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# Pharmacological investigation of Polyherbal formulation on Carbon tetrachloride ($CCl_4$)-induced liver damage in wistar rats

**Hardik Soni**[1*], Priyanka Desai[2], Natvarlal Patel[2] and Ghanshyam Patel[1]

[1]*Vasu Research Centre, A Division of Vasu Healthcare Pvt. Ltd., 896/A, G.I.D.C., Makarpura, Vadodara – 390 010, Gujarat, India.*
[2]*Department of Pharmacognosy and Phytochemistry, Shri B. M. Shah College of Pharmaceutical Education and Research, Dhansura road, College campus, Modasa – 383 315, Gujarat, India.*

## Corresponding author*:

Hardik Soni
Asst. Manager, R&D
Vasu Research Centre (A Division of Vasu Healthcare Pvt. Ltd.)
896/A, G.I.D.C., Makarpura, Vadodara- 390010, Gujarat, India.
Tel.: 91-265-2657701, 2657702, Fax: 91-265-2647331
E-mail: hsoni@vasuresearch.com

## Abstract

**Objective:** To investigate effect of Polyherbal formulation on Carbon tetrachloride ($CCl_4$)-induced liver damage in wistar rats.

**Methods:** Wistar albino rats weighing 180-230 g either sex were used. The selected animals were divided in to four groups where each group consisted of six animals. Experimentally liver damage was produced by intra-peritoneal administration of $CCl_4$ and olive oil mixture (1:1 v/v) (1 mL/kg, once daily, i.p.) for 7 days. Test Drug, Polyherbal formulation was administered orally for 7 consecutive days at 3 mL/kg, once daily. On 8$^{th}$ day, Blood samples were collected to evaluate different serum biochemical parameters like Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Total bilirubin and Total protein. Liver from animals of each group was dissected out for histopathological examination.

**Statistical analysis:** Statistical calculation were done by analysis of variance (ANOVA) followed by *post hoc* Dunnett's test, with significant level of $p<0.05$.

**Results and Discussion:** Polyherbal formulation showed significant effect on activity levels of serum AST, ALT, ALP and total bilirubin level while comparing test group to disease control group. It also showed significant elevation in decreased level of serum total protein. Pre-treatment of Polyherbal formulation restored the hepatic architecture and protected the liver tissue from fatty degenerative changes by preventing the toxic chemical reaction induced by $CCl_4$.

**Conclusion:** Finding of this study concludes that Polyherbal formulation (Vasuliv Syrup) has promising hepatoprotective activity against $CCl_4$-induced liver damage. It can be employed as safe and effective treatment for hepato-toxicity or liver damage.

**Keywords:** Polyherbal formulation, $CCl_4$-induced liver damage, Hepatotoxicity, Hepato-protective

## 1. Introduction

Liver is a versatile organ of the body that regulates body's highly complex internal chemical environment. It plays a major role in detoxification and excretion of many endogenous and exogenous toxic compounds. Even little impairment to its function may lead to serious implications and even death. Management of liver diseases is still a challenge to the modern scientific community[1,2]. Long term use of such conventional drugs is associated with known side effects like gastrointestinal disturbances, allergic skin rashes and nausea[3].

In recent years, the usage of herbal drugs for the treatment of liver diseases has increased all over the world [4]. The herbal drugs are believed to be safe and free from serious adverse reactions, as they are obtained from nature. Also, the limited therapeutic success of modern medicine has steered the increase in the usage of alternative medicine including herbal preparations [5].

Polyherbal formulation used in this study, is an Ayurvedic proprietary formulation which contains extract of *Glycyrrhiza glabra* (Yashtimadhu) root [6], *Phyllanthus niruri* (Bhumyamalaki) whole plant [7], *Boerhaavia diffusa* (Punarnava) root [8], *Eclipta alba* (Bhringraj) whole plant [9], *Terminalia belerica* (Bibhitaki) fruit [10], *Terminalia chebula* (Haritaki) fruit [11], *Picrorhiza kurroa* (Kutaki) root [12], *Tephrosia purpurea* (Sharpunkh) whole plant [13], *Cichorium intybus* (Kasni) seed [14] and *Andrographis paniculata* (Kalmegh) aerial [15]. It has already been proven as standardized Ayurvedic Polyherbal formulation [16]. It is manufactured and marketed by Vasu Healthcare Pvt. Ltd., Vadodara with the name "Vasuliv Syrup". Ingredients of Polyherbal formulation are well reported in Ayurvedic literature and scientific research publications as liver functions stimulant, hepato-protective and anti-oxidant. However, no such evidence was found which proves the

efficacy of their combination.

In the present study, an attempt was made to investigate effect of this Polyherbal formulation on Carbon tetrachloride (CCl4)-induced liver damage in wistar rats.

## 2. Materials and methods

### 2.1 Test drug and experimental dose

Polyherbal formulation (Brand Name: Vasuliv Syrup) was received from Vasu Healthcare Pvt. Ltd., Vadodara, Gujarat and used for evaluation of acute toxicity study and hepato-protective activity. For acute toxicity study 2000mg/kg and 5000mg/kg single dose was administered orally. For hepato-protective activity, dose of the test drug was fixed by extrapolating the human dose to laboratory animals, based on body surface area ratio as per the table of Paget and Barnes [17]. Test drug was administered at 3 mL/kg/day (p.o). Silymarin was used as a standard drug and was administered at 25 mg/kg/day (p.o).

### 2.2 Experimental animals

Healthy Wistar albino rats, weighing 180-230 g of either sex were used for the acute toxicity study and hepato-protective activity. The animals were housed in a three rats per polypropylene cages, maintained under controlled temperature (22±2°C) and humidity (55±5%) with 12:12 h light and dark cycle. Animals had free access to standard pellet diet and purified drinking water *ad libitium*. All protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) of Shri B. M. Shah College of Pharmaceutical Education and Research, Modasa, Gujarat, India (IAEC Protocol No.: IAEC/BMCPER/04/2010-11).

### 2.3 Acute toxicity study

Healthy Wistar albino rats (180 - 230 g) were divided into 2 groups of 3 animals each. The animals had free access to water and food throughout the experiment, except for the fasting period before the oral administration of the single dose of the Polyherbal formulation. The Polyherbal formulation was administered as it is by gavages (orally) at single dose of 2000 mg/kg to 1$^{st}$ group and single dose of 5000 mg/kg to 2$^{nd}$ group. The general behavior and mortality of the rats was continuously monitored for 1 h after dosing periodically during first 24 h (with special attention given during the first 4 h.) and then daily for a total of 14 days. Changes in the normal activity of rats, sign and symptoms of toxicity and mortality were monitored and recorded. Acute toxicity study was carried out as per OECD Guidelines 423 [18].

### 2.4 Experimental design

The selected animals were divided into four groups where each group consisted of six animals.

Group-I (NC): Served as normal control and received vehicle

Group-II (DC): Served as disease control received $CCl_4$ and olive oil mixture (1:1 v/v) at the dosage of 1 mL/kg, once daily, i.p.

Group-III (TD): Served as test drug treated group and received Polyherbal syrup formulation (3 mL/kg, once daily, p.o.) + $CCl_4$ and olive oil mixture (1:1 v/v) (1 mL/kg, once daily, i.p.)

Group-IV (SD): Served as standard drug treated group and received Silymarin (25 mg/kg, once daily, p.o.) + $CCl_4$ and olive oil mixture (1:1 v/v) (1 mL/kg, once daily, i.p.)

#### 2.4.1 $CCl_4$-induced liver damage in wistar rats

Experimentally liver damage was produced by intra-peritoneal administration of $CCl_4$ and olive oil mixture (1:1 v/v) (1 mL/kg, once daily, i.p.) for 7 days [19]. Test Drug was administered orally for 7 consecutive days 1 h prior to administration of $CCl_4$. On 8$^{th}$ day, the rats were anesthetized and sacrificed. Blood samples were collected to evaluate different bio-chemical parameters. Livers of different group were dissected out and preserved in 10% formalin solution for histo-pathological examination.

#### 2.4.2 Estimation of serum biochemical parameters

The activity levels of hepato-specific marker enzymes viz, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) in serum were estimated by the method of Reitman and Franke [20], and the activity level of Alkaline phosphatase (ALP) in serum was estimated by the method of King [21]. The level of total bilirubin in serum was estimated by the method of Dangerfield and Finlayson [22]. Serum total protein was estimated using Biuret method[23].

#### 2.4.3 Histopathology of liver

Under anesthesia, Liver from animals of each group was removed after sacrificing. They were preserved group wise in bottle containing 10% formalin solution and immediately processed by paraffin technique. Section of approximately 5µm thickness was cut and stained by hematoxylin and eosin (H&E). Sections were examined under microscope to evaluate structural changes.

### 2.5 Statistical analysis

Analysis was done with the help of standard statistical software, Graph pad prism version 5. Results were expressed as Mean ± Standard Error of Mean (SEM). Different groups were compared with analysis of variance (ANOVA) followed by *post hoc* Dunnett's test. A $p<0.05$ was considered as statistically significant.

## 3. Results

### 3.1 Acute toxicity study

The animals were observed for mortality and other toxic symptoms for 14 days of observation period. No toxic symptoms and mortality were found in both the dose level during this study.

### 3.2 Effect of Polyherbal formulation on serum biochemical parameters

The activity levels of serum AST, ALT and ALP were taken as indices for hepato-toxicity induced by $CCl_4$. The disease control group showed significant increases in AST, ALT, ALP activities and total bilirubin level when compared to the normal control group (Table 1). Treatment of Polyherbal formulation showed significantly decreases in AST, ALT, ALP activities and total bilirubin level when compared to disease control group. Level of serum total protein in disease control group was significantly decreased due to toxicant ($CCl_4$) treatment when compared to normal control. Treatment of Polyherbal formulation showed significant elevation in decreased level of serum total protein. Standard drug treated group showed significant alteration in all serum biochemical parameters as anticipated (Table 1).

**Table 1: Effect of Polyherbal formulation on serum biochemical parameters**

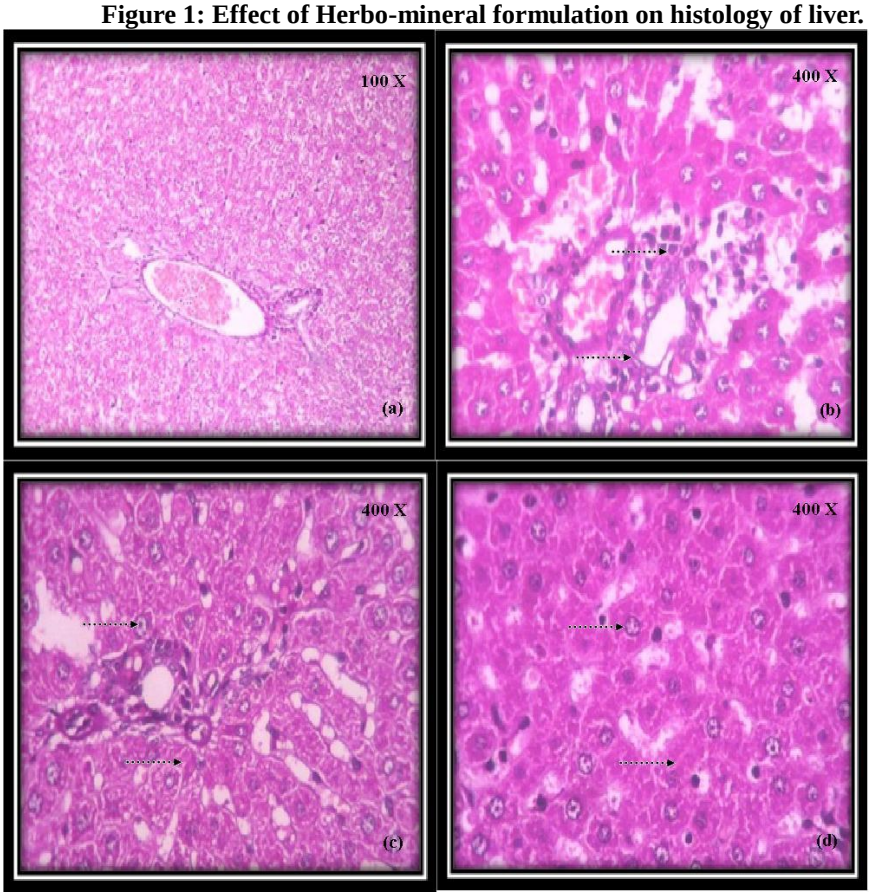
| Groups | AST (IU/L) | ALT (IU/L) | ALP (IU/L) | Total bilirubin (mg/dL) | Total protein (g/dL) |
|---|---|---|---|---|---|
| NC | 46.17±2.33 | 34.99±1.88 | 26.01±1.12 | 1.22±0.05 | 7.83±0.42 |
| DC | 132.29±1.80$^{\#\#\#}$ | 119.47±2.14$^{\#\#\#}$ | 112.29±1.26$^{\#\#\#}$ | 2.30±0.12$^{\#\#}$ | 3.34±0.12$^{\#\#\#}$ |
| TD | 53.96±2.19$^{**}$ | 42.28±0.82$^{***}$ | 44.57±1.13$^{***}$ | 1.38±0.13$^{**}$ | 6.41±0.37$^{**}$ |
| SD | 42.58±3.86$^{***}$ | 36.97±1.66$^{***}$ | 42.58±2.10$^{***}$ | 1.20±0.04$^{**}$ | 7.57±0.29$^{***}$ |

All the values are expressed as mean ± SEM (n=6). $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ when compared to normal control (NC) group. $^{**}p< 0.01$, $^{***}p< 0.001$when compared to disease control (DC) group.

### 3.3 Histopathological findings

The photomicrograph of the liver section of normal control (NC) shows normal histological architecture having hepatic cells with well-preserved cytoplasm and prominent nucleus (Figure 1-a) as compared to disease control (DC) showing distortion in the arrangement of the hepatic cells with few perivascular vacuoles (Figure 1-b). Photomicrograph of the liver section of test drug treated (TD) group shows well preserved hepatocytes which are arranged like normal. Few Kupffer cells appear around the sinusoids (Figure 1-c). Photomicrograph of the liver section from standard drug treated (SD) group is showing normal histological architecture and well preserved hepatocytes and sinusoids (Figure 1-d).

**Figure 1: Effect of Herbo-mineral formulation on histology of liver.**




(a) Photomicrograph of the liver section of normal control (NC) showing normal histological architecture; (b) Photomicrograph of the liver section of disease control (DC) showing distortion in the arrangement of the hepatic cells with few perivascular vacuoles as indicate by arrow; (c) Photomicrograph of the liver section of Polyherbal formulation treated (TD) showing well preserved hepatocytes which are arranged normally. Few Kupffer cells appear around the sinusoids as indicate by arrow; (d) Photomicrograph of the liver section of standard drug treated (SD) showing normal histological architecture and well preserved hepatocytes and sinusoids as indicate by arrow.

## 4. Discussion

Measurement of enzyme levels in the body fluids is useful in identifying and quantifying of any disease state. An obvious sign of hepatic injury is leakage of cellular enzymes into serum. The enzymes AST and ALT are important cellular enzymes [24], which catalyze reversibly and bring the amino acid into the Krebs's cycle. The ALP can transfer phosphorous group / remove 5'-phosphate group from DNA and RNA. It also removes phosphate group from nucleotide and protein. This enzyme is most active at alkaline pH hence the name was obtained[25].

In the present study, elevated levels of serum AST, ALT and ALP in disease control animals showed the damage of liver tissue and changes in cell permeability that allow AST, ALT and ALP to leak into serum [26]. The decreased level of serum AST, ALT and ALP in test drug treated group showed that test drug was able to repair the hepatic injury or restoring the cellular permeability (Table 1).

Serum bilirubin is one of the most sensitive tests employed in the diagnosis of hepatic diseases. Bilirubin is the end product of "heme" catabolism. Damage to the liver is impairing its ability to excrete bilirubin or obstruct the excretory ducts of the liver [27]. Elevated level of total bilirubin in $CCl_4$ treated rats is due to $CCl_4$ toxicity. It is resulted in hyperbilirubinemia. The significant reduction in the level of serum total bilirubin of test drug treated rats suggested the hepatoprotective potential of it.

Liver has significant role in synthesis of protein like albumin [28]. The decreased level of serum total protein in $CCl_4$ treated animals indicates chronic liver damage and it reflect as hypo-proteinemia. Treatment with Polyherbal formulation successfully maintained normal level of serum total protein against $CCl_4$ induced liver damage.

Treatment of Polyherbal formulation restored the hepatic architecture and protected the liver tissue from fatty and degenerative changes, by preventing the toxic chemical reaction induced by $CCl_4$.

The various active constituents present in Polyherbal formulation like andrographolide from *Andrographis paniculata* [15], gallic acid from *Terminalia belerica* and *Terminalia chebula* [10,11], punarnavoside from *Boerhaavia diffusa* [8] can be thought of playing an important role in regulating the liver function and in providing protection against $CCl_4$.

## 5. Conclusion

On the basis of study data, it can be concluded that Polyherbal formulation (Vasuliv Syrup) has promising hepatoprotective activity against $CCl_4$-induced liver damage. It can be employed as safe and effective treatment for hepato-toxicity or liver damage.

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