

# Acetaminophen-induced hepato- and nephrotoxicity and amelioration by hydroalcoholic polyherbal formulation in experimental rodents

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## Abstract

**Objective:** The objective of this study was to evaluate hepato- and nephroprotective potential of extracts of polyherbal formulations against acetaminophen (paracetamol [PCM])-induced dysfunction in experimental rodents. **Materials and Methods:** Acute and subacute toxicity study of hydroalcoholic polyherbal formulation (HAF) was performed according to the OECD guidelines. Sprague Dawley female rats were grouped into three containing six animals each for acute toxicity study. For subacute toxicity study, animals were observed periodically for the symptoms of toxicity and death within 24 h and then daily for 14 days. Acetaminophen-induced hepato- and nephrotoxicity models were used for this study. Hepatotoxicity and nephrotoxicity were performed it to control group rats received normal saline (p.o.) per day for 7 days. Hepatotoxicity and nephrotoxicity induced by acetaminophen (PCM) were administered at a dose of 750 mg/kg/day/oral for 7 day and Groups III & IV were treated with PCM (750 mg/kg/day/oral) and HAF of doses 200 and 400 mg/kg/day/oral for 7 days respectively. The liver weight, kidney weight, liver function test, and kidney function test were evaluated along with histopathological investigation in various experimental groups of rats. **Results:** It was observed that the PCM treatment induced significant elevation ( $P < 0.001$ ) in creatinine, kidney weight, liver weight, and liver functions such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and triglycerides. Treatment of HAF of doses 200 and 400 mg/kg/d (p.o) for 7 day on experimental rats recorded significant decrement (up to  $P < 0.001$ ) in creatinine, kidney weight, liver weight, and liver functions such as ALT and AST. The doses of 400 mg/kg/body weight/oral of HAF were found significant when compare with at doses of 200 mg/kg/body weight/oral. A histological observation of liver and kidney tissues provides positive response on experimental groups having PCM + HAF 400 mg/kg-induced model and significant data also correlate the biochemical parameters. **Conclusions:** This finding powerfully supports that polyherbal formulation acts in the liver and kidney as a potent scavenger of free radicals to prevent the toxic effects of PCM. The biochemical and histopathological parameters of polyherbal formulation validate its ethnomedicinal uses and polyphenolic presence.

**Key words:** Biochemical, histopathology, nephrotoxicity

## INTRODUCTION

Paracetamol (PCM) (acetaminophen) is one of the most commonly used analgesic-antipyretic drugs worldwide, and in most countries, it is available without a prescription.<sup>[1]</sup> PCM overdose is known to cause hepatotoxicity and numerous studies about PCM-induced hepatotoxicity, and its mechanisms are available in the literature.<sup>[2-4]</sup> Significant PCM-induced hepatotoxicity usually triggers nephrotoxicity.<sup>[5]</sup> Renal insufficiency is reported to occur in 1–2% of patients exposed to PCM.<sup>[6]</sup> After oral administration, about 63% of PCM is

metabolized through glucuronidation and 34% through sulfation primarily in the liver. The water-soluble metabolites consisting of these metabolic pathways are excreted through the kidney. N-acetyl-p-benzoquinone is a reactive intermediate that occurs

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**Table 1:** Composition of polyherbal formulation

Name of drug	Authentication number	Part used	Quantity (in parts)
<b>Formulation (HAF)</b>			
<i>Bergenia ciliata</i>	NISCAIR/RHMD/CONSULT/2016/2976-03-5	Roots	2 part
<i>Pedaliium murex</i>	NISCAIR/RHMD/CONSULT/2016/2976-03-1	Fruits	2 part
<i>Tribulus terrestris</i>	NISCAIR/RHMD/CONSULT/2017/3050-77-6	Fruits	2 part
<i>Sphaeranthus indicus</i>	NISCAIR/RHMD/CONSULT/2016/2976-03-4	Flowers	2 part
<i>Tinospora cordifolia</i>	NISCAIR/RHMD/CONSULT/2016/2976-03-2	Stem	2 part
<i>Piper longum</i>	NISCAIR/RHMD/CONSULT/2016/2976-03-3	Fruits	1 part

HAF: Hydroalcoholic polyherbal formulation

**Table 2:** Acute toxicity study of polyherbal formulation (HAF)

Acute toxicity study sign	HAF		
	F 1 (300 mg/kg) b.w. (n=1)	F 2 (2000 mg/kg b.w. (n=1)	F3 (2000 mg/kg b.w. (n=4)
Lacrimation	No	No	No
Salivation	No	No	No
Piloerection	No	No	No
Drowsiness	No	No	No
Tremors	No	No	No
Convulsion	No	No	No
Skin	Normal	Normal	Normal
Body weight (g)	120	118	117
Food consumption	Normal	Normal	Normal
Water consumption	Normal	Normal	Normal
Mortality	No	No	No

F: Female rat (Sprague Dawley rat), HAF: Hydroalcoholic polyherbal formulation

when oxidization of <5% of PCM takes place by the microsomal P-450 enzyme system.<sup>[7]</sup> Acute toxicity of acetaminophen in the liver is mediated by cytochrome P450 mixed function oxidase and toxicity is enhanced by compounds which induce the enzyme. Acute renal cortical toxicity is mediated by two or three enzyme systems: Cytochrome-P450 m.f.o., prostaglandin endoperoxide synthetase, and possibly deacetylase.<sup>[8,9]</sup> Chronic toxicity manifested by analgesic nephropathy with renal inner medullary necrosis is mediated by prostaglandin endoperoxide synthetase, and toxicity is enhanced by salicylates. The present study sought to investigate the hepato- and nephroprotective capacity of polyherbal formulation (hydroalcoholic polyherbal formulation [HAF]) which is combination of *Bergenia ciliata*, *Pedaliium murex*, *Tribulus terrestris*, *Tinospora cordifolia*, *Sphaeranthus indicus*, and *Piper longum*.<sup>[10,11]</sup>

## MATERIALS AND METHODS

### Collection, Preparation and Extraction of Herbal Samples

All the medicinal plant materials were collected from different geographical areas of districts Fatehpur, Deoria, and Agra, Uttar Pradesh, India. All the medicinal plants

were authenticated from the National Institute of Science Communication and Information Research, New Delhi, India, under the supervision of scientist Dr. Sunita Garg with different authentication numbers [Table 1].

### Plant Extraction

Selected parts of the plants were used for extraction and then dried for 2 weeks under shade at room temperature, subjected to size reduction with a crusher and then passed through sieve no. 40 to get uniform powder.<sup>[12,13]</sup> Around 250 g of individual powdered plant material was subjected to extraction with solvent such as petroleum ether (for the purpose of defatting) and alcohol (60%). The hydro-alcoholic (40:60) extracts were subjected for maceration process of cold extraction. Each extract was then distilled to dryness under reduced pressure using rotatory evaporator to yield the respective dried extracts.

### Herbal Formulations

The amount of composition of powder extracts contained the ethanolic (60%) extracts was used for the preparation of polyherbal formulation as mentioned in Table 1. The standardization of the polyherbal formulation was tested as

**Table 3:** Summary of clinical sign and mortality

Treatment	Dose (mg/kg) b.w.	Dose volume (ml)	Number of animals	Sex	Clinical sign	Mortality
Sighting study step-I	300	1	01	F	N	0 out 1
Sighting study step-II	2000	1	01	F	N	0 out 1
Main study	2000	1	04	F	N	0 out 4

N: Normal, F: Female

**Table 4:** Hematological and biochemical parameters of animals during subacute toxicity study

Parameters	Clinical parameters	Extracts values of polyherbal formulation
		HAF (200 mg/kg) body weight
Body weight	Pre-treatment	158±6.44
	Post-treatment	162±5.91
HB (g%)	Pre-treatment	12.80±0.92
	Post-treatment	12.30±0.85
RBC count	Pre-treatment	6.40±0.30
	Post-treatment	6.40±0.17
WBC count	Pre-treatment	9.30±0.6
	Post-treatment	8.90±0.27
Urea	Pre-treatment	29.50±1.71
	Post-treatment	34.00±1.27
Creatinine	Pre-treatment	0.80±0.10
	Post-treatment	0.90±0.02
SGOT	Pre-treatment	41.10±2.64
	Post-treatment	42.80±2.90
SGPT	Pre-treatment	40.50±3.05
	Post-treatment	45.30±2.95
Blood sugar	Pre-treatment	94.50±2.72
	Post-treatment	90.50±1.89

Statistical data expressed in mean±SEM ( $n=6$ ). SEM: Standard error of the mean, HAF: Hydroalcoholic polyherbal formulation, HB: Hemoglobin, RBC: Red blood cell, WBC: White blood cell, SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase

per the WHO guidelines for the quality control of herbal materials.<sup>[14-16]</sup>

### Animal Ethical Committee Approval

The study was performed after permission of the Institutional Animal Ethical Committee (IAEC) of Anand College of Pharmacy, Agra, Uttar Pradesh, India (CPCSEA/IAEC/ACP/2017/15). All animals were housed in facilities approved by international guidelines.

### Acute and Subacute Oral Toxicity Studies

Acute toxicity study was performed according to the OECD

guidelines No. 420. Sprague Dawley female rats were grouped in to three containing six animals. Animals were observed periodically for the symptoms of toxicity and death within 24 h and then daily for 14 days. HAF was administered orally at a single dose level of 300 mg/kg/b.w to one animal for sighting study step-I. In Sighting study step-II, HAF was administered orally at a single dose level of 2000 mg/kg/body weight/oral to single animal, The altered autonomic effects (lacrimation, salivation, piloerection) central nervous system effect (tremors, convulsion, drowsiness) skin, body weight, food consumption, water consumption and mortality [Table 3] were observed. For the final study, HAF was administered at a dose of 2000 mg/kg/body weight to group of four animals. The summary of clinical sign and mortality of HAF is shown in Table 3. The maximum dose tested (2000 mg/kg) for LD<sub>50</sub>. From the LD<sub>50</sub>, doses such as 1/5<sup>th</sup> and 1/10<sup>th</sup> were selected and considered for further study, i.e., 200 and 400 mg/kg body weight. The subacute toxicity studies of the formulations were determined as per the OECD guidelines. Sprague Dawley female rats were grouped for daily oral administration of HAF at individual doses of 200 and 400 mg/kg/b.w for 28 consecutive days.<sup>[17,18]</sup> The haematological and biochemical parameters were found significant [Table 4] during sub-acute toxicity studies.

### Experimental Design

The Wister albino rats of 9–12 weeks old weighing about 150–200 g were randomly divided into the following four groups, with each group containing five rats. The animals were maintained under standard environmental condition (23–25°C, 12 h/12 h light/dark cycle) and had free access to standard pellet diet, water *ad libitum*. The animals were acclimatized to the laboratory environment for a week before the of the start study.<sup>[19,20]</sup> Group (I) treated as control, Group (II) treated as (experimental Group) liver and kidney toxicities induced by PCM (750 mg/kg body weight/oral), Group (III) treated as combination doses of PCM (750 mg/kg/body weight/oral) + HAF (200 mg/kg/body weight/oral), Group (VI) Treated as combination doses of PCM (750 mg/kg/body weight/oral) + HAF (400 mg/kg/body weight/oral). [Tables 5 and 6]. The groups were housed separately in different cages for 7 days.<sup>[21-25]</sup>

### Biochemical Studies

After collection of blood, animals were sacrificed by cervical dislocation under mild ether anesthesia, and liver and kidneys

**Table 5: Effect of HAF on liver function of paracetamol-induced liver injury rat model**

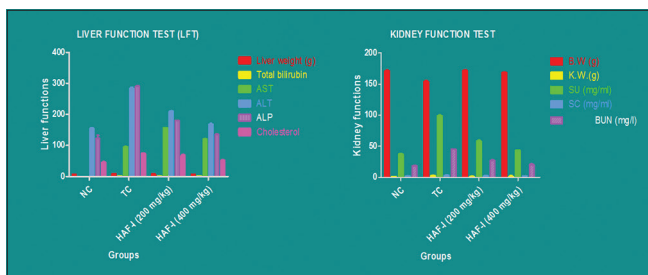
Groups	Liver weight (g)	Total bilirubin (mg %)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Cholesterol (mg/dl)
NC	6.35±0.13	0.52±0.10	95.4±2.50	154.5±2.30	122.67±9.95	45.02±2.1
TC	8.0±0.17	1.05±0.50	205±1.02	283.96±3.20	290±2.00	74.50±0.52
HAF-I (200 mg/kg)	7.48±1.25 <sup>a</sup>	0.82±0.04 <sup>a</sup>	157±0.24 <sup>b</sup>	210.20±1.25 <sup>b</sup>	180.1±0.25 <sup>a</sup>	67.45±3.25 <sup>b</sup>
HAF-I (400 mg/kg)	6.42±0.24 <sup>a</sup>	0.60±0.15 <sup>a</sup>	120±1.50 <sup>a</sup>	168.26±2.25 <sup>a</sup>	135.5±0.70 <sup>b</sup>	52.25±2.20 <sup>b</sup>

NC: Normal control, TC: Toxic control, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase. In this data, each group contain six animals. The Neuman Kaul's test was performed between control versus PCM-induced group and PCM versus respective polyherbal-treated groups. Where a is highly significant ( $P < 0.0001$ ), b is significant ( $P < 0.001$ ), and Ns is non-significant ( $P > 0.05$ ). PCM: Paracetamol, HAF: Hydroalcoholic polyherbal formulation

**Table 6: Effect of polyherbal formulation (HAF) on renal function test**

Groups	B.W (g)	K.W. (g)	SU (mg/ml)	SC (mg/ml)	BUN (mg/l)
NC	171±1.18	1.6±0.03	37.21±0.99	1.71±0.72	18.43±0.65
TC	153.8±1.74	2.4±1.02	99.12±1.05	3.11±0.75	45.20±0.50
HAF-I (200 mg/kg)	171.6±1.435 <sup>a</sup>	1.65±0.20 <sup>a</sup>	58.45±1.05 <sup>b</sup>	2.10±0.62 <sup>a</sup>	26.90±0.95 <sup>a</sup>
HAF-I (400 mg/kg)	168.6±0.979 <sup>a</sup>	1.32±1.25 <sup>b</sup>	43.32±0.38 <sup>b</sup>	1.75±0.25 <sup>b</sup>	20.4±1.20 <sup>a</sup>

BW: Body weight, KW: Kidney weight, SU: Serum urea, SC: Serum creatinine, BUN: Blood urea nitrogen. In this data, each group contain six animals. The Neuman Kaul's test was performed between control versus PCM-induced group and PCM Versus respective poly herbal treated groups. Where a is highly significant ( $P < 0.0001$ ), b is significant ( $P < 0.001$ ), And Ns is non-significant ( $P > 0.05$ ). HAF: Hydro-alcoholic polyherbal formulation, PCM: Paracetamol



**Figure 1:** In this data, each group contain six animals. The Neuman Kaul's test was performed between control versus paracetamol (PCM)-induced group and PCM versus respective polyherbal-treated groups. Where a is highly significant ( $P < 0.0001$ ), b is significant ( $P < 0.001$ ), and Ns is non-significant ( $P > 0.05$ ). BW - Body weight, KW - Kidney weight, SU - Serum urea, SC - Serum creatinine, BUN - Blood urea nitrogen

were harvested, rinsed in saline, and stored at  $-80^{\circ}\text{C}$  until further biochemical analysis.<sup>[26]</sup> Liver function tests and renal function tests were measured to observe the oxidative stress level in serum.<sup>[27-32]</sup>

## Histological Analysis

### Histopathological studies of the liver

Liver tissues were cut in small pieces and immersed in neutral buffered formalin for 24h. The fixed tissues were processed routinely, embedded in paraffin, sectioned, deparaffinized, and rehydrated using the standard techniques. The extent of PCM -induced necrosis was evaluated by assessing the morphological changes in the liver sections stained with hematoxylin and eosin (H and E), using standard techniques

[Figure 1].

### Histopathological studies of the kidney

Pieces of the kidney from each group were fixed immediately in 10% neutral formalin for a period of at least 24 h, dehydrated in graded (50–100%) alcohol, embedded in paraffin wax, cut into 4–5  $\mu\text{m}$  thick sections, and stained with H and E. The sections were evaluated for the pathological symptoms of nephrotoxicity such as necrosis, fatty infiltration, fibrosis, lymphocyte infiltration, and blood vessel congestion [Figure 2].

## RESULTS AND DISCUSSION

### Acute and Subacute Toxicity Studies

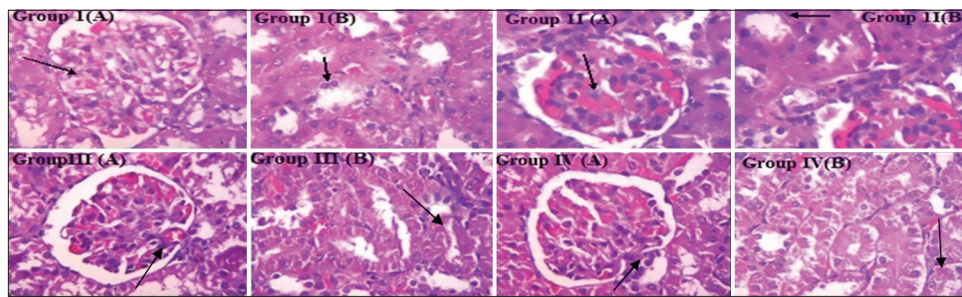
No abnormality and sign of toxicity produced at a dose of 2000 mg/kg body weight. The HAF was found to be safe up to a dose of 2000 mg/kg body weight and the data are shown in Tables 2 and 3. The doses of 200 mg/kg and 400 mg/kg body weight were selected for formulation based on the results of acute and subacute toxicity study.

### Experimental Design

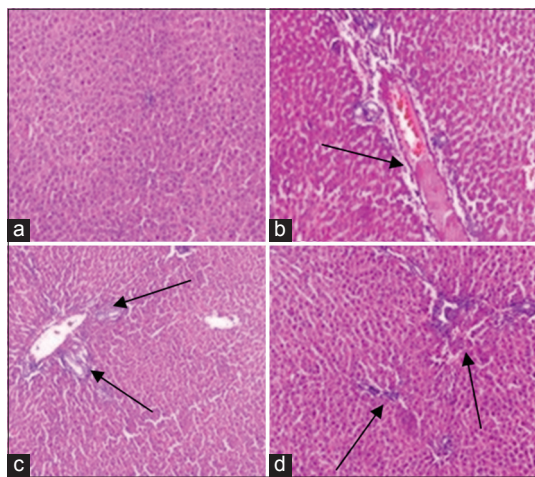
#### Quantification of liver tests

There were no significant changes in bilirubin values among all the experimental groups studied. Aspartate amino-transferase, alanine aminotransferase, and alkaline phosphatase levels increased in toxic control group while treatment resulted in decreasing these values and the





**Figure 2:** Histopathology of negative and positive control group in glomerulus and renal tubules. Group I negative control showed intact architecture of renal parenchyma. In glomerulus [Group IA, Arrow], Bowman’s space and mesangial cells appeared intact. In intact renal tubules [Group IB, Arrow], blood vessels and interstitium were unremarkable. Group II: Positive control, i.e., PCM treated showed focally distorted renal parenchyma architecture [mainly tubules]. In glomerulus [Group IIA, Arrow]: Intact Bowman’s space, extravasation of erythrocytes seen, Mesangial cells appear increased. Most of the renal tubules showed degenerative changes [Group IIB, Arrow]. Blood vessels and interstitium were unremarkable. Group III: Treatment done with HAF-I 200 mg/kg body weight, p.o. showed intact architecture of renal parenchyma. In glomerulus [Group IIIA, Arrow], +: Intact Bowman’s space and extravasation of erythrocytes were seen; mesangial cells appear increased. Few renal tubules show degenerative changes [Group IIIB, Arrow]. Blood vessels and interstitium were unremarkable. Group IV: Treatment done with HAF-I 400 mg/kg, p.o. showed intact architecture of renal parenchyma. In glomerulus [Group IVA, Arrow], extravasation of erythrocytes was seen; mesangial cells appear increased. Few renal tubules show degenerative changes [Group IVB, Arrow]. Blood vessels and interstitium were unremarkable



**Figure 3:** (a) Control group showing central vein surrounded by hepatic cord of cell, (b) paracetamol-induced liver necrosis and disruption with massive fatty changes, ballooning degeneration, and loss of cellular boundaries, (c) Group C shows mild congestion in CV, less fatty changes, and mild necrotic cells with minimal inflammatory conditions on administration of hydroalcoholic polyherbal formulation doses of 200 mg/kg p.o. for 7 days, and (d) animals shows regeneration of hepatocytes around CV, near normal liver architecture with administration on hydroalcoholic polyherbal formulation doses of 400 mg/kg p.o. for 7 days

maximum decrease was found in HAF 400 mg/kg treated group [Table 5].

**Quantification of kidney test**

Table 6 shows the renal protective effect of therapy, indicating the maximum decrease in blood urea nitrogen (BUN) and serum creatinine with HAF 400 mg/kg group.

HAF 200 mg/kg group also showed a decline in BUN and creatinine levels as compared to toxic control group, but they were more than HAF 400 mg/kg dose group.

**Histopathological Study**

**Histopathological studies of the liver**

Histopathological study shows the protective activity of HAF. This included the histological changes in the liver architecture such as architecture of hepatic lobules, swelling of liver cells, fatty change, focal necrosis, inflammatory cell infiltration around portal area, kuffar cells, and hyperplasia. The healing effect of HAF-400 mg/kg body weight produces more significant results when compared to HAF-200 mg/kg [Figure 3].

**Histopathological studies of the kidney**

A histopathological study shows the curative and protective activity of HAF.

**CONCLUSIONS**

Overdose of PCM causes toxicity in human beings as well as in rats and produce liver and kidney damage. Thus, PCM is a suitable experimental toxin to induce hepatic and renal damage. As we gone through various studies on the treatment of kidney and renal disorders, we can conclude that herbal plants and its polyherbal formulation play a unique and important role in the liver and kidney-related medication. The phytoconstituents obtained from different plants of herbal formulation contain phenolics, tannins, flavonoids, and

others mainly responsible for hepato- and nephroprotective activity. The aim of this research is to record experimental correlations with medicinal folk-lore for curing hepato- and nephroprotective and phytochemicals presented in the polyherbal formulation (HAF). And also, we have attempted to use our best endeavors of indigenous herbs to alternative medicine of liver and renal damage. From this study, it is clear that the HAF possesses significant hepato- and nephroprotective activity.

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