Evaluation of Hepatoprotective Activity of Polyherbal Formulation in Paracetamol Induced Hepatotoxicity in Rats

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ABSTRACT
The objective of the present study therefore was to assess the Hepatoprotective Effect of Polyherbal Formulation in Paracetamol induced Hepatotoxicity in Rats. In these models of Hepatotoxicity, 42 male adult male wistar rats were divided in to 7 groups. Group-1 and Group-2 served as a Normal control and positive control respectively, Group-3 served as a standard group (Liv. 52 5.2 ml/kg) and Group-4, 5, and 6 group received polyherbal formulation with the dose of 100 mg/kg, 300 mg/kg and 500 mg/kg respectively prior to PCM and Group-7 received polyherbal formulation followed by Paracetamol treatment. After the experimental period blood sample were collected for biochemical parameters. Paracetamol treatment caused hepatotoxicity as evidenced by marked elevation in AST, ALT, LDH, and total bilirubin. Co-administration of polyherbal syrup with Paracetamol showed decrease in AST, ALT, LDH, and total bilirubin. It was observed that Polyherbal syrup containing Allium sativum, Ocimum sanctum, Curcuma longa, Aloe barbadensis conferred hepatoprotective and anti-oxidant activities by biochemical observation against Paracetamol induced hepatotoxicity in rats. In the near future polyherbal syrup could lead to discovery of novel herbal combination drug for treatment of hepatotoxicity.

Keywords: Allium sativum, Aloe barbadensis, Curcuma longa, Hepatoprotective activity, LDH, UIV 52, Total bilirubin.

INTRODUCTION
Hepatotoxicity refers to any disorder of the liver which includes the following conditions: steatosis or fatty deposits in the liver, fibrosis or scarring of the liver, hepatitis or inflammation of the liver, cirrhosis where scarring and inflammation spread through the liver and irreversibly disrupt its shape or function causing permanent cell damage and ultimately liver failure and leading to liver cancer which causes ultimately liver failure and death. Hepatotoxicity is caused by various toxicants such as paracetamol, certain chemotherapeutic agents, carbon tetrachloride, thioacetamide, chronic alcohol consumption and microbes. Most of the population in world is suffering from liver disease. About 20,000 deaths found every year due to liver disorders. Female to male ratio of hepatotoxicity was found to be 1.4:1 at age 39. The prevalence of various types of hepatotoxicity for jaundice, acute hepatitis, cholestatis, cholestatic hepatitis, acute and chronic liver injury was found to be 60.4%, 41.4%, 15.5%, 24.1%, 4.5% and 8.6% respectively. Several different substances can be toxic to the liver such as Viruses (Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis D), Obesity, Chronic or long-term alcohol use, Genetic defects (Haemochromatosis), Drugs like PCM, Aspirin, Ibuprofen, amiodarone, isoniazid, methotrexate, valproic acid, rifampin, etc., Poisons like CCl4, trichloroethylene, etc., Autoimmune Disorder, Herbs like Cascara, mahuang, kava etc., Hormones like birth control pills, anabolic steroids.

There are no specific allopathic medicines use as hepatoprotective but there are some drugs used to treat the symptoms of hepatotoxicity such as colestyramine, ursodeoxycholic acid, cetirizine, naltrexone, spironolactone, furesemide, propranolol, loratidine, vasopressin having certain disadvantage such as Diarrhoea, constipation, flatulence, abdominal discomfort, variable response, and may aggravates encephalopathy.

Herbal-based therapeutics for liver disorders has been in use in India for a long time and has been popularized world over by leading pharmaceuticals. The use of natural remedies for the treatment of liver diseases has a long history, starting with the Ayurvedic treatment, and extending to the Chinese, European and other systems of traditional medicines. The 21st century has seen a paradigm shift towards therapeutic evaluation of herbal products in liver disease models by carefully synergizing the strengths of the traditional systems of medicine with that of the modern concept of evidence based medicinal evaluation, standardization and randomized placebo controlled clinical trials to support clinical efficacy.

Liver protective herbal drugs contains a variety of chemical constituents like phenol, coumarins, lignans, essential oil, monoterpenes, Carotenoids, glycosides, flavonoids, lipids, alkaloids and xanthenes, which are responsible for the hepatoprotective activity.

In the traditional Indian system of medicine plant formulation and combined extract of plants are used as a drug of choice rather than individual drug. Various herbal formulations are well known for their Hepatoprotective...
effect.\textsuperscript{11} In recent years, there is growing interest in polyherbal formulation for the treatment of various diseases and it has enormously increased worldwide. Polyherbal formulations have shown curative effect on liver diseases and disorders in rats.\textsuperscript{12}

While searching for hepatoprotective drug in natural product, highly encouraging result was obtained in our laboratory with Polyherbal formulation of Momordica charantia and Azadirachta indica A. Juss (neem).\textsuperscript{13} The present investigation is undertaken to study Hepatoprotective effect of polyherbal formulation (Allium sativum, Rubus fruticosus, Curcuma longa, Viscum articulatum) on paracetamol induced Hepatotoxicity in rats.

**MATERIALS AND METHODS**

**Collection and authentification of plant material**

All the plants were collected from the market and authenticated by Dr. Bimal S. Desai at the Botanical Department, Agriculture University, Navsari.

**Preparation of polyherbal formulation**

All the procured and authenticated drugs were dried in shade and crushed using grinder and passed through mesh no.80. The individual drugs were weighed as per quantity required. The drugs were mixed. The mixed formulation was unloaded, weighed, and packed in labeled plastic bags.

**Preparation of extract**

The ingredients of the polyherbal formulation were extracted in methanol at 50 - 60°C in a soxhlet apparatus separately. The extract was concentrated to dryness in a water bath at controlled temperature. The dried methanolic extracts of polyherbal formulation were used for further treatment.

**Preparation of hydro alcoholic syrup**

The simple syrup was prepared as per standard IP procedure. Extracts were mixed with the simple syrup with constant stirring. 8-10% of alcohol was added in these mixtures for complete dissolution of a extract of the powder. Volume was adjusted with simple syrup.

**Phytochemical Screening**

Standard methods were used for preliminary phytochemical screening of methanolic extracts, to know the nature of phytoconstituents present in it.

**Animals**

Studies were carried out using wistar either sex albino rats weighing between 200-220g. They were procured from Jay foundation research, Vapi and Flair lab Surat. Animals were approved from the IAEC to carry out project study used for the hepatoprotective study. The animals were grouped housed in poly propylene cages with not more than six animals per cage and maintain edunder standard laboratory conditions: temperature (at 24° ± 2°C) relative humidity (55 ± 5%) with light/dark cycle (12/12 hours). They were allowed free access of standard pellet diet and water ad libitum. The rats were aclimatised to laboratory condition for 10 days before the commencement of experiment. Animals were approved by the Institutional Animal Ethics Committee and conducted according to the regulations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).\textsuperscript{14}

**Acute Toxicity Study**

Acute toxicity study was determine as per OECD Guideline 423.\textsuperscript{15}

Three female rats were being taken in each group. Then initial weight was measured. The polyherbal formulation was administered in the form of syrup prepared in purified water. Initially the drug was administered in a dose of 2000 mg/kg. Mortality and body weight was checked continuously for initial 4 hrs & intermittenly for next 6 hrs & then again at 24 hour and 48 hour. Changes in behavior and body weight were observed. The dose was increased to 5000 mg/kg if the unwanted effects do not appear with 2000 mg/kg.\textsuperscript{15} and then grooming, hyperactivity, sedation, loss of righting reflex, convulsion parameters were measured.

**Experimental Design**

Healthy albino wistar rats (200-250 gm) of male sex were housed under uniform husbandry conditions and will be given pelleted diet and water ad-libitum. The animals were housed at a temperature of 25±2°C with a 12:12 hrs light and dark cycle will be followed.

Seven groups each containing of six rats was allotted to different groups. Group 1 (control) was treated with vehicle only. Group 2 received vehicle and paracetamol (2g/kg p.o) daily for 7 days. Group 3 received paracetamol (2g/kg p.o) after seventh day and LIV 52(5.2 ml/kg body weight p.o) daily for 14 days. Group 4 received PCM (2g/kg p.o) daily from seventh day + poly herbal formulation (100 mg/kg p.o) daily for 14 days. Group-5 received PCM (2g/kg p.o) from seventh day + poly herbal formulation (300 mg/kg p.o) daily for 14 days. Group-6 received PCM (2g/kg p.o) from seventh day + poly herbal formulation (500 mg/kg p.o) daily for 14 days. Group 7 received PHF for 14 days and received PCM for first 7 days. All the animals were sacrificed on day 15\textsuperscript{th} and the blood samples was collected by retro orbital puncture method into sterilized dry centrifuge tubes. Blood allowed to coagulate for 30 min at 37°C. The serum were separated at 2500 rpm for 10 min. Serum were collected and investigated for AST, ALT, ALP, Total Bilirubin.\textsuperscript{15}

**Statistical analysis**

The data was expressed as Mean ± Standard Error of Mean (SEM). One way analysis of variance (ANOVA) was applied to test the significance of difference between average physical parameters, serum parameters and functional parameters of different groups with correction.
for multiple comparisons by the Dunnet test. p - value less than 0.05 was considered statistically significant. The entire statistical analysis was performed using statistical package called Graph pad Instate Software.

RESULTS AND DISCUSSION

The present study was carried out on methanolic extract of all four plants used in polyherbal formulation, extracted in the Parul Institute of Pharmacy & Research, vadodara.

Identification Tests for Major Constituents

Methanolic extract of individual plants of PHF was found to have following constituents as shown in Table 1.

Acute Toxicity Study

Acute toxicity was performed at dose 2000mg/kg and changes were not observed in skin, fur, eyes and mucous membranes. Tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma were not observed. Changes in body weight were not observed. No deaths were observed.

Biochemical Parameters

Aspartate Amino transfarase (AST)

In Paracetamol treated group of animals the concentration of AST was considerably increased than the normal animals (group 1) which indicates severe hepatotoxicity. Treating with the polyherbal syrup (group 4, 5, 6, 7) showed significant decrease (p<0.01) in concentration of AST compared to Paracetamol treated group (group 2). Standard group showed significant decreased (p<0.01) in concentration of AST compared to disease control group.

All the values are expressed as mean ± SEM (n=6) in each group. Where, **p < 0.01 when compared to Paracetamol treated group (i.e group II) ANOVA followed by dunnette test.

Table 1: Phytochemical screening of Polyherbal formulation

<table>
<thead>
<tr>
<th>Types of Phytochemical Constituents</th>
<th>Methanolic Extract of Allium sativum</th>
<th>Methanolic Extract of Ocimum sanctum</th>
<th>Methanolic Extract of Curcuma longa</th>
<th>Methanolic Extract of Aloe barbadensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Protein</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Coumarin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Symbols ‘++’ indicates presence of the phytochemical in high concentration; ‘+’ indicates presence in moderate concentration; and ‘+’ indicates presence in trace concentration and ‘-’ indicates absence of the stated phytochemical.

Table 2: Effect of Polyherbal formulation on serum AST level in hepatotoxic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>AST (mg/dL) (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>74.34 ± 1.87</td>
</tr>
<tr>
<td>2</td>
<td>Positive control</td>
<td>143.99 ± 3.65</td>
</tr>
<tr>
<td>3</td>
<td>Standard group</td>
<td>80.44 ± 1.98 **</td>
</tr>
<tr>
<td>4</td>
<td>Test group I (100mg/kg)</td>
<td>117.77 ± 3.40 **</td>
</tr>
<tr>
<td>5</td>
<td>Test group II (300mg/kg)</td>
<td>110.89 ± 3.12 **</td>
</tr>
<tr>
<td>6</td>
<td>Test group III (500mg/kg)</td>
<td>94.48 ± 2.45 **</td>
</tr>
<tr>
<td>7</td>
<td>Treatment group</td>
<td>111.24 ± 0.43 **</td>
</tr>
</tbody>
</table>

Figure 1: Effect of Polyherbal syrup on AST
**Alanine Amino transfarase (ALT)**

In Paracetamol treated group of animals the concentration of ALT was considerably increased than the normal animals (group 1) which indicates severe hepatotoxicity. Treating with the polyherbal syrup (group 4, 5, 6, 7) showed significant decrease (p<0.01) in concentration of ALT compared to paracetamol treated group (group 2). Standard group showed significant decreased (p<0.01) in concentration of ALT compared to disease control group. All the values are expressed as mean ± SEM (n=6) in each group. Where, **" p < 0.01 when compared to Paracetamol treated group (i.e group II) ANOVA followed by dunnet test.**

**Table 3: Effect of Polyherbal formulation on serum ALT level in hepatotoxic rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALT (IU/L) (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>82.46 ± 1.93**</td>
</tr>
<tr>
<td>2</td>
<td>Positive control</td>
<td>123.56 ± 2.70</td>
</tr>
<tr>
<td>3</td>
<td>Standard group</td>
<td>95.78 ± 3.41**</td>
</tr>
<tr>
<td>4</td>
<td>Test group I (100mg/kg)</td>
<td>112.57 ± 2.86**</td>
</tr>
<tr>
<td>5</td>
<td>Test group II (300mg/kg)</td>
<td>105.96 ± 3.73**</td>
</tr>
<tr>
<td>6</td>
<td>Test group III (500mg/kg)</td>
<td>99.30 ± 2.68**</td>
</tr>
<tr>
<td>7</td>
<td>Treatment group</td>
<td>108.5 ± 0.65**</td>
</tr>
</tbody>
</table>

**Alkaline Phosphatase (ALP)**

In paracetamol treated group of animals the concentration of ALP was considerably increased than the normal animals (group 1) which indicates severe hepatotoxicity. Treating with the polyherbal syrup (group 4, 5, 6, 7) showed significant decrease (p<0.01) in concentration of ALP compared to paracetamol treated group (group 2). Standard group showed significant decreased (p<0.01) in concentration of ALP compared to disease control group. All the values are expressed as mean ± SEM (n=6) in each group. Where, **" p < 0.01 when compared to Paracetamol treated group (i.e group II) ANOVA followed by dunnet test.**

**Table 4: Effect of Polyherbal formulation on serum ALP level in hepatotoxic rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALP (U/L) (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>28.34 ± 1.95**</td>
</tr>
<tr>
<td>2</td>
<td>Positive control</td>
<td>111.92 ± 4.77</td>
</tr>
<tr>
<td>3</td>
<td>Standard group</td>
<td>75.59 ± 3.81**</td>
</tr>
<tr>
<td>4</td>
<td>Test group I (100mg/kg)</td>
<td>93.10 ± 1.88**</td>
</tr>
<tr>
<td>5</td>
<td>Test group II (300mg/kg)</td>
<td>84.75 ± 2.94**</td>
</tr>
<tr>
<td>6</td>
<td>Test group III (500mg/kg)</td>
<td>76.96 ± 2.63**</td>
</tr>
<tr>
<td>7</td>
<td>Treatment group</td>
<td>89.31 ± 0.63**</td>
</tr>
</tbody>
</table>

**Total Bilirubin (TBL)**

In paracetamol treated group of animals the concentration of total bilirubin was considerably increased than the normal animals (group 1) which indicates severe hepatotoxicity. Treating with the polyherbal syrup (group 4, 5, 6, 7) showed significant decrease (p<0.01) in concentration of total bilirubin compared to paracetamol treated group (group 2).
Standard group showed significant decreased \((p<0.01)\) in concentration of bilirubin compared to disease control group. All the values are expressed as mean ± SEM \((n=6)\) in each group. Where, ```p < 0.01 when compared to Paracetamol treated group (i.e group II) ANOVA followed by dunnet test.

**Figure 4:** Effect of polyherbal syrup on Total bilirubin

Paracetamol is a commonly used as analgesic and antipyretic drug and is safe in therapeutic doses but produces fatal hepatic necrosis with toxic doses. Paracetamol being a drug capable of causing liver disorders if overdoses are consumed. The covalent binding of \(N\)-acetyl-Pbenzoquinoneimine, an oxidation product of paracetamol, to sulphydryl groups of protein resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver as the cause of hepatotoxicity have been reported earlier.\(^{16,17}\) In the present study PCM was found to cause a significant increase in the liver weight, which is due to blocking of secretion of hepatic triglycerides into the plasma.\(^{18}\)

It is well documented that PCM is biotransformed under the action of Microsomal cytochrome-P450 of liver to reactive metabolites.\(^{19,20}\) These free radicals bind covalently to unsaturated lipid membrane, provoking a sharp increase of lipid peroxides followed by pathological changes such as elevated levels of serum marker enzymes like AST, ALT and ALP , decreased protein synthesis, triglyceride accumulation , and finally hepatocyte damage.\(^{21}\) This suggests that PCM induces liver injury by sharing a common property of free radical mechanism. Significant reduction of these enzyme levels by treatment with polyherbal formulation is indicative of its hepatoprotective effect.

Serum bilirubin is one of the most sensitive tests employed in the diagnosis of hepatic diseases. Hyperbilirubinemia was observed due to excessive heme destruction and blockage of biliary tract. As a result of blockage of the biliary tract there is a mass inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged and dead hepatocytes.\(^{22}\)

Administration of polyherbal formulation decreased the level of bilirubin is indicative of its hepatoprotective effect.

Protein metabolism is a major project of liver and a healthy functioning liver is required for the synthesis of serum proteins except for the \(\gamma\) globulins. Hypoproteinemina is a feature of liver damage due to significant fall in protein synthesis. Albumin is decreased in chronic liver disease and is generally accompanied by an increase in the \(\beta\) and \(\gamma\) globulins as a result of production of IgG and IgM.\(^{23}\) Hypoproteinemina was observed after paracetamol ingestion but the trend turns towards normal after polyherbal formulation treatment.

Lactate dehydrogenase is localized in the cytoplasm of cells and thus is extruded into the serum when cells are damaged. The measurement of total lactate dehydrogenase can be useful when only a specific organ such as the liver is known to be involved. Lactate dehydrogenase is a sensitive intracellular enzyme which increases in serum is also an indication of liver cell damage. Significant reduction of LDH levels by treatment with polyherbal formulation is indicative of its hepatoprotective effect.

Histopathological profile of control animals showed normal hepatocytes. The section of liver of control group of animals exhibited severe intense congestion, hydropic degeneration and occasional necrosis. The liver sections of LIV 52 treated animals showed normal hepatic architecture with few fatty globules. The liver sections of animals treated with polyherbal formulation showed normal hepatic cords and absence of severe congestion and occasional necrosis indicating pronounced protection of hepatocytes by paracetamol induced hepatic damage.

Thus the present study indicates that the polyherbal formulation prevents and treats the hepatotoxicity induced by PCM in rats.

**CONCLUSION**

From the result of the present study, it can be concluded that the polyherbal formulation possessed a significant hepatoprotective activity against PCM induced hepatotoxicity in rats, as evident by physical, biochemical, histological and functional parameters.

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