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### Comparative evaluation of hepatoprotective activity of polyherbal formulations PHF-A and PHF-B in rats

Sandhya Desai\*, Bhaskar Gavitre, Mahesh Gite, Akbar Ahmad, Yogesh More, Pooja Telang.

K.M.Kundnani College Of Pharmacy, 23,  
Jote Joy Building, Rambhau Salgaonkar Marg, Cuffe Parade,  
Colaba, Mumbai-400 005

#### Abstract

*The hepatoprotective effects of two polyherbal preparations viz., PHF-A and PHF-B containing constituents with potential hepatoprotective activity were evaluated using Paracetamol-induced hepatotoxicity. The treatment effect on lipid peroxidation (LPO) and levels of the associated antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) were investigated in rats. In Paracetamol-induced hepatotoxicity liver damage was induced in Wistar rats by administering Paracetamol (835 mg/kg i. p.) on 7<sup>th</sup> day. The standard drug Silymarin (25 mg/kg), PHF-A (3 dose levels i. e. 200, 400 and 600 mg/kg) and PHF-B (3 dose levels i. e. 200, 400 and 600 mg/kg) were administered orally to the respective groups once daily for 7 days. The levels of marker enzymes (SGOT, SGPT and ALP), and proteins [albumin (Alb), globulin and total proteins (TP)] were assessed in serum. The effects of PHF-A on antioxidant enzymes SOD, CAT, GPX and lipid peroxidation (LPO), were assayed in liver homogenates to evaluate antioxidant activity. PHF-A (200 mg/kg), PHF-B (200 mg/kg), and silymarin elicited a significant hepatoprotective activity by lowering the levels of serum marker enzymes and lipid peroxidation and elevating the levels of SOD, CAT, GPX, Alb and TP in a dose dependant manner. The present findings suggest that the hepatoprotective effect of PHF-A and PHF-B in Paracetamol-induced damage might be due to inhibition of Cytochrome P-450 activity or prevention of inactivation of antioxidant enzymes.*

**Key words:** PHF-A, PHF-B Silymarin, Paracetamol, Antioxidant, Hepatoprotective.

#### INTRODUCTION

Herbal medicines as hepatoprotective agents are widely available and are prescribed for the treatment of many different types of liver disorders.[1] Herbal preparations known as "Phytopharmaceuticals" or "Phytomedicine" are preparations made from different parts of plants. They come in different formulations and dosage forms including tablets, capsules, elixirs, powders, extracts, tinctures, creams and parenteral preparations. Herbal products in the crude

state are also used. A large number of plants and formulations have been claimed to have hepatoprotective activity. In India more than 87 plants are used in 33 patented and proprietary polyherbal formulations. Polyherbal formulations reputed to have hepatoprotective activity that are available on the Indian market comprise about one hundred Indian medicinal plants.[2]

Hepatotoxicity implies chemical-driven liver damage. The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents when taken in overdoses and occasionally when introduced within therapeutic ranges may injure the organ. More than 900 drugs have been implicated in causing liver injury[3] and it is one of the most common reasons for a drug to be withdrawn from the market.

Several mechanisms are responsible for either inducing hepatic injury or worsening the damage process. Many chemicals damage mitochondria, an intracellular organelle that produces energy. Its dysfunction releases excessive amount of oxidants which in turn injures hepatic cells. Activation of some enzymes in the cytochrome P-450 system such as CYP2E1 also leads to oxidative stress. Injury to hepatocyte and bile duct cells lead to accumulation of bile acid inside the liver. This promotes further liver damage[4].

In spite of the tremendous advances made in allopathic medicine, no effective hepatoprotective medicine is available. Plant drugs are known to play a vital role in the management of liver diseases. There are numerous plants and polyherbal formulations claimed to have hepatoprotective activities. Nearly 150 phytoconstituents from 101 plants have been claimed to possess liver protecting activity [2,5]. At the same time, surprisingly, we do not have readily available satisfactory plant drugs or formulations to treat severe liver disease.

Polyherbal formulation PHF-A and PHF-B contains various active constituents, having potential for hepatoprotective activity. Hence it was considered worthwhile to evaluate their efficacy and toxicity to determine their potency & safety. The Polyherbal formulations-

PHF-A (composed of *Phyllanthus niruri*, *Cichorium intybus*, *Boerhaavia diffusa*, *Eclipta alba*, *Tinospora cordifolia*, *Tecomella undulate*, *Andrographis paniculata*, *Berberis aristata*, *Solanum nigrum*, *Embelia ribes*, *Picrorrhiza kurroa*, *Fumaria parviflora*) and PHF-B (composed of *Tinospora cordifolia*, *Embllica officinalis*, *Withania somnifera*, *Curcuma longa*, *Glycyrrhiza glabra*, *Bacopa monnieri*, *Terminalia chebula*, *Terminalia arjuna*, *Asparagus racemosus*, *Aloe barbadensis*) has been selected to evaluate their hepatoprotective activity in paracetamol-induced hepatotoxicity in rats.

Treatment with PHF-A showed significant reduction in the serum enzymes level in dose dependant manner, more effectively as compared to PHF-B. In case of total protein and Bilirubin PHF-A showed better efficacy than PHF-B. Treatment with PHF-A raised the levels of SOD, CAT and GPx more effectively than PHF-B against the paracetamol-induced oxidative stress, it is suggestive of hepatoprotective activity through antioxidant mechanism. Pretreatment with PHF-A showed better results with respect to histopathological changes as compared to PHF-B. Meanwhile, the results of the acute toxicity test, for PHF-A and PHF-B indicate that they are relatively safe and/or non-toxic to rats.

The findings of these experimental animal studies indicate that PHF-A possesses potential hepatoprotective activity as compared to PHF-B.

## MATERIALS AND METHODS

### Animals

Albino rats of Wistar strain (weighing 100-200 g) of either sex, obtained from Bharat Serum and Vaccines, Thane, India were housed under standard conditions of temperature ( $24 \pm 1^{\circ}\text{C}$ ), relative humidity ( $65 \pm 10\%$ ), 10-h light and 14-h dark cycle and fed with standard pellet diet (Chakan Mill Ltd, Pune, India) with water *ad libitum*. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee (Approval number of project: 080906 and Registration Number of institute: 25/1999/CPCSEA) and were in accordance with the guidelines of the CPCSEA, Ministry of Forests and Environment, Government of India. The animals were deprived of food for 24 hour before experimentation but allowed free access to water throughout.

### Drugs and Chemicals

Poly Herbal Formulations PHF-A and PHF-B were a gift sample from Om Pharmaceuticals, Bangalore. The dry powder of PHF-A and PHF-B were reconstituted using 0.5% w/v Sodium Carboxy Methyl Cellulose (CMC) to get  $1 \text{ mg ml}^{-1}$  of PHF-A and PHF-B respectively. The suspension was freshly prepared before use. Paracetamol and Silymarin from Sigma Chemical Co, St Louis, MO, USA., and all other chemicals, reagents used were of analytical grade.

### Acute toxicity studies

**1. PHF-A:** Based on % of active constituents present in powdered PHF-A suspended in distilled water using 1 % Sod.CMC, 6 Albino Wistar rats (3 females + 3 males) weighing in the range of 100-200 gm, were orally administered the suspension at a dose of 2000 mg/kg. The rats were critically observed for clinical signs, gross behavioral changes and mortality if any, following the administration of suspension at different time intervals like 30 min, 1hr, 2hr, 4hr, 24hr, 48hr, 72hr upto a period of 14 days.

The study was carried out according to OECD Guidelines No.423.

**2. PHF-B:** Based on % of active constituents present in powdered PHF-B suspended in distilled water using 1 % Sod.CMC, 6 Albino Wistar rats (3 females + 3 males) weighing in the range of 100-200 gm, were orally administered the suspension at a dose of 2000 mg/kg. The rats were critically observed for clinical signs, gross behavioral changes and mortality if any, following the administration of suspension at different time intervals like 30 min, 1hr, 2hr, 4hr, 24hr, 48hr, 72hr upto a period of 14 days.

The study was carried out according to OECD Guidelines No.423.

### Paracetamol-induced hepatotoxicity in rats

Rats were divided into nine groups of six animals each viz: control (untreated) group, standard drug group, toxicant group, treatment group PHF-A (3 dose levels i. e. 200, 400 and 600 mg/kg) and PHF-B (3 dose levels i. e. 200, 400 and 600 mg/kg). The standard drug Silymarin (25 mg/kg) and the test drugs were administered orally once daily for 7 days to the respective groups. On 7<sup>th</sup> day Paracetamol (835 mg/kg) was injected intraperitoneally to all the groups except in control group.

Group-1: Served as normal control, orally received pure water once daily for 7 days. On 7<sup>th</sup> day intraperitoneally received isotonic normal saline (10 ml/kg).

Group-2: Served as toxicant control, orally received pure water once daily for 7 days. On 7<sup>th</sup> day intraperitoneally received Paracetamol (835 mg/kg).

Group-3: Received standard drug Silymarin 25 mg/kg orally once daily for 7 days. On 7<sup>th</sup> day intraperitoneally received Paracetamol (835 mg/kg).

Group-4: Received test drug PHF-A ( 200 mg/kg p. o. ) once daily for 7 days. On 7<sup>th</sup> day intraperitoneally received Paracetamol (835 mg/kg).

Group-5: Received test drug PHF-A ( 400 mg/kg p. o.) once daily for 7 days. On 7<sup>th</sup> day intraperitoneally received Paracetamol (835 mg/kg).

Group-6: Received test drug PHF-A ( 600 mg/kg p. o.) once daily for 7 days. On 7<sup>th</sup> day intraperitoneally received Paracetamol (835 mg/kg).

Group-7: Received test drug PHF-B ( 200 mg/kg p. o. ) once daily for 7 days. On 7<sup>th</sup> day intraperitoneally received Paracetamol (835 mg/kg).

Group-8: Received test drug PHF-B ( 400 mg/kg p. o.) once daily for 7 days. On 7<sup>th</sup> day intraperitoneally received Paracetamol (835 mg/kg).

Group-9: Received test drug PHF-B ( 600 mg/kg p. o.) once daily for 7 days. On 7<sup>th</sup> day intraperitoneally received Paracetamol (835 mg/kg).

After 24 hrs of paracetamol administration animals in all the groups were humanely sacrificed with ether and 4ml of blood was withdrawn by cardiac puncture and allowed to clot for 30 mins at room temperature. The serum was separated by using refrigerated centrifuge and used for the assay of marker enzymes viz., SGOT, SGPT, ALP, TP, Alb, Globulin and Bilirubin. The livers were dissected out immediately, washed with ice-cold saline and 10% homogenates in Phosphate buffer solution (pH 7.4) were prepared. Liver homogenate was used for the assay of lipid peroxidation (LPO) while some fraction of homogenates were centrifuged at 7000 g for 10 min at 4<sup>o</sup>C using refrigerated centrifuge, and the supernatants were used for the assay of Superoxide Dismutase (SOD) Catalase (CAT), Glutathione peroxidase (GPx). Some portion of liver from each group was aseptically excised and stored in 10% formalin for histopathological studies.

*Statistical analysis.* All the values are expressed as mean  $\pm$  S.E.M. The results were analyzed statistically by Analysis of Variance (ANOVA) followed by Dunnett's test. *P* values <0.05 were considered significant.

## RESULTS AND DISCUSSION

Acute oral toxicity did not show any toxic or deleterious effects upto 2000 mg/ kg p.o. dose indicating low toxicity of the PHF-A and PHF-B at high doses.

### *Hepatoprotective activity*

The hepatoprotective effect of PHF-A and PHF-B are shown in Table 1 and 2. PHF-A and PHF-B treatment at all dose levels showed significant reduction in the serum SGOT and SGPT level in dose dependant manner. PHF-A showed significant reduction in the serum ALP level in dose dependant manner while PHF-B showed its maximum effect at 400 mg/kg (Table 1). The Total protein in PHF-A and PHF-B treated groups showed increase upto dose of 400 mg/kg as compared to toxicant group while PHF-A shows maximum effect on Albumin and Bilirubin levels at a dose of 400 mg/ kg and PHF-B shows its maximum effect at 200 mg/kg (Table 1). Since oxidative stress contribute to the development of paracetamol-induced hepatotoxicity, the levels of liver antioxidant enzymes SOD, CAT and GPx, were significantly diminished in the paracetamol-intoxicated group as compared with normal control. The PHF-A treated groups near normalized the levels of these enzymes as compared to PHF-B (Table 2).

**Table 1: Effect of PHF-A and PHF-B on biochemical serum parameters in Paracetamol-induced hepatotoxicity**

Treatment groups and dose (mg/kg)	SGOT (IU/l)	SGPT (IU/l)	ALP (IU/l)	Total Protein g/dl	Albumin g/dl	Globulin g/dl	Bilirubin mg/dl
<b>Control</b>	208.30 ±9.51	71.67 ±6.84	662.00 ±12.99	6.27 ±0.15	3.80 ±0.15	2.47 ±0.20	0.45 ±0.03
<b>Toxicant</b>	473.20 ±16.05 <sup>aa</sup>	153.80 ±5.15 <sup>aa</sup>	819.20 ±13.35 <sup>aa</sup>	5.28 ±0.13 <sup>aa</sup>	2.90 ±0.13 <sup>a</sup>	2.38 ±0.21	0.72 ±0.03 <sup>aa</sup>
<b>Standard 25</b>	269.00 ±8.37 <sup>**</sup>	72.50 ±3.40 <sup>**</sup>	669.80 ±27.91 <sup>**</sup>	5.92 ±0.08 <sup>*</sup>	3.55 ±0.04 <sup>**</sup>	2.37 ±0.08	0.47 ±0.02 <sup>**</sup>
<b>PHF-A 200</b>	323.50 ±20.66 <sup>**</sup>	88.33 ±4.29 <sup>**</sup>	689.50 ±32.10 <sup>**</sup>	5.65 ±0.18	2.93 ±0.20	2.72 ±0.16	0.63 ±0.04
<b>PHF-A 400</b>	315.00 ±13.40 <sup>**</sup>	77.67 ±3.80 <sup>**</sup>	684.00 ±33.89 <sup>**</sup>	5.85 ±0.12	3.40 ±0.13 <sup>*</sup>	2.45 ±0.16	0.45 ±0.03 <sup>**</sup>
<b>PHF-A 600</b>	296.80 ±17.71 <sup>**</sup>	73.33 ±4.01 <sup>**</sup>	680.20 ±12.23 <sup>**</sup>	5.72 ±0.14	3.03 ±0.09	2.68 ±0.16	0.60 ±0.04
<b>PHF-B 200</b>	358.70 ±13.21 <sup>**</sup>	92.50 ±8.56 <sup>**</sup>	706.80 ±24.56 <sup>**</sup>	5.67 ±0.13	3.53 ±0.10 <sup>**</sup>	2.13 ±0.20	0.48 ±0.04 <sup>**</sup>
<b>PHF-B 400</b>	311.00 ±14.34 <sup>**</sup>	80.67 ±3.05 <sup>**</sup>	675.30 ±17.61 <sup>**</sup>	5.78 ±0.16	3.20 ±0.12	2.58 ±0.20	0.50 ±0.06 <sup>**</sup>
<b>PHF-B 600</b>	304.70 ±19.46 <sup>**</sup>	74.50 ±6.39 <sup>**</sup>	686.00 ±16.16 <sup>**</sup>	5.47 ±0.26	2.88 ±0.12	2.58 ±0.22	0.57 ±0.04

N = 6 ; Each data suggest Mean ± SEM; One-way ANOVA followed by Dunnett's test is applied for statistical analysis, ; \*\* Significant at p <0.01, \* Significant at p < 0.05; When Drug treated groups were compared with toxicant group.; aa Significant at p <0.01, a Significant at p < 0.05; When Paracetamol group compared with Normal group

Biochemical observations were further substantiated by histopathological studies. The liver sections of animals treated with Paracetamol (**Figure 2**) showed multifocal moderate to marked necrosis and mildly multifocal mild periportal lymphocytic infiltration with marked lobular disarray. Silymarin treated animal livers exhibited almost normal histology with minimal diffuse granular degeneration and mildly multifocal mild periportal lymphocytic infiltration (**Figure 3**). Compared with the lesions observed in the Galactosamine group, the lesions noted in livers of PHF-A 200 treated animals were of a multifocal mild degree necrosis, (**Figure 4**). The liver sections of this group showed moderate diffuse granular degeneration and minimal to mild lobular disarray. The livers in the PHF-A 400 group (**Figure 5**) showed multifocal minimal degree necrosis and mild diffuse granular degeneration. The PHF-A 600 group (**Figure 6**) showed mild diffuse granular degeneration, indicating dose related hepatoprotection,

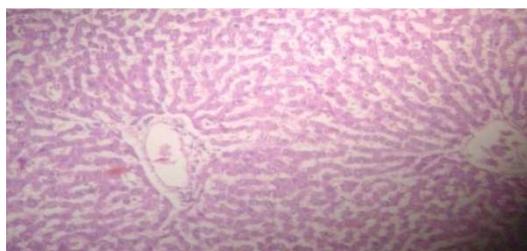
While PHF-B 200 treated animals were of a mild necrosis, (**Figure 7**). The liver sections of this group showed moderate diffuse granular degeneration and minimal lobular disarray. The livers in the PHF-B 400 group (**Figure 8**) showed multifocal minimal degree necrosis and mild diffuse

granular degeneration. This PHF-A 600 group (**Figure 9**) showed minimal diffuse granular degeneration, supporting dose dependent hepatoprotective activity of PHF-A and PHF-B.

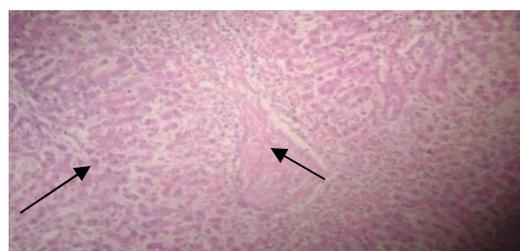
**Table 2: Effect of PHF-A and PHF-B on biochemical liver parameters in Paracetamol-induced hepatotoxicity**

Treatment groups and dose (mg/kg)	SOD u/mg protein	CATALASE u/mg protein	GPx u/mg protein	Lipid Peroxidation	
				(nmoles of MDA/g liver weight)	% Inhibition
<b>Control</b>	13.41 ± 0.17	0.4827 ±0.02	13.01 ± 0.12	85.89 ±3.51	37.31
<b>Toxicant</b>	2.53 ± 0.08 <sup>aa</sup>	0.0873 ±0.01 <sup>aa</sup>	2.19 ± 0.06 <sup>aa</sup>	137.00 ±4.12 <sup>a</sup>	0.00
<b>Standard 25</b>	6.73 ± 0.18 <sup>**</sup>	0.1627 ±0.003 <sup>**</sup>	6.15 ± 0.12 <sup>**</sup>	100.26 ±2.54 <sup>*</sup>	26.82
<b>PHF-A 200</b>	4.63 ± 0.09 <sup>**</sup>	0.1129 ±0.002 <sup>*</sup>	3.41 ± 0.07 <sup>**</sup>	132.19 ±4.69 <sup>*</sup>	3.51
<b>PHF-A 400</b>	5.26 ± 0.16 <sup>**</sup>	0.1255 ±0.002 <sup>**</sup>	4.08 ± 0.11 <sup>**</sup>	121.38 ±3.98 <sup>*</sup>	11.40
<b>PHF-A 600</b>	6.10 ± 0.07 <sup>**</sup>	0.1457 ±0.002 <sup>**</sup>	4.96 ± 0.14 <sup>**</sup>	117.90 ±3.21 <sup>*</sup>	13.94
<b>PHF-B 200</b>	4.36 ±0.12 <sup>**</sup>	0.1100 ±0.001	3.04 ±0.05	130.96 ±2.87	4.39
<b>PHF-B 400</b>	5.09 ±0.07 <sup>**</sup>	0.1200 ±0.001 <sup>**</sup>	3.77 ±0.10	114.89 ±4.62 <sup>*</sup>	16.64
<b>PHF-B 600</b>	5.92 ±0.10 <sup>**</sup>	0.1400 ±0.002 <sup>**</sup>	4.42 ±0.07	107.10 ±3.96 <sup>*</sup>	21.20

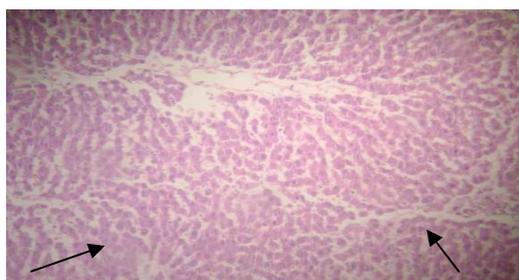
N = 6 ; Each data suggest Mean ± SEM ; One-way ANOVA followed by Dunnett's test is applied for statistical analysis.; \*\* Significant at p <0.01, \* Significant at p < 0.05; When Drug treated groups were compared with toxicant group.; aa Significant at p <0.01, a Significant at p < 0.05; When Paracetamol group compared with Normal group



**FIGURE 1. NORMAL CONTROL**



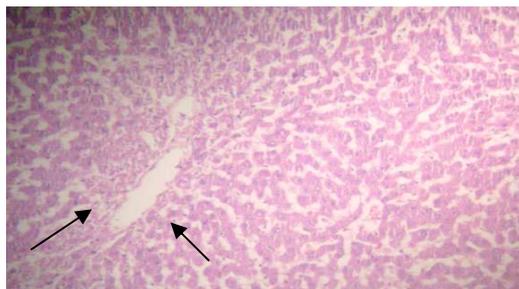
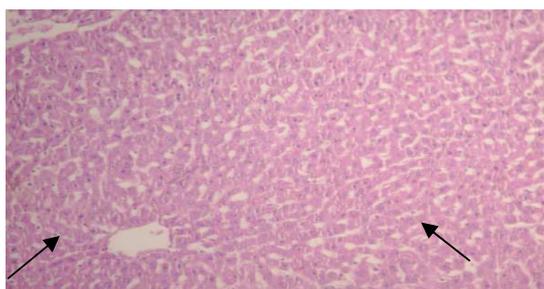
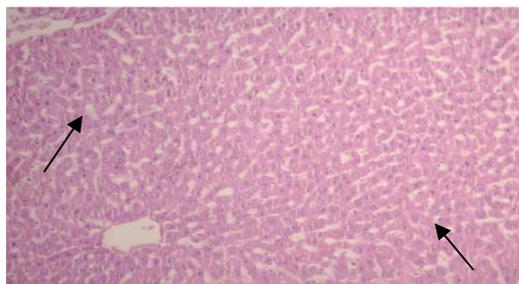
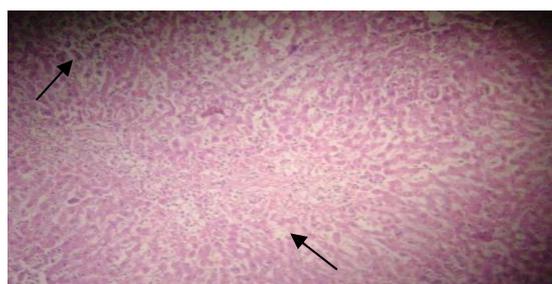
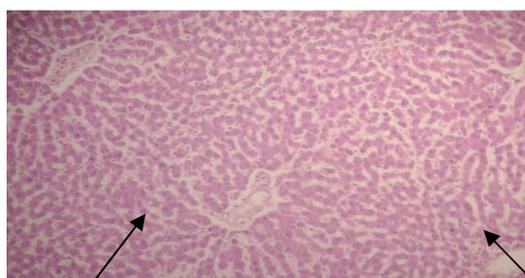
**FIGURE 2. TOXICANT CONTROL**



**FIGURE 3. SILYMARIN TREATED (25 mg/kg)**



**FIGURE 4. PHF-A TREATED (200mg/kg)**

**FIGURE 5. PHF-A TREATED (400mg/kg)****FIGURE 6. PHF-A TREATED (600 mg/kg)****FIGURE 7. PHF-B TREATED (200mg/kg)****FIGURE 8. PHF-B TREATED (400mg/kg)****FIGURE 9. PHF-B TREATED (600 mg/kg)**

Paracetamol is an antipyretic and analgesic drug, which is widely used to cure fever, headache and other pains, and is readily available without prescription. When taken at toxic doses, it becomes a potent hepatotoxin, generating fulminated hepatic and renal tubular necrosis which is lethal in humans and experimental animals.[6] The laboratory features of hepatotoxicity induced by Paracetamol resemble other kinds of acute inflammatory liver disease with prominent increase in SGOT, SGPT, and ALP levels.[7]

The metabolic activation and biochemical mechanisms of hepatotoxicity induced by Paracetamol have been reviewed, and it has been shown that overdose of Paracetamol can deplete glutathione stores, leading to accumulation of N-acetyl-p-benzoquinone-imine (NAPQI), mitochondrial dysfunction, the development of acute hepatic necrosis[8], liver function failure and death in human as well as experimental animals[9]. Extent of hepatic damage is assessed by the level of released cytoplasmic enzymes (SGOT, SGPT and ALP) in circulation[10]. Paracetamol at therapeutic dose is primarily metabolized and detoxified by glucuronidation and sulphation, and subsequently followed by renal excretion.[11] However, when Paracetamol is taken at toxic doses, the compound is converted to a toxic form NAPQI, which is an electrophilic intermediate oxidized by cytochrome P-450 and converted to a highly reactive and toxic metabolite as in cases of Paracetamol overdose.[12] NAPQI can rapidly react with glutathione (GSH) and lead to a 90% total hepatic GSH depletion in cells and mitochondria, which can result in hepatocellular death and mitochondrial dysfunction. In addition, NAPQI can increase the formation of ROS and reactive nitrogen species (RNS) such as superoxide anion, hydroxyl radical, and hydrogen

peroxide, and nitro oxide and peroxyxynitrite, respectively. Excess levels of ROS and RNS can attack biological molecules such as DNA, protein, and phospholipids, which leads to lipid peroxidation, nitration of tyrosine, and depletion of the antioxidant enzymes (SOD, CAT, GPx) that further results in oxidative stress[13] NAPQI can also induce DNA strand breaks and promote apoptosis and necrosis in Paracetamol-induced hepatotoxicity.[14] Previous studies have demonstrated that oxidative stress is a major mechanism in the development of Paracetamol-induced hepatotoxicity.[15, 16, 17]

PHF-A treatment more effectively reduces the increased serum enzyme levels as compared to PHF-B. It also showed better efficacy in reduced levels of antioxidant enzyme levels as compared to PHF-B. Both PHF-A and PHF-B were found to have promising hepatoprotective activity at doses of 200mg/kg, 400mg/kg and 600mg/kg. Improved hepatoprotective activity of PHF-A could be either due to: Inhibition of Cytochrome P-450 activity, prevention of inactivation of antioxidant enzymes, prevention of process of lipid peroxidation or free radical scavenging activity. Pretreatment with PHF-A and PHF-B, for 7 days reduced the histopathological damage associated with hepatotoxicity from paracetamol-intoxicated treatment. However PHF-A showed more effective results as compared to PHF-B.

### CONCLUSION

On the basis of results obtained, it can be concluded that PHF-A, a Polyherbal formulation possesses potent hepatoprotective activity as compared to PHF-B. Detailed studies are required to understand the exact mechanism of action responsible for hepatoprotection.

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