CHAPTER 5

HEPATOPROTective ACTIVITY OF MUCUNA PRURIENS AND PHYLLANTHUS NIRURI ON ACETAMINOPHEN INTOXICATED RATS
Use of herbal drugs in the treatment and management of liver has a long tradition. Natural remedies represent $1.8 billion market in the United States, and a single herbal preparation, silymarin, which is used almost exclusively for liver diseases, amounts to $180 million in Germany alone [1]. Marketing of herbals tripled between 1992 and 1996 [1], and nearly a third of outpatients attending liver clinics use these products [2]. This is reflected in the internet home pages of hepatitis foundations. Herbal products have been classified as food supplements and thus are exempt from regulations on quality control and proof of efficacy that governs standard pharmaceuticals.

Liver disease, especially viral hepatitis, occurs predominantly in the developing world [3] with an enormous impact on both public health and economics. Liver plays an important role in drug elimination and detoxification, but in turn, it can be subject to damage by xenobiotics. Liver damage may also be caused by alcohol consumption, malnutrition, infection, anemia and certain medications. Currently available drugs have little effect on the treatment of liver illness, which creates a demand to develop new drugs. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices and in traditional system of medicine in India. However, we do not have satisfactory remedy for serious liver disease; most of the herbal drugs speed up the natural healing process of liver. So the search for effective hepatoprotective drug continues.

Aerobic organs such as the liver employ a battery of defence mechanisms, such as antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)) to prevent or mitigate oxidative tissue damage. These radicals, which react with cell membranes and thus induced lipid peroxidation or cause inflammation, have been implicated as important pathological mediators in many clinical disorders such as heart disease, diabetes, gout and cancer [4-6]. A major defence mechanism is the antioxidant enzymes, which convert active oxygen molecules into non-toxic compounds [7,8].

5.1. MATERIALS AND METHODS

5.1.1. TEST COMPOUNDS
The methanol extracts of the seeds of *Mucuna pruriens* (*MEMP*) and *Phyllanthus niruri* (*MEPN*) at the doses of 125mg and 250mg/kg b.w.

### 5.1.2. ANIMALS

Male Wistar Albino rats weighing 150 – 175g was used in the present investigation and they were procured from Ghosh Enterprises, Kolkata (W.B.), India. The animals were fed standard pellet diet, Hindustan Lever Ltd., Kolkata (W.B.), and water *ad libitum*. All the animals were acclimatized for a week before starting the experiment.

### 5.1.3. EXPERIMENTAL PROCEDURE

Healthy Albino rats were divided into seven groups of six animals each (*n = 6*).

- **Group I**, received normal saline only (0.9%NaCl w/v, 5ml/kg).
- **Group II**, received Acetaminophen (500mg/kg dissolved in 10% PEG, i.p.)
- **Group III**, received Acetaminophen (500mg/kg) + *MEMP* (125mg/kg)
- **Group IV**, received Acetaminophen (500mg/kg) + *MEMP* (250mg/kg)
- **Group V**, received Acetaminophen (500mg/kg) + *MEPN* (125mg/kg)
- **Group IV**, received Acetaminophen (500mg/kg) + *MEPN* (250mg/kg) and
- **Group VII**, received Acetaminophen (500mg/kg) + Silymarin (25mg/kg, suspended in 1% CMC, i.p.). for 7 days [9].

### 5.1.4. BIOCHEMICAL ESTIMATIONS

The blood was obtained from all animals by puncturing retroorbital plexus. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and utilized for the estimation of various biochemical parameters like SGOT [10], SGPT [10], ALP [11], Serum bilirubin [12] uric acid [13] and total proteins [14].

#### 5.1.4.1. Estimation of Serum Glutamate Oxaloacetate Transaminase (SGOT)

**Reagents:**

a) Substrate buffer:

\[ K_2HPO_4 \text{------------- 1.0g} \]
KH$_2$PO$_4$ -------------- 0.2g  
Oxoglutaric acid ------- 0.03g  
L-Aspartic acid --------- 1.78g  
GDW --------------------- 100ml  
pH adjusted to 7.4 with 0.4N NaOH.

b) 0.4N NaOH:

1.6g of NaOH was dissolved in 100ml GDW.

c) Ketone reagent:

0.02g of 2,4-dinitrophenylhydrazine in 100ml N HCl.

d) Standard Oxaloacetic acid:

Stock standard – 0.0264g of oxaloacetic acid in 100ml buffer solution.

Buffer solution: KH$_2$PO$_4$ ------- 2.69g  
K$_2$HPO$_4$ ------- 13.97g  
GDW ---------- 1000ml  

Working standard – 10ml Stock standard in 100ml buffer solution.

**Procedure:**

Preparation of diluted serum: Fresh blood was allowed to clot and serum was collected. Serum was diluted with physiological saline in 1:10 ratio.

0.2-MI diluted serum was taken in a tube marked ‘Sample’ and to it 1.0ml substrate buffer solution was added, mixed properly and incubated at 37°C for 1h. In a tube marked ‘Blank’, 0.2ml diluted serum was taken and to it 1.0ml substrate buffer solution was added. Mixed well. No incubation was done. In tubes marked ‘Standard’, different volumes of working standard solution were taken viz., 0.0ml, 0.1ml, 0.2ml, and 0.3ml. Then GDW were added to make up the volume of each tube containing 2.2ml. To the ‘Sample’, ‘Blank’, and ‘Standard’ tubes 1.0ml ketone reagent was added and kept at room temperature for 15 minutes. To tubes ‘Sample’ and ‘Blank’, 5.0ml and to ‘Standard’ 10ml of 0.4 N NaOH was added respectively, mixed thoroughly and kept at room temperature for 20 minutes. Optical densities of samples and standards were measured at 530nm setting the Spectrophotometer ‘0’ with blank. A standard curve was plotted with standard values and the unit of enzyme present per 100ml of serum sample was calculated from the curve.

5.1.4.2. Estimation of Serum Glutamate Pyruvate Transaminase (SGPT)
Reagents:

a) Substrate buffer:

- K$_2$HPO$_4$ ------------ 1.0g
- KH$_2$PO$_4$ ------------ 0.2g
- Oxoglutaric acid ------- 0.03g
- DL-Alanine ------------ 1.78g
- GDW ----------------- 100ml

b) 0.4N NaOH:

- 1.6g of NaOH was dissolved in 100ml GDW.

c) Ketone reagent:

- 0.02g of 2,4-dinitrophenylhydrazine in 100ml N HCl.

d) Standard Oxaloacetic acid:

- Stock standard – 0.0264g of oxaloacetic acid in 100ml buffer solution.
- Buffer solution: KH$_2$PO$_4$ --------- 2.69g
  - K$_2$HPO$_4$ ------- 13.97g
  - GDW ---------- 1000ml

- Working standard – 10ml Stock standard in 100ml buffer solution.

Procedure:

In a tube marked ‘Sample’ 1.0ml substrate buffer solution was taken and to it 0.2ml diluted serum (prepared as in SGOT) was added, mixed by inversion and incubated at 37°C for 1h. In a tube marked ‘Blank’, 0.2ml diluted serum was taken and to it 1.0ml substrate buffer solution was added. Mixed well. No incubation was done. In tubes marked ‘Standard’, different volumes of working standard solution were taken viz., 0.0ml, 0.1ml, 0.2ml, and 0.3ml. Then GDW were added to make up the volume of each tube containing 2.2ml. To the ‘Blank’, ‘Sample’ and ‘Standard’ tubes, 1.0ml ketone reagent was added and kept at r.t. for 15min. To tubes ‘Sample’ and ‘Blank’, 5.0ml; and to ‘Standard’ 10ml of 0.4N NaOH was added, mixed thoroughly and kept at room temperature for 20 minutes. Optical densities of samples and standards were measured at 530nm setting the Spectrophotometer ‘0’ with blank. A standard curve was plotted with standard values and the unit of enzyme present per 100ml of serum sample was calculated from the curve.

5.1.4.3. Estimation of Serum alkaline phosphatase (SALP)
-nitrophenyl phosphate used as the substrate for the determination of ALP activity. The enzymatic product liberated is -nitrophenol, which in the presence of NaOH forms a yellow anion.

Reagents

a) Tris-buffer --------------- 500ml
b) -nitrophenyl phosphate solution – 1.0ml
c) NaOH (3N) --------------- 100ml

Procedure

Two tubes were marked ‘Control’ and ‘Sample’. To both the tubes 0.1ml -nito phenyl phosphate and 0.8ml Tris-buffer were added and incubated at 37°C for 10 minutes. Preparation of diluted serum: 0.1ml serum was diluted in 0.5ml GDW. 0.1ml of diluted serum was added to ‘Sample’ tube and again incubated at 37°C for 30 minutes. To both ‘Sample’ and ‘Control’ tubes 0.5ml (3N) NaOH was added. Then to ‘Control’ tube 0.1ml diluted serum and 1.4ml of Tris-buffer were added. A blank was prepared with 0.1ml diluted serum and 1.4ml of Tris-buffer. Contents of each tube were properly mixed and optical densities were measured at 405nm in the Spectrophotometer against blank and results were calculated.

5.1.4.4. Estimation of Serum bilirubin

Total serum bilirubins consists of conjugated (mostly with glucuronic acid) and free form. Conjugated and total serum bilirubin concentration was measured. The unconjugates bilirubin content was determined by subtracting conjugated bilirubin from total bilirubin.

Reagents

a) Diazoo reagent:
   Solution (i): Sulphanilic acid ------ 1g
   Conc. H₂SO₄ ------- 15ml
   GDW up to ----------- 1000ml
   Solution (ii): Sodium nitrite ------- 0.5g
   GDW up to ----------- 10ml
   Solution (i) and (ii) were mixed just before the experiment.

b) Diazoo blank: 1.5ml HCl
c) Absolute methanol
d) Standard bilirubin:

Stock standard solution: 1mg in 10ml CHCl₃. Stored in dark at 0°C.

Working standard solution: 5ml of stock was diluted to 10ml with methanol.

**Procedure**

Four test tubes were marked ‘Sample’, ‘Sample control’, ‘Standard’ and ‘Standard control’. Preparation of ‘Sample’ and ‘Sample control’: In both the tubes 2.7ml of GDW and 0.1ml serum was added. 0.7ml of diazo blank were added respectively in ‘Sample’ and ‘Sample control’ tubes. Preparation of ‘Standard’ and ‘Standard control’: In both the tubes 2.7ml of GDW and 0.1ml of working standard solution were added. 0.7ml of diazo blank were added in ‘Standard’ and ‘Standard control’ tubes respectively. Solutions of all the tubes were mixed thoroughly and kept in dark for 30min. Optical densities of ‘Standard’, ‘Standard control’, ‘Sample’ and ‘Sample control’ were measured within 10min by setting the Spectrophotometer with water (used as blank) at 540nm.

5.1.4.5. Estimation of Serum total proteins

**Reagents**  

a) CuSO₄ reagent:

Solution (i): Na₂CO₃ -------- 20g  
NaOH -------------- 4g  
Rochelle salt ------- 0.2g

Solution (ii): CuSO₄.5H₂O ------ 0.5%  
50ml of solution (i) and 1.0ml of solution (ii).

b) Folin-Ciocalteau phenol reagent: diluted with equal volume (1:1) of water.

c) Bovine serum albumin (BSA): 1mg/ml.

**Procedure**

Two tubes were taken and marked ‘Sample’ and ‘Standard’. To these tubes 0.2ml of serum and 0.2ml of standard solution (BSA) were added respectively. In another tube marked ‘Blank’, 1.0ml GDW was taken. In tubes, ‘Sample’ and ‘Standard’, 0.8ml of GDW was added. In all the three tubes 4.0ml of CuSO₄ reagent was added and the tubes were kept at r. t. for 10min. 0.5ml Folin reagent was added in all the tubes and the tubes were kept in dark for 30min. Optical densities were measured at 660nm. The amount or proteins present in the sample was calculated and expressed as g of protein/100ml of serum.

5.1.4.6. Estimation of Serum uric acid
The serum uric acid was measured by the method of Oser.

Reagents

a) Tungstic Acid Reagent:
   - Na-tungstate -------------------- 50 ml
   - Sulphuric Acid ------------------- 50 ml
   - A few drops of Phosphoric Acid
   - GDW ----------------------------- 800 ml

b) Phosphotungstate Reagent:
   - Stock Solution: 50g Na-Tungstate, 400ml GDW and 40ml ortho-H$_3$PO$_4$ were refluxed for 2 h. Cooled and transferred to a brown bottle.
     The volume was made up to 500ml with GDW.
   - Working Solution: 10ml stock was diluted to 100ml before use.

c) Na$_2$CO$_3$ -------------------------- 10 %

d) Standard Uric Acid:
   - Stock Standard Solution: 84.1 mg dissolved in 100 ml GDW
   - Working Standard Solution: 0.05, 0.1, 0.15 and 0.2 ml of stock were taken in different tubes and volume was made up to 10ml with GDW.

Procedure

In a tube 9.0ml dilute tungstic acid, 0.1ml serum and 0.9ml GDW were taken. All additions were performed keeping the tube in shaking condition. The tubes were centrifuged. 6ml of supernatant, 6.0ml of working standard solution and 6.0ml GDW were taken in tubes marked “Sample”, “Standard” and “Blank” respectively. In all three tubes 1.2 ml Na$_2$CO$_3$ and 1.2 ml diluted phosphotungstate reagent were added, mixed properly and kept at 25°C for 30 minutes. Optical densities were measured at 700nm within 15 minutes and the amount of uric acid present per sample was calculated from the readings and expressed as mg of uric acid/100ml serum.

5.1.5. Determination of In vivo Antioxidant Activity

After collection of blood samples the rats were sacrificed and their livers excised, and divided into two parts. One part of liver was rinsed in ice cold normal saline followed by 0.15 M Tris-HCl buffer and processed for the estimation of lipid peroxidation [15]. A portion of homogenate after precipitating proteins with Trichloric acetic acid (TCA) was used for the estimation of glutathione [16]. The rest of the homogenate was centrifuged at
15000 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of SOD [17] and CAT [18] activities. Another part of the liver was used for histopathology.

5.1.5.1. Determination of Lipid peroxidation

Lipid peroxidation was estimated by the method of Fraga et al. This represents the in vivo lipid peroxides formed in the tissues.

Briefly, to 0.5mL tissue homogenate, 0.5mL saline and 1.0mL 10% TCA were added, mixed well and centrifuged at 3000 rpm for 20 minutes. To 1.0mL of the protein-free supernatant, 0.25mL of thiobarbituric acid (TBA) reagent was added, the contents were mixed well and boiled for 1 h at 95°C. The tubes were then cooled to room temperature under running water and absorption measured at 532nm.

5.1.5.2. Determination of reduced GSH

The tissue GSH was determined by the method of Beutler and Kelly. Virtually all the non-protein sulfhydryl groups of tissues are in the form of reduced GSH.

Briefly, 0.2 ml of tissue homogenate was mixed with 1.8 ml of EDTA solution. To this 3.0 ml precipitating reagent (1.67g of metaphosphoric acid, 0.2g of EDTA disodium salt, 30g sodium chloride in 1000ml of distilled water) was added, mixed thoroughly and kept for 5 min before centrifugation. To 2.0ml of the filtrate, 4.0ml of 0.3-M disodium hydrogen phosphate solution and 1.0ml of DTNB (5,5'-dithio bis 2-nitro benzoic acid) reagent were added and read the absorbance at 412nm.

5.1.5.3. Assay of SOD

The activity of SOD in tissue was assayed by the method of Kakkar et al. Briefly, the assay mixture contained 1.2ml sodium pyrophosphate buffer (pH 8.3, 0.025mol/L), 0.1ml phenazine methosulphate (186mmol/L), 0.3ml NBT (300mmol/L), 0.2ml NADH (780mmol/L) and approximately diluted enzyme preparation and water in a total volume of 3ml. After incubation at 30°C for 90 sec, the reaction was terminated by the addition of 1.0ml of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4.0ml n-butanol. The color intensity of the chromogen in the butanol layer was measured at 560nm against n-butanol.

5.1.5.4. Assay of CAT

Catalase was assayed according to the method of Maehly and Chance. Briefly, the estimation was done spectrophotometrically following the decrease in absorbance at 230nm.
The tissue was homogenized in M/150 phosphate buffer (pH 7.0) at 1–4°C and centrifuged at 5000 rpm. The reaction mixture contained 0.01 M phosphate buffer (pH 7.0), 2mM H₂O₂ and the enzyme extract. The specific activity of catalase is expressed in terms of units/mg protein. A unit is defined as the velocity constant per second.

5.1.6. **HISTOPATHOLOGY**

Initially the liver samples were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h. Paraffin sections were taken at 5mm thickness, processed in alcohol-xylene series and were stained with alum hemotoxylin and eosin [19]. The sections were examined microscopically for histopathological changes.

5.1.7. **STATISTICAL ANALYSIS**

The experimental results were expressed as the mean ± S.D. The level of significance was evaluated using Student’s $t$-test. $P$ value of < 0.05 was considered as statistically significant.

5.2. **RESULTS**

The results of biochemical parameters revealed the elevation of enzyme level in Acetaminophen treated group, indicating that Acetaminophen induces damage of the liver (Table 1). There was a significant ($P < 0.01$) increase in the SGOT, SGPT, SALP, bilirubin and total protein levels and decrease ($P < 0.05$) in the uric acid level in Acetaminophen control animals when compared with normal animals. The enzyme levels were almost restored to more or less normal on administration of *MEMP* and *MEPN* at the doses of 125mg and 250mg/kg (Groups III to VI, Table 1) and Silymarin at the dose of 25mg/kg (Group VII, Table 1).

It was observed that the size of the liver was enlarged in Acetaminophen intoxicated rats but it was brought back to near normal in drug treated groups (Groups III to VII, Table 1). A significant ($P < 0.001$) in liver weight supports this finding.

The effects of *MEMP* and *MEPN* on rat liver lipid peroxidation, glutathione, and antioxidant enzyme (SOD and CAT) levels are shown in Fig. 1 to 4. In our study, significant ($P < 0.01$) elevation in the levels of end products of lipid peroxidation in Acetaminophen-treated animals was observed. The increase in malondialdehyde (MDA) in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of
antioxidant defence mechanisms. Treatment with \textit{MEMP} and \textit{MEPN} at the doses of 125mg and 250mg/kg significantly ($P < 0.05$) prevented the increase in TBARS levels and brought them near to normal level (Tab 2 and Fig 3).

Liver enzymatic and nonenzymatic antioxidant levels were significantly ($P < 0.05$) decreased in Acetaminophen treated rats when compared with normal group. GSH, SOD, and CAT levels were significantly ($P < 0.05$) increased in \textit{MEMP} and \textit{MEPN} treated groups (Fig 1,2 and 3). The effects of \textit{MEMP} and \textit{MEPN} were compared with that of standard reference drug Silymarin.

5.3. DISCUSSION

Acetaminophen, a widely used nonprescription analgesic-antipyretic drug, is safe when used within therapeutic doses. When taken in larger doses, it becomes a potent hepatotoxin, generating fulminated hepatic and renal tubular necrosis which is lethal in humans and many species of animals [20-22]. The laboratory features of hepatotoxicity induced by acetaminophen resemble other kinds of acute necroinflammatory liver disease with prominent increase in serum transaminase enzymes and histological changes [21,22]. The histopathological appearances of the liver biopsy or autopsy revealed a variably extensive centrizonal necrosis without steatosis and with a relatively light inflammatory infiltration [20]. Acetaminophen is primarily metabolized by sulphation and glucuronidation to unreactive metabolites, and then activated by the cytochrome P-450 system to result in liver injury [23]. The characteristic zone 3 necrosis of acetaminophen appears to be produced by an electrophilic metabolite of the drug ($N$-acetyl-$p$-benzoquinonimine, \textit{NAPQI}). \textit{NAPQI} is initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid [24]. However, when the rate of \textit{NAPQI} formation exceeds the rate of detoxication by GSH, \textit{NAPQI} will oxidize tissue macromolecules, such as lipids or protein thiols, and alter the homeostasis of calcium after depleting GSH. Lipid peroxidation has been postulated to be the destructive process in liver injury due to acetaminophen administration [25]. The coincidence of antioxidant activity and liver tissue protective effects after acetaminophen administration suggest that both free radical generation and lipid peroxidation may be involved in this kind of drug injury process.

This present study evaluates the antihepatotoxic effects of \textit{MEMP} and \textit{MEPN} in acetaminophen induced liver toxicity. Acute administration of Acetaminophen produced a
marked elevation of the serum activity enzymes of SGOT, SGPT, SALP, serum bilirubin and total proteins (Group II) when compared with that of the normal group (Group I). Treatment with \textit{MEMP} and \textit{MEPN} at doses of 125mg/kg and 250mg/kg for 7 days significantly reduced the elevated levels of the enzymes.

The elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver [26]. Serum ALP, and bilirubin level on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure [27]. Treatment with \textit{MEMP} and \textit{MEPN} decreased the serum levels of GOT, GPT towards the respective normal value that is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by Acetaminophen. The above changes can be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchyma cells. Effective control of alkaline phosphate (ALP) and bilirubin levels points towards an early improvement in the secretary mechanism of the hepatic cell.

Lipid peroxidation has been postulated as being the destructive process in liver injury due to acetaminophen administration [25]. Present study exhibits the in the levels of TBARS in liver of rat treated with Acetaminophen were observed. The increase in TBARS levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. Treatment with \textit{MEMP} and \textit{MEPN} significantly reversed these changes. Hence it may be possible that the mechanism of hepatoprotection of \textit{MEMP} and \textit{MEPN} is due to its antioxidant effect.

Glutathione is one of the most abundant tripeptide, nonenzymatic biological antioxidant present in the liver. Its functions are concerned with the removal of free radical species such as hydrogen peroxide, superoxide radicals, alkoxy radicals, and maintenance of membrane protein thiols and as a substrate for glutathione peroxidase (GPx) and GST [28]. The present study reveals that the decreased level of GSH has been associated with an enhanced lipid peroxidation in Acetaminophen treated rats. Administration of \textit{MEMP} and \textit{MEPN} significantly increased the level of glutathione in a dose dependent manner. Increase in serum activity of superoxide dismutase (SOD) is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in liver injury [29,30].
SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide, hence diminishing the toxic effect caused by this radical. In the present study, it was observed that the \textit{MEMP} and \textit{MEPN} caused significant increase in the hepatic SOD activity of the paracetamol induced liver damage rats. It means that the \textit{MEMP} and \textit{MEPN} can reduce reactive free radicals that might lessen oxidative damage to the tissues and improve the activities of the hepatic antioxidant enzyme.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissue and the highest activity is found in the red cells and in liver. CAT decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals [31]. Therefore, the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. Administration of \textit{MEMP} and \textit{MEPN} increased the activities of SOD and CAT in Acetaminophen induced liver damage rats to prevent the accumulation of excessive free radicals and protects the liver from Acetaminophen intoxication.

From the above discussion it can be considered that the \textit{MEMP} and \textit{MEPN} exhibit significant antihepatotoxic activity as it reduces cell membrane disturbances induced by Acetaminophen \textit{in vivo}. 
Fig 1: Section of liver tissue of normal control rats (H & E, 100x)

Fig 2: Section of liver tissue of acetaminophen-treated control rats
(H & E, 100x)
Fig 3: Section of liver tissue of methanol extract of *Mucuna pruriens* (MEMP) treated (dose-125mg/kg) rats (H & E, 100x)

Fig 4: Section of liver tissue of methanol extract of *Mucuna pruriens* (MEMP) treated (dose-250mg/kg) rats (H & E, 100x)
Fig 5: Section of liver tissue of methanol extract of *Phyllanthus niruri* (MEPN) treated (dose-125mg/kg) rats (H & E, 100x)

![Image of liver tissue from methanol extract of Phyllanthus niruri treated with 125mg/kg dose.](image)

Fig 6: Section of liver tissue of methanol extract of *Phyllanthus niruri* (MEPN) treated (dose-250mg/kg) rats (H & E, 100x)

![Image of liver tissue from methanol extract of Phyllanthus niruri treated with 250mg/kg dose.](image)
Fig 7: Section of liver tissue of Silymarin (standard) treated (20mg/kg) rats
(H & E, 100x)
Table 1: Effect of methanol extracts of *Mucuna pruriens* (*MEMP*) and *Phyllanthus niruri* (*MEPN*) on biological profile and liver weight in rats

(Values are mean ± S.D., 6 animals in each group.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
<th>Bilirubin (mg%)</th>
<th>Total Protein (mg/dL)</th>
<th>Uric acid (mg/dL)</th>
<th>Liver wt. (wt/100g b.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (NaCl 0.9% w/v), 5ml/kg.</td>
<td>41.12 ± 2.22</td>
<td>45.32 ± 3.88</td>
<td>124.21 ± 1.99</td>
<td>1.21 ± 0.77</td>
<td>6.43 ± 0.34</td>
<td>6.41 ± 0.45</td>
<td>3.81 ± 1.09</td>
</tr>
<tr>
<td>Acetaminophen (500mg/kg)</td>
<td>117.67 ± 4.96</td>
<td>135.11 ± 4.10</td>
<td>173.46 ± 2.01</td>
<td>3.72 ± 0.67</td>
<td>9.80 ± 0.87</td>
<td>8.89 ± 0.91</td>
<td>6.43 ± 0.49</td>
</tr>
<tr>
<td>Acetaminophen + <em>MEMP</em> (500mg/kg + 125mg/kg)</td>
<td>80.83 ± 7.22b</td>
<td>72.45 ± 3.90b</td>
<td>152.77 ± 3.66b</td>
<td>1.59 ± 0.66b</td>
<td>7.11 ± 0.44b</td>
<td>6.50 ± 0.34b</td>
<td>4.66 ± 0.27b</td>
</tr>
<tr>
<td>Acetaminophen + <em>MEMP</em> (500mg/kg + 250mg/kg)</td>
<td>65.33 ± 5.92b</td>
<td>48.44 ± 1.99b</td>
<td>140.05 ± 2.10b</td>
<td>1.00 ± 0.31b</td>
<td>6.49 ± 0.64b</td>
<td>6.44 ± 0.56b</td>
<td>3.83 ± 0.25b</td>
</tr>
<tr>
<td>Acetaminophen + <em>MEPN</em> (500mg/kg + 125mg/kg)</td>
<td>77.15 ± 6.51b</td>
<td>69.83 ± 5.88b</td>
<td>143.22 ± 5.63b</td>
<td>1.62 ± 0.55b</td>
<td>6.98 ± 1.03b</td>
<td>7.22 ± 1.55b</td>
<td>4.14 ± 0.88b</td>
</tr>
<tr>
<td>Acetaminophen + <em>MEMP</em> (500mg/kg + 250mg/kg)</td>
<td>52.33 ± 8.47a</td>
<td>40.08 ± 8.17a</td>
<td>127.68 ± 3.44a</td>
<td>1.12 ± 0.41a</td>
<td>6.11 ± 1.07a</td>
<td>6.30 ± 1.11a</td>
<td>3.22 ± 1.01a</td>
</tr>
<tr>
<td>Acetaminophen + Silymarin (500mg/kg + 25mg/kg)</td>
<td>61.2 ± 4.66b</td>
<td>37.72 ± 6.91b</td>
<td>146.00 ± 5.02b</td>
<td>1.09 ± 0.45b</td>
<td>6.08 ± 0.40b</td>
<td>5.88 ± 0.33b</td>
<td>4.01 ± 0.26b</td>
</tr>
</tbody>
</table>

Values are statistically significant at $^{b}P < 0.05$ compared with control.
Table 2: Effect of methanol extracts of *Mucuna pruriens* (*MEMP*) and *Phyllanthus niruri* (*MEPN*) on TBARS, GSH, SOD and CAT levels in rats

(Values are mean ± S.D., 6 animals in each group.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TBARS (nM MDA/100g protein)</th>
<th>GSH (mg/100mg protein)</th>
<th>SOD (Units/mg protein)</th>
<th>CAT (Units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (NaCl 0.9% w/v), 5ml/kg.</td>
<td>0.91 ± 0.24</td>
<td>44.72 ± 4.02</td>
<td>12.33 ± 0.54</td>
<td>322.83 ± 8.22</td>
</tr>
<tr>
<td>Acetaminophen (500mg/kg)</td>
<td>1.77 ± 0.18</td>
<td>10.61 ± 3.88</td>
<td>2.90 ± 0.66</td>
<td>189.22 ± 11.57</td>
</tr>
<tr>
<td>Acetaminophen + <em>MEMP</em> (500mg/kg + 125mg/kg)</td>
<td>1.25 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.54 ± 5.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.75 ± 0.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>199.45 ± 18.47&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acetaminophen + <em>MEMP</em> (500mg/kg + 250mg/kg)</td>
<td>1.14 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.34 ± 5.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.14 ± 1.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>256.78 ± 22.71&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acetaminophen + <em>MEPN</em> (500mg/kg + 125mg/kg)</td>
<td>1.19 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.43 ± 4.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.54 ± 1.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>215.66 ± 18.92&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acetaminophen + <em>MEMP</em> (500mg/kg + 250mg/kg)</td>
<td>1.10 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.18 ± 5.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.44 ± 1.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>232.31 ± 51.99&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acetaminophen + Silymarin (500mg/kg + 25mg/kg)</td>
<td>0.78 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.87 ± 4.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.58 ± 1.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>279.09 ± 22.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are statistically significant at <sup>b</sup><em>P</em> < 0.05 compared with control.
Fig 1: Effect of methanol extracts *Mucuna pruriens* (MEMP) and *Phyllanthus niruri* (MEPN) on biochemical profile.

- SGOT
- SGPT
- SALP

- Normal
- Acetaminophen (500mg/kg)
- Acetaminophen + MEMP (125mg/kg)
- Acetaminophen + MEPN (125mg/kg)
- Acetaminophen + Silymarin (25mg/kg)

The graph shows the biochemical profiles under different conditions, with significance denoted by letters a and b.
Fig 2: Effect of methanol extracts of *Mucuna pruriens* (MEMP) and *Phyllanthus niruri* (MEPN) on biochemical profile

![Graph showing the effect of methanol extracts of *Mucuna pruriens* (MEMP) and *Phyllanthus niruri* (MEPN) on biochemical profile.](image-url)

- **Bilirubin**
  - Normal
  - Acetaminophen (500mg/kg)
  - Acetaminophen + MEMP (125mg/kg)
  - Acetaminophen + MEPN (125mg/kg)
  - Acetaminophen + Silymarin (25mg/kg)
  - Acetaminophen + MEPN (250mg/kg)

- **Total protein**
  - Normal
  - Acetaminophen (500mg/kg)
  - Acetaminophen + MEMP (125mg/kg)
  - Acetaminophen + MEPN (125mg/kg)
  - Acetaminophen + Silymarin (25mg/kg)
  - Acetaminophen + MEPN (250mg/kg)

- **Uric acid**
  - Normal
  - Acetaminophen (500mg/kg)
  - Acetaminophen + MEMP (125mg/kg)
  - Acetaminophen + MEPN (125mg/kg)
  - Acetaminophen + Silymarin (25mg/kg)
  - Acetaminophen + MEPN (250mg/kg)
Fig 3: Effect of methanol extracts of *Mucuna pruriens* (MEMP) and *Phyllanthus niruri* (MEPN) on weight of the liver

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver weight (in grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>Acetaminophen (500mg/kg)</td>
<td></td>
</tr>
<tr>
<td>Acetaminophen + MEMP (125mg/kg)</td>
<td></td>
</tr>
<tr>
<td>Acetaminophen + MEMP (250mg/kg)</td>
<td></td>
</tr>
<tr>
<td>Acetaminophen + MEPN (125mg/kg)</td>
<td></td>
</tr>
<tr>
<td>Acetaminophen + MEPN (250mg/kg)</td>
<td></td>
</tr>
<tr>
<td>Acetaminophen + Silymarin (25mg/kg)</td>
<td></td>
</tr>
</tbody>
</table>

**Legend:**
- Normal
- Acetaminophen (500mg/kg)
- Acetaminophen + MEMP (125mg/kg)
- Acetaminophen + MEMP (250mg/kg)
- Acetaminophen + MEPN (125mg/kg)
- Acetaminophen + MEPN (250mg/kg)
- Acetaminophen + Silymarin (25mg/kg)
Fig 4: Hepatoprotective effect of methanol extracts of *Mucuna pruriens* (MEMP) and *Phyllanthus niruri* (MEPN) on Thiobarbituric acid reactive substances levels
Fig 5: Hepatoprotective effect of methanol extracts of *Mucuna pruriens* (MEMP) and *Phyllanthus niruri* (MEPN) on reduced Glutathione levels

![Graph showing the hepatoprotective effect of methanol extracts of *Mucuna pruriens* (MEMP) and *Phyllanthus niruri* (MEPN) on reduced Glutathione levels. The x-axis represents different treatments, and the y-axis represents Glutathione levels in mg/100g protein. The treatments include Normal, Acetaminophen (500mg/kg), Acetaminophen + MEMP (125mg/kg), Acetaminophen + MEMP (250mg/kg), Acetaminophen + Silymarin (25mg/kg), and Acetaminophen + MEMP (125mg/kg). The graph indicates significant differences between the groups, with some treatments showing a protective effect against reduced Glutathione levels.]

Legend:
- Normal
- Acetaminophen (500mg/kg)
- Acetaminophen + MEMP (125mg/kg)
- Acetaminophen + MEMP (250mg/kg)
- Acetaminophen + Silymarin (25mg/kg)
- Acetaminophen + MEMP (125mg/kg)
Fig 6: Hepatoprotective effect of methanol extracts of *Mucuna pruriens* (MEMP) and *Phyllanthus niruri* (MEPN) on Superoxide dismutase levels.
Fig 7: Hepatoprotective effect of methanol extracts of *Mucuna pruriens* (MEMP) and *Phyllanthus niruri* (MEPN) on Catalase levels

![Graph showing the hepatoprotective effect of methanol extracts of *Mucuna pruriens* (MEMP) and *Phyllanthus niruri* (MEPN) on Catalase levels. The x-axis represents various treatments, and the y-axis represents U/mg protein. Different treatments include Normal, Acetaminophen (500mg/kg), Acetaminophen (125mg/kg), Acetaminophen (250 mg/kg), Acetaminophen + MEMP (125mg/kg), Acetaminophen + MEMP (250 mg/kg), Acetaminophen + MEPN (125mg/kg), Acetaminophen + MEPN (250mg/kg), and Acetaminophen + Silymarin (25mg/kg). The graph indicates significant differences between treatments, with some treatments showing a protective effect on Catalase levels.]
5.4. REFERENCES


