

**TO STUDY THE EFFECT OF POLY HERBAL FORMULATION FOR
PARACETAMOL INDUCED HEPATOTOXICITY**

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ABSTRACT

Hepatotoxicity is an incessant disease that can make significant results going from extreme metabolic irregularities passing. Hepatotoxicity is brought about by free revolutionaries in most of cases. Numerous biochemical exercises depend on free extremists, and they are a significant part of oxygen consuming life and digestion. The pathogenecity of significant ailments like malignant growth, rheumatoid joint inflammation, the maturing system, and cardiovascular sickness has been connected to receptive oxygen species-interceded oxidative harm to macromolecules like lipids, proteins, and DNA. Cancer prevention agents have been displayed to safeguard cells from free extreme harm by impeding the oxidation cycle through revolutionary rummaging activity.

KEY WORDS: Poly Herbal, Formulation, Hepatocytes, Liver.

INTRODUCTION

To manage receptive oxygen species (ROS) and their results, liver cells have a scope of compensatory components, including the development of cell reinforcement proteins including superoxide dismutase (Turf), catalase, and glutathione peroxidase (GSHPx). The chemical cell reinforcement framework [Cu-Zn, Mn-Grass, catalase, GSHPx, and GSH reductase (GR)] works by eliminating ROS straightforwardly or successively, ending their movement. Oxidative harm is brought about by awkwardness between oxidative powers and cell reinforcement safeguard components, and it has been connected to diseases including atherosclerosis, diabetes, malignant growth, and liver cirrhosis.

In sound settings, ROS is consistently created and effectively eliminated by numerous intracellular and extracellular cancer prevention agent systems. Harm to cell macromolecules (DNA, lipids, and protein) and other little cancer prevention agent atoms is normal when ROS age is uncontrolled. The superoxide anion revolutionary O₂⁻, hydrogen peroxide (H₂O₂), alkoxy (RO), peroxy (ROO), hydroxyl extremist (Goodness), and hypochlorous corrosive are the main ROS (HOCl). Nitric oxide (NO) and peroxy nitrite, two non-oxygen species that exist as responsive nitrogen species (RNS), have huge bioactivity. The free extreme response is a critical component in different organic frameworks. The age of free extreme intermediates, which causes the disturbances, is a huge kind of reaction among various types of synthetic prompted hurt.

Most of hepatotoxic substances make lipid peroxidation and other oxidative harm liver cells. The liver has an unmistakable digestion and assumes a basic part in the leeway of synthetic compounds from the entrance course, making it helpless against drug poisonousness, xenobiotic harmfulness, and oxidative pressure. The cytochrome p-450 and GSH-peroxidase catalysts are associated with two distinct metabolic cycles in the liver.

HEPATOPROTECTIVE AGENTS:

Natural ingredients were frequently used to relieve or heal sickness. Throughout the evolution of human society, the interaction between humans and plants has been extremely intimate. There has been a continual interest in medications from the plant world as our understanding of human ailments has grown.

According to recent studies, there are no effective medications for treating liver problems. Corticosteroids or immunosuppressive medicines in large dosages are the sole medications utilized. These might work by bolstering the body's defense mechanisms. The liver illness, particularly jaundice, is left untreated to heal on its own over time. During this time, further strain is avoided on the liver, which is already impaired. However, several plants are utilized and reported to be effective as liver protectors in Ayurveda and traditional medicine.

Hepatoprotective properties have been investigated in a wide range of herbal and synthetic medicines and formulations.

LIVER AND HEPATOTOXICITY:

The liver is the greatest organ in the body and furthermore works as an organ. A rosy earthy colored organ with four curves isn't generally a similar size or structure. The typical human liver weighs 1.44-1.66 kg (3.2-3.7 lb).

It is situated in the right upper quadrant of the stomach pit, under and associated with the stomach. It has two major blood veins, one named the hepatic conduit and the other called the entrance vein. The hepatic course conveys blood from the aorta, though the entryway vein conveys blood from the entire gastrointestinal framework, as well as the spleen and pancreas. These blood channels split into vessels, which in this manner branch out into lobules. A huge number of hepatic cells, which are the center metabolic cells, make up every lobule. The liver's utilitarian units are called lobules. Through its various and enhanced jobs, it contributes fundamentally to the upkeep of the inside climate .

The liver's primary functions include:

- A significant portion of amino acid production.
- The liver is involved in glucose metabolism in numerous ways:
- Gluconeogenesis.
- Gluconeogenesis (the breakdown of glycogen into glucose).
- Glycogenesis (the process of converting glucose into glycogen) (muscle tissues can also do this).
- The liver is in charge of protein metabolism, synthesis, and breakdown.
- The liver is also involved in lipid metabolism in numerous ways:
- Cholesterol production
- Triglyceride generation (lipogenesis) (fats).

- The liver produces the majority of lipoproteins.
- Coagulation factors I (fibrinogen), II (prothrombin), V, VII, IX, and X are all produced by the liver.
- The liver is the primary location of red blood cell synthesis in the first trimester fetus.
- The liver generates and excretes bile (a yellowish liquid) essential for emulsifying fats and aids in the absorption of vitamin K from the food by the 32nd week of pregnancy. Some bile goes straight to the duodenum, while others are retained in the gallbladder.
- Insulin-like growth factor 1, a polypeptide protein hormone that plays a key role in childhood growth and continues to have anabolic effects in adulthood, is also produced by the liver.
- Glucose (in the form of glycogen), vitamin A (1–2 years' supply), vitamin D (1–4 months' supply), vitamin B12 (1–3 years' supply), iron, and copper are all stored in the liver.
- The liver generates albumin, the primary osmolar component of blood serum; the reticuloendothelial system of the liver includes numerous immunologically active cells, serving as a'sieve' for antigens delivered to it via the portal system.

When stimulated by renin, an enzyme produced when the kidney detects low blood pressure, the liver produces angiotensinogen, a hormone that is responsible for boosting blood pressure.

Any injury to the liver or impairment of its functioning has negative consequences. Liver illnesses (such as jaundice) are widespread conditions that plague humans, yet there is currently no treatment accessible in allopathy.

The liver is an important organ for detoxication and disposal of endogenous chemicals.

It is repeatedly and widely exposed to xenobiotics, hepatotoxins, and chemotherapeutic drugs, all of which affect its functioning. Toxic substances, excessive alcohol intake, and infections are the major causes of liver damage.

LIVER DISEASES:

- Hepatitis: Inflammation of the liver caused mostly by viruses (viral hepatitis), but also by liver toxins (e.g., alcoholic hepatitis), autoimmunity (autoimmune hepatitis), and inherited disorders.
- Alcoholic liver: This phrase refers to any hepatic symptom of excessive alcohol intake, such as fatty liver disease, alcoholic hepatitis, and cirrhosis.
- Hepatic steatosis (fatty liver disease): Large vacuoles of triglyceride fat build in liver cells in this reversible disease. Non-alcoholic fatty liver disease (NAFLD) is a group of conditions linked to obesity, metabolic syndrome, and other factors. Inflammation of the liver (steatohepatitis) and, finally, cirrhosis can result from fatty liver.
- Cirrhosis: This is the creation of fibrous tissue (fibrosis) in the place of liver cells that have perished owing to a number of factors such as viral hepatitis, excessive alcohol intake, and other kinds of liver toxicity. Chronic liver failure is caused by cirrhosis.
- Hepatocellular carcinoma and/or cholangiocarcinoma are the most prevalent kinds of primary liver cancer; angiosarcoma and hemangiosarcoma of the liver are rarer variants. (Many liver tumors are secondary lesions that have spread from original cancers in the gastrointestinal system and other organs such the kidneys, lungs, breast, and prostate.)
- Primary biliary cirrhosis: A devastating autoimmune disease affecting the bile capillaries.

HEPATOTOXIC AGENTS:

Compound liver harm is a customary event. The sort of the hepatotoxic substances, the idea of the harm, the instrument of the hepatotoxic outcomes, openness conditions, clinical and social significance are variables to consider.

Hepatotoxins can be tracked down in nature as plant, contagious, or bacterial metabolic items, or as minerals. Many are drug or substance area items (Mill operator et al., 1976). Others are

modern results or waste things that might gain admittance to people through contamination of the climate.

CLASSIFICATION OF HEPATOTOXIC AGENTS:

Hepatic harm can be brought about by two unique sorts of synthetic compounds. One bunch of synthetic substances is basically harmful, implying that its hepatotoxicity is a fundamental element to which most uncovered individuals are defenseless. They're named genuine Characteristic or Unsurprising Hepatotoxins. Specialists that exclusively cause hepatic harm make up different classes. People are bizarrely touchy. Non-Unsurprising or Quirky substance hepatic harm is the name given to this sort of hepatic injury.

INTRINSIC HEPATOTOXINS

a. Direct Hepatotoxins (Toxipathic)

This category includes drugs and their direct metabolic products that cause direct harm to the hepatocyte's plasma membrane, endoplasmic reticulum, or other organelles. This class's prototype is carbon tetrahydrofuran (CCl₄). BrCCl₃, CHCl₃, Iodoform, various haloalkanes, and elemental phosphorus are the other direct hepatotoxins.

b. Indirect Hepatotoxins (Tropopathic)

These substances are antimetabolites and similar chemicals that cause liver damage by interfering with a specific metabolic route or activity, or by distorting individual cell molecules. Indirect hepatotoxins appear to cause structural damage as a result of metabolic lesions. They disrupt the hepatocyte's metabolic architecture by interfering with a specific metabolic route or causing selective damage to a cell component or hepatic function.

There are two types of indirect hepatotoxins:

(i).Cytotoxic indirect Hepatotoxins

It will cause cytotoxicity by interfering with metabolic pathways or processes required for hepatocyte integrity.

Experimental Toxins: Ethionine, Dialkylnitrosamines, Bromobenzene,
Galactosamine, Thioacetamide.

Natural Hepatotoxins: Afalotoxins, Mycotoxins, Pyrrolidizine alkaloids,
Mushroomtoxin.

Medicinal Agents: Antibiotics (Tetracycline), antimetabolites
(Methotrexate), Mithramycin, Mercaptopurine,
Urethane) and analgesics like acetaminophen.

(ii).Cholestatic indirect Hepatotoxins:

These are intrinsic toxins that cause jaundice or decreased liver function by interfering with bile flow selectively without causing additional hepatocyte damage. Icerogen, C-197 alkaloids, anabolic and contraceptive steroids are among examples.

RESEARCH METHODOLOGY

SELECTION OF ANIMALS:

This study employed Swiss albino mice measuring 20-25 g and Albino Wistar rats weighing 150-250 g of either sex, aged 4 months. The experimental animals were kept in polypropylene cages and were subjected to conventional care (12hour light/dark cycles, 25^oC, and 35-60% humidity). Ad libitum access to standard pelletized feed and tap water was supplied.

After five days, the animals are divided into four groups, each of which contains three animals, and each group is given a single dose of polyherbal concoction. Individual animals were monitored for any harmful effects or mortality after dosing at least once during the first 30 minutes, occasionally during the first 24 hours, with specific attention paid during the first 4 hours, and daily thereafter for a total of 14 days.

EVALUATION OF HEPATO PROTECTIVE ACTIVITY:

CCl₄ induced hepatotoxicity in rats:

The rats were divided into nine groups at random.

Group 1: The normal control group was given 1% gum accasia for 7 days.

Group 2: Toxin control group received 1 percent gum accasia for 6 days before receiving a single intraperitoneal dose of CCl₄ 1.5mlkg in ground nut oil (1:1 ratio).

Group 3: The standard control group was given silymarin (50 mg/kg/day) orally for 7 days and then a single dosage of CCl₄ (1.5 ml in ground nut oil, 1:1) 30 minutes after the silymarin was given.

Group 4 (Low dose): Group 4 got a single dose of CCl₄ 1.5mlkg⁻¹ in ground nut oil 30 minutes after receiving PHF-I (100mg/kg) orally for 7 days.

Group 5 (Medium dose) was given a single dose of CCl₄ 1.5mLkg⁻¹ in ground nut oil 30 minutes after receiving PHF-I (200mg/kg) orally for 7 days.

Group 6 (High dose) received a single dose of CCl₄ 1.5mLkg⁻¹ in ground nut oil 30 minutes after receiving PHF-I (400mg/kg) orally for 7 days and a single dose of PHF-I (400mg/kg) orally for 7 days.

Group 7 (Low dose): Group 4 got a single dose of CCl₄ 1.5mLkg⁻¹ in ground nut oil 30

minutes after receiving PHF-II (100mg/kg) orally for 7 days and a single dose of PHF-II (100mg/kg) orally for 7 days.

Group 8 (Medium dose): Group 5 was given daily doses of PHF-II (200mg/kg) orally for 7 days, followed by a single dose of CCl₄ 1.5mLkg⁻¹ in ground nut oil 30 minutes after the PHF-II was given.

Group 9 (High dose): Group 6 got a single dose of CCl₄ 1.5mLkg⁻¹ in ground nut oil 30 minutes after receiving PHF-II (400mg/kg) orally for 7 days, followed by a single dose of PHF-II (400mg/kg) orally for 7 days.

Rats were deprived of food overnight and sacrificed by decapitation at the end of the experiment. Blood samples were taken and left to coagulate for 30-40 minutes. Serum was separated and washed with phosphate buffer saline after centrifugation at 370C to estimate various biochemical parameters, while liver tissue was separated and washed with phosphate buffer saline to estimate various biochemical parameters (0.05M, ph7.4). After that, the liver tissue was chopped into little pieces and homogenized in an ice cold phosphate buffer saline solution (0.05M, ph7.4)

Using a tissue homogenizer, 1:9 (w/v) (10%) entire homogenate was obtained. For the measurement of malondialdehyde, a portion of the homogenate was combined with an equivalent volume of 10% Trichloroacetic acid (TCA). The homogenate was centrifuged for 30 minutes at 8000 rpm in a Remi cold centrifuge. The supernatant was separated and utilized to estimate anti-oxidant levels in all tissues, including catalase, glutathione, and reduced glutathione.

RESULTS AND DISCUSSION

EFFECT OF POLY HERBAL FORMULATION S-I & II ON HEPATOCYTES OF LIVER:

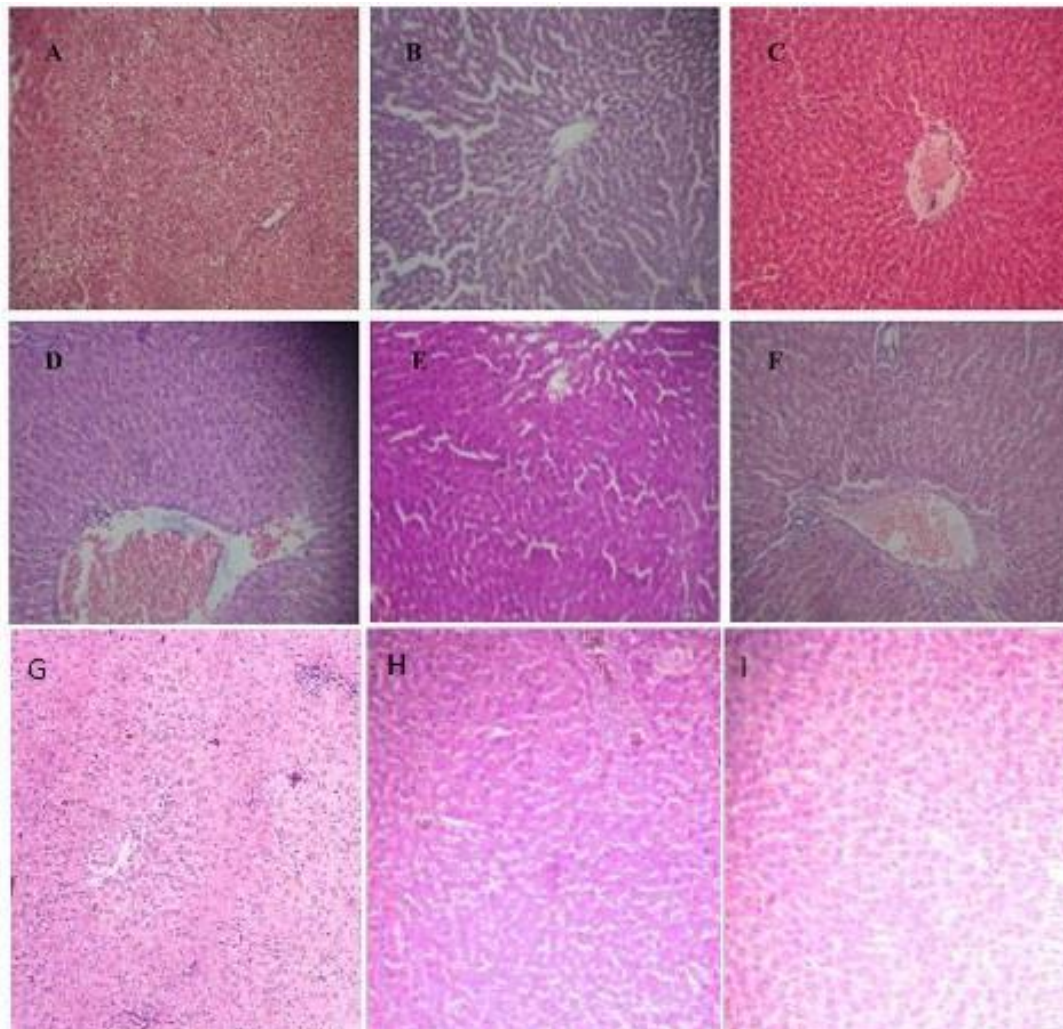


Fig.No. 1: (A) Control Liver: Individual hepatocytes appeared normal indicated by black arrow Portal region indicated by red arrow which appeared normal. Sinusoidal spaces appeared normal. Degenerative and inflammatory changes were not observed. Tissue is stained with Haematoxylin and Eosin at magnification 80X.

(B) Hepatic degeneration or individual hepatocyte degeneration was seen in the CCl₄-treated liver, as indicated by the black arrow. At a magnification of 100X, the tissue is stained with Haematoxylin and Eosin.

(C). Individual hepatocytes in the Silymarin+CCl₄-treated liver appeared normal (red arrow) each portal zone appeared to be normal. Spaces that appeared to be sinusoidal appeared to be

normal. At a magnification of 100X, the tissue is stained with Haematoxylin and Eosin.

(D).PHF-I 100mg/kg + CCL4: Multiple necrosis foci in the periportal area of the liver were seen (arrow).

(E) Inflammatory cells were not seen in these necrotic foci. At a magnification of 100X, the tissue is stained with Haematoxylin and Eosin.

(F) Hepatocytes appeared normal in the presence of PHF-I 200mg/kg + CCL4. Inflammatory cells were not seen in these necrotic foci. At a magnification of 100X, the tissue is stained with Haematoxylin and Eosin.

Hepatocytes appeared normal in the presence of PHF-I 400mg/kg +CCL4. Inflammatory cells were not seen in these necrotic foci. At a magnification of 100X, the tissue is stained with Haematoxylin and Eosin.

(G).PHF-II 100mg/kg + CCL4: Multiple necrosis foci in the periportal area of the liver were seen (arrow).

(H) Inflammatory cells were not seen in these necrotic foci. At a magnification of 100X, the tissue is stained with Haematoxylin and Eosin.

(I)Hepatocytes appeared normal in the presence of PHF-II 200mg/kg + CCL4. Inflammatory cells were not seen in these necrotic foci. At a magnification of 100X, the tissue is stained with Haematoxylin and Eosin.

(J) Hepatocytes appeared normal in the PHF-II 400mg/kg +CCL4 group. Inflammatory cells were not seen in these necrotic foci. At a magnification of 100X, the tissue is stained with Haematoxylin and Eosin.

EVALUATION OF POLY HERBAL FORMULATION S-I & II FOR

PARACETAMOL INDUCED HEPATOTOXICITY

Effect of Poly herbal formulation - I & II on serum profile in paracetamol induced hepatotoxicity in rats:

Table No. 1: Effect of Poly herbal formulation - I & II on Serum profile:

Groups	Treatment	SGOT (IU/dL)	SGPT (IU/dL)	ALP (IU/L)	Total bilirubin (mg/dL)	Total protein (g/dL)
I	Control treated with 1% gum acacia	15.6±0.7	24.5±1.89	21.6±1.86	1.39±0.24	8.2±0.58
II	PCM (3mg/kg)	24.3±0.35*	38.5±1.23*	33.5±1.25*	3.55±0.66*	5.7±0.32*
III	Silymarin (25mg/kg)+ PCM (3mg/kg)	16.8±0.53**	26.3±21.9*	21.4±1.6**	1.9±0.68**	7.5±0.23**
IV	PHF-I (100mg/kg)+ PCM (3mg/kg)	19.5±0.7#	30.2±0.22#	33.2±0.25#	2.65±0.56#	6.5±0.47#
V	PHF-I (200mg/kg)+ PCM (3mg/kg)	17.7±0.8##	26.8±1.88#	27.61±1.82##	1.77±0.54##	7.9±0.47##
VI	PHF-I (400mg/kg)+ PCM (3mg/kg)	15.7±1.7##	22.8±0.88#	6.21±0.57#	1.21±0.53##	9.9±0.44##
VII	PHF-II (100mg/kg)+ PCM (3mg/kg)	42.36±4.6#	63.65±3.22#	32.65±0.25#	3.54±0.57#	5.15±1.22#
VIII	PHF-II (200mg/kg)+ PCM (3mg/kg)	36.54±1.7##	59.54±2.85##	39.12±1.88##	2.96±0.54##	6.79±1.46##
IX	PHF-II (400mg/kg)+ PCM (3mg/kg)	32.58±2.7##	49.64±1.84##	45.23±0.85##	1.17±0.25##	7.9±0.48##

*p0.001 when compared to the normal Control group, **p0.0001 when compared to the Paracetamol treated group, #p0.001 when compared to the Paracetamol group, ##p0.001 when compared to the Paracetamol group by utilizing one way ANOVA followed by DUNNETT's multiple comparison test.

SGOT, SGPT, ALP, total bilirubin, and total protein levels were determined to be 15.6 0.5 iu/dL, 24.5 1.89 iu/dL, 1390.24 mg/dL, and 8.2 0.54 g/dL in the control group I, respectively. The levels of SGOT, SGPT, AL,P, and total bilirubin were all higher in the PCM-treated group II, whereas total protein levels were lower. In the PHF-I-treated groups (IV, V, and VI), there was dosage-dependent protection, with the highest dose (400mg/kg) having a good effect against PCM-induced intoxication. The conventional medication silymarin provided protection against PCM-induced intoxication in group III animals. The PHF –I-treated groups (VII, VIII, and IX) showed dosage-dependent protection against PCM-induced intoxication, with the 400mg/kg dose providing the best protection. PHF – I was the more active of the two formulations.

CONCLUSION

Hepatotoxicity was produced using CCl₄ to determine hepatoprotective activity, and liver function was assessed by quantifying the levels of serum enzymes such as ALT, AST, and ALP. These enzyme levels in serum may rise with the severity of liver damage during hepatic injury. The increased levels of these enzymes in the CCl₄-treated group correlated to the toxin's significant liver damage.

PHF-I and II (100, 200, and 400 mg/kg) showed considerable hepatoprotective effect against CCl₄-induced damage insult in a dose-dependent manner in the current investigation. Carbon tetrachloride causes hepatotoxicity by activating metabolic pathways, resulting in selective toxicity in liver cells that leads to metabolic dysfunction. Carbon tetrachloride is metabolically activated by cytochrome P450 in the endoplasmic reticulum to form a trichloromethyl free radical (ccl₃), which reacts with cellular lipids and proteins in the presence of oxygen to cause lipid peroxidation, resulting in changes in the endoplasmic reticulum and other membrane structures, loss of metabolic enzyme activation, reduced protein synthesis, and elevation of serum transaminases,. Lipid peroxidation is reduced under the effect of a specific extract due to the scavenging action of the phytoconstituents present.

Alkaline phosphatase is a membrane-bound glycoprotein enzyme found in abundance in the sinusoids and endothelium. This enzyme primarily enters the liver through the bones. Because it is eliminated in the bile, hepatobiliary disorders cause an increase in serum levels. The

current study's findings suggest that PHF-I and II phytocostituents may protect the hepatic plasma membrane from CCl₄-induced damage, resulting in lower ALP levels.

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