Assessment of Hepatoprotective activity of a novel polyherbal formulation against D-galactosamine and paracetamol induced hepatic toxicity in experimental animals

B.R.V. Bhargavi, C.Rajaram*, S. Nelson Kumar, R.Manohar, S.Kalpana

Department of Pharmacology, P. Rami Reddy Memorial College of Pharmacy, Kadapa - 516 003, Andhra Pradesh, India.

Professor & Head, Department of pharmacy practice, P. Rami Reddy Memorial College of Pharmacy, Kadapa - 516 003, Andhra Pradesh, India.

Corresponding Author: Dr. C. Rajaram
rajaram212121@yahoo.co.in

ABSTRACT

The objective of the present study was to evaluate the protective effect of a polyherbal formulation developed against paracetamol and D-galactosamine -induced hepatotoxicity in wistar rats. The research was conducted in two separate 10 and 14 day investigations against paracetamol and D-galactosamine, respectively. There were five distinct treatment groups for the animals. A toxicant group in two experiments received paracetamol 750 mg/kg orally every 72 hours for 10 days and D-galactosamine 400 mg/kg intravenously as a single dose. The test formulation was administered at doses of 100 mg/kg/day and 200 mg/kg/day. In addition to the D-galactosamine given to the toxicant group, test formulations were also administered to the treatment groups. The biochemical evaluation demonstrated that both paracetamol and galactosamine caused hepatotoxicity in the toxicant groups. Nevertheless, treatment with polyherbal formulation (PHF) significantly ($P < 0.001$, vs. toxicant) diminished the levels of SGOT, SGPT, serum bilirubin, and ALP, and decreased lipid peroxidation. In addition, treatment with the test formulation increased serum albumin and GSH levels significantly (P vs 0.001 vs. toxicant) compared to toxicant groups. On the premise of these studies and comparative evaluation, it can be concluded that PHF demonstrated hepatoprotective activity against paracetamol and D-galactosamine at 100 mg/kg and 200 mg/kg, respectively.

Keywords: Polyherbal formulation, Hepatoprotective, Paracetamol

Introduction

One of the leading causes of morbidity and mortality worldwide is liver disease. One of the main causes of liver illnesses and the reason for an increase in hospital admissions is drug-induced hepatic toxicity [1]. Scientific research has demonstrated the effectiveness of natural plant compounds in treating and preventing liver diseases [2]. To prevent and cure liver conditions, we developed a novel polyherbal formulation. It contains aqueous extracts from the leaves of *Pulcaria wightiana*, *Barleria Gibsonii*, *Cleome gynandra*, *Terminalia chebula*, *Emblica officinalis* and *Azadirachta indica*. The goal of the current investigation was to assess PHF ability to protect the liver against paracetamol and D-galactosamine-induced liver toxicity in wistar rats.

Materials and Methods

Experimental Animals

Wistar albino male rats weighing between 200 and 250 g were used in the research. Food pellets (Pavan Agro, Bangalore) and water were provided ad libitum, and the animals were housed in a clean, humid environment typical of a laboratory. The study was approved by the PRRM College of Pharmacy's Institutional Animal Ethics Committee (1423/PO/Re/S/11/CPCSEA).

Paracetamol induced hepatic toxicity

Animals were randomly divided into five groups (n = 6/group) and treated as follows:

Group 1 (control): Normal saline, 1 ml/kg for 10 days

Group 2 (toxicant): Paracetamol 750 mg/kg p.o. every 72 h for 10 days

Group 3 (standard): Liv52 p.o. for 10 days along with paracetamol 750 mg/kg p.o. every 72 h for 10 days

Group 4 (formulation dose 1): PHF, 100 mg/kg p.o. for 10 days, along with paracetamol 750 mg/kg p.o. every 72 h for 10 days

Group 5 (formulation dose 2): PHF, 200 mg/kg p.o. for 10 days along with paracetamol 750 mg/kg p.o. every 72 h for 10 days.

D-galactosamine induced hepatic toxicity

Animals were randomly divided into five groups (n = 6/group) and treated as follows:

Group I (control): Normal saline, 1 ml/kg for 14 days

Group II (toxicant): D-galactosamine 400 mg/kg i.p. single dose

Group III (standard): Liv52 p.o. for 14 days, D-galactosamine 400 mg/kg i.p. the single dose was co-administered on 14th day

Group IV (formulation dose 1): PHF, 100 mg/kg p.o. for 14 days, D-galactosamine 400 mg/kg i.p. the single dose was co-administered on 14th day

Group V (formulation dose 2): PHF, 200 mg/kg p.o. for 14 days, D-galactosamine 400 mg/kg i.p. the single dose was co-administered on 14th day

Biochemical and histopathological evaluation

Serum was extracted by drawing blood from the tail vein of animals at 27°C after 48 hours' therapy was finished, letting it clot, and then centrifuging it. The animals were then sacrificed, and liver tissue was extracted for histological examination and lipid peroxidation/glutathione (GSH) level assessment. Serum levels of SGOT, SGPT, albumin, bilirubin, and ALP were all measured biochemically using commercially available kits.

Results and Discussion

Paracetamol induced hepatic toxicity

Table 1 displays the impact of various therapies on liver biochemical markers. In the toxicant group, paracetamol caused hepatic toxicity as evidenced by significantly ($P < 0.001$, vs. toxicant) elevated levels of SGOT, SGPT, serum bilirubin, and ALP; increased lipid peroxidation; and decreased serum albumin and tissue glutathione peroxidase (GSH). On the other hand, when comparing the control and toxicant groups, Liv52 showed statistically significant ($P < 0.001$) improvements in these measures.

Serum SGOT and SGPT levels were significantly decreased ($P < 0.001$) following treatment with Polyherbal formulation at 100 mg/kg and 200 mg/kg compared to the toxicant group.

The blood SGOT levels decreased more at the 200 mg/kg dose ($P < 0.001$) compared to the 100 mg/kg dose. Similar reductions in elevated blood ALP and bilirubin levels were observed after treatment with formulation at doses of 100 and 200 mg/kg ($P < 0.001$ vs. toxicant). While paracetamol treatment dramatically decreased blood albumin and tissue GSH levels in comparison to the control group ($P < 0.001$ vs. control), PHF treatment considerably increased both ($P < 0.001$ vs. toxicant). Paracetamol (toxicant) treated groups had higher levels of malondialdehyde (MDA) in their hepatic tissue compared to controls ($P < 0.001$), indicating lipid peroxidation. However, the increased MDA level was dramatically decreased ($P < 0.001$ vs. toxicant) after treatment with the PHF. The histological findings [Figure 1] were consistent with the biochemical indicators.

Table 1: Effect of PHF on hepatic biomarkers in different treatment groups against paracetamol-induced hepatic toxicity

Parameters	**Control**	**Toxicant**	**Liv 52**	**PHF (100 mg/kg)**	**PHF (200 mg/kg)**
SGOT (IU/L)	155.2 ± 15.841	380.2±15.419[a]	183.1±16.168[b]	251.8 ± 15.978[b]	224.6 ± 16.942[b]
SGPT (IU/L)	172.7± 7.301	341.5±16.910[a]	161.9±17.753[b]	250.7 ± 15.693[b]	219.2 ± 11.81[b]
Albumin (mg/dL)	0.89 ± 0.03337	3.14±0.02915[a]	0.98±0.04781[b]	1.12 ± 0.03851[b]	0.87 ± 0.03055[b]
Bilirubin (mg/dL)	0.89±0.029	2.19±0.16[a]	1.16±0.031[b]	1.18±0.06[b]	.98±0.031[b]
ALP (IU/L)	174.9 ± 15.852	341.4±16.357[a]	182.6± 6.005[b]	244.3 ± 17.887[b]	234.6 ± 11.54[b]
GSH (nmol/mg)	7.32±0.21	5.65±0.2[a]	6.92±0.16[b]	6.54±0.16[b]	6.99±0.21[b]
MDA (nmol/mg)	0.171±0.01	0.81±0.01[a]	0.20±0.02[b]	0.24±0.1[b]	0.22±0.12[b]

a = $p < 0.001$, when compared to normal animals, b = $p < 0.001$, when compared to control animals, SGOT: Serum glutamate oxaloacetate transferase, SGPT: Serum glutamate pyruvate transferase, ALP: Alkaline phosphatase, GSH: Glutathione, MDA: Malondialdehyde

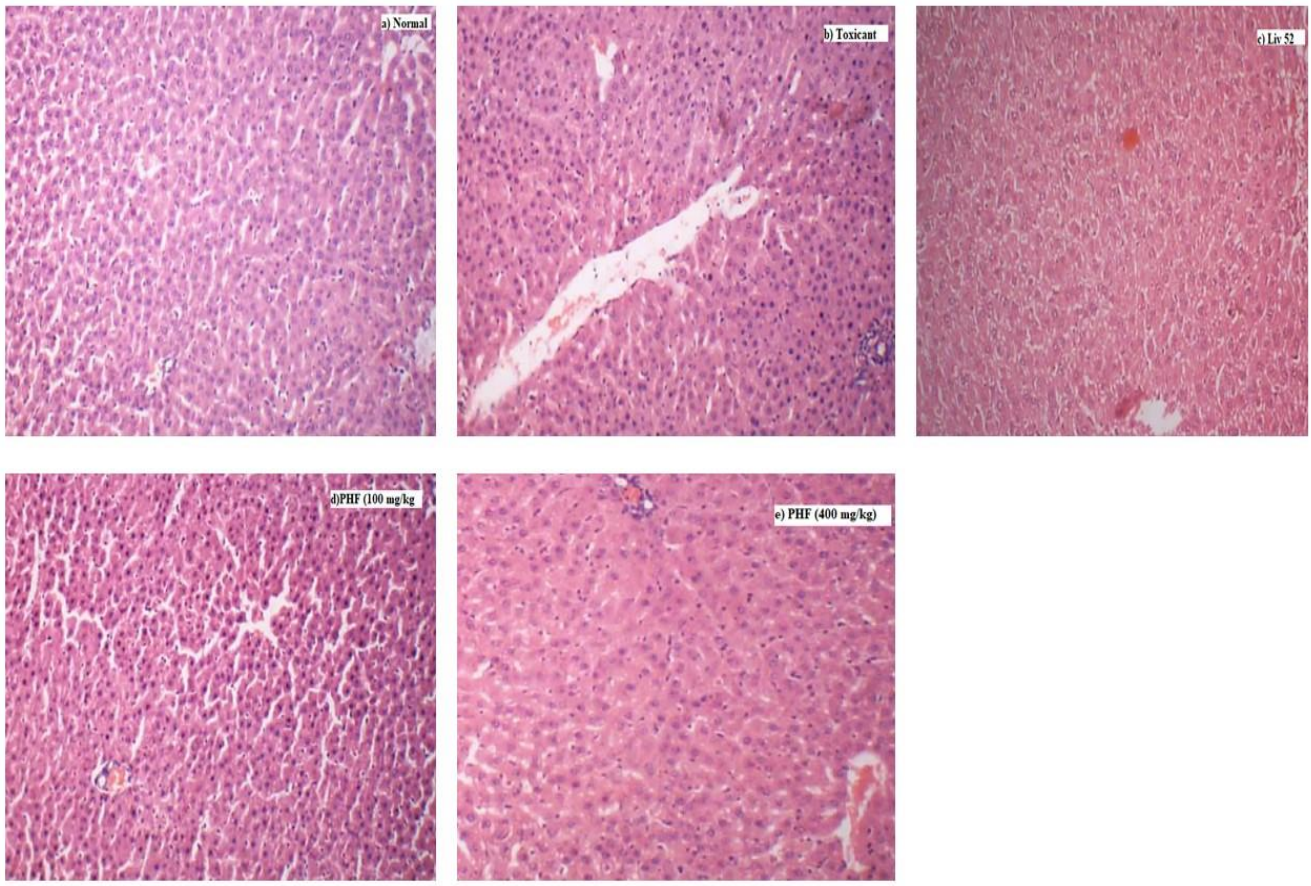

Figure 1: Histology of hepatic tissue of different treatment groups against paracetamol-induced hepatic toxicity. (a) Normal group with normal histological features, (b) toxicant group necrotic areas and vacuole formation, (c) Normal architecture of hepatic tissue in animals treated with standard Liv 52 (d) and (e) showing almost normal histology after treatment with at 120 mg/kg and 240 mg/kg, respectively.

D-galactosamine induced hepatic toxicity

Table 2 shows biochemical estimates of response for several treatment groups against D-galactosamine-induced hepatotoxicity. The toxicant group had considerably ($P < 0.001$, vs. toxicant) higher SGOT, SGPT, serum bilirubin, and ALP levels, while the control group had significantly ($P < 0.001$, vs. toxicant) decreased SGOT, SGPT, serum bilirubin, and ALP levels. D-galactosamine significantly decreased GSH levels and increased lipid peroxidation in the treated rats. The increased levels of SGOT, SGPT, serum bilirubin, alanine aminotransferase (ALP), and tissue malondialdehyde (MDA) were considerably ($P < 0.001$) decreased after treatment with standards. At 100 and 240 milligrams per kilogram, PHF significantly decreased ($P < 0.001$) SGOT and SGPT levels compared to the toxicant group. Both SGOT and SGPT levels reduced more noticeably ($P < 0.001$) at the higher 200 mg/kg dose compared to the lower 100 mg/kg dose. Similar reductions in elevated blood ALP and bilirubin levels were observed after treatment with PHF at doses of 100 and 200 mg/kg ($P < 0.001$ vs. toxicant). The higher dose of polyherbal formulation (200 mg/kg) was associated with a relatively superior reduction in bilirubin levels. Serum albumin and tissue glutathione

peroxidase levels both increased after further treatment with PHF ($P < 0.001$) compared to toxicant. Treatment with PHF at 100 and 200 mg/kg significantly reduced elevated lipid peroxidation assessed as liver tissue MDA level ($P < 0.001$ vs. toxicant). The outcomes of biochemical estimations agreed with histopathological findings [Figure 2].

PHF contains a variety of well-known herbal medications. According to Bishayi et al., *T. cordifolia* extract therapy may be the key treatment for enhancing immune system and CCl4-induced liver impairment [3]. It has also been demonstrated that *P. niruri* and *A. indica* are protective against drug-induced hepatic oxidative stress [4,5]. It has also been shown that andrographolide, the main active antihepatotoxic component of *A. paniculata*, lowers the levels of hepatic enzymes caused by CCl4 [6]. Similar to *C.gynandra*, and *p.wightiana* has also been linked to hepatoprotective activity, which is explained by its strong antioxidant and membrane stabilizing properties.[7] It has been discovered that *E. officinalis*, *B.gibsoni*i extract lowers increased levels of liver biomarkers, suggesting that the extract may prevent the production of fibrosis in rats.[8] *T. belerica* has been proven to have protective effects on microsomal lipid peroxidation and triglycerides in the liver, which point to a healing role in the liver damage process.[9] *P. kurroa* possesses hepatoprotective action, much like all of the other ingredients. Additionally, it drastically decreased the lipid content of the liver more than the usual dose of the well-known hepatoprotective silymarin[10] All of these data point to formulation 8060's potent hepatoprotective effect against the two toxicants.

Table 2: Effect of PHF on hepatic biomarkers in different treatment groups against D-galactosamine induced hepatic toxicity

Parameters	**Control**	**Toxicant**	**Liv 52**	**PHF (100 mg/kg)**	**PHF (200 mg/kg)**
SGOT (IU/L)	97.37±5.89	420.5±12.28 [a]	143.6±6.11 [b]	327.6±8.16	191.9±8.02
SGPT (IU/L)	76±5.0	301±14	103±6.9 [b]	229±7.1 [b]	124±7.5 [b]
Albumin (mg/dL)	6.22±0.42	4.15±0.28[a]	6.09±0.37[b]	5.88±0.52[b]	5.13±0.3[b]
Bilirubin (mg/dL)	0.03083±0.005	0.08217±0.003 [a]	0.03083±0.004 [b]	0.04417±0.002 [b]	0.03483±0.002 [b]
ALP (IU/L)	165.2±7.31	332.9±0.10 [a]	191.8±6.51 [b]	294.4±8.94 [b]	240.1±10.26 [b]
GSH (nmol/mg)	9.854±1.002	5.35±0.64 [a]	10.52±0.64 [b]	9.516±0.41 [b]	10.58±0.69 [b]
MDA (nmol/mg)	0.448±0.07	1.288±0.14 [a]	0.4384±0.05 [b]	0.718±0.059 [b]	0.4924±0.11 [b]

a = $p < 0.001$, when compared to normal animals, b = $p < 0.001$, when compared to control animals, SGOT: Serum glutamate oxaloacetate transferase, SGPT: Serum glutamate pyruvate transferase, ALP: Alkaline phosphatase, GSH: Glutathione, MDA: Malondialdehyde

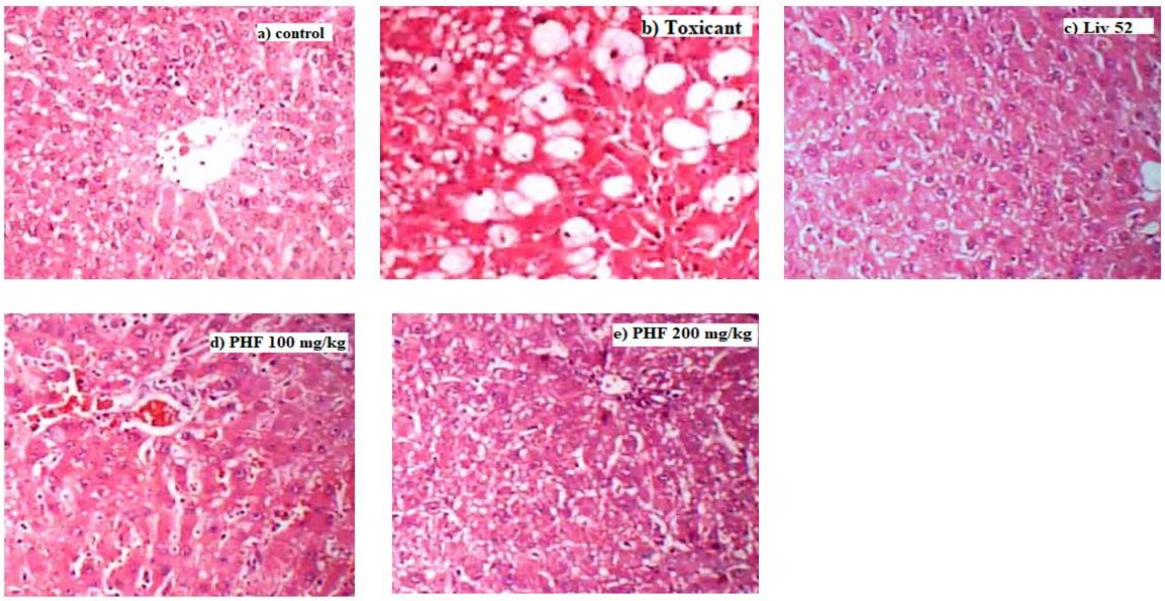

Figure 2: liver tissue histology in relation to D-galactosamine-induced liver damage in various treatment groups. Following treatment with PHF at 100 mg/kg and 200 mg/kg, respectively, the control group (a) displayed normal histological features, the toxicant group (b) necrotic regions and vacuole formation, (c) In rat given regular Liv 52 therapy, the typical architecture of the liver tissue was observed and the experimental groups (d) and (e) displayed nearly normal histology.

Conclusion

The novelb polyherbal possesses good Hepatoprotective activity.

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Nil.

Conflicts of interest

There are no conflicts of interest

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