF helped to uplift the GSH depletion induced by APAP.
Table 8: Effect of treatment with ethanol extract MA & BF the renal intracellular GSH activity (µg/mg protein), GPx (nmol of GSH oxidized/min/mg protein), CAT (U/mg protein), SOD (units/mg protein) & TBARS (nM/mg of protein) in rats with APAP-induced nephrotoxicity

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 Control (NaCl 0.9% w/v)</th>
<th>Group 2 APAP (750 mg/kg)</th>
<th>Group 3 MA (250mg/kg) + APAP (750 mg/kg)</th>
<th>Group 4 MA (500 mg/kg) + APAP (750 mg/kg)</th>
<th>Group 5 MA (500 mg/kg) only</th>
<th>Group 6 BF (250 mg/kg) + APAP (750mg/kg)</th>
<th>Group 7 BF (500 mg/kg) + APAP (750mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH</td>
<td>10.32±0.62</td>
<td>4.34±0.16 a,**</td>
<td>7.62±0.25 b,*</td>
<td>9.68±0.25 b,**</td>
<td>10.06±0.24</td>
<td>7.58±0.23 b,*</td>
<td>8.54±0.28 b,**</td>
</tr>
<tr>
<td>SOD</td>
<td>4.02±0.04</td>
<td>3.62±0.19 a,**</td>
<td>3.80±0.30 b,*</td>
<td>3.74±0.23 b,**</td>
<td>3.54±0.16</td>
<td>3.56±0.25 b,*</td>
<td>3.34±0.31 b,**</td>
</tr>
<tr>
<td>GPx</td>
<td>9.74±0.61</td>
<td>7.42±0.14 a,**</td>
<td>8.22±0.54 b,*</td>
<td>9.19±0.36 b,**</td>
<td>9.21±0.32</td>
<td>8.22±0.32 b,*</td>
<td>9.76± 0.15 b,**</td>
</tr>
<tr>
<td>CAT</td>
<td>26.32±2.42</td>
<td>9.04±0.44 a,**</td>
<td>19.38±1.15 b,*</td>
<td>24.24±1.06 b,**</td>
<td>25.26±1.16</td>
<td>20.08±0.42 b,*</td>
<td>25.06±1.23 b,**</td>
</tr>
<tr>
<td>TBARS</td>
<td>130.82±4.62</td>
<td>194.26±10.26 a,**</td>
<td>165.52± 6.44 b,*</td>
<td>137.65±5.52 b,**</td>
<td>129.16± 4.52</td>
<td>161.52±6.52 b,*</td>
<td>136.76± 6.53 b,**</td>
</tr>
</tbody>
</table>

Values are expressed mean ± S.D for six rats in each group. a As compared with control, b As compared with APAP, ** represents P<0.001, * represents P<0.01.
Renal SOD activity was decreased significantly (P<0.001) in the APAP treated (group II) animals as compared to the normal group. Treatment with the extract (250 & 500 mg/kg) (Groups III, IV) significantly (P<0.01 & P<0.001 respectively) raised the SOD levels as compared to the APAP induced (Group II) animals. A similar behavior was observed when BF extract was treated on the animal, which significantly (P<0.01 & P<0.001 respectively) change in the SOD levels. The pictorial representation of the same is shown in figure 17.

Figure 17: Effect of treatment with ethanol extract of MA & BF on renal SOD (units/mg protein) activity in rats with APAP-induced nephrotoxicity

Data are expressed as mean ± S.D., (n = 6). a As compared with control, b As compared with APAP, **represents P<0.001 , *represents P<0.01.

The GSH level of APAP and extract treated animals are presented in Table 8. The GSH level reduced significantly (P<0.001) as compared to the Group I. However, on treatment with ethanol extracts of MA and BF, the GSH level was found to be enhanced significantly (P<0.01 & P<0.001), as shown in figure 13.
The decreased GPx activity as a result of the treatment with APAP was also restored by the ethanol extracts of MA and BF (P<0.001) for Groups IV & VII as compared to the normal group.

The activity of CAT in the APAP treated group was significantly (P<0.001) decreased when compared to the normal animals (Group I). Treatment with the ethanol extracts of MA and BF at two different doses (250 mg/kg and 500 mg/kg; (Group III, IV and VI, VII) significantly (P<0.01 & P<0.001) prevented the decrease in the level of catalase activity compared to the APAP induced rat (Group II). Whereas the observation on CAT has shown a distinct difference when treated with extract of MA alone and nearly exhibiting a similar behavior as that of control (Group I) and shown in figure 18.

Figure 18: Effect of treatment with ethanol extract of MA & BF the renal intracellular GSH activity (µg/mg protein), GPx (nmol of GSH oxidized/min/mg protein) & CAT (U/mg protein) in rats with APAP-induced nephrotoxicity

(Data are expressed as mean ± S.D., (n = 6). a As compared with control, b As compared with APAP, ** represents P<0.001 , * represents P<0.01).
The TBARS level was found to be increasing during APAP administration in Group II significantly (P<0.001). However, on treatment with ethanol extracts of MA and BF, the TBARS level was found to be reduced significantly (P < 0.01 & P <0.001) as shown in figure 19, in Groups III, IV and VI, VII as compared to the induced group (Group II).

![Figure 19](image_url)

**Figure 19:** Effect of treatment with MA & BF on the renal TBARS (nM/mg of protein) level in rats with APAP-induced nephrotoxicity

Data are expressed as mean ± S.D., (n = 6). a As compared with control, b As compared with APAP, **represents P<0.001, represents P<0.01.

Rats treated with plant extract of MA alone (group 5) has shown similar effect on SOD, CAT, GSH, GPx and TBARS levels when compared to control (Group I).
## 6.5 EFFECT OF ETHANOLIC EXTRACT OF MA AND BF ON HEMATOLOGICAL PARAMETERS

Table 9: Effect of treatment with ethanolic extract of MA & BF on the blood hematological parameters MCH (pg), MCHC (g/dl), Gran (%), MCV (femto litre/cell), PCV (%), PLC ($\times 10^3/\mu L$) & TLC ($\times 10^3/\mu L$) in rats with APAP-induced nephrotoxicity

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 Control (NaCl 0.9% w/v)</th>
<th>Group 2 APAP (750mg/kg)</th>
<th>Group 3 MA (250mg/kg) + APAP (750mg/kg)</th>
<th>Group 4 MA (500mg/kg) + APAP (750mg/kg)</th>
<th>Group 5 MA (500mg/kg) only</th>
<th>Group 6 BF (250mg/kg) + APAP (750mg/kg)</th>
<th>Group 7 BF (500mg/kg) + APAP (750mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCH</td>
<td>20.23±1.65</td>
<td>16.26±3.45 a,**</td>
<td>18.53±1.32 b,**</td>
<td>15.66±2.62 b,**</td>
<td>20.43±3.43</td>
<td>18.12±2.76 b,*</td>
<td>15.23±3.18 b,**</td>
</tr>
<tr>
<td>MCHC</td>
<td>35.06±4.64</td>
<td>27.67±3.34 a,**</td>
<td>29.14±2.08 b,*</td>
<td>31.43±4.32 b,**</td>
<td>33.12±4.23</td>
<td>30.28±4.32 b,*</td>
<td>31.34±5.23 b,**</td>
</tr>
<tr>
<td>Gran</td>
<td>16.27±1.46</td>
<td>10.89±0.84 a,**</td>
<td>11.43±2.32 b,*</td>
<td>14.32±2.32 b,**</td>
<td>15.52±2.54</td>
<td>12.12±1.87 b,*</td>
<td>15.76±2.29 b,**</td>
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<tr>
<td>PLC</td>
<td>485.52±26.36</td>
<td>406.12±23.75 a,**</td>
<td>445.87±32.32 b,*</td>
<td>458.32±28.45 b,**</td>
<td>480.32±25.32</td>
<td>440.32±22.28 b,*</td>
<td>462.87±25.32 b,**</td>
</tr>
<tr>
<td>MCV</td>
<td>59.26±2.48</td>
<td>61.76±3.25 a,**</td>
<td>56.22±3.56 b,*</td>
<td>52.09±5.22 b,**</td>
<td>53.28±3.28</td>
<td>50.34±4.16 b,*</td>
<td>48.34±3.56 b,**</td>
</tr>
<tr>
<td>PCV</td>
<td>62.96±2.26</td>
<td>55.87±2.86 a,**</td>
<td>58.12±3.45 b,*</td>
<td>56.82±3.12 b,**</td>
<td>61.25±4.45</td>
<td>58.89±3.28 b,*</td>
<td>55.76±4.72 b,**</td>
</tr>
</tbody>
</table>

Values are expressed mean ± S.D for six rats in each group. a As compared with control, b As compared with APAP, ** represents P<0.001, * represents P<0.01.
The hematological parameter, MCH, when induced with APAP has been reduced. But when treated with different doses (250mg/kg, 500mg/kg) of ethanolic extract of MA along with APAP, has found to be increasing in Group III and Group IV significantly (P<0.001 and P<0.01). When the rats are treated with Ethanol extract of MA alone in Group V has exhibited a similar activity as that of control, as shown in Table 9 and in figure 20. MCH in Group VI with dose of ethanolic extract of BF with APAP has been enhanced than Group II significantly (P<0.001).

![Figure 20: Effect of treatment with ethanol extract of MA & BF on the blood Hematological parameters [MCH (pg), MCHC (g/dl) & Gran (%), in rats with APAP-induced nephrotoxicity](image)

Data are expressed as mean ± S.D., (n = 6). a As compared with control, b As compared with APAP, **represents P<0.001, *represents P<0.01.

Further, in the APAP treated group (Group II), the level of MCHC has decreased significantly (P<0.001) when compared with control (Group I) (Figure 20). By the administration of ethanolic extract of MA at two different doses (250 & 500 mg/kg) these levels returned to normal significantly (P<0.01 & P<0.001). By the
administration of ethanol extract of BF (250 mg & 500 mg), the MCHC level increased significantly (P<0.001 & P<0.01 respectively) (Figure. 20).

APAP caused a significant (P<0.001) decrease in the Gran levels as given in Table 9 resulting in acetaminophen associated nephropathy. Administration of ethanolic extract of MA significantly (P<0.001) increased the Gran values recorded for APAP nephrotoxicity and also caused a significant (P<0.01) increase in Gran level in APAP and ethanolic extract of BF treated animals (Group VI and VII). These are also inferred from the figure 20.

Figure 21: Effect of treatment with ethanol extracts of MA & BF on the blood Hematological parameter (PLC (×10^3/µL) in rats with APAP-induced nephrotoxicity

Data are expressed as mean ± S.D., (n = 6). a As compared with control, b As compared with APAP, **represents P<0.001 , *represents P<0.01.

APAP induced animals in Group II have shown a decrease in PLC level when compared with control in Group I significantly (P<0.001). When they are
treated with ethanolic extract of MA in doses of 250 & 500 mg/kg with APAP in Group III and IV have found to be increasing with respect to Group II significantly (P<0.001 and P<0.01). PLC in Group IV, where ethanolic extract of MA is administered alone has shown a similar activity with that of control in Group I.

Animals administered with ethanolic extract of BF in doses of 250 & 500 mg/kg with APAP in Group VI and VII also found to be increasing significantly when compared with group II (P<0.001 and P<0.01) as shown in figure 21.

Further, in the APAP treated group (Group II), the level of MCV increased significantly (P<0.001) when compared with control (Group I). However, the administration of ethanolic extract of MA (Groups III & IV) reversed the significant (P<0.001) increase in MCV levels as given in Table 9 and represented in figure 22. By the administration of ethanol extract of BF at two different doses (250 & 500 mg/kg) these levels returned to normal significantly (P<0.01 & P<0.001) in Group VI and VII.

APAP caused a significant (P<0.001) decrease in the PCV levels (Figure. 22) resulting in acetaminophen induced nephrotoxicity. Administration of ethanolic extract of MA significantly (P<0.001) increased the PCV values recorded for APAP nephrotoxicity and also caused a significant (P<0.01) increase in PCV levels in APAP treated animals (Group VI and II) when treated with BF
Effect of treatment with ethanol extracts of MA & BF the blood hematological parameter MCV (femtolitre/cell) & PCV (%) in rats with APAP-induced nephrotoxicity

Data are expressed as mean ± S.D., (n = 6). a As compared with control, b As compared with APAP, **represents P<0.001, *represents P<0.01.

DISCUSSION

Acetaminophen overdose is often linked to many metabolic disorders including serum electrolyte, urea and creatinine dearrangements. Increased concentration of serum urea and creatinine are considered for investigating drug induced nephrotoxicity in animals and man (Rai et al., 2006). The reason behind acetaminophen toxicology is the CYP-mediated conversion of acetaminophen to a highly reactive quinone imine, N-acetyl-p-benzoquinone imine. The fundamental role of NAPQI in the toxicity of acetaminophen has been supported by many subsequent studies (Dahlin et al., 1984, Streeter et al., 1984, Lowry et al., 1951). Blood urea nitrogen is found in the liver protein that is derived from diet or tissue sources and is
normally excreted in the urine. In renal disease, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance (Mayne et al., 1994). Elevation of urea and creatinine levels in the serum was taken as the index of nephrotoxicity (Anwar et al., 1999, Bennit et al., 1982, Ali et al., 2001). Creatinine, on the other hand, is mostly derived from endogenous sources by tissue creatinine breakdown (Mayne et al., 1994).

Thus serum urea concentration is often considered a more reliable renal function predictor than serum creatinine. In the present study, administration of APAP to rats resulted in nephrotoxic condition and development of oxidative stress damage in renal tissues. In this study, APAP induced nephrotoxicity showed a significant (P<0.01) increase in the serum urea and creatinine concentrations in group 2 (APAP induced) rats when compared to the normal group (Group 1).

Oral administration of plant extract significantly (P<0.001) increased the uric acid level in groups 3 and 4 respectively, when compared to the APAP induced rats (Group 2).

Thus, oxidative stress and lipid peroxidation are early events related to radicals generated during the hepatic metabolism of APAP. Also the generation of reactive oxygen species has been proposed as a mechanism by which many chemicals can induce nephrotoxicity (Somani et al., 2000). Previous studies have clearly demonstrated that acute APAP overdose increases the lipid peroxidation and suppresses the antioxidant defense mechanisms in renal tissue (Abdel-Zaher et al., 2007). However, in the APAP treated animals, the TBARS levels are increased significantly, when compared to control rats. On administration of ethanol extract of MA and BF, the levels of TBARS decreased significantly when compared to APAP induced rats.
During kidney injury, superoxide radicals are generated at the site of damage and modulate SOD and CAT, resulting in the loss of activity and accumulation of superoxide radical, which damages kidney. SOD and CAT are the most important enzymes involved in ameliorating the effects of oxygen metabolism (Linares et al., 2006). The present study also demonstrated that acute APAP overdose resulted in a decrease in the SOD, CAT and GST activities, when compared to control rats. It is due to enhanced lipid peroxidation or inactivation of the antioxidative enzymes. When the rats were treated with the ethanol extract of MA and BF, the reduction of SOD, CAT and GST activity increased significantly when compared to the induced group (P<0.001) (Group 2).

Current evidence suggests that intracellular GSH plays an essential role in detoxification of APAP and prevention of APAP-induced toxicity in the liver and kidney (Newton et al., 1996, Richie et al., 1992). However, APAP was found to increase the microsomal superoxide and hydrogen peroxide production in mice. The generation of the reactive oxygen species appears as an early event which precedes intracellular GSH depletion and cell damage in APAP hepatotoxicity (Manov et al., 2003). APAP administration also caused a significant decrease in GSH content. Administration of ethanol extract of MA and BF helped to increase the GSH depletion induced by APAP.

Other nephroprotective medicinal plants have been reported to inhibit xenobiotic-induced nephrotoxicity in experimental animal models due to their potent anti-oxidant or free radicals scavenging effects (Devipriya et al., 1999, Annie et al., 2005). In addition, alkaloids have also been reported to strongly inhibit lipid peroxidation induced in isolated tissues via its antioxidant activity (Kumaran et al., 2007). The protection offered by the extract could have been due to the presence of flavonoids and alkaloids (Lucia et al., 2007).
The activity elicited by the extract might be due to its ability to activate antioxidant enzymes. The findings suggest the potential use of the ethanol extract of MA as a novel therapeutically useful nephroprotective agent. Therefore, further studies to elucidate their mechanisms of action should be conducted to aid the discovery of new therapeutic agents for the treatment of renal diseases.

The various blood cells (erythrocytes, leucocytes, and platelets) are produced at a turnover rate of about 1 to 3 million per second in a healthy human adult and this value could be altered in certain physiological or pathological states including hemolytic anemia or suppressive inflammation (Guyton, 1991). Certain drugs including alkylating cytotoxic agents could also affect blood formation rate and the normal range of hematological parameters (Adeneye et al., 2008). Treatment with APAP oral dose significantly increased the MCV level. After administration of MA and BF extract this level decreased significantly compared to the APAP induced group, whereas the levels of granulocyte, PCV, MCH, MCHC, Gran and PLC were decreased significantly in the APAP treated group, compared to the control group. However after administration of MA and BF extract these levels are significantly increased compared to the APAP treated group. However this study shows that the BF extract could contain candidate molecules reversing the hematotoxic effect of acetaminophen, with ensuing improvement of hematopoiesis.

The recorded hematotoxicity could be secondary to the deleterious effect of acetaminophen on organs of hematopoeisis in the body which include liver and kidneys. Literature has shown acute or chronic large dose acetaminophen to be associated with overproduction of a highly reactive intermediate, N-acetyl-\(p\)-benzoquinone-imine (NAPQI), which covalently bound to macromolecules of renal tissues (Prescott, 1980; Fored et al., 2001) resulting in acetaminophen-associated nephropathy (Emeigh Hart et al., 1996).
However, oral treatment with MA and BF extract reversed the significant decrease in the PCV, PCV, MCH, MCHC and Gran value recorded for acetaminophen hematotoxicity and also have caused a significant (P<0.001, P<0.001) dose related increase in the MCV. This study showed that the extract could contain active biological principle(s) reversing the hematotoxic effect of acetaminophen, with subsequent enhancement of hematopoiesis. The biological principle(s) could also be mediating hematopoietin-like effect or enhancing the release of hematopoietin from hematopoetic organs such as the kidneys or liver.

6.6 HISTOPATHOLOGICAL STUDIES OF APAP INDUCED NEPHROTOXICITY IN ALBINO RATS AFTER TREATMENT WITH ETHANOLIC EXTRACTS OF MELIA AZADIRACHTA (MA) AND BORRASSUS FIABELLIFER (BF)

The biochemical results were also confirmed by the histological pattern of normal kidney showing normal tubular brush borders and intact glomeruli and Bowman’s capsule [Plate 1.A]. Severe tubular necrosis and degeneration is shown in the renal tissue on treatment with acetaminophen [Plate 1.B]. The rats treated with ethanolic extract of Melia Azadirachta (250 mg/kg body weight) with APAP (750 mg/kg) showed normal tubular pattern with a mild degree of swelling, necrosis and degranulation [Plate 1.C]. Treatment with the ethanolic extract of MA (500 mg/kg body weight) with APAP 750 mg/kg of ameliorated the toxic manifestations in the kidney [Plate 1.D].
Plate 1.A: Normal kidney of rat

Plate 1.B: APAP (750 mg/kg) induced
Plate 1.C: APAP (750 mg/kg) + MA (250mg/kg)

Plate 1.D: APAP (750 mg/kg) + MA (500mg/kg)
Rats administered with MA (500 mg/kg) alone did not show any significant changes in the renal tissues [Plate 1.E].

The rats treated with ethanol *Borrassus Fiabellifer* (BF) extract (250 mg/kg body weight) showed normal tubular pattern with a mild degree of swelling, necrosis and degranulation [Plate 2(A)]. Treatment with the ethanolic extract of BF (500 mg/kg body weight) ameliorated the toxic manifestations in the kidney [Plate 2(B)]. APAP-induced nephrotoxicity was evidenced by biochemical measurements and histopathological changes that coincide with the observations of other investigators (Gardner *et al.*, 2002; Newton *et al.*, 1983; Trumper *et al.*, 1998). The biochemical results were also confirmed by the histological findings which showed preservation of the glomeruli and the surrounding Bowman’s capsule and mildly swollen tubules. Other nephroprotective medicinal plants have been reported to inhibit xenobiotic-induced nephrotoxicity in experimental animal models due to their potent anti-oxidant or free radicals scavenging effects (Devipriya *et al.*, 1999, Annie *et al.*, 2005).
Plate 2.A: APAP (750 mg/kg) + BF (250mg/kg)

Plate 2.B: APAP (750 mg/kg) + BF (500mg/kg)