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# Scientific validation of polyherbal hepatoprotective formulation against paracetamol induced toxicity

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ABSTRACT

**Objective:** This polyherbal formulation has been traditionally used in the Indian system of medicine as a chief formulation for the treatment of hepatic diseases as hepatoprotective. The aim of the study was to study hepatoprotective activity which will be scientific validation of traditional knowledge claimed about this polyherbal formulation. **Methods:** Hepatotoxicity was induced by administration of paracetamol (300mg/kg) to the animals. The levels of liver enzymes (SGOT, SGPT, Alkaline phosphatase, Serum Bilirubin), lipid profiles (triglyceride, cholesterol, HDL, LDL), creatinine, urea levels and histopathological parameters were measured in order to evaluate hepatoprotective activity of polyherbal formulation. **Results:** The polyherbal formulation produced a significant hepatoprotective activity of the decoction of polyherbal formulation. The polyherbal formulation (PHF = 1) shows good hepatoprotective activity by lowering the levels of SGOT, alkaline phosphatase, bilirubin parameters ($P<0.05$), lipid profiles – cholesterol, triglyceride, LDL and histopathological evaluations shows that PHF = 1 and PHF = 3 formulations have significantly hepatoprotective activity ($P<0.05$). **Conclusions:** The study validates that polyherbal formulation has a good hepatoprotective activity. Further standardization processes may be performed in order to make it a beneficial hepatoprotective formulation.

## 1. Introduction

Liver disease is the major problem worldwide, as liver has paramount importance due to its functions involving metabolic functions, detoxification of xenobiotics, drugs, chronic alcoholism, in the alteration of divers homeostatic mechanisms may lead to serious liver disease [1]. The serious adverse effects of the synthetic and conventional drugs protest their uses and support the scope of traditional medicines again worldwide [2]. Natural remedies from medicinal plants are considered to be effective and safe alternative treatment for liver toxicity [3]. Herbal based natural products are the key aliments in the treatment of liver diseases [4].

The present study deals with the hepatoprotective activity of this polyherbal hepatoprotective formulation (PHF) containing eight medicinal plants mentioned in Indian ancient literature *Ayurveda*. The hepatoprotective activity of PHF is explored against paracetamol (PCM) because of its liver dysfunction activity [5]. Polyherbal hepatoprotective formulation consist dried powders of medicinal plants (50 gm. each crude drug) [6].

## 2. Materials and methods

### 2.1. Plant material

The plant materials used for preparation of a polyherbal formulation includes Emblica officinalis (Fruits), Terminalia chebula (Fruits), Terminalia bellirica (Fruits), Picrorhiza kurroa (Rhizomes), Tinospora cordifolia (Stem), Swertia chirata (Entire herb), Azadirachta indica (Bark), Adhatoda vasica (Stem bark) had been provided and authenticated by Department of Botany [Voucher specimens (DNM01 – DNM08)], CSIR – CIMAP Lucknow.

2.2. Chemicals All the materials used for this experiment were of analytical grade. Paracetamol (*E. Merck*), Liv –

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52 (Himalaya Herbal Healthcare), Diagnostic kits for the estimation of serum glutamicoxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), serum bilirubin, blood urea, serum creatinine, cholesterol, triglyceride, HDLc and LDLc were supplied by Merck Specialties Pvt. Ltd, Germany. Standard orogastric cannula was used for oral drug administration.

### 2.3. Test Animals

Mus muscullus species female mice weighing 17-22 gms. were obtained from "JEEVANIKA", CSIR-CIMAP Lucknow. The animals were keep in polypropylene cages under standard conditions (Temperature of 22 ± 3 0C with 50 - 70% relative humidity and 12 : 12 hrs. on light and dark cycles). The animals were fed with pellet diet (M/s Hindustan Lever Ltd., Bombay, India) and water ad libitum. All study protocols conducted in accordance with guidelines and approved by animal ethics committee CSIR - CIMAP, Lucknow. (Ref - DN01 Date - 13/10/2011).

### 2.4. Prparation of polyherbal drug extract

The plant materials were coarsely grounded separately then mixed and extracted as decoction [7]. The slurry was filtered with muslin cloth followed by whattman filter paper and subjected to Rota vapor distillation unit (BUCHI Switzerland). Solvents are removed and syrupy solution is obtained. The syrupy solution is transferred to Petri plates and place over water bath at 40 - 45 0C Temp. till dryness. This dried polyherbal crude extract was brownish black in colour and total quantitative yield of decoction was 12.80% (w/w) [8].

### 2.5. Phytochemical screening

The preliminary phytochemical screening was done by following standard qualitative chemical tests for phytoconstituents and the hydroalcoholic extract showed that alkaloid, glycoside, anthraquinones glycoside, phenolic & tannins, steroids, flavonoid, saponin, carbohydrate, reducing sugars are present in the decoction.

### 2.6. Experimental design and drug treatment

All the animals were randomly divided into six groups of six mice each and groups were aligned as follows:-

Group 1 Vehicle control
Group 2 Received PCM
Group 3 Received positive control (Liv - 52) + PCM
Group 4 Received PCM + PHF = 1 (300 mg/kg body weight)
Group 5 Received PCM + PHF = 2 (400 mg/kg body weight)
Group 6 Received PCM + PHF = 3 (500 mg/kg body weight)

The mice of all groups except group 1 received PCM at a dose of 300 mg/kg body weight i.p. on 8th day. The polyherbal formulation was dissolved in distilled water and given orally through intragastric tube daily in the morning for seven days.

### 2.7. Collection of tissue sample and serum

On the 8th day, after 2:30 hrs of toxin induction about 500 μl of blood was collected through retro orbital plexus of the mice using non heparinized hematocrit capillaries. The animals were sacrificed by cervical dislocation and livers were excised and transferred into ice cold containers. The blood was allowed to clot at room temperature for 30 min. and then kept at 4℃ for 30 minutes [9]. After 30 min. the blood samples were removed from refrigerator and allowed to compatible to room temperature. After that the serum samples are centrifuged at 2000 rpm for 15 minutes. The supernatant containing the serum was collected and stored at -20℃ [10].

### 2.8. Biochemical parameters and histological evaluation

Biochemical parameters SGOT, SGPT, Alkaline phosphatase [11], Cholesterol, Triglyceride, Blood urea, HDLc, LDLc, Serum Creatinine, and Bilirubin direct were evaluated. The excised livers were washed with phosphate buffer, dried with the help of tissue paper and transferred into 10% formalin fixation solution for 48h [12]. The paraffin embedding was done and 5 μm thick sections were taken in a microtome. Hematoxyline and eosin was used for staining and slides were examined for histopathological changes under microscope (40,100 and 200X) [13].

### 2.9. Statistical analysis

All the data are represented as Mean ± S.E.M. (six animals/group) and comparison between groups are done by One-way ANOVA followed by Dunnett's multiple comparison test *at $P<0.01$, **at $P<0.05$, ***at $P>0.05$ was considered significant.

## 3. Results

The significant liver damage was observed when PCM - treated group (PCM 300mg/kg) and vehicle control group compared (at $P<0.01$). All groups were treated with PCM to induce hepatotoxicity except vehicle control group showed significantly increased level of liver enzymes - SGOT, SGPT, Alkaline phosphatase, Serum Bilirubin. The pretreatment with the polyherbal formulation has shown good hepatoprotective activity at the PHF = 1.

SGOT level in the present test is decreased in dosing

**Table 1**

Effects of Polyherbal formulation (PHF) on the activities of enzymes and the levels of bilirubin, urea and creatinine in serum [14]

| Groups | AST (U/L) | ALT (U/L) | ALP (U/L) | Bilirubin (mg/dl) | Urea (mg/dl) | Creatinine (mg/dl) |
|---|---|---|---|---|---|---|
| (1) Vehicle control | 20.43±2.11 | 19.84±10.61 | 107.5±13.64 | 0.046±0.01 | 43.38±3.86 | 0.19±0.04 |
| (2) PCM –treateda | 98.07±31.21* | 108.61±57.28* | 145.4±16.12*** | 0.089±0.02* | 74.39±7.74* | 0.35±0.09* |
| (3) PCM+ Positive control (Liv 52)b | 72.98±20.03*** | 72.35±22.75*** | 136.02±9.98** | 0.079±0.01*** | 59.11±4.13* | 0.25±0.01* |
| (4) PCM +PHF = 1 (300 mg/kg) b | 40.28±6.55*** | 81.26±16.99*** | 108.1±10.65* | 0.059±0.02*** | 44.87±2.06* | 0.23±0.2* |
| (5) PCM+PHF = 2 (400 mg/kg) b | 76.36±26.55*** | 30.65±14.85*** | 128.9±13.18*** | 0.06±0.01** | 59.93±5.36* | 0.21±0.01* |
| (6) PCM+PHF = 3 (500 mg/kg) b | 68.50±24.38*** | 60.63±26.61* | 119.52±6.74*** | 0.088±0.02** | 68.65±5.67*** | 0.21±0.01* |

All values are expressed as Mean ± SEM, N=6. a–as compared to control group, b–as compared to PCM – treated group. Analysis by One–way ANOVA followed by Dunnet's test. Significant *at $P$<0.01, **at $P$<0.05, ***at $P$>0.05.

**Table 2**

Effects of Polyherbal formulation (PHF) on lipid profile **[15]**

| Groups | Triglyceride (mg/dl) | Cholesterol (mg/dl) | HDLc (mg/dl) | LDLc (mg/dl) |
|---|---|---|---|---|
| (1) Vehicle control | 14.61±5.24 | 55.44±6.63 | 48.89±13.47 | 0.36±0.04 |
| (2) PCM – treateda | 49.42±7.22* | 126.1±12.70* | 115.1±17.25* | 0.51±0.05* |
| (3) PCM+ Positive control (Liv 52) b | 41.97±16.86*** | 82.51±4.99* | 84.44±19.09** | 0.41±0.05** |
| (4) PCM +PHF = 1 (300 mg/kg) b | 41.58±12.75*** | 119.7±11.91*** | 95.11±19.82*** | 0.42±0.03** |
| (5) PCM+PHF = 2 (400 mg/kg) b | 32.22±8.14*** | 95.66±6.69* | 105.3±21.25*** | 0.48±0.05*** |
| (6) PCM+PHF = 3 (500 mg/kg) b | 41.08±12.93*** | 91.24±5.86* | 85.33±13.28** | 0.37±0.07** |

All values are expressed as Mean ± SEM, N=6. a–as compared to control group, b–as compared to PCM – treated group. Analysis by One–way ANOVA followed by Dunnet's test. Significant *at $P$<0.01, **at $P$<0.05, ***at $P$>0.05.

groups PHF = 1, PHF = 2 and PHF = 3.But dosing PHF = 1 is significantly comparable to dosing group PCM – treated ($P$<0.05) which is more clinically important. The serum SGPT level in the dosing groups PHF = 3 is statically significant as compared to PCM – treated group ($P$<0.01).

The serum alkaline phosphatase level was deceased in the dose group PHF = 1 and dosing group PHF = 3, which is comparable to PCM – treated group ($P$<0.01) (Table 1). The bilirubin total test the level of bilirubin is significantly decreased in dosing groups PHF = 2 and PHF = 3 which is comparable to the PCM – treated group ($P$<0.05). Statically significant decrease in the serum urea level in the dosing group PHF = 1and PHF = 2 have been most comparable to the PCM – treated group ($P$<0.01).

Serum creatinine level in the dosing groups positive control, PHF = 1, PHF = 2, PHF = 3 are significantly low as compared with PCM – treated group ($P$<0.01) (Table 1). It was observed that the serum triglyceride level of all three test dosing groups was deceased significantly and comparable to the PCM – treated group ($P$<0.05). The cholesterol level was decreased in dosing groups PHF = 2, PHF = 3 and these are comparable with PCM – treated group ($P$<0.01) (Table 2). The HDL Cholesterol level was decreased in all three test dosing groups but the dosing group PCM – treated and dosing group PHF = 3 was statically comparable ($P$<0.05). The LDL Cholesterol level was decreased in all three test dosing groups but the dosing groups PHF = 1 and PHF = 3 is statically comparable with PCM – treated group ($P$<0.05) and found that it may be clinically significant (Table 2).

### *3.1. Histopathological evaluation*

Histopathological study of livers of vehicle control group showed normal hepatocellular architecture (figure 1) [18]. Livers challenged with PCM showed disarrangement of normal hepatic cells with massive interlobular necrosis, inflammatory infiltration of lymphocytes and fatty changes (figure 2) [19]. The Polyherbal formulation (PHF = 1 and PHF = 2) treated mice exhibited significant protection against PCM intoxication as evident by presence of normal hepatic cords and absence of necrosis with minimal inflammatory conditions around the central vein (figure 3, 4). However, moderate protection was observed in case of other dose (PHF = 3) group animals (figure 5). In the liver section of Liv – 52 treated rats Normal hepatocytes and lobular structure was observed in hepatocytes. Which may be due to the effective mechanisms of Liv – 52 (figure 6).

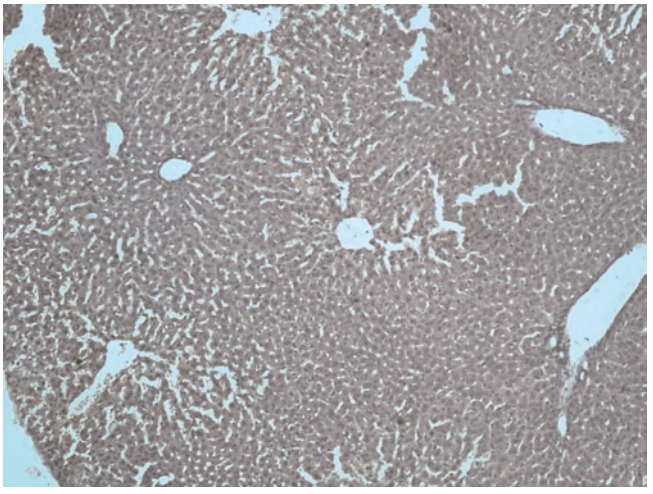

**Fig. 1**. Hepatocytes of the normal control group showed a normal lobular architecture of the liver [16].

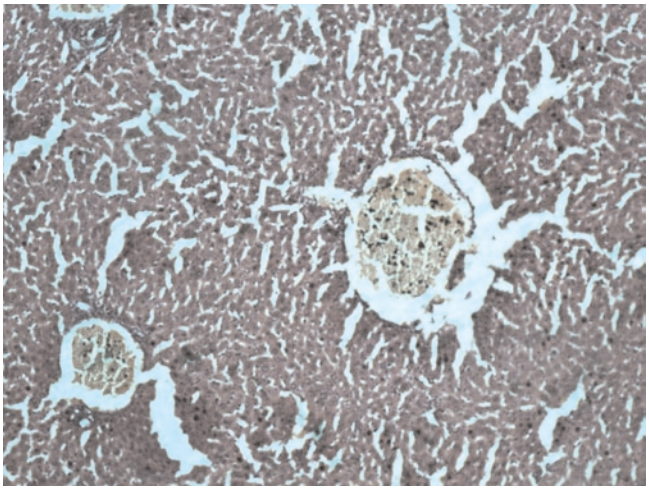

**Fig. 2.** Hepatocytes of the PCM treated group showed liver cell necrosis and inflammation also observed in the centrilobular region with portal triaditis.

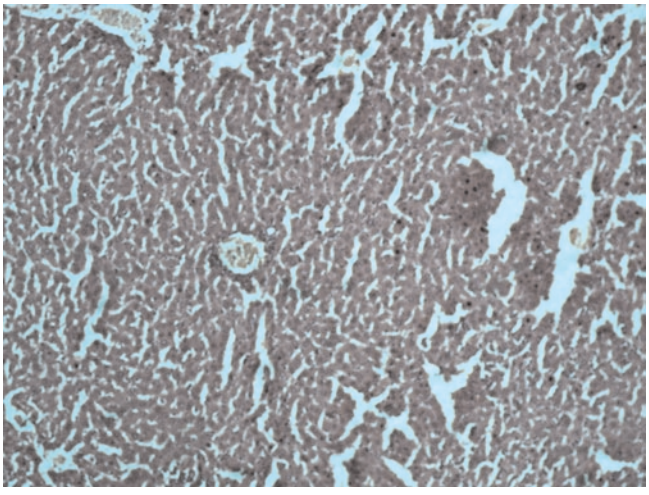

**Fig. 3.** Hepatocytes of the PHF = 1 pretreated group showed minimal inflammation with moderate portal triaditis and their lobular architecture was normal.

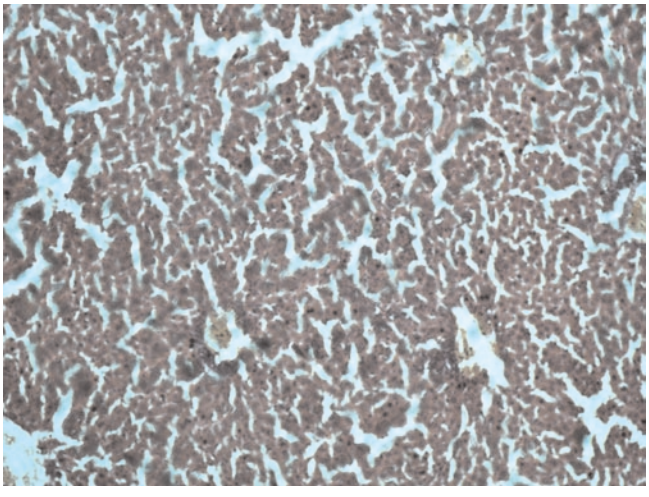

**Fig. 4.** Hepatocytes of the PHF = 2 pretreated group showing partial protection of hepatocytes, tiny focal necrosis with mild portal inflammation.

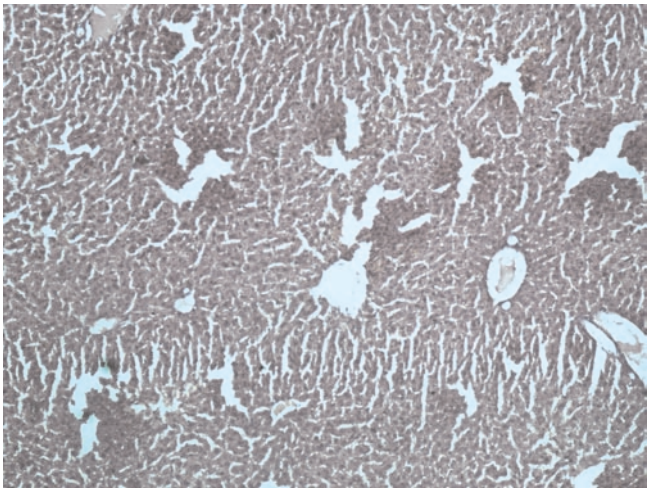

**Fig. 5.** Hepatocytes of the PHF = 3 pretreated group showed moderate portal triaditis and their lobular architecture was normal.

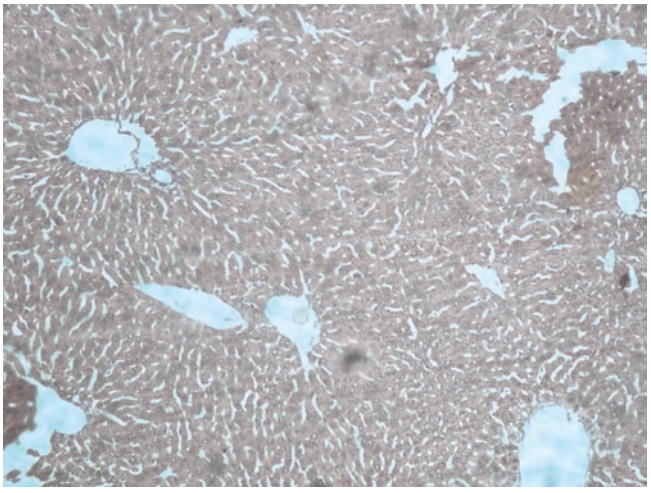

**Fig. 6.** Hepatocytes of the Liv - 52 pretreated group showed normal hepatocytes and their lobular architecture was normal [17].

## 4. Discussion

In the present study liver enzymes level, lipid profiles and liver histopathology of the rats were evaluated in order to explore the combined effect of the selected medicinal drugs used for formation of this experimental polyherbal formulation.

The drug metabolizing enzymes are largely present in the hepatic microsomes [20]. The experimental model of paracetamol (APAP) induced hepatotoxicity in rats represents the adequate model for the screening of hepatoprotective drugs [21]. In this model the metabolism by conjugation becomes saturated and excess APAP is oxidatively metabolized by the CYP enzymes (CYP2E1, 1A2, 2A6, 3A4) to a reactive toxic metabolite N -acetyl-p-benzoquinone-imine (NAPQI). NAPQI has an extremely short half-life and is rapidly conjugated with glutathione a sulfhydryl donor and is renally excreted. Under conditions of excessive NAPQI formation or reduction in glutathione stores by approximately 70%, NAPQI covalently binds to the cysteinyl sulfhydryl groups of cellular proteins forming

NAPQI-protein adducts [22]. The abnormal elevation of liver enzymes (AST, ALT and ALP) level in serum is indication of loss of functional integrity of hepatic cell membranes and cellular leakage. An elevated level of AST in the serum is not specific of the hepatic disease, is used mainly to diagnosis and to verify the course of this disease with other enzyme like ALT and ALP [23]. The depletion of elevated serum glutamate pyruvate transaminase (SGPT 40.28± 6.55 U/L) level together with the suppression in the serum urea (44.87±2.06 mg/dl) level of rats treated with polyherbal formulation suggests the possibility of the present polyherbal formulation being able to stabilize biliary dysfunction of rat liver during chronic hepatic injury with paracetamol. The accumulation of triglyceride (TG) in liver of paracetamol treated rats is not due to the interference with the formation of TG by the liver, but it is due to the inhibition or destruction of TG secreting mechanism. The observations showed restoration of the paracetamol changes in the lipid profile especially of triglyceride (32.22±8.14 mg/dl) and low density lipoprotein cholesterol serum level (LDLc). The histopathological examination of damaged hepatocytes i.e., cell necrosis (Figure 2) was observed to be decreased in the PHF = 1 treatment (Figure 3) which may be the indicative of the efficacy of present polyherbal formulation. In the cholesterol level it was observed that there is decreased in dosing groups PHF = 2, PHF = 3 and these are comparable with PCM - treated group ($P$<0.01) and HDL Cholesterol level was decreased in all three test dosing groups but the dosing group PCM - treated and dosing group PHF = 3.

The hepatoprotective activity of polyherbal formulation was observed in PCM induced liver toxicity in mice. These investigations validate the use of this polyherbal formulation as hepatoprotective in Indian system of medicine. To make beneficial use of this polyherbal formulation it needs further studies in terms of standardization and quality control.

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## Conflict of interest statement

We declare that we have no conflict of interest.

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