Phytochemical analysis and hepatoprotective effect of polyherbal formulation on CCl₄ induced hepatotoxicity in mice

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Abstract: The potent phytotherapeutic modalities against the hepatotoxicity have motivated us to explore numerous plants and polyherbal preparations because conventional drug discovery is more expensive and tedious. So, this study was conducted to evaluate the hepatoprotective potential of a polyherbal formulation (PHF), comprising of Solanum nigrum, Silybum marianum, Artemisia absinthium, Achillea millifolium and Cichorium intybus against carbon tetrachloride (CCl₄) induced hepatotoxicity in experimental rats. CCl₄ intoxication induced vacuole formation and fast degeneration so selective liver enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin in rat’s plasma, as well as liver histological architecture, were used to evaluate the effect of herbal treatments with different doses (ranging 100-500 mg/kg) for two weeks. Statistical analysis showed that PHF significantly (P<0.05) improved the level of liver enzymes as well as improved the liver architecture comparative to control groups. It could be concluded from current findings that PHF prepared from Solanum nigrum, Silybum marianum, Artemisia absinthium, Achillea millifolium and Cichorium intybus have some hepatoprotective activities.

Keywords: Drugs, Ploy herbal formulation, intoxication, hepatoprotective activities, hepatitis, medicinal plants.

INTRODUCTION

The liver is the main organ which plays an important role in detoxification and metabolism of various compounds i.e. carbohydrates, proteins and lipids. It also secretes bile that has a significant role in digestion. The role played by the liver makes it vulnerable to a persistent exposure to noxious agents resulting in its dysfunction (Kumar et al., 2012). At present approximately 12% of adults (more than 20 million) have gallbladder stones and approximately 2 to 3% of adults (about 5.5 million of people) have chronic liver diseases (CLD) or cirrhosis (Udompap et al., 2015). The current burden of these diseases required more efforts for their prevention and control. This can be achieved by understanding these ailments on the basis of biomedical research. To lessen the toxicity and side effects associated with available treatments, the development of PHF is needed to manage liver disorders.

CCl₄ is widely used to induce liver injury in rats. Impairment of protective cellular mechanism is associated with hepatotoxicity. Liver toxicity is caused by CCl₄ that results in fatty degeneration, necrosis, and fibrosis that leads to liver cirrhosis. Exposure to CCl₄ from any means (oral, parenteral or inhalation), results in hepatocellular carcinomas. In various studies, the toxic effects of CCl₄ were also noted. Generation of free radicals by CCl₄ is among various mechanisms that result in oxidative damage to liver cells (Alam et al., 2018). Many natural compounds and medicinal plants showed protective effects against liver toxicity caused by CCl₄ (Kamalakannan et al., 2005) and in many cases, this protective response was due to their antioxidant properties (Lin et al., 2000).

Therefore, the exploration of such active ingredients from plants has become more significant (Mukherjee et al., 2006). A single plant may not have all desired ingredients to treat a liver injury caused by CCl₄. Henceforth the current study was carried out to assess the possible beneficial protective effects of PHF confirmed by estimation of various parameters viz. histological and morphological parameters. The result was compared with silymarin (a well-known potent antioxidant) that is widely used for the treatment of various hepatic disorders (Dixit et al., 2007).

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MATERIALS AND METHODS

Plant material and preparation of Poly-herbal Formulation (PHF)
Selected Medicinal plants including Solanum nigrum, Silybum marianum, Atremesia absinthium, Achillea millefolium and Cichorium intybus used in this study were obtained from local market of Rawalakot (AJ&K). Plants were identified and authenticated by a Taxonomist from Department of Botany, The University of The Poonch Rawalakot Azad Jammu & Kashmir-Pakistan. Extract of each plant was prepared by using 70 % hydro-alcohol as a solvent and then 20 gram powders of each plant (100 gram of total) was sonicated twice with 200 mL of hydro-alcohol for 48 hours at room temperature. To get a final volume of 100 mL PHF, the solution was filtered by Whatman filter paper and then evaporated by using rotary evaporator under reduced pressure.

Phytochemical analysis
Phytochemical constituents like carbohydrates, alkaloids, glycosides, saponins, steroids, flavonoids and tannins, of selected medicinal plants, were screened using standardized protocol (Vaghasiya et al., 2011).

Experimental design and animals grouping
Male Swiss albino mice of 5 to 6 weeks old with weight 28± 2 g were obtained from animal house of Government College University, Faisalabad-Pakistan and kept for a week under standard conditions i.e. fed with pelleted diet and water ad libitum, with 12 hours dark and 12 hours light rhythm, sultriness 30-70 % and temperature 21°C before commencement of the experiment. CCl₄ was used for persuading hepatic injury in all animal groups except group I. Liquid paraffin was used for thinning of CCl₄ at a ratio of 1:2, given intra-peritoneal at a dose of 2 mL/kg body weight using Ha et al., (2005) method. Mice were divided into 6 groups (n=36) as Group I (n=6): Control animals (Normal) given carboxymethyl cellulose (1mL/kg of 1% w/v, P/O); Group II (n=6): Intoxicated Control (CCl₄,1mL/kg, i/P); Group III (n=6): Standard drug (Silymarin 200 mg/kg, P/O); Group IV (n=6): PHF extract (500 mg/kg, P/O); Group V (n=6): PHF extract (250 mg/kg, P/O) and Group VI (n=6): PHF extract (100 mg/kg, P/O). Based on consequences of previous studies on mice treatment plan was adjusted for 14 days (Pandey et al., 2011) and Standard doses of Silymarin and PHF extract were given orally daily for 2 weeks to respective groups.

Cytotoxic studies
Cytotoxic studies were done by increasing the dose of the sample in rats. At a dose of 1000 mg/kg toxic clinical features appeared and mice died in 12 hours.

Biochemical and histopathological study
Blood samples were obtained from the common carotid artery. The serum was separated by centrifugation and used for the assay of ALT, ALP, AST and bilirubin.

After collection of the blood sample, mice were killed and the liver was separated for histological analysis. The liver samples were preserved in 10% buffered formalin (7.2 pH), processed, embedded in paraffin wax and sections were stained with hematoxylin and eosin (H & E). Histological examination was carried out on stained sections with a microscope (Olympus CX23) to see the arrangement of hepatic lobules, fatty changes and infiltration of inflammatory cell.

Ethical approval
This study was approved by the Departmental Ethical Committee of Medical and Health Sciences Faculty, University of Poonch, Rawalakot, Azad Kashmir.

STATISTICAL ANALYSIS
Statistical analysis was done by ANOVA, followed by Dunnett’s post or Turkey-Kramer test using SPSS 20. All the data are expressed as means ± standard deviation. Recurrence examination was done using Fisher’s test. The difference was significant at the level of p <0.05.

RESULTS
Phytochemical analysis
Phytochemical screening of PHF showed the presences of various active ingredients as shown in table 1.

Biochemical analysis
Results showed that PHF induction significantly (p<0.05) reduced the level of serum Bilirubin, ALT, AST and ALP in all CCl₄ intoxicated groups after treating with specific doses of PHF and Silymarin as compare to non-treated group (table 2; figs. 1 & 2).

Histopathological analysis
Histological examination of liver sections showed that intoxication with CCl₄ induced severe morphological changes (fig. 3 B) as compared to sections taken from the non-intoxicated group (fig. 3A). Further, it was reported that treatment of animals with silymarin and different doses of PHF significantly improved the liver architecture (fig. 3C-3F) and these morphological observations in all groups of this study were in line with the findings of serum ALT, AST, ALP and bilirubin.

DISCUSSION
The liver can be damaged by various drugs and chemicals. In this study, CCl₄ was selected as hepatotoxicant to induce liver damage. The primary aim of this study is to assess the hepatoprotective activity of PHF (containing 5 different plants) at different doses against CCl4 induced liver damage.
Table 1: Phytochemical analysis of the PHF

<table>
<thead>
<tr>
<th></th>
<th>Glycosides</th>
<th>Alkaloids</th>
<th>Flavonoids</th>
<th>Saponins</th>
<th>Carbohydrates</th>
<th>Tannins</th>
<th>Steroids</th>
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<tr>
<td>Solanum nigrum</td>
<td>+++</td>
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<td>++</td>
<td>+++</td>
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<tr>
<td>Silybum marianum</td>
<td>++</td>
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<td>+++</td>
<td>++</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Cichorium intybus</td>
<td>_</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>_</td>
<td>_</td>
<td>_</td>
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<tr>
<td>Artemisia absinthium</td>
<td>_</td>
<td>_</td>
<td>+++</td>
<td>+++</td>
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<td>_</td>
<td>_</td>
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<tr>
<td>Achillea millefolium</td>
<td>+++</td>
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<td>+++</td>
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Table 2: Effect of Polyherbal formulation and silymarin on serum biochemical parameters in mice subjected to CCl4 induced toxicity.

<table>
<thead>
<tr>
<th>Group</th>
<th>Group I: Control</th>
<th>Group II: CCl4 (1 mL/kg)</th>
<th>Group III: Silymarin (200mg/kg)+CCl4</th>
<th>Group IV: PHF (500 mg/kg)+ CCl4</th>
<th>Group V: PHF (250 mg/kg)+ CCl4</th>
<th>Group VI: PHF (100 mg/kg)+ CCl4</th>
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<tbody>
<tr>
<td>ALT (U/I)</td>
<td>40.2±2.99</td>
<td>374.4±15.42</td>
<td>41.2±2.79</td>
<td>44.6±2.42</td>
<td>134.8±11.46</td>
<td>182.6±7.12</td>
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<tr>
<td>AST (U/I)</td>
<td>46.8±1.47</td>
<td>269.2±9.02</td>
<td>46.4±4.22</td>
<td>47±2.10</td>
<td>71.6±8.69</td>
<td>169.2±15.10</td>
</tr>
<tr>
<td>ALP (U/I)</td>
<td>128.2±6.68</td>
<td>313.8±4.62</td>
<td>165.6±10.74</td>
<td>168.4±10.21</td>
<td>186.2±9.66</td>
<td>220±7.07</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>0.416±0.07</td>
<td>2.532±0.11</td>
<td>0.438±0.08</td>
<td>0.420±0.08</td>
<td>0.520±0.05</td>
<td>0.702±0.12</td>
</tr>
</tbody>
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Fig. 1: After 14 days level of Serum Bilirubin after PHF treatment as compared to standard drug and to the control group

Fig. 2: After 14 days levels of liver enzymes on PHF treatment as compared to standard drug and to the control group

Fig. 3: Microphotomicrograph (10x) and (40x) showing histopathological profile of the livers of mice. (A). Normal Control, Liver treated with distilled water showed normal cellular with well-preserved cytoplasm, prominent nucleolus and central vein (B). CCl4 Control shows severe liver damage i.e. steatosis, necrosis and infiltration by inflammatory cells (C). Silymarin and (D). PHF 500mg/kg, treated cell is quite similar to control group (E). PHF 250mg/kg and (F). PHF 100mg/kg showed moderate changes than those treated with CCl4 only.
CCl₄ is metabolized in the liver by cytochrome p450 in mitochondria and endoplasmic reticulum and converted into highly reactive free radicals namely trichloromethyllthionitrate intermediate peroxidation of cell membranes and ultimately lead to necrosis of the cells (Vlacheva-Kuzmanova et al., 2004). In viral hepatitis, liver damage is practically identical to that of CCl₄ (Abere et al., 2010). When hepatocytes are damaged due to any cause, an increased level of enzymes i.e. AST, ALT, ALP etc. are released from damaged hepatocytes into the blood. Similarly, when hepatocytes are damaged by CCl₄, it also raises the serum level of AST, ALT, ALP and bilirubin (Alkreathy et al., 2004). Level of these enzymes is also used as biomarkers for assessing the therapeutic role of studied medicinal plants (Tapsell et al., 2006). Cell injury is also evident in the histopathological examination as there is marked micro-vascular stenosis and macro-vesicular congestion (Khan et al., 2013). PHF has the ability to reverse or to protect CCl₄ induced hepatotoxicity in mice.

The CCl₄ treated animals (group II) exhibited an increase in the levels of bilirubin, AST, ALT and ALP as compared to normal control group, representing hepatocellular damage. The group V and VI treated with 250mg/kg and 100 mg/kg respectively decreased the level of CCl₄ induced level of bilirubin, AST, ALT and ALP in a dose-dependent manner as compared to group II (only CCl₄ treated animals). Group IV (treated with PHF 500mg/kg) showed almost the same level of the hepatic enzyme as compared to group I (normal control treated with distilled-water) comparable to Group III (silymarin treated).

The histopathological examination also corresponds to the level of hepatic enzymes in various groups of study. Phytochemical analysis of the plant's extracts showed the presence of glycosides, flavonoids, alkaloids, saponins, tannins, steroids and carbohydrates. This constituent may be possibly responsible for the hepatoprotective activity of PHF and is expected due to its ability to the inflammatory responses, in combination with the ability to scavenge free radicals.

CONCLUSION

The study showed that PHF provides a significant protection against liver injury caused by CCl₄ bearing a potential for clinical application in the treatment of liver diseases. The therapeutic use of herbs by developing PHF is effective, safe and affordable, and hopefully, it will bring improvement in the alternative management of liver disease.

REFERENCES


