Phytochemical analysis and hepatoprotective effect of polyherbal formulation on CCl_4 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*, *Atrmesia absinthium*, *Achillea millifolium* and *Cichorium intybus* against carbon tetrachloride (CCl_4) induced hepatotoxicity in experimental rats. CCl_4 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*, *Atrmesia absinthium*, *Achillea millifiloium* 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.

CCl_4 is widely used to induce liver injury in rats. Impairment of protective cellular mechanism is associated with hepatotoxicity. Liver toxicity is caused by CCl_4 that results in fatty degeneration, necrosis, and fibrosis that leads to liver cirrhosis. Exposure to CCl_4 from any means (oral, parenteral or inhalation), results in hepatocellular carcinomas. In various studies, the toxic effects of CCl_4 were also noted. Generation of free radicals by CCl_4 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 CCl_4 (Kamalakkannan *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 Formation (PHF)

Selected Medicinal plants including *Solanum nigrum, Silybum marianum, Atrmesia absinthium, Achillea millifiloium* 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 powder 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 21ºC before commencement of the experiment. CCl_4 was used for persuading hepatic injury in all animal groups except group I. Liquid paraffin was used for thinning of CCl_4 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 ($CCl_4$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 CCl_4 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 CCl_4 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, CCl_4 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

	Glycosides	Alkaloids	Flavonoids	Saponins	Carbohydrates	Tannins	Steroids
Solanum nigrum	+++	+++	++	+++	_	++	+++
Silybum marianum	+++	+	+++	++	_	_	_
Cichorium intybus	_	++	+++	+++	_	+++	_
Artemisia absinthium	_	_	+++	+++	_	+++	_
Achillea millifolium	+++	_	++	+++	_	++	_

Table 2: Effect of Polyherbal formulation and silymarin on serum biochemical parameters in mice subjected to CCl_4 induced toxicity.

	Group	ALT (U/l) Mean ± SE	AST (U/l) Mean ± SE	ALP (U/l)Mean ± SE	Bilirubin (mg/dl) Mean ± SE
Group I	Control	40.2±2.99	46.8±1.47	128.2±6.68	0.416±0.07
Group II	CCl_4 (1 mL/kg)	374.4±15.42	269.2±9.02	313.8±4.62	2.532±0.11
Group III	Silymarin (200mg/kg)+CCl_4	41.2±2.79	46.4±4.22	165.6±10.74	0.438±0.08
Group IV	PHF (500 mg/kg)+ CCl_4	44.6±2.42	47±2.10	168.4±10.21	0.42±0.08
Group V	PHF (250 mg/kg)+ CCl_4	134.8±11.46	71.6±8.69	186.2±9.66	0.52±0.05
Group VI	PHF(100 mg/kg)+ CCl_4	182.6±7.12	169.2±15.10	220±7.07	0.702±0.12

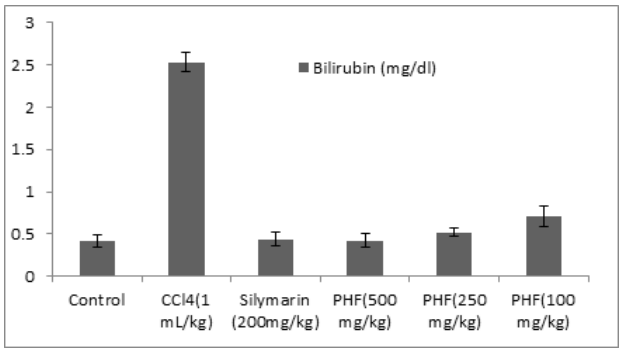

Fig. 1: After 14 days level of Serum Bilirubin after PHF treatment as compared to standard drug and to the control group

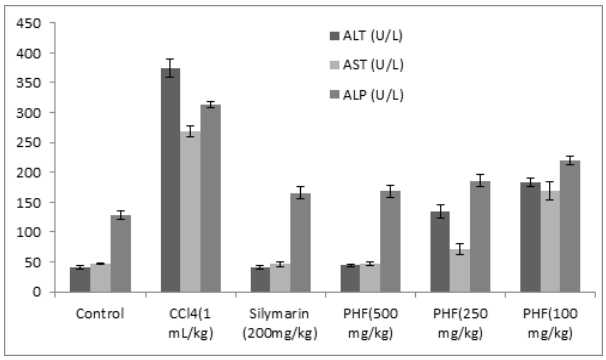

Fig. 2: After 14 days levels of liver enzymes on PHF treatment as compared to standard drug and to the control group

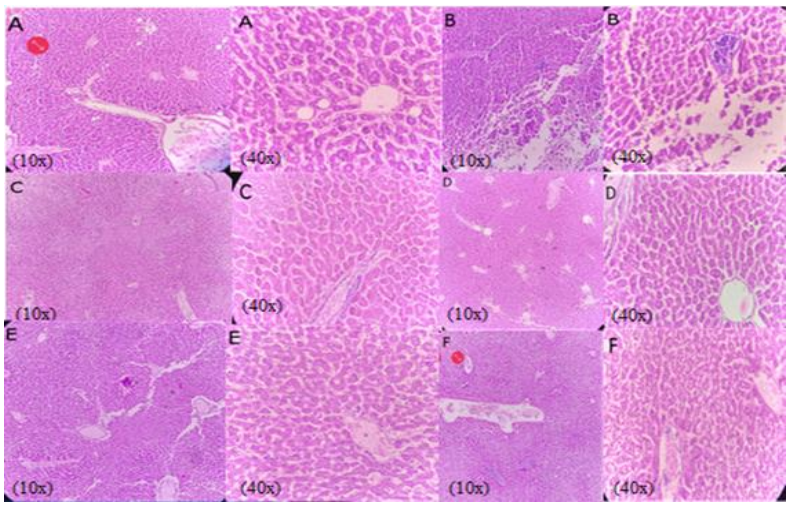

Fig. 3: Microphotomicrograph (10x) and (40x) showing histopathological profile of the livers of mice. (A). Normal Control, Liver treated with distilled water showed normal cellular with well-preserved cytoplasm, prominent nucleolus and central vein (B). CCl_4 Control shows severe liver damage i.e. steatosis, 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 CCl_4 only.

CCl_4 is metabolized in the liver by cytochrome p450 in mitochondria and endoplasmic reticulum and converted into highly reactive free radicals namely trichloromethylthatinitiate lipid 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 CCl_4 (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 CCl_4, 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-vesicular steatosis and macro-vesicular congestion (Khan *et al*., 2013). PHF has the ability to reverse or to protect CCl4 induced hepatotoxicity in mice.

The CCL_4 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 CCl_4 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 CCl_4 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.

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