Introduction

Human body is very complex in its structure and possesses so many organs and other glands, these all are equally important for the survival, out of these liver plays superior role. It is the 2nd largest organ in our body after skin. It has to perform a wide range of functions, including removal of toxic materials from the body and converting them into non toxic materials. Apart from this function liver also synthesize protein, and do production of biochemical necessary for digestion. Present generation is fast moving generation, the people want fast result and fast relief from pain. Due to excess consumption of N.S.A.I.D. and alcohol liver is getting more harm and developing in to liver disease. According to W.H.O. about 18,000 people die every year due to liver disease. The common disease of liver are the cirrhosis, inflammation, cholestasis, and hepatitis, portal hypertension, hepatic encephalopathy, and others. Liver diseases are because of toxic chemicals, excess consumption of alcohol, infection and auto immune disorders. The main reason for the liver damage is prolonged drug therapy, excessive use of paracetamol, diclofenac, nimesulide and other pain killer, alcoholism, exposure to certain xenobiotic, pollutants and certain disease state have been reported to affect liver functioning. Lipid peroxidation and other oxidative damage are the mainly responsible for the damage of liver cell. The liver damage is one of the main leading problems in India which may be metabolic disorder to even mortality. To cure the liver disease there are several synthetic and semi synthetic medicines are available in the market to but they are not as safe as herbal medicine. Plants have been most reliable source of medicine, not from today but since the dawn of civilization. The traditional medicine system of India is one of the strongest medicine systems in the world. It provides identification and utilization of different plant species. The Indian subcontinent is enriched by verity of flora, both aromatic and medicinal plants. This is due to the diverse climatic condition available in India, ranging from deserts to swamps. Numerous types of herbs have been well recognized and catalogued by botanist from the high ranges of Himalaya.

Paracetamol is probably the most versatile and widely used analgesic and antipyretic drug worldwide. It has excellent safety profile in therapeutic dose, but hepatotoxicity can be developed with overdose. The major target organ in paracetamol poisoning is the liver and the primary lesion is acute centrilobular hepatic necrosis. When taken in over dose it causes the production of reactive metabolites N-acetyl p-benzoquinonimine. Hepatotoxicity is the result of formation of the toxic metabolites.

*Madhuca indica* is common tree in India and it belonging to the family Sapotaceae, having tremendous therapeutic potentials but due to unawareness in people it has not been fully utilized. *Madhuca indica* is a large, shady, deciduous tree up to 18 m high with short bole, spreading branches and large rounded crown. Bark is grey to black with vertical cracks. Previous Phytochemical studies on *Madhuca indica* included the characterization of Sapogenin, triterpenoids, steroids, saponin, flavonoids and glycosides *Madhuca indica* is having several pharmacological activities like Antidiabetic, anti inflammatory effect, analgesic, anti pyretic, anti asthmatic, anti ulcer, anti cancer, hepatoprotective activity, and anti bacterial.

Keywords: *Madhuca indica*, mahua, hepatoprotective, paracetamol, liver disease.
Identification and Authentication of plant material

Madhuca indica (mahua) were collected from the Raisen road, Bhopal, Madhya Pradesh in the month of January-February 2012 and authenticated by Dr. Jia Ul Hasan, faculty of botany, Safia College of Science, Bhopal and voucher specimen (322/bot/safia/2012) was deposited in T.I.T. College of pharmacy Anand Nagar Bhopal, India.

Extraction of Madhuca indica leaf

Madhuca indica leaves were screened to get the better quality, then rough and poor leaves were removed from the collection. The remaining leaves were air dried to prevent the loss of active constituents, and then they were crushed manually by hand to obtain the coarse powder. The powder was then passed to 40 mesh size sieve and extracted with-

1. Hydro alcoholic solution in ratio of 70 percent water and 30 percent ethanol.
2. Extraction using 95 % ethanol

Both extractions were performed using soxhlet extraction apparatus at 40°C until the completion of extraction cycle. After extraction process extract were dried initially at room temperature and then at the water bath to evaporate the solvent and to obtain the dry extract. The extract was stored in well closed container and store in the vacuum desiccator.

Drugs and chemicals

Standard drug- Standard hepatoprotective drug silymarin was obtained as a gift sample from the sapience laboratory Bhopal.

Paracetamol- have been obtained as a gift sample from sapience laboratory Bhopal.

Diagnostic reagents kit - diagnostic reagent kits for Alkaline Phosphate, Bilirubin, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) were purchased from market which was manufactured by Span diagnostics Ltd. Surat, India.

Animals for the study

Wistar rats of both sexes ranging from 90-150 gm were obtained from the animal house of sapience laboratory (CPCSEA Approved and ISO 9001:2008 Certified) Bhopal, Madhya Pradesh, India. They were maintained under the standard laboratory conditions in standard polypropylene cages for 12 h light/dark cycle and provided food and water ad libitum. The experimental protocol was approved by institutional animal ethics committee registration no. 1413/a/11 (CPCSEA)

Acute toxicity study and effect of extract on liver

The acute toxicity studies were carried out in adult albino rats weighing 90-150 gm, by up and down method as per OECD 425 guidelines. Overnight fasted animals received test drug (both ethanolic and hydro alcoholic) at a dose of 100, 200, 400, 800, 1000, 2000 mg/kg body weight orally. Then the animals were observed continuously once in a half an hour for next 4 hours and then after 24 hours for general behavior, neurological and autonomic profiles and to find out mortality. The extracts were found safe up to dose of 2000 mg/kg body weight and none of the animal was died, so out of them 200 and 400 mg/kg dose were selected for the administration. There is little modification in the protocol; here for the better evaluation of hepatotoxicity one group is prepared only for extract and its histopathology will be studied at the last. This group is given the ethanolic extract of Madhuca indica at 400 mg/kg dose.

Administration of hepatotoxins in animals

For the study of hepatoprotective effect of Madhuca indica leaf extract, hepatic injury in all groups except in standard control were induced by oral administration of paracetamol (500 mg/kg body weight) once in a day for 7 days.

Assessment of hepatoprotective activity

In order to assess the hepatoprotective activity of leaf extract of Madhuca indica the Wistar rats of both sexes weighing 90-150 gm. were maintained in animal care facility and they were divided into 8 groups of 6 animals each and provided a dose of 200 and 400 mg/kg body weight by oral route.

Groups for experiments

Group 1:- Control group- receive only normal diet and water.

Group 2:- Treat group, which receive only extract at 200 mg/kg body weight for 7 days (for toxicity study only).

Group 3:- Toxic group-Received Paracetamol 500 mg/kg body weight on daily basis for 7 days.

Group 4:- Standard group- Received silymarin 100 mg/kg and paracetamol 500 mg/kg body weight once daily for 7 days.

Group 5:- Received ethanolic extract of Madhuca indica 200 mg/kg and paracetamol 500 mg/kg body weight once daily for 7 days.

Group 6:- Received the ethanolic extract of Madhuca indica 400 mg/kg and paracetamol 500 mg/kg body weight once daily for 7 days.

Group 7:- Received hydro alcoholic extract of Madhuca indica 200 mg/kg and paracetamol 500 mg/kg body weight once daily for 7 days.

Group 8:- Received hydro alcoholic extract of Madhuca indica 400 mg/kg and paracetamol 500 mg/kg body weight once daily for 7 days.

Paracetamol was administered at the morning and extract of Madhuca indica was given at evening.
Collection of Blood
After 24 hours of last treatment, the rats were anaesthetized and blood samples from each animal of all groups were collected by retro-orbital plexus puncture in sterilized centrifuge tubes. The blood samples were then allowed to coagulate at 30°C for 45 minutes. Serum portion was separated from each sample by centrifugation at 25000 rpm for 20 minutes.8

Biochemical analysis of serum samples
Serum samples collected from different groups were analyzed for Aspartate Transaminase (AST), Alkaline Transaminase (ALT) Alkaline Phosphate (ALP) and Total Bilirubin using procedure and packaged kits made by Span diagnostics Ltd. Surat, India. The absorption was recorded using given nm in spectrophotometer.

Liver Histopathology
10% formalin was freshly prepared and the right liver lobe of all groups were fixed in it for 48 hours and subsequently dehydrated in alcohol, cleared with xylene and embedded in paraffin wax, and then section have been prepared.

Statistical analysis
Results were expressed as means of SEM (n=6). Statistical analysis was performed with one way ANOVA followed by Turkey-Kramer multiple comparison test. P value less than 0.05 was considered to be statistically significant (P<0.05).

RESULTS

Acute Toxicity
The acute oral toxicity studies showed no animal died even at 2000 mg/kg and hence the ethanolic and hydro alcoholic extract of *Madhuca indica* leaf was treated as non toxic. Therefore as per CPCSEA guidelines 420 it was thought that 2000 mg/kg was the LD50 cut off dose. And out of them 200 and 400 mg/kg body weight dose has been selected for the oral administration to the experimental rats. The histopathology of extract treated rat doesn’t show any important abnormality in structure except accumulation of fatty materials.

Ascites formation
There was ascites formation (figure 1) in the paracetamol treated toxic group, which is usually serous fluid which is a pale yellow and clear fluid. The most common cause of ascites is advanced liver disease or cirrhosis. Approximately 80% of the ascites cases are thought to be due to cirrhosis.

![Figure 1: Ascites formation due to the Paracetamol hepatotoxicity](image)

Serum biochemical parameters
Different serum biochemical parameters have been performed for the evaluation of hepatoprotective activity of *Madhuca indica* ethanolic and hydro alcoholic leaf extract. The parameters include SGOT, SGPT, ALP and total serum Bilirubin. The values thus obtained are compared with the control, toxic and standard group and the result was satisfactory and hence it was safe to use as a hepatoprotective. The results are presented in table 1.

Histopathology
The histopathology of varies group shows the variation in the structure and the composition in the cellular content when they are compare with the control and silymarin treated groups. The result thus obtained showed the significantly improvement in the groups which were treated with ethanolic and hydro alcoholic extract of *Madhuca indica* leaf extract, but group treated with the hydro alcoholic extract gives more satisfactory result than the ethanolic group. Histopathology of liver is shown in figure 2.

<table>
<thead>
<tr>
<th>Table 1: Effect of ethanolic and hydro alcoholic leaf extract of <em>Madhuca indica</em> against PCM induced hepatotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (mg/kg)</td>
</tr>
<tr>
<td>Vehicle Control</td>
</tr>
<tr>
<td>Extract treated</td>
</tr>
<tr>
<td>Toxic PCM</td>
</tr>
<tr>
<td>Standard</td>
</tr>
<tr>
<td>PCM + ET 200</td>
</tr>
<tr>
<td>PCM + H.A.200</td>
</tr>
<tr>
<td>PCM + H.A.400</td>
</tr>
</tbody>
</table>

* E.T. = Ethanolic Extract. H.A. = Hydro alcoholic extract. PCM = Paracetamol
**DISCUSSION**

Paracetamol is one of pharmacological tools used to produce liver damage in animal model. Its hepatotoxic action is being with the changes in endoplasmic reticulum which results in loss of metabolic enzyme located in the intracellular structure. Ascorbic acid is formed as a metabolite of glucose and galactose in rat liver microsomes via the glucuronic acid pathway and is excreted in urine. The hepatoprotective potentials of the plant depends on its chemical constituents presents in it, and upon its ability in reducing the harmful effects caused by the hepatotoxins.

The hepatoprotective mechanism of the extract of the *Madhuca indica* is due to more than one reason. Antioxidant, scavenging and regulators of intracellular content of the glutathione may be the possible mechanism by which plant show hepatoprotective activity. Flavonoid is one of the main constituents present in the *Madhuca indica* leaf extract and it has been well known that the flavonoid is having potent effects as the hepatoprotective agent.9, 10 Oxidative stress usually develops when the pro-oxidative action of an inducer exceed the antioxidant capacity of the defence system. The another possible mechanism of action of the extract of *Madhuca indica* may be as a cell membrane stabiliser and permeability regulator that prevent hepatotoxic agent to cross across the cell membrane, another mode of action may be as a promoters of the rRNA synthesis, they help in the synthesis in the protein in the liver cell and they usually increase the rate of new cell generation and as to repair the old and damage cell inside the hepatic cell.

**CONCLUSION**

The ethanolic and hydro alcoholic extract of *Madhuca indica* at the increasing dose showed a significance reduction in the liver toxicity, which was induced by the paracetamol. The hydro alcoholic extract comparatively reduced the hepatotoxicity by ethanolic extract, and more potent as hepat protective. The result was satisfactory when compare with then silymarin the standard drug. The serum biochemical parameter and the histopathology of liver slide also stand with the result as the extract is useful in the treatment in the hepatotoxicity and other associated liver problems.
Acknowledgements: I am thankful to my family and friends Renuka Shukla, Jyoti Sahu, Anuradha Patel, Deep Chand and sapience laboratory Bhopal.

REFERENCES


7. OECD guidelines for the testing of chemicals, acute oral toxicity test- up and down procedure (UDP) 425 adopted 3 october 2008.


About Corresponding Author: Mr. Pushpendra K. Patel

Pushpendra K. Patel graduated from the CSVTU University from Raipur and he completed post graduation, having specialization in Pharmacology and has presented major project on phytochemical screening and evaluation of the hepatoprotective activity of *Madhuca indica*. 