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Pharmacological Evaluation of Hepatoprotective Activity of Indian Medicinal Plants *Cordia Myxa* and *Canscora Diffusa* on Wistar Albino Rats

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Abstract

Two plants, Cordia myxa and Canscora diffusa, which were claimed to possess hepatoprotective activity, were selected. The selected plants were authenticated by an expert botanist. In the current study, herbal extraction involving solvents of decreasing polarity (petroleum ether, ethyl acetate, and hydroalcoholic) was used to extract bioactive compounds from the plants of Cordia Myxa and Canscora diffusa in polyherbal preparations. A phytochemical evaluation was performed, including an acute toxicity study and hepatoprotective activity induced by CCl_4 and paracetamol. The current investigation showed that polyherbal formulations at doses of 100, 200, and 400 mg/kg significantly reduced the severity of CCl_4-induced and paracetamol-induced liver damage. By activating metabolism, it leads to selective toxicity in liver cells and impairment of metabolic activity. In conclusion, both the extract and the prepared formulation had significant liver-protective effects. This is evident from the evaluation of several important biochemical parameters in selected experimental animals. These parameters are generally related to liver function and damage. There was a marked improvement in response to treatment. The results indicate that the formulation is effective in protecting liver cells from damage or facilitating recovery. The results observed decrease in liver enzyme levels of ALT, AST and bilirubin and it indicates reduction in liver stress and injury. It also confirms the tested formula's beneficial effects on overall liver health. These findings highlight its potential therapeutic value in the management of liver disorders.

Keywords: *Cordia myxa*, *Canscora diffusa*, hepatoprotective, polyherbal formulation, phytochemical evaluation

INTRODUCTION

Ayurveda is a centuries-old system of medicine with a long history of practice. Ayurveda is not just a compilation of medicinal plants; it offers a holistic framework for health and well-being. This is commonly known as Ayurvedic medicine firmly rooted in ancient Vedic teachings. Ayurveda, one of the oldest healing traditions, has its origins in India thousands of years ago. This knowledge has been carefully preserved and passed down through generations. Today, Ayurveda remains a vital part of health and wellness practices. Ayurveda is known as the "Mother of All Healing". Its significant impact is evident in many practices of natural and holistic medicine. Its timeless principles continue to shape wellness practices worldwide, providing a strong foundation for natural healing systems across cultures [1].

Ayurveda employs two primary approaches to drug formulation: single herb and polyherbal formulation (PHF). PHF is a traditional therapeutic

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strategy that combines multiple medicinal herbs to enhance their therapeutic efficacy. This practice, often referred to as polypharmacy or polyherbalism, aims to create more effective treatments by leveraging the unique properties of each herb. By blending these herbs, practitioners seek to achieve synergistic health benefits that surpass those of a single herb alone [2].

The liver is an essential organ that performs a wide range of crucial functions, including metabolism, bile secretion for digestion, and the storage of nutrients like vitamins and glycogen. Additionally, it plays a key role in detoxification by filtering harmful substances from the blood, thereby maintaining overall health and well-being. When damaged, these essential functions can be impaired. The liver is constantly exposed to toxins from the intestines, which are delivered through the portal vein. This organ constantly works to filter out harmful substances and uphold overall health. Disruptions in its functions can significantly impair the body's ability to process and eliminate toxins [3, 4].

Carbon tetrachloride (CCl_4) toxicity is influenced by both dosage and exposure duration. Lower doses can disrupt calcium (Ca^{2+}) homeostasis, cause lipid peroxidation, and release cytokines. These changes may trigger apoptosis, but cellular regeneration is possible. However, higher doses or prolonged exposure can lead to severe liver damage, including fibrosis, cirrhosis, or even cancer. Understanding dosage and exposure time is crucial for assessing the risk and severity of CCl_4 toxicity [5, 6].

Paracetamol (acetaminophen) is an antipyretic analgesic that effectively reduces fever and pain. However, excessive doses can lead to acute liver damage and hepatocyte necrosis. It's a widely used experimental model for studying drug-induced liver damage.

At therapeutic doses, paracetamol is primarily metabolized into glucuronic or sulfated derivatives, which are excreted. A portion is also converted into reactive intermediates, detoxified through glutathione conjugation. Excessive paracetamol undergoes oxidation via the cytochrome P450 system, particularly CYP2E1, producing N-acetyl-p-benzoquinone (NAPQI) [7].

High NAPQI and depleted glutathione can lead to harmful effects like protein adduct formation, mitochondrial dysfunction, and increased oxidative stress. These can cause liver cell necrosis.

This study assessed hepatoprotective activity using carbon tetrachloride (CCl_4) and paracetamol-induced hepatotoxicity in rats at various dosing intervals [8].

MATERIAL AND METHODS

Collection of Plant Material

In October 2019, leaves of *Cordia myxa* and *Canscora diffusa* were collected from Moolchand Phoolchand Herbal Store in Bhopal, Madhya Pradesh. Dr. Saba Naaz, Head of the Department of Botany at Saifia Science College, Bhopal, identified the leaves. The identified specimens were then submitted to the Department of Botany for preservation. These specimens are documented and stored as voucher specimens with the following numbers: *Cordia myxa* (414/Saif/Sci/Clg/Bpl) and *Canscora diffusa* (415/Saif/Sci/Clg/Bpl). These voucher specimens ensure that the samples are recognized for future research and study.

Pharmacognostic Studies

Macroscopic Characteristics

A thorough macroscopic examination was conducted on the leaves of *Cordia myxa* and *Canscora diffusa*. This analysis focused on observing the physical characteristics and attributes of the leaves. This assessment focused on various characteristics, including the shape and size of the leaves, as well as their surface features and texture. Additionally, observations were made regarding the color, consistency, odor, and taste of the leaves. These aspects are crucial for identifying and differentiating plants and understanding their potential applications in herbal medicine and other fields [9].

Microscopic Characteristics

Leaves of *Cordia myxa* and *Canscora diffusa* were collected for analysis. To prepare the samples, the sections were first cleared using chloral hydrate to remove any unwanted materials. They were then stained with a safranin staining solution to enhance visibility under the microscope. Subsequently, the samples were mounted in glycerin to maintain their structural integrity for further examination. This preservation method ensured that the specimens remained suitable for detailed analysis. This same procedure was applied to assess the microscopic characteristics of the powdered material derived from both *Cordia myxa* and *Canscora diffusa*. This thorough process helps in understanding the anatomical features of the plants, which can be crucial for further research and applications [10].

Preparation of the Extract

The leaves of *Cordia myxa* and *Canscora diffusa* were first washed thoroughly with tap water to remove any contaminants. The leaves were washed and then allowed to air-dry completely. Once dried, they were ground into a fine powder for further analysis. This powdered material was stored in airtight bottles to maintain its integrity.

To prepare the extracts, ten grams of the dried powder underwent a defatting process using petroleum ether. After this step, extraction was performed using methanol in a Soxhlet apparatus. This method allowed for efficient extraction of the desired compounds from the plant material. Once the extraction was completed, the solvent was evaporated until fully dry, resulting in a concentrated crude extract. This process ensured that all solvent residues were removed, leaving behind the desired compounds. This extract was then stored in an airtight bottle at a temperature of 4°C to preserve its properties.

The hydroalcoholic extracts obtained from *Cordia myxa* and *Canscora diffusa* had a yield ratio of 60:40. These hydroalcoholic extracts were utilized throughout the entire study, serving as the primary materials for further analysis and experimentation.

Preparation of Hydroalcoholic Extracts

The powdered hydroalcoholic extracts of *Cordia myxa* and *Canscora diffusa* leaves were subjected to solvent extraction using appropriate methods.

Preparation of Extract (Hydroalcohol Methanol and Water 60:40)

Approximately 500 grams of powdered material were extracted using the maceration method, employing about 5.0 liters of purified water. This extraction process lasted for seven days. After this period, the liquid extract, known as the menstruum, was separated from the solid material by filtering through a muslin cloth.

The residual solid, or marc, was washed with approximately 500 milliliters of fresh solvent to ensure maximum extraction. This wash was also filtered through muslin cloth and combined with the initial menstruum. Subsequently, the solvent was removed using a rotary flash evaporator set at approximately 45ºC ± 5ºC. This controlled temperature facilitated efficient solvent removal while preserving the integrity of the extract. The resulting extract was then concentrated further until a dry residue was obtained, which was stored in a desiccator over anhydrous sodium sulfate to prevent moisture absorption.

The percentage yield of the extract was determined based on the weight of the air-dried powdered material. This calculation provided insight into the efficiency of the extraction process. Additionally, the extract was analyzed for its color, consistency, and overall yield, providing valuable insights into its properties and potential applications [11].

Phytochemical Analysis

Qualitative Phytochemical Analysis

Preliminary chemical tests were carried out for hydroalcoholic extracts of *Cordia Myxa* and *Canscora diffusa* to identify different phytoconstituents, such as alkaloids, flavonoids, proteins, amino acids, terpenoids, and glycosides [12, 13].

Acute Toxicity Studies

Acute toxicity studies for the two polyherbal preparations were conducted according to the OECD 423 guidelines.

Principle of the Test

Groups of animals of the same sex were given fixed doses in a sequential manner. This approach ensured consistent administration across the test subjects. The doses used were 5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg, with the possibility of considering an additional fixed dose of 5000 mg/kg. The initial dose level was selected based on findings from a preliminary sighting study. This ensured that the dosing was informed by prior observations. This initial dose is anticipated to induce some signs of toxicity while preventing severe adverse effects or mortality.

Clinical signs and conditions related to pain, suffering, and the risk of death are thoroughly outlined in a separate OECD Guidance Document based on the observed signs of toxicity or mortality. Additional groups of animals may receive adjusted fixed doses. This approach allows for the assessment of varying dose levels in response to the initial findings. This process continues until a dose is found that either causes observable toxicity or results in no more than one death. This helps identify the threshold at which adverse effects occur. The study will determine the threshold at which effects are observed, or when no adverse effects are seen at the highest dose, or if mortality occurs at the lowest dose tested.

Selection of Animals

This study utilized Swiss Albino mice weighing between 20 and 25 grams. Additionally, Albino Wistar rats, weighing 150 to 250 grams and of either sex, aged four months, were also included in the research. The animals were housed in polypropylene cages and kept under standard environmental conditions, including a 12-hour light/dark cycle, with the temperature maintained at 25 ± 3ºC and humidity levels between 35–60%. The animals were given free access to standard pelletized feed and tap water throughout the study, ensuring their nutritional needs were met.

After a period of five days, the chosen animals were separated into four distinct groups, each consisting of three animals. Each group was injected with a specific fixed single dose of a polyherbal preparation. Following the administration of the dose, the animals were carefully observed on an individual basis. They were monitored at least once within the first 30 minutes following the injection. This observation period allowed for the assessment of any immediate reactions. Observations continued periodically throughout the initial 24 hours, with particular focus on the critical first 4 hours.

Following the initial observation period, the animals were examined daily for a duration of 14 days. This monitoring was essential to identify any potential toxic effects or signs of mortality resulting from the polyherbal preparation. Such a thorough approach ensured that any adverse reactions were documented and assessed effectively.

Evaluation of the Hepatoprotective Activity

CCl_4 Induced Hepatotoxicity in Rats

Rats were randomly assigned to six different groups for the study:

- *Group 1:* This constituted the normal control group. The rats in this group received 1% gum acacia for 7 days without any additional treatments.
- *Group 2:* The control group received 1% gum acacia for 6 days. On the 7th day, they were given a single dose of CCl4 at 1.5 ml/kg in a 1:1 mixture with groundnut oil via intraperitoneal injection.
- *Group 3:* The standard control group received oral treatment with silymarin at a dosage of 50 mg/kg/day for 7 days. On the 7th day, 30 minutes after administering silymarin, a single dose of CCl_4 (1.5 ml in a 1:1 mixture of groundnut oil) was given.

- *Group 4 (Low dose):* This group was treated daily with polyherbal formulation (PHF) at a low dose of 100 mg/kg, orally, for 7 days. On the 7th day, 30 minutes after the PHF administration, the rats received a single dose of CCl4 (1.5 ml/kg in a 1:1 mixture of groundnut oil).
- *Group 5 (Medium dose):* In this group, rats were given PHF-I at a medium dose of 200 mg/kg daily for 7 days. On the 7th day, 30 minutes after the PHF-I administration, they were injected with CCl4 (1.5 ml/kg in groundnut oil).
- *Group 6 (High dose):* The high dose group received PHF-I at 400 mg/kg daily for 7 days. On the 7th day, 30 minutes after administering PHF-I, a single dose of CCl_4 (1.5 ml/kg in groundnut oil) was administered.

All groups receiving CCl_4 were dosed on the 7th day to evaluate the protective effects of the different treatments against CCl_4-induced toxicity.

At the conclusion of the experimental period, the rats were fasted overnight to prepare for the procedure. They were then sacrificed through decapitation. Blood samples were collected right after the procedure and were permitted to clot for 30 to 40 minutes. After clotting, serum was separated through centrifugation at 37°C. This serum was subsequently used to estimate various biochemical parameters [14].

Subsequently, the liver tissue was carefully extracted and rinsed with phosphate-buffered saline (0.05 M, pH 7.4) to remove any residual blood or impurities. The liver was then minced into small fragments to facilitate further processing. These fragments were homogenized in ice-cold phosphate-buffered saline (0.05 M, pH 7.4) using a tissue homogenizer. This process resulted in a 1:9 (w/v) dilution, creating a 10% whole homogenate of liver tissue for subsequent analysis.

A portion of the homogenate was mixed with an equal volume of 10% trichloroacetic acid (TCA) to enable the estimation of malondialdehyde levels. This combination facilitated the analysis of lipid peroxidation. The mixture was then subjected to centrifugation using a Remi Cool centrifuge at a speed of 8000 rpm for 30 minutes. Following centrifugation, the supernatant was carefully separated from the pellet. This process ensured the isolation of the liquid portion for further analysis. This supernatant was utilized to assess the antioxidant levels of various enzymes, including catalase, glutathione, and reduced glutathione, across all tissue samples. This method facilitated a detailed evaluation of the antioxidant capacity in the tissues. It provided valuable insights into the tissues' ability to combat oxidative stress.

Paracetamol Induced Hepatotoxicity in Rats

Male rats were divided into six groups, with each group consisting of six rats.

Group I was administered 0.1 ml of 2% gum acacia daily for seven days.

Group II acted as the disease control group. In this group, hepatotoxicity was induced by administering a single oral dose of paracetamol at 3 g/kg.

Group III served as a standard and received N-acetyl cysteine (450 mg/kg b.wt, p.o) for seven days, once daily.

Groups IV, V, and VI received low-dose (100 mg/kg), medial dose (200 mg/kg), and high dose (400 mg/kg) of polyherbal formulation once daily for seven days, p.o., respectively.

All animals in Groups III–VI received a single dose of paracetamol (3 g/kg, p.o) one hour before the test dose on the first day. The dosing was administered to all animals at the same time for seven days.

After 24 h of the last dose, that is, on the 8th day, all animals were subjected to ether anesthesia. Blood was collected through an orbital puncture. The samples were then allowed to clot for 60 minutes at room temperature. Serum was separated by centrifugation at 4000 rpm for 15 min and used for the estimation of serum parameters like SGPT, SGOT, serum bilirubin and serum albumin the animals were sacrificed with light ether and liver tissue was separated, and washed with phosphate buffer saline (0.05M, ph7.4). Liver tissue was later removed, minced into small pieces, and homogenized. The homogenate was centrifuged in a cooled centrifuge at 8000 rpm for 30 minutes. This process facilitated the separation of the components within the sample. The supernatant was separated and utilized to measure the antioxidant levels of various enzymes, including catalase, glutathione, and reduced glutathione, in all the tissues. This analysis provided insights into the antioxidant capacity present in the samples [15].

Bio Chemical Estimation

Analytical methods used in this study: In the present study, the following serum biochemical parameters were selected to assess liver function in rats. The following parameters were evaluated. Assay of alanine aminotransferase (SGPT) or (ALT), assay of asparate aminotransferase, assay of serum alkaline phosphatase, total protein estimation, creatinine, alkaline phosphates (alp), catalase [16].

RESULTS AND DISCUSSION

Yield of Plant Extracts

The total yield of the plant extracts varied depending on the solvents used in this study. The alcoholic extracts of *Cordia myxa* and *Canscora diffusa* produced higher yield (8.67 and 9.11 mg/g). Generally, the extraction efficiency of a specific component depends on the polarity of the extraction medium. Additionally, it is influenced by the ratio of solute to solvent (Table 1).

Table 1. Yield of plant extracts.

Solvent Extracts	***Cordia myxa (g)***	***Canscora diffusa (g)***
Petroleum ether extract	2.66	2.9
Ethyl acetate extract	4.6	4.13
Hydroalcoholic extract	8.76	9.11

Phytochemical Screening of Plant Extracts

Phytochemical screening of the extracts from *Cordia myxa* and *Canscora diffusa* indicated the presence of several bioactive compounds. The analysis revealed that these extracts contained carbohydrates, proteins, and amino acids. Additionally, alkaloids, glycosides, and phenolics/tannins were identified. Flavonoids, saponins, fixed oils/fats, and steroids were also present in the extracts. This diverse array of phytochemicals suggests that both plants possess significant potential for various therapeutic applications (Table 2).

Table 2. Preliminary phytochemical screening of *Cordia myxa*.

S.N.	**Phytoconstituents**	**Test Name**	**Hydroalcoholic Extract**
1	Alkaloids	Wagner's test	–(ve)
2	Carbohydrates	Fehling's test	+(ve)
3	Flavonoids	Lead acetate	+(ve)
		Alkaline reagent test	+(ve)
4	Proteins & amino acids	Precipitation test	+(ve)
5	Phenols	Ferric chloride test	+(ve)
6	Diterpenes	Copper acetate test	+(ve)
7	Saponins	Foam test	+(ve)

Table 3. Preliminary phytochemical screening of *Canscora diffusa*.

S.N.	Phytoconstituents	Test Name	Hydroalcoholic Extract
1	Alkaloids	Wagner's test	–(ve)
2	Carbohydrates	Fehling's test	+(ve)
3	Flavonoids	Lead acetate	+(ve)
		Alkaline reagent test	+(ve)
4	Proteins & amino acids	Precipitation test	+(ve)
5	Phenols	Ferric chloride test	+(ve)
6	Diterpenes	Copper acetate test	+(ve)
7	Saponins	Foam test	+(ve)

The results of the phytochemical tests demonstrated that the extracts of *Cordia myxa* and *Canscora diffusa* contain a variety of bioactive compounds. Specifically, the analysis indicated the presence of carbohydrates, flavonoids, proteins and amino acids, phenols, diterpenes, saponins, and alkaloids in these extracts. Furthermore, the phytochemical analysis revealed that all polar compounds, as well as those soluble in methanol and aqueous solutions, were found in the extracts derived from the leaves of *Canscora diffusa*. This extensive profile of phytochemicals suggests that both plants may offer significant therapeutic potential (Table 3).

Acute Toxicity Studies

(a) Acute toxicity studies were carried out following the OECD Guideline No. 423, which provides a standardized approach for assessing the toxicity of substances.

(b) During these acute toxicity studies, no instances of mortality were observed at a dose level of 2,000 mg/kg. As a result, doses corresponding to one-fifth, one-tenth, and one-twentieth of the 2,000 mg/kg dose – specifically, 100 mg/kg, 200 mg/kg, and 400 mg/kg – were established as the effective doses (ED50 values) for evaluating the herbal formulation. This systematic approach helps in determining the safety and efficacy of the herbal preparation.

Evaluation of Poly Herbal Formulation Against CCL_4 Induced Hepatotoxicity

Body Weights and Organ Weights

The body and liver weights of rats that received CCl_4 treatment (Group II) were significantly lower ($p < 0.05$) compared to those in the normal control group (Group I). However, treatment with the polyherbal formulation at three different dosage levels significantly ($p < 0.05$) enhanced both body weight and liver weights, bringing them close to normal levels. This indicates that the polyherbal formulation has a protective effect against CCl_4-induced damage, promoting recovery in the treated rats (Figure 1).

Table 4. Effect of poly herbal formulation on body weight and organ weight in CCl_4 intoxicated rats.

Groups	Treatment	Initial Body wt (Gms)	Final body wt (Gms)	Liver wt (Gms)
Group-I	Control treated with 1% gum acacia	200 ± 5.2	199 ± 7.73	6.94 ± 1.05
Group-II	CCl_4 (3 mg/kg)	206 ± 3.97^	151 ± 8.50^	3.56 ± 2.36^
Group-III	Silymarin (25 mg/kg) + CCl_4 (3 mg/kg)	204.5 ± 7.68*	198 ± 4.49*	6.34 ± 1.3
Group-IV	PHF (100 mg/kg) + CCl_4 (3 mg/kg)	203 ± 3.30 *	162 ± 3.90**	5.84 ± 1.34*
Group-V	PHF (200 mg/kg) + CCl_4 (3 mg/kg)	208 ± 7.04 *	172.56 ± 8.07**	5.81 ± 2.7*
Group-VI	PHF (400 mg/kg + CCl_4 (3 mg/kg)	205 ± 2.32*	182 ± 2.81**	6.18 ± 1.25*

*Notes: Values are presented as mean ± SEM, and the data were analyzed using one-way analysis of variance (ANOVA). This was followed by Dunnett's multiple comparison test for further evaluation. **$p < 0.01$ and *$p < 0.05$ as compared with CCl_4 group; ^$p < 0.05$ as compared with control group (Table 4).*

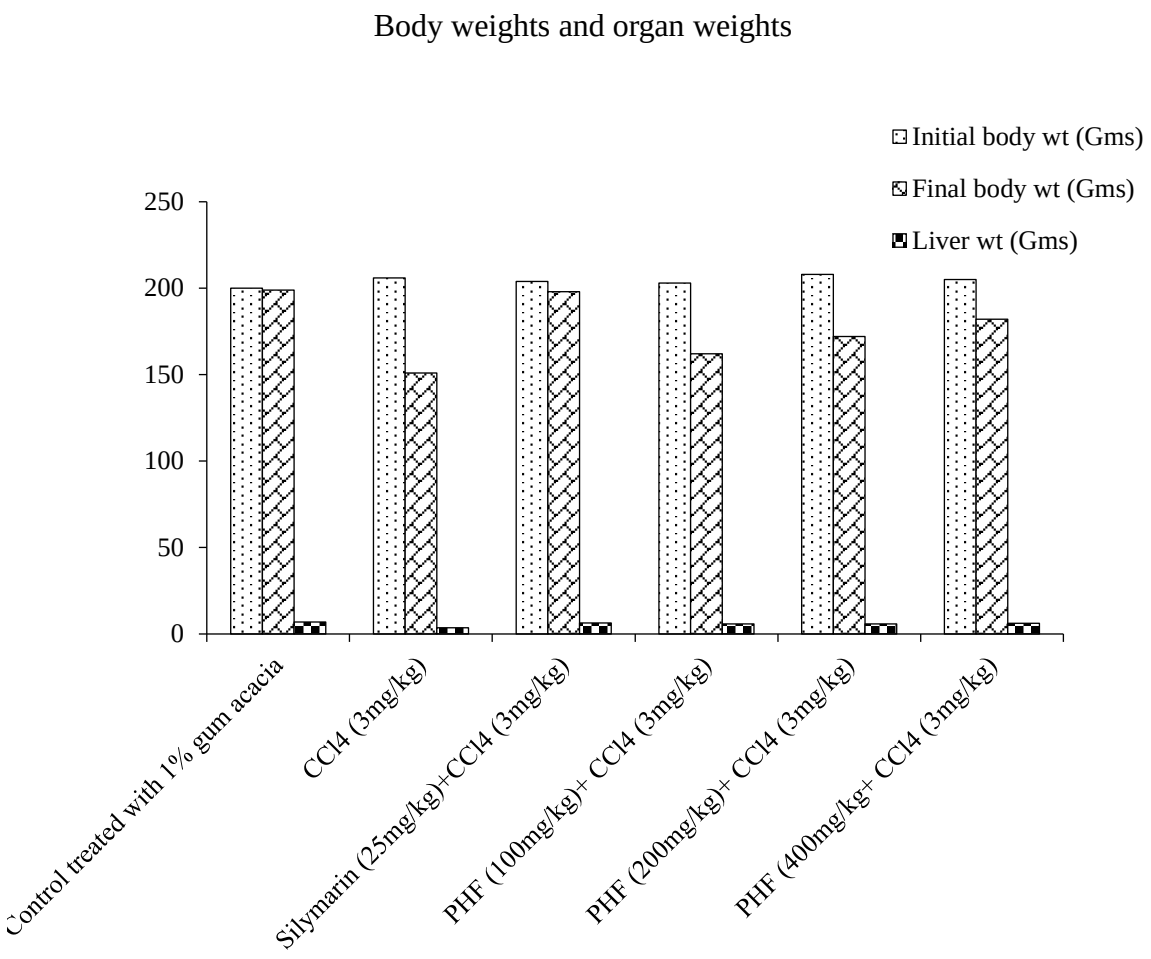

Figure 1. Effect of polyherbal formulation on organ and body weight in CCl_4 intoxicated rats.

Hematological Parameters

The hematological parameters in the toxin control group showed significant alterations when compared to those in the normal control group. Specifically, the leukocyte (WBC) count was notably increased, while the red blood cell (RBC) count and hemoglobin levels were significantly decreased in the toxin control animals. These changes indicate that CCl_4 exposure adversely affects blood parameters, reflecting potential hematological disturbances caused by the toxin (Table 5).

Treatment with the polyherbal formulation led to a significant increase in hemoglobin (Hb) levels and red blood cell (RBC) counts. In contrast, in animals intoxicated with CCl_4, the administration of the polyherbal formulation effectively prevented hemolysis and significantly elevated both Hb content and RBC counts. Additionally, the white blood cell (WBC) count was also restored to levels closer to normal when compared to the toxin control group. These findings suggest that the polyherbal formulation has a beneficial effect on hematological parameters in CCl_4-induced toxicity (Figure 2).

The values are reported as mean ± SEM. For data analysis, one-way ANOVA was employed to assess the differences among groups. This was followed by Dunnett's multiple comparison test to identify specific group differences. Statistical significance is denoted as $^{**}p < 0.01$ and $^{*}p < 0.05$ when compared with the CCl_4 group. Additionally, $^{\wedge}p < 0.05$ indicates significance in comparison to the control group. These values reflect the reliability of the findings and highlight the significance of the observed differences between the treatment groups (Table 6).

Table 5. Effect of polyherbal formulation on hematological parameters in CCl_4 intoxicated rats.

Groups	Treatment	Hb (g%)	RBC (million cells/Cmm)	WBC (thousands/Cmm)
Group-I	Control treated with 1% gum acacia	11.11 ± 0.1	4.57 ± 0.71	11.47 ± 0.74
Group-II	CCl4 (3 mg/kg)	8.24 ± 0.1^	3.4 ± 0.6^	15.54 ± 0.19^
Group-III	Silymarin (25 mg/kg) + CCl_4 (3mg/kg)	9.32 ± 0.31*	3.22 ± 0.47*	12.99 ± 0.14*
Group-IV	PHF (100 mg/kg) + CCl_4 (3 mg/kg)	10.32 ± 0.14*	3.88 ± 0.97 *	13.14 ± 0.44 *
Group-V	PHF (200 mg/kg) + CCl_4 (3 mg/kg)	10.87 ± 0.11*	4.41 ± 0.08 *	13.77 ± 0.2 *
Group-VI	PHF (400 mg/kg + CCl_4 (3 mg/kg)	11.78 ± 1.11*	4.88 ± 1.77*	12.47 ± 2.2*

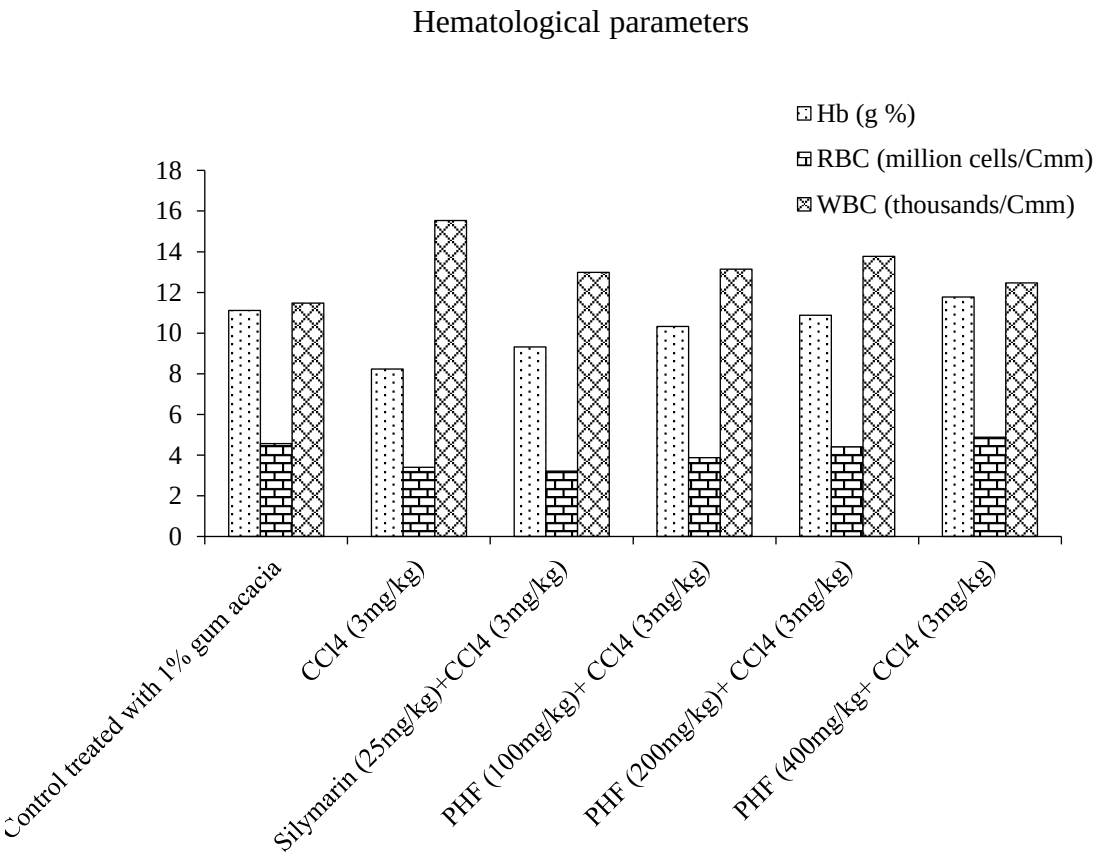

Figure 2. Illustrates the impact of the polyherbal formulation on hematological parameters in rats that have been intoxicated with CCl_4. The data presented in this figure demonstrate how the treatment influences various blood parameters, highlighting the potential protective effects of the polyherbal formulation against CCl_4-induced hematological alterations.

Serum Biochemical Parameters

The influence of the polyherbal formulation on liver enzymes, including SGPT (alanine aminotransferase), SGOT (aspartate aminotransferase), and ALP (alkaline phosphatase), was evaluated in rats exposed to liver damage caused by CCl_4. These enzymes serve as vital markers to assess liver health and the extent of injury (Figure 3).

The impact of the polyherbal formulation on total protein, bilirubin, and creatinine levels was assessed in rats that experienced liver damage due to CCl_4 exposure. These parameters are important indicators of liver and kidney function (Table 7).

Table 6. Effect of the polyherbal formulation on SGPT, SGOT, and ALP in CCl_4 intoxicated rats.

Groups	Treatment	SGPT IU/L	SGOT IU/L	ALP IU/L
Group-I	Control treated with 1% gum acacia	63.77 ± 6.5	100.01 ± 0.57	181 ± 0.66
Group-II	CCl_4 (3 mg/kg)	179 ± 4.81^	188.1 ± 0.6^	362.2 ± 0.33^
Group-III	Silymarin (25 mg/kg) + CCl_4 (3 mg/kg)	119 ± 8.88*	110 ± 0.47*	274 ± 0.24*
Group-IV	PHF (100 mg/kg) + CCl_4 (3 mg/kg)	170.5 ± 4.77 *	148 ± 0.55 *	314.2 ± 0.11 *
Group-V	PHF (200 mg/kg) + CCl_4 (3 mg/kg)	147 ± 6.57 *	128 ± 0.07 *	304.7 ± 0.4 *
Group-VI	PHF (400 mg/kg + CCl_4 (3 mg/kg)	141 ±5.55*	121 ± 0.78*	268.7 ± 2.7*

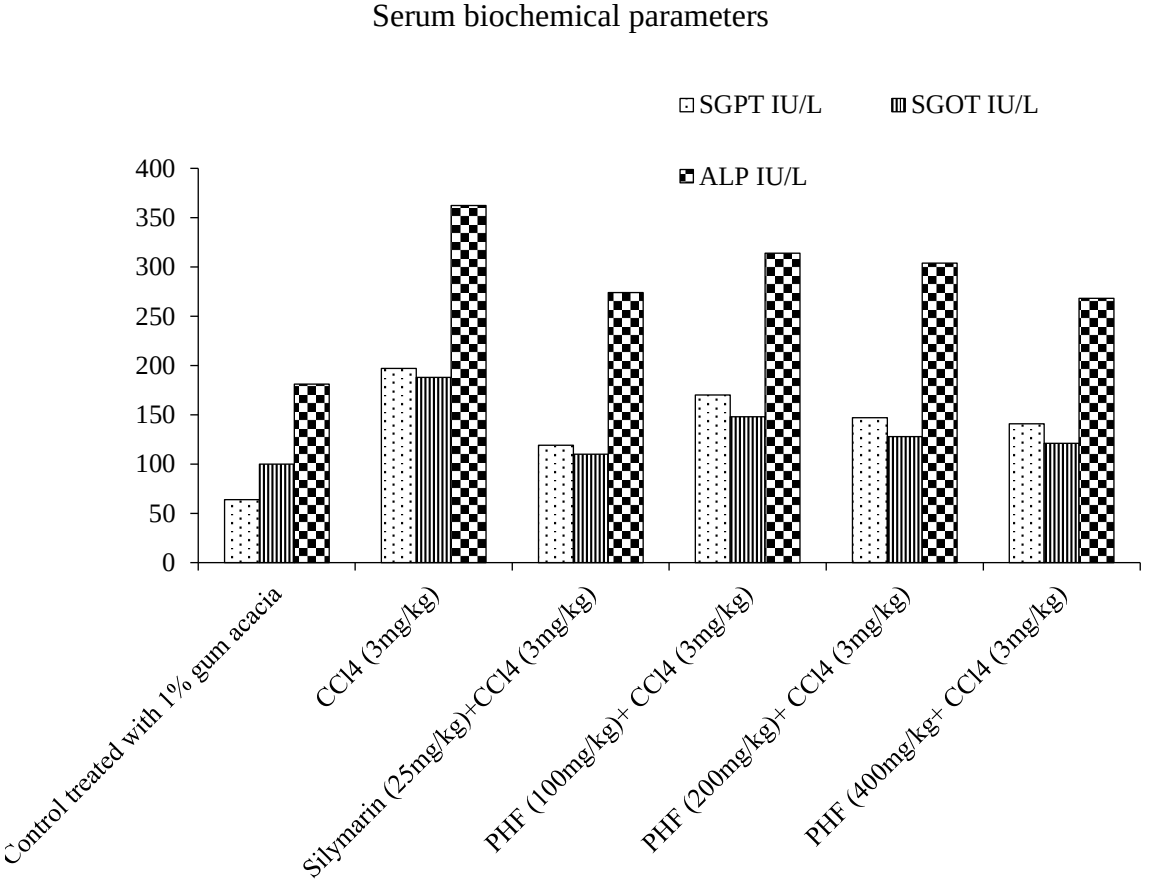

Figure 3. Serum biochemical parameters.

Table 7. Effect of polyherbal formulation on liver and kidney markers in CCl_4-induced toxicity.

Groups	Treatment	Total Protein (g/dl)	Bilirubin (mg/dl)	Creatinine (mg/dl)
Group-I	Control treated with 1% gum acacia	9.41 ± 21	1.36 ± 0.04	6.33 ± 0.04
Group-II	CCl_4 (3 mg/kg)	6.3 ± 0.44	3.66 ± 0.17	4.77 ± 0.17
Group-III	Silymarin (25 mg/kg) + CCl_4 (3 mg/kg)	8.11 ± 3.3*	1.8 ± 0.03	5.11 ± 0.23
Group-IV	PHF (100 mg/kg) + CCl_4 (3 mg/kg)	5.11 ± 0.02 *	1.90 ± 0.12	4.11 ± 0.11
Group-V	PHF (200 mg/kg) + CCl_4 (3 mg/kg)	7.44* ± 0.02 *	1.75 ± 0.12	6.01 ± 0.12
Group-VI	PHF (400 mg/kg + CCl_4 (3 mg/kg)	8.11 ± 4.1*	1.58 ± 0.11	6.91 ± 0.01

The biochemical markers in the serum, including SGOT, SGPT, ALP, ALT, AST, and bilirubin, showed a significant ($p < 0.05$) increase in the toxin control group compared to the normal control group. Additionally, the total protein levels were notably lower in the toxin control group than in the normal group ($p < 0.05$). When rats were treated with the polyherbal formulation before CCl_4 exposure,

it significantly ($p < 0.05$) prevented the drop in protein levels, leading to an increase in these levels in a dose-dependent manner. The highest dose of the formulation effectively counteracted the effects of CCl_4 intoxication, preserving plasma protein levels and providing protective benefits.

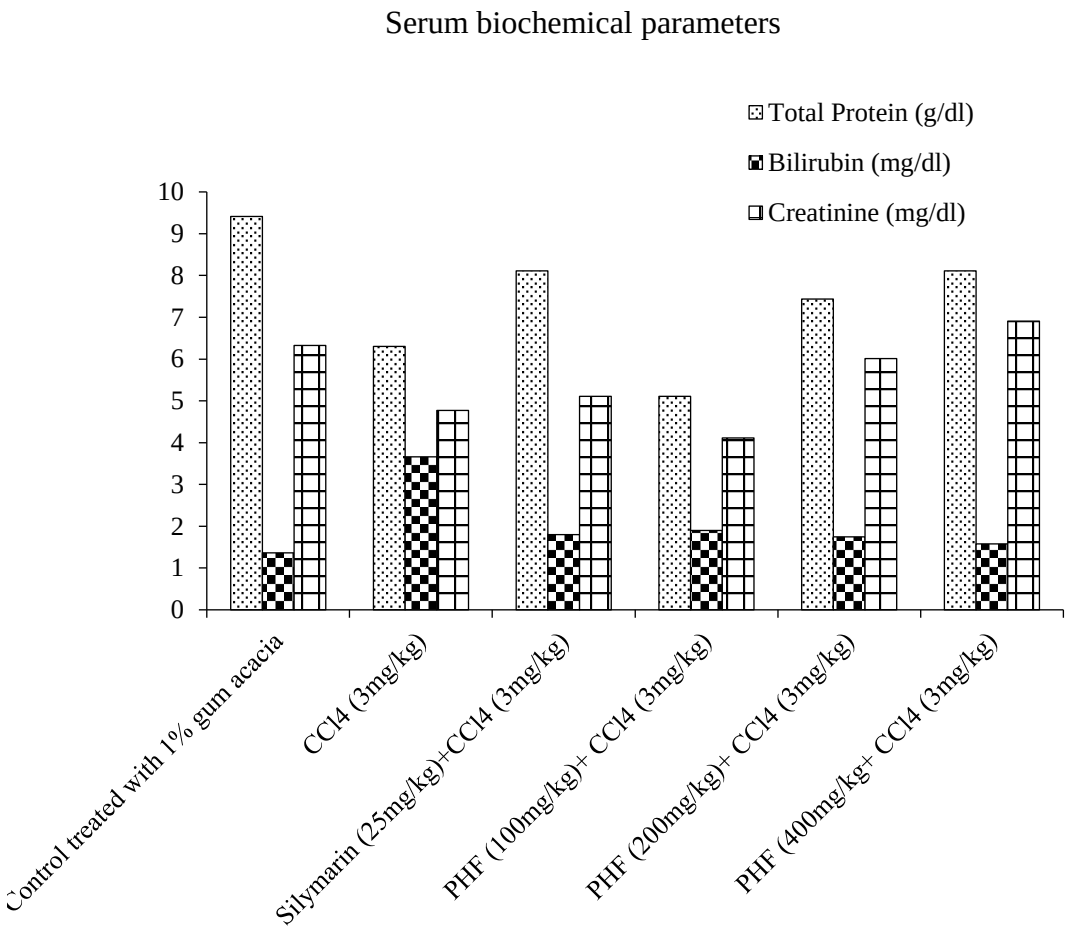

Figure 4. Illustrates the effect of the polyherbal formulation on serum bilirubin and creatinine levels in rats exposed to CCl_4 intoxication. The data shows how the administration of the polyherbal formulation influenced these parameters, helping to assess the protective effects against liver and kidney damage caused by CCl_4. Elevated bilirubin and creatinine levels, often markers of liver and kidney dysfunction, were significantly reduced with the polyherbal treatment, indicating its potential therapeutic benefits in counteracting the toxic effects of CCl_4.

The groups treated with polyherbal formulation against CCL_4 intoxication significantly ($p < 0.05$) reduced the rise in SGPT, SGOT and serum bilirubin levels in a dose dependent manner. Treatment with silymarin (25 mg/kg) also inhibited the increase in SGPT, SGOT, ALP, and serum bilirubin levels.

The results clearly indicated that the herbal formulation possessed hepatoprotective activity in a dose-dependent manner, with a high dose producing better activity. Among the formulations, this formulation produced the best activity (Figure 4).

Effect of Poly Herbal Formulation of Hepatic Antioxidant Parameters

In group I, the normal MDA, GR, catalase, and GSH levels were found to be 71.23 ± 0.22, 33.41 ± 0.87, 51.21 ± 0.11 and 42.73 ± 0.60, respectively. In group II treated with single dose CCL_4 the MDA and GR levels were raised to 138.11 ± 0.47 nm/g and 47.22 ± 0.21 μ/ml, respectively.

In group III treated with silymarin (25 mg/kg), MDA, GR, Catalase and GSH levels remained near normal levels and were significantly protected ($P < 0.05$). The groups (IV, V, and VI) treated with 100, 200 and 400 mg/kg doses showed dose-dependent protection against MDA, GR, catalase, and GSH against CCl_4 induced intoxication (Table 8).

Table 8. Effect of polyherbal formulation on MDA, GR, catalase, and GSH in CCl_4 Induced Hepatotoxicity in rats.

Groups	Treatment	MDA nm/g	GR u/ml	Catalase K/min	GSH Mcg/ml
Group-I	Control treated with 1% gum acacia	71.23 ± 0.22	33.41 ± 0.87	51.21 ± 0.11	42.73 ± 0.60
Group-II	CCl_4 (3 mg/kg)	138.11 ± 0.47	47.22 ± 0.21	40.94 ± 0.24	30.14 ± 0.22
Group-III	Silymarin (25 mg/kg) + CCl_4 (3mg/kg)	66.41 ± 0.71	27.74 ± 0.22	59.78 ± 0.54	37.61 ± 0.41
Group-IV	PHF (100 mg/kg) + CCl_4 (3mg/kg)	129.11 ± 0.44	36.22 ± 0.71	44.35 ± 0.22	29.31 ± 0.22
Group-V	PHF (200 mg/kg) + CCl_4 (3mg/kg)	105.55 ± 2.4	30.92 ± 0.55	56.85 ± 0.24	33.15 ± 0.41
Group-VI	PHF (400 mg/kg + CCl_4 (3mg/kg)	93.11 ± 0.47	23.55 ± 0.11	68.47 ± 0.47	39.15 ± 0.55

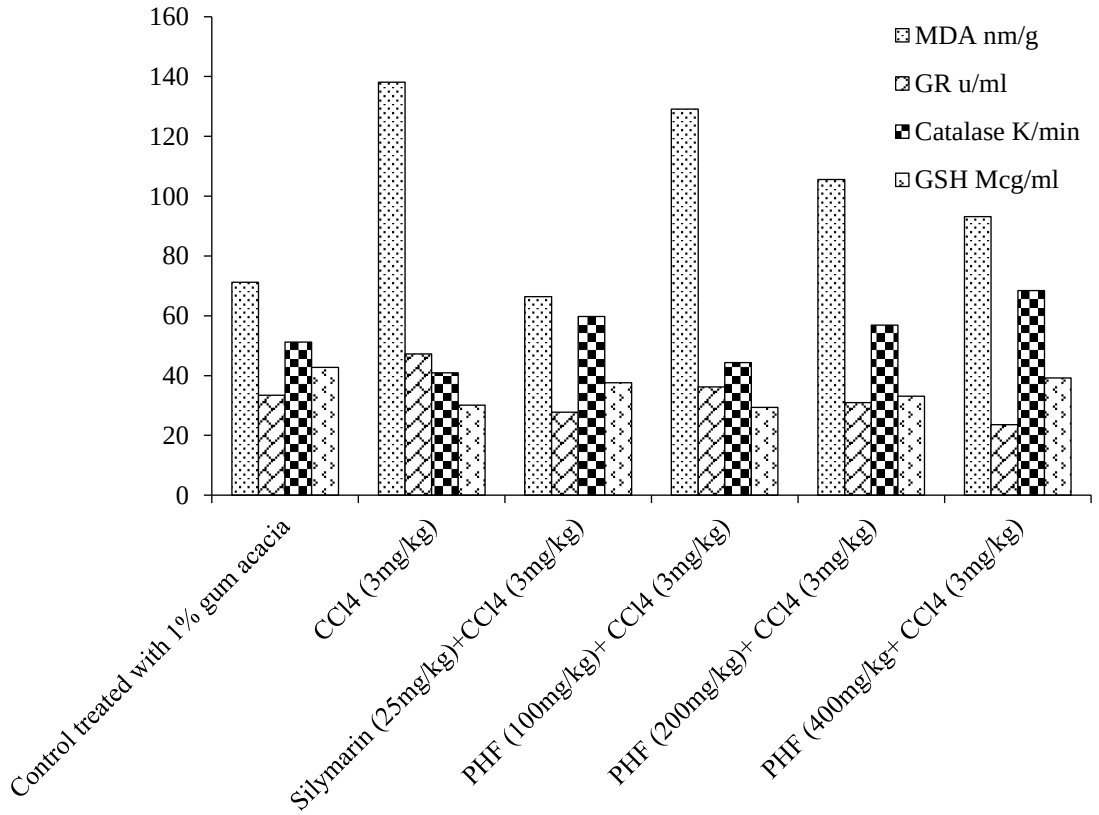

Figure 5. Effect of the polyherbal formulation on MDA, GR, catalase, and GSH in CCl_4 induced hepatotoxicity studies.

The results clearly indicated that the formulation possessing hepatoprotective activity showed better activity in a dose-dependent manner and was comparable to that of the standard drug silymarin (Figure 5).

Evaluation of Poly Herbal Formulation for Paracetamol Induced Hepatotoxicity

Effect of the polyherbal formulation on serum profile in paracetamol-induced hepatotoxicity in rats.

In control group I, the levels of SGOT, SGPT, ALP, total bilirubin, and total protein were measured at 14.6 ± 0.2, 19.4 ± 1.1, 20.4 ± 1.8, 1.23 ± 0.23, and 7.4 ± 0.11, respectively. In group II, which was treated with PCM, the levels of SGOT, SGPT, ALP, and total bilirubin increased, while the total protein

levels decreased (Table 9). This indicates a significant alteration in liver function due to the treatment (Figure 6).

Table 9. Effect of poly herbal formulation on serum profiles.

Groups	Treatment	SGOT (IU/dL)	SGPT (IU/dL)	ALP (IU/L)	Total Bilirubin (mg/dL)	Total Protein (g/dL)
Group-I	Control treated with 1% gum acacia	14.6 ± 0.2	19.4 ± 1.1	20.4 ± 1.8	1.23 ± 0.23	7.4 ± 0.11
Group-II	PCM (3 mg/kg)	21.4 ± 0.4	33.7 ± 0.1	32.4 ± 0.5	3.44 ± 0.63	4.9 ± 0.31
Group-III	Silymarin (25 mg/kg) + PCM (3 mg/kg)	15.7 ± 0.7	21.3 ± 0.7	20.1 ± 0.2	1.3 ± 0.55	6.41 ± 0.47
Group-IV	PHF (100 mg/kg) + PCM (3 mg/kg)	17.4 ± 0.4	25.3 ± 0.4	32.4 ± 0.9	2.87 ± 0.61	5.7 ± 0.43
Group-V	PHF (200 mg/kg) + PCM (3 mg/kg)	16.5 ± 0.7	21.4 ± 0.4	26.5 ± 0.7	1.75 ± 0.22	7.8 ± 0.78
Group-VI	PHF (400 mg/kg + PCM (3 mg/kg)	14.9 ± 0.8	18.7 ± 0.2	5.9 ± 0.5	1.11 ± 0.51	8.6 ± 0.55

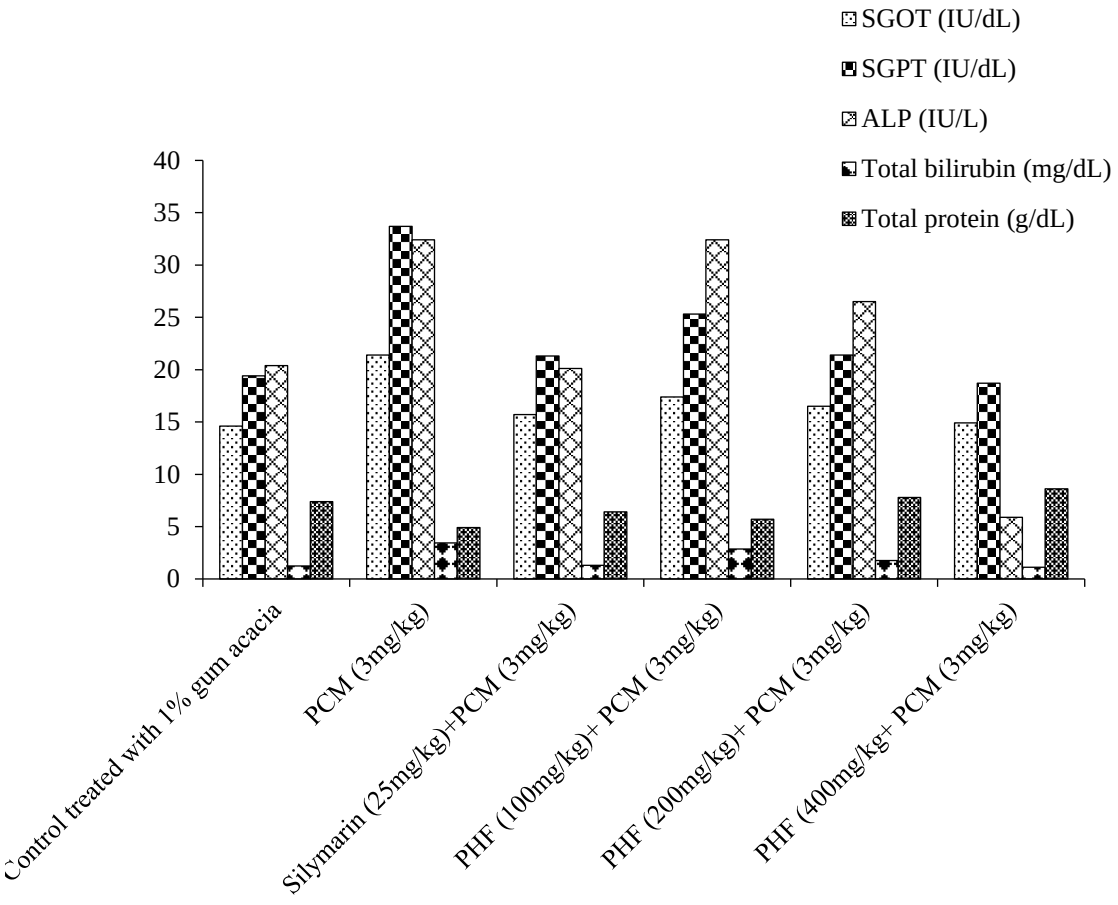

Figure 6. Effect of polyherbal formulation on serum profile.

CONCLUSION

According to the results of this experiment, the toxicity and side effects of peracetamol and CCl_4-induced effects were greatly reduced by polyherbal formulations at doses of 100, 200, and 400 mg/kg.

Carbon tetrachloride produces hepatotoxicity, which is a selective toxicity that affects liver cells and impairs their ability to metabolize substances. Finally, the hepatoprotective effect of the prepared formulation and extracted material was demonstrated by assessing several biochemical parameters in the chosen animals.

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