# HEPATOPROTECTIVE EFFECT OF *INDIGOFERA PROSTRATA* IN PARACETAMOL INDUCED AND ALCOHOL INDUCED LIVER INJURY IN EXPERIMENTAL RATS

**Mahendar Boddupally[1*], S. Shobha Rani[2]**

**Abstract**

To evaluate the hepatoprotective activity of Methanolic extract of *Indigofera prostrata* against paracetamol and alcohol induced liver injury in experimental rats. Methanolic extract of *Indigofera prostrata*at doses of 200mg/kg and 400mg/kg body weight, p.o., was administered for 15 days in paracetamol (500gm/kg, orally) and 30% alcohol (1.5 ml/rat / twice a day) for 15 days intoxicated rats and compared with silymarine (25 mg/kg, p.o.) treated rats. Biochemical parameters, alkaline phosphatase (ALP), serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and total bilirubin (TB) levels were recorded to investigate the degree of improvement in the conditions of the rabbits. The liver was removed, washed with normal saline and preserved in 10% formalin and used in histopathological studies of hepatic architecture by microscopy. Phytochemical screening of the extract was also carried out. The levels of biochemical parameters were increased in paracetamol and alcohol intoxicated rats when compared with the normal group. The extract, at doses of 200 and 400mg/kg, exhibited significant ($p < 0.001$) reduction in biochemical parameters (ALP, SGOT, SGPT and TB). Hepatoprotective activity was also confirmed by histopathological findings. Furthermore, the phytochemical profile of the extract revealed the presence of tannins, alkaloids, saponins and flavonoids. These results suggest that *Indigofera prostrata* extract possesses significant hepatoprotective effect against paracetamol and alcohol induced hepatotoxicity and this may be due to the presence of flavonoids and tannins.

**Key words**:*Indigofera Prostrate*, hepatoprotection, alkaline phosphatase (ALP), serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT)

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## INTRODUCTION

As hepatic cells have a strong capacity for self-regeneration and reparative activity, liver disease advances gradually and typically remains asymptomatic for lengthy periods of time. The liver's main job is to metabolise poisons, including those from drugs and natural compounds. Additionally, the liver makes bile, proteins, clotting factors, glycogen, triglycerides, and cholesterol, and even little liver injury can have an impact on the entire body. [1]

The most commonly used analgesic-antipyretic is paracetamol-acetaminophen, which is safe up to a dose of 4g/day in divided doses. When the dose is exceeded, toxic metabolites are formed by cytochrome p-450, which further causes paracetamol to induce hepatotoxicity in the absence or decreased production of glutathione and cytochrome. [2, 3, 4]

The main contributors to toxicants-induced hepatotoxicity nowadays include excessive alcohol consumption, non-steroidal anti-inflammatory drug use, and painkiller use, which is associated with a higher incidence rate. Alcoholic liver disease, which is brought on by portal alcohol concentration and its metabolic effects, is the primary cause of liver-related mortality worldwide. [5,6] Steatosis, as well as steatohepatitis, fibrosis, and potentially cirrhosis, affect 90% of alcoholics who consume more than 16g of alcohol per day on average.[6,7] These damages are typically only discovered later, are difficult to regulate, and cannot be repaired. [5]

Natural remedies are frequently investigated since they have fewer side effects and have a more effective long-term healing process, particularly in the case of liver illness where they block fibrogenesis and tumour growth factor, get rid of infections, and protect against oxidative stress. [8] With an overdose, paracetamol absorption rises and the glucuronidation pathway of metabolism becomes more saturated, which boosts the generation of reactive metabolites. This dangerous byproduct of paracetamol mediates the hepatotoxic action that causes cell damage. A damaged liver causes cellular leakage and a loss of functional integrity, which raises serum enzyme levels. [2, 3, 4] The alcohol-induced liver disease comprises increased gut-derived lipopolysaccharide (LPS) entry into the liver. LPS/TLR-4 activates Kupffer cells and produce TNF-α and various other cytokines and growth factors, acts on alcohol exposed hepatocytes and induce apoptosis. [6]

The diverse genus *Indigofera* has demonstrated distinctive qualities that make it an intriguing option as a potential perennial crop. Particularly, there is wide variation among species with a variety of distinctive traits. Variations in pericarp thickness, fruit type, and flowering shape are a few instances of this variety. It has demonstrated the potential for mixed smallholder systems with at least one other species as well as a resilience that permits steady nitrogen uptake despite changing environmental circumstances. The purpose of the current investigation was to determine whether *Indigofera prostrata* might protect experimental rats' livers from harm caused by alcohol and paracetamol.

## MATERIALS AND METHODS

### Chemicals

The entire chemicals used were of analytical grade. The chemicals and reagents used were purchased from Sigma Aldrich (St. Louis, MO, USA).

### Collection of Plant material and authentication

Whole Plant *Indigofera prostrata* were obtained from the local places of Tirupati, AP. The Plant was authenticated by Dr. K. Madhava Chetty M.Sc., M.Ed., M.Phil., Ph.D., PG DPD.,

### Extraction by Maceration

Fresh *Indigofera prostrata*whole plant was dried in the shade after being water-washed to remove contaminants like dirt and other impurities. To get a consistent, coarse powder, these dry whole plant were crushed and sieved. One kg of powdered plant material was weighed, immersed in methanol, and left to macerate for seven days while being occasionally stirred. The solvent was filtered on the eighth day by squeezing it through a muslin cloth, and it was then heated to 40°C in a rotary evaporator to evaporate. The end product was placed in a desiccator to get rid of any remaining methanol. For additional research, the dried methanolic extract of *Indigofera prostrata*(MEIP) was placed in an airtight container and kept in a dry location.

### Preliminary Phytochemical Analysis

All the extract/fractions of *Indigofera prostrata*were analyzed for their primary and secondary metabolites to confirm the presence of various primary metabolites, such as carbohydrates, amino acids, proteins, and lipids, and secondary metabolites, such as alkaloids, tannins, phenols, flavonoids, saponins, steroids, glycosides, and resins, according to standard methods.

**Animals**

Male Wistar Rats of 180-200mgs, housed maximum of 6 per cage in a polypropylene cage with maintained room temperature of 20.2- 23.5ºC, relative humidity of 30 - 70% with 12h fluorescent light and 12h dark cycle were used in the studies. Animals were fed with rodent feed and purified water that was provided ad libitum. Animals were kept in sterilized rice husk beddings changed along with the cage twice a week during acclimatization and entire experimental study period. The study protocol was approved by IAEC with Ethical No: **45/SRCP/CPCSEA/2021**

**Evaluation of hepatoprotective activity: [9]**

**Experimental Design**

**Evaluation of hepatoprotective activity in alcohol-induced hepatotoxicity:**

Wistar Albino Rats (Wistar Strain) of either sex weighing 150-200g were selected and divided intofive groups of six animals each. Group I: Vehicle treated rats were kept on normal diet and served as control for 15 days. Group II: Rats orally received 30% alcohol (1.5 ml/ Rat / twice a day) for 15 days. Group III: Rats orally received Silymarin (25 mg/kg b.w/day) and alcohol as group II, for 15 days. Group IV: Rats orally received MEIP (200 mg/kg b.w/day) and alcohol as group II, for 15 days. Group V: Rats orally received MEIP (400 mg/kg b.w/day) and alcohol as group II, for 15 days.

**Evaluation of hepatoprotective activity in Paracetamol induced hepatotoxicity.**

In case of paracetamol-induced hepatotoxicity, the rats were divided into 5 groups of 6 rats each: Group I: Vehicle treated rats were kept on normal diet and served as control for 15 days. Group II: Rats received paracetamol (500 mg/kg b.w/day, orally) for 15 days. Group III: Rats received Silymarin (25 mg/kg b.w/day, orally) and paracetamol as group II, for 15 days. Group IV: Rats received MEIP (200 mg/kg b.w/day, orally) and paracetamol as group II, for 15 days. Group V: Rats received MEIP (400 mg/kg b.w/day, orally) and paracetamol as group II, for 15 days.

This period of treatment, the rats were maintained under normal diet and water. The blood was collected from the retro orbital plexus of the rats of all groups 24 h after the last dose administration, under light anesthetic ether. The blood samples are centrifuged at 3000rpm for 30min to separate the serum. The serum was analyzed for various biochemical parameters such as SGOT, SGPT, ALP, BIT, BID and ALB. Liver was dissected out and subjected for morphological study such as liver weight and liver volume of each animal. Further the liver was placed in 10% formalin solution for histopathological study.

**Estimation of serum bio-chemical parameters [11, 11, 12**]

Different biochemical parameters were estimated like SGOT, SGPT, ALP, bilirubin and albumin

**Assessment of antioxidant parameters**

Hepatic tissues of rats were homogenized (10%) in phosphate buffer (pH 7.4) with a Potter-Elvenhjem glass homogenizer. The homogenate was centrifuged at 12,000 rpm for 20 min at 4 °C to obtain post mitochondrial supernatant (PMS) and it was used for the estimation of Lipid peroxidation (LPO), The activity of catalase (CAT), superoxide dismutase (SOD), and Reduced glutathione (GSH), in the PMS of liver was measured by the methods described by Aebi [13], Kakkar et al. [14], and Upadhyay [15].

**Histopathological studies**

For histopathological studies, the slices of liver from each group were preserved in 10% buffered neutral formalin (pH 7.4). The tissues were mounted in the laboratory by embedding paraffin sections of 5–10µ size. These sections were then stained with haemotoxyline and eosin dye. The degree of necrosis was expressed as the mean of 10 high power fields (HPFs), chosen at random and classified on a scale of 0–5 (no hepatocyte necrosis, 0; necrosis in few hepatocytes, 1; necrosis in more than 10% but less than 24% of hepatocytes, 2; necrosis in more than 25% but less than 39% of hepatocytes, 3; necrosis in more than 40% but less than 49% of hepatocytes, 4; and necrosis in more than 50% of hepatocytes, 5) as per Silva et al. [16].

## RESULTS AND DISCUSSION

**Table 1:** Results of Phytochemical screening

| S. No | Name of the Phytochemical | MEIP |
|---|---|---|
| 1. | Carbohydrates | + |
| 2. | Amino acids | + |
| 3. | Proteins | + |
| 4. | Alkaloids | + |
| 5. | Cardiac glycosides | + |
| 6. | Triterpenoids | + |
| 7. | Saponins | + |
| 8. | Flavonoids | + |
| 9. | Phenolic compounds | + |
| 10. | Tannins | + |
| 11. | Steroids | + |
| 12. | Gums | - |

Where, + means positive and - means negative.

In the present study, the investigation of Methanolic extraction *Indigofera prostrata*revealed the presence of various presences of various phytoconstituents like flavonoids, phenolic compounds, triterpenoids, tannins, saponins, amino acids, proteins, Steroids, Gums and carbohydrates results were showed in **Table 1**

**Alcohol induced hepatotoxicity**

**Liver weight and liver volume:**
Alcohol treatment in rats resulted in enlargement of liver which was evident by increase in the liver weight and volume. The groups treated with Silymarin showed good restoration of liver weight and liver volume where as test groups treated with MEIP showed significant effect on liver weight and liver volume compared to toxic control group results were showed in **Table 2**.

**Table 2:** Liver weight and liver volume in alcohol induced hepatotoxic rats

| Group | Liver weight gm/100gm | Liver volume ml/100gm |
|---|---|---|
| **Control** | 3.68±0.18 | 6.95±0.65 |
| **Toxic control** | 4.82±0.16 | 8.95±0.65 |
| **Silymarin (10mg/kg)** | 3.81±0.48** | 7.23±0.72** |
| **MEIP(200mg)** | 4.21±0.13* | 7.58±0.81** |
| **MEIP(400mg)** | 4.11±0.43** | 7.48±0.65** |

Values are expressed as mean±SEM; n=6
* p≤0.05, **p≤0.01 and***P<0.001. Comparison with toxic control

**Bio chemical parameters:**
**Effect of MEIP on SGOT, SGPT & ALP levels in alcohol induced hepatotoxic rats:**
Rats treated with alcohol developed a significant hepatic damage observed as elevated serum levels of hepatospecific enzymes like SGPT, SGOT and ALP when compared to normal control. Treatment with Silymarin had showed good protection against alcohol induced toxicity to liver. Groups treated with MEIP showed significant effect which can be comparable with toxic control. Dunnet's test indicates a significant reduction in elevated serum enzyme levels with extract treated animals compared to toxic control animals results were showed in **Table 3 & Figure 1**.

**Table 3:** SGOT, SGPT & ALP levels in alcohol induced hepatotoxic rats

| Group | SGOT (IU/L) | SGPT (IU/L) | ALP (IU/L) |
|---|---|---|---|
| **Control** | 113.15±5.05 | 106.11±4.13 | 130.75±3.97 |
| **Toxic control** | 396.93±0.93 | 391.77±7.87 | 378.62±3.59 |
| **Silymarin** | 148.22±1.79** | 135.39±6.88** | 181.04±11.49** |
| **MEIP (200mg)** | 205.24±3.56** | 190.47±8.11** | 220.35±10.05** |
| **MEIP (400mg)** | 177.30±9.56** | 162.25±3.67** | 206.69±2.18** |

Values are expressed as mean±SEM; n=6
* p≤0.05, **p≤0.01 and***P<0.001.Comparison with toxic control

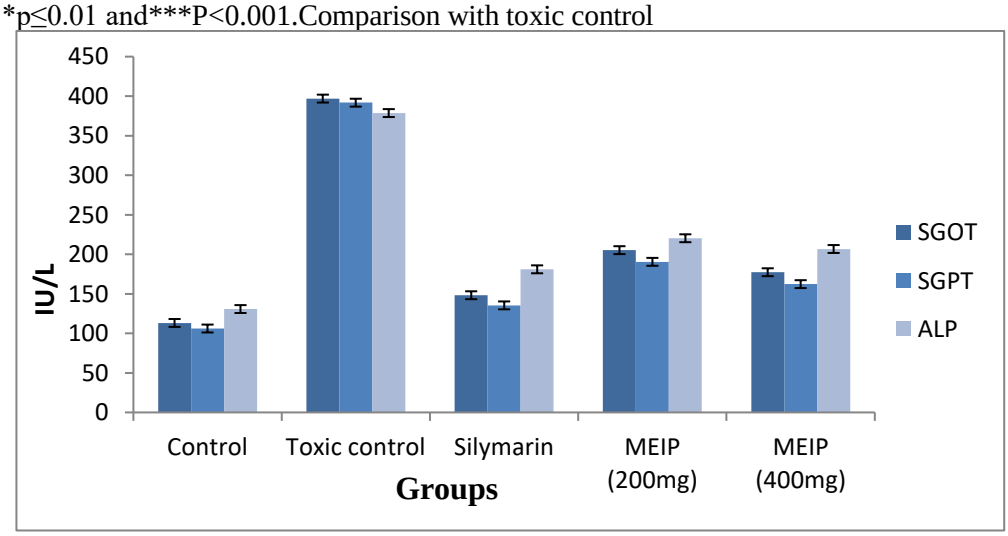


**Figure 1:** SGOT, SGPT & ALP levels in alcohol induced hepatotoxic rats

The total bilirubin concentration was found to increase in animals with liver damage by alcohol. In standard group, Silymarin administration reduced total bilirubin and animals treated with MEIP have exhibited dose dependent significant reduction in total bilirubin compared to toxic control group.Alcohol treated groups significantly elevated direct bilirubin concentration in animals by inducing hepatic damage compared to normal animals. But treatment with standard drug Silymarin showed good reduction in direct bilirubin concentration. Groups treated with MEIP significantly reduced direct bilirubin level in respective groups. Induction of liver damage by administration of alcohol significantly reduced serum albumin level in positive control group animals when compared to normal animals. But the treatment with Silymarin has shown significant increase while MEIP have shown dose dependent increase in serum albumin level compared to toxic control group. Results were showed in **Table 4.**

**Table 4:**BIT, BID & ALB levels in alcohol induced hepatotoxic rats

| Group | BIT (mg/dl) | BID (mg/dl) | ALB(g/dl) |
|---|---|---|---|
| **Control** | 0.63±0.09 | 0.30±0.06 | 4.64±0.22 |
| **Toxic control** | 2.04±0.15 | 1.98±0.17 | 2.18±0.11 |
| **Silymarin** | 0.75±0.07** | 0.41±0.02** | 4.38±0.47** |
| **MEIP (200mg)** | 1.03±0.05** | 0.82±0.05** | 3.81±0.29** |
| **MEIP (400mg)** | 0.92±0.05** | 0.68±0.05** | 4.13±0.14** |

Values are expressed as mean±SEM; n=6
* p≤0.05, **p≤0.01 and***P<0.001. Comparison with toxic control

**Table 5:** Effect of MEIP on SOD, CAT, GSH and LPO in alcohol induced hepatotoxic rats

| Groups and treatments | SOD (Units/mg tissue protein) | CAT (µ mol $H_2O_2$/mg tissue protein) | GSH (µg/mg tissue protein) | LPO (µ mole/mg tissue protein) |
|---|---|---|---|---|
| **Control** | 4.45± 0.56 | 18.38 ± 2.21 | 5.65 ± 0.065 | 28.43± 1.65 |
| **Toxic control** | 1.89 ± 0.058# | 8.11 ± 2.67# | 1.95 ± 0.19# | 83.7 ± 3.17# |
| **Silymarin** | 4.14 ± 0.77*** | 15.15 ± 3.47*** | 3.87 ± 0.13*** | 36.5 ± 1.76*** |
| **MEIP (200mg)** | 2.45 ± 0.754* | 11.20 ± 2.58* | 2.15 ± 0.48 | 53.12 ± 5.49** |
| **MEIP (400mg)** | 3.712 ± 0.056** | 13.59 ± 3.42*** | 2.48 ± 0.69* | 40.6 ± 4.52*** |

Values are expressed as mean ± SEM of 6 rats in each group.
P values: #<0.001 compared with respective normal control group I.
P values: *<0.05, **<0.01, ***<0.001 compared with group II (alcohol induced hepatotoxic rats).

**Histopathological studies of the liver in alcohol induced hepatotoxic rats:**

Normal control group Section studied shows normal architecture. Microvascular steato is occasionally observed. No ductal growth, necrosis, or fibrosis was visible. (Impression: Normal liver). Alcohol treated group Section shows liver tissue with centrizonal necrosis and lymphocytic infiltration. Other areas show microvesicular fatty change. No fibrosis.Silymarin + Alcohol treated group Section studied shows liver parenchyma with intact architecture. Few of the central veins and sinusoids show dilatation with focal congestion. Also seen are mild stromal inflammatory infiltration comprising of lymphocytes and macrophages within the periportal and focal midzonal areas. (Impression: Mild liver injury). MEIP (200mg) + Alcohol treated group Sections show liver tissue with mild necrosis of hepatocytes in the central zone. Portal areas show mild lymphocytic collections. Lobules show microvesicular fatty change. MEIP (400mg) + Alcohol treated group Section shows liver with mild distortion of architecture. There is microvesicular fatty change and focal collections of lymphocytes, histiocytes with necrosis of cells. (Impression: focal liver injury).Results were showed in **figure 2.**

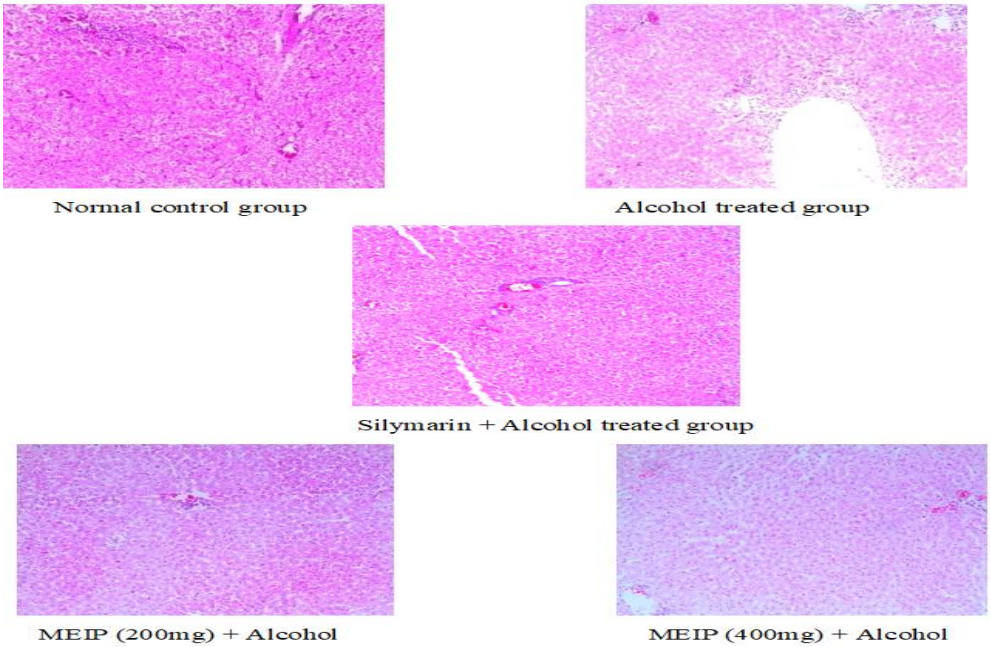


**Figure 2:** Histopathological studies of the liver in alcohol induced hepatotoxic rats

**Paracetamol induced hepatotoxicity:**

**Liver weight and liver volume**

Paracetamol treatment in rats resulted in enlargement of liver which was evident by increase in the liver weight and volume. The groups treated with Silymarin showed good restoration of liver weight and liver volume where as test groups treated with MEIP showed significant effect on liver weight and liver volume compared to toxic control group.Results were showed in **Table 6.**

**Table 6:**Liver weight and liver volume in paracetamol induced hepatotoxic rats

| Group | Liver weight gm/100gm | Liver volume ml/100gm |
|---|---|---|
| **Control** | 3.11±0.11 | 5.6±0.15 |
| **Toxic control** | 4.55±0.18 | 9.65±0.18 |
| **Silymarin** | 3.23±0.12** | 6.06±0.10** |
| **MEIP(200mg)** | 3.7±0.10** | 7.05±0.08** |
| **MEIP(400mg)** | 3.5±0.16** | 6.57±0.08** |

Values are expressed as mean±SEM; n=6

* p≤0.05, **p≤0.01 and***P<0.001. Comparison with toxic control

**Bio chemical parameters:**

**Effect of MEIP fruits on SGOT, SGPT & ALP levels in paracetamol induced hepatotoxic rats**

Rats treated with paracetamol developed a significant hepatic damage observed as elevated serum levels of hepatospecific enzymes like SGPT, SGOT and ALP when compared to normal control. Treatment with Silymarin had showed good protection against Alcohol induced toxicity to liver. Groups treated with MEIP showed significant effect which can be comparable with toxic control. Dunnet's test indicates a significant reduction in elevated serum enzyme levels with extract treated animals compared to toxic control animals. Results were showed in **Table 7.**

**Table 7:**SGOT, SGPT & ALP levels in paracetamol induced hepatotoxic rats

| Group | SGOT (IU/L) | SGPT (IU/L) | ALP (IU/) |
|---|---|---|---|
| **Control** | 109.20±2.66 | 96.49±5.35 | 96.68±5.21 |
| **Toxic control** | 388.98±2.49 | 302.46±7.49 | 379.94±4.79 |
| **Silymarin** | 123.86±4.67** | 106.83±2.21** | 128.64±1.87** |
| **MEIP(200mg)** | 178.46±3.72** | 171.92±1.83** | 191.19±11.51** |
| **MEIP(400mg)** | 152.71±2.23** | 141.81±6.41** | 156.16±6.20** |

Values are expressed as mean±SEM; n=6

* p≤0.05, **p≤0.01 and***P<0.001. Comparison with toxic control

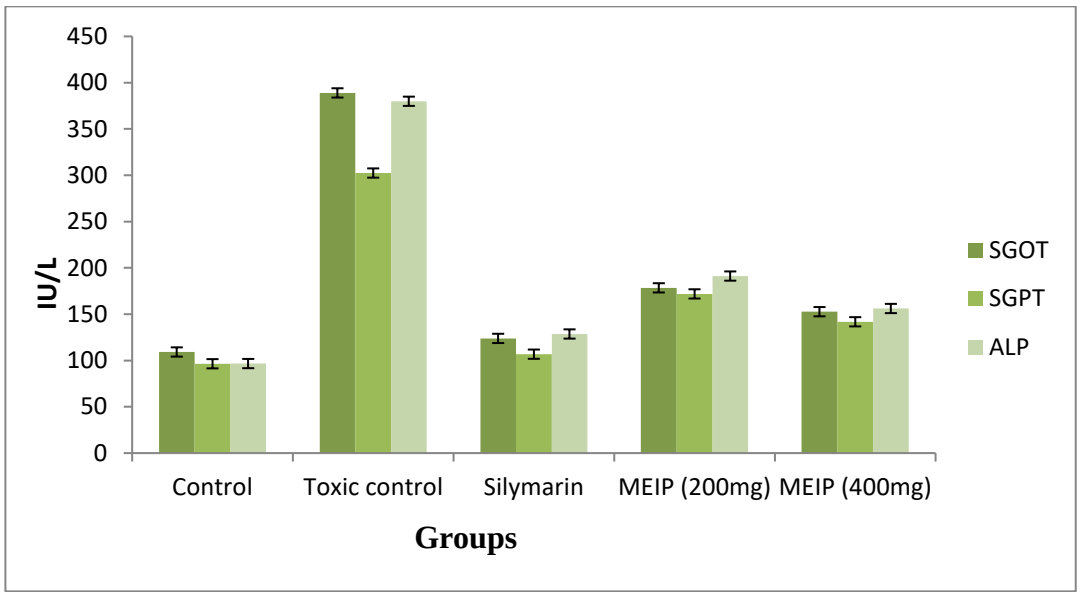


**Figure 3:** SGOT, SGPT & ALP levels in paracetamol induced hepatotoxic rats

The total bilirubin concentration was found to increase in animals with liver damage by paracetamol. In standard group, Silymarin administration reduced total bilirubin and animals treated with MEIP have exhibited dose dependent significant reduction in total bilirubin compared to toxic control group.Paracetamol treated groups significantly elevated direct bilirubin concentration in animals by inducing hepatic damage compared to normal animals. But treatment with standard drug Silymarin showed good reduction in direct bilirubin concentration. Groups treated with MEIP significantly reduced direct bilirubin level in respective groups. Induction of liver damage by administration of paracetamol significantly reduced serum albumin level in positive control group animals when compared to normal animals. But the treatment with Silymarin has shown significant increase while MEIP have shown dose dependent increase in serum albumin level compared to toxic control group. Results were showed in **Table 8.**

**Table 8:**BIT, BID & ALB levels in paracetamol induced hepatotoxic rats

| Group | BIT (mg/dl) | BID (mg/dl) | ALB(g/dl) |
|---|---|---|---|
| **Control** | 0.66±0.03 | 0.29±0.01 | 4.67±0.25 |
| **Toxic control** | 2.95±0.48 | 2.08±0.32 | 2.38±0.07 |
| **Silymarin** | 0.75±0.01** | 0.39±0.02** | 4.41±0.43** |
| **MEIP(200mg)** | 1.16±0.03** | 0.96±0.01** | 3.75±0.04** |
| **MEIP(400mg)** | 0.99±0.05** | 0.67±0.01** | 4.10±0.16** |

Values are expressed as mean±SEM; n=6
* p≤0.05, **p≤0.01 and***P<0.001. Comparison with toxic control

**Table 9:** Effect of MEIP on SOD, CAT, GSH and LPO in paracetamol induced hepatotoxic rats

| Groups and treatments | SOD (Units/mg tissue protein) | CAT ( µ mol $H_2O_2$/mg tissue protein) | GSH (µg/mg tissue protein) | LPO (µ mole/mg tissue protein) |
|---|---|---|---|---|
| **Control** | 4.45± 0.56 | 18.38 ± 2.21 | 5.65 ± 0.065 | 28.43± 1.65 |
| **Toxic control** | 1.89 ± 0.058# | 8.11 ± 2.67# | 1.95 ± 0.19# | 83.7 ± 3.17# |
| **Silymarin** | 4.14 ± 0.77*** | 15.15 ± 3.47*** | 3.87 ± 0.13*** | 36.5 ± 1.76*** |
| **MEIP (200mg)** | 2.76 ± 0.43* | 12.21 ± 2.98* | 2.76 ± 0.76 | 51.12 ± 6.43** |
| **MEIP (400mg)** | 3.82 ± 0.65** | 13.87 ± 2.63*** | 3.18 ± 0.87* | 39.26 ± 3.24*** |

Values are expressed as mean ± SEM of 6 rats in each group.
P values: #<0.001 compared with respective normal control group I.
P values: *<0.05, **<0.01, ***<0.001 compared with group II (alcohol induced hepatotoxic rats).

**Histopathological studies of the liver in paracetamol induced hepatotoxic rats**

Normal control groupSection studied shows normal architecture. Microvascular and occasional microvascular steato seen. No fibrosis, Necrosis and ductal proliferation seen. (Impression: Normal liver). Paracetamol treated group Section studied shows lobular architecture. Necrosis & patchy steatosis is seen. Lymphocytic infiltrate are seen in periportal area. Fibrosis/necrosis is seen. (Impression: Acute liver injury).Silymarin + Paracetamol treated group Section studied shows lobular architecture. Microvascular and occasional. Microvascular steatosis seen. No fibrosis, Necrosis and ductal proliferation seen. (Impression: Mild liver injury).MEIP (200mg) + Paracetamol treated groupLiver shows normal architecture and lobules show microvesicular fatty change. There are focal lymphocytic collections and necrotic liver cells. No fibrosis.(Impression: Mild hepatic injury).MEIP (400mg) + Paracetamol treated groupLiver shows normal architecture. Other areas show microvesicular fatty change. No fibrosis. (Impression: Mild hepatic injury).results were showed in **figure 4.**

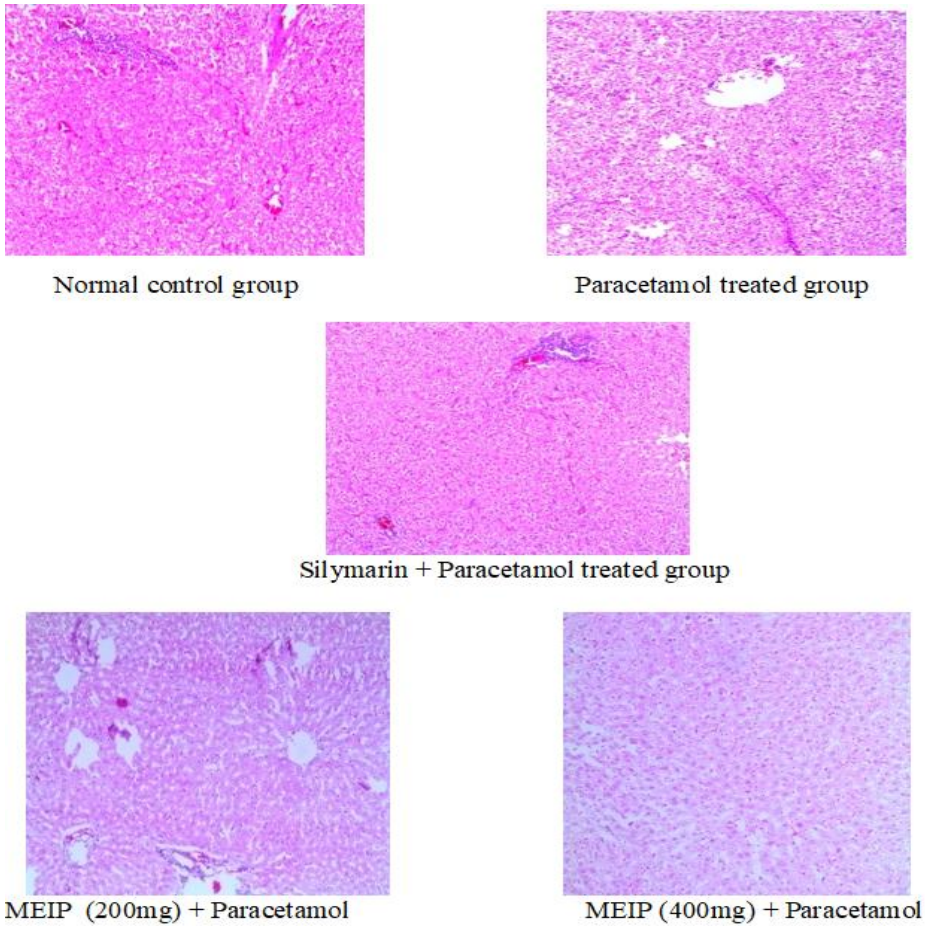


**Figure 4:** Histopathological studies of the liver in paracetamol induced hepatotoxic rats

## DISCUSSION

In the present study the effect of MEIP was evaluated for its hepatoprotective activity. The MEIP were observed for the presence of alkaloids, glycosides, carbohydrates, flavonoids, saponins, steroids and tannins. Alcohol and paracetamol treatment in rats resulted in enlargement of liver which was evident by increase in the liver weight and volume. The groups treated with Silymarin showed good restoration of liver weight and liver volume whereas test groups treated with MEIP showed significant effect on liver weight and liver volume. Rats treated with alcohol and paracetamol developed a significant hepatic damage observed as elevated serum levels of hepatospecific enzymes like SGPT, SGOT and ALP when compared to normal control. Treatment with Silymarin had showed good protection against alcohol and paracetamol induced toxicity to liver. Groups treated with MEIPshowed significant effects.

The total bilirubin concentration was found to increase in animals with liver damage by alcohol and paracetamol. In standard group, Silymarin administration reduced total bilirubin and animals treated with MEIP have exhibited dose dependent significant reduction in total bilirubin. Alcohol and

paracetamol treated groups significantly elevated direct bilirubin concentration in animals by inducing hepatic damage compared to normal animals. But treatment with standard drug Silymarin showed good reduction in direct bilirubin concentration. Groups treated with MEIP significantly reduced direct bilirubin level in respective groups. Induction of liver damage by administration of alcohol and paracetamol significantly reduced serum albumin level in positive control group animals when compared to normal animals. But the treatment with Silymarin has shown significant increase while MEIP have shown dose dependent increase in serum albumin levels. The effect of MEIP was further evaluated by the histopathological studies where the liver sections of groups treated with MEIP showed less hepatic damage.

Paracetamol is a strong analgesic and antipyretic drug; it works by inhibiting prostaglandin synthesis in the central nervous system [17, 18]. This drug can be purchased freely at a low price, so its use is often not according to the rules. At high doses exceeding therapeutic doses can cause toxicity, with side effects of liver and kidney injuries [19]. The absorption of PCM through the gastrointestinal tract is very fast; the first cross-metabolism occurs in the cells of the intestinal lumen and is conjugated in the liver. In therapeutic dose, PCM is converted by drug metabolizing enzymes to water-soluble metabolites and secreted in the urine. The toxic dose of PCM caused the depletion of GSH resulting in accumulation of NAPQI which then covalently binds to the cysteinyl sulfhydryl groups of cellular proteins forming NAPQI-protein adducts [20].This event results liver cell injuries [21] In our study, the animals group with induced an oral multiple doses administration of PCM 500 mg/kg/d for ten day was hepatotoxic in rats as shown by the significant ($P<0.05$) increase in plasma ALT and AST activities levels [22]. The high concentrations of serum liver enzymes indicate hepatocyte damage because these enzymes are located in the cell cytoplasm and are released into the bloodstream following hepatic cell damage [23]. This is causing the leaking of cellular enzymes and can be measured in the serum [24].

Ethanol is a unique substance with a rapid action owing to its solubility in both water and lipids, which is absorbed from the stomach and intestine and then rapidly diffuses into the blood circulation, where it is dispersed throughout the body. Hepatocellular necrosis is caused by alcohol use, resulting in an increase in serum marker enzymes released into the bloodstream [25]. Elevated SGOT, SGPT, ALP, BIT, BID, ALB levels are prominent biomarkers of hepatic damage [26]. It also increases the production of reactive oxygen species inside the living system, since ethanol is extensively metabolised by the microsomal oxidising system to acetaldehyde and ultimately to acetate through cytochrome P450 [27]. Steatosis is the most significant alteration that happens in the liver following alcohol use. As a result of redox state imbalance, lipid peroxidation occurs [28]. In organisms, GSH is an essential antioxidant capable of reducing damage produced by reactive oxygen species. MDA is produced by lipid peroxidation and is a sign of oxidative stress. Free radicals and oxidative stress caused by ethanol promote MDA overproduction and GSH depletion [29]. Excessive free radicals are likely to elicit Kupffer cells, which can regulate the inflammatory process in the liver by releasing TNF-alpha and other pro-inflammatory cytokines. Increased pro-inflammatory mediators and cytokines (e.g., TNF-α, IL-1 β, and IL-6) aggravated in ethanol-induced groups may have been through NF-κB activation. The potent effect of AAE reduced free radicals and apoptotic properties by the enhanced antioxidant capacity against oxidative stress induced by alcohol for hepatoprotection.

The histopatological alteration includes necrosis, degeneration, haemorrhage, and congestion. The major vein becomes severely congested, and capillaries become blocked. This obstruction results in blood building up in the sinusoids and veins, which explains why the PCM group has liver edoema, which makes the size and weight larger and heavier. Every parameter in the PCM group differs significantly from the control group in comparison. Necrotic centrolobular histopathological alterations are the most typical ones seen in hepatotoxicity paracetamol. [30]. Severe was seen in the PCM group, and there was mild hemorrhage in the MEIP group treated [31].

## CONCLUSION

The present study reveals that the, preliminary phytochemical estimation of MEIP revealed the presence of tannins, flavonoids, glycosides, alkaloids, steroids, carbohydrates and saponins. On the basis of the results obtained in the present study, it is concluded that both the MEIP exhibits significant hepatoprotective activity. However, the exact mechanism responsible for activities is currently unclear. Therefore, further investigations need to be carried out to isolate and identify specific compounds present in the plant extract responsible for these activities and exact mechanism.

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