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Research Article

Protective Effect of Polyherbal Formulation, Against CCl_4-Induced Liver Injury

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ABSTRACT:
Objective: The aim of the present study was to investigate the hepatoprotective effect of a polyherbal syrup formulation containing extracts of *Tridax procumbens* Linn. *Piper betal*, *Cichorium intybus*, *Moringa olifera*, and *Chrysanthum indicum* against CCl_4-induced hepatotoxicity in rats.
Methods: Hepatotoxicity was induced in rats by i.p. injection of CCl_4 (50% CCl_4/olive oil; 2 mL/kg) 3 times a week for 8 weeks. Polyherbal syrup (PHS) or Silymarin was administered along with CCl_4 and the biochemical parameters like aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were estimated. Furthermore, biomarkers of oxidative stress such as MDA levels, Glutathione contents, SOD and catalase activity, proinflammatory cytokines such as TNF-α and IL-6 levels in liver tissue were estimated.
Results: Treatment with polyherbal syrup significantly ($P<0.001$) reduced the elevated levels of AST, ALT and ALP as compared to CCl_4 control rats. The treatment with PHS also showed a significant increase in glutathione contents, SOD and catalase activity and a decrease in MDA, proinflammatory cytokines such as TNF-α and IL-6 levels as compared to CCl_4 control rats.
Conclusion: The finding of present study indicates that polyherbal syrup formulation showed a protective effect against CCl_4-induced liver injury. These results support the traditional use of *Tridax procumbens* Linn. *Piper betal*, *Cichorium intybus*, *Moringa olifera*, and *Chrysanthum indicum* in the treatment of liver disorders. Our findings confirm that the formulation was found to be effective pharmacologically at higher dose against carbon tetrachloride induced hepatotoxic models and were comparable to that of Silymarin.

Key words: CCL_4, Hepatotoxicity, oxidative stress, proinflammatory cytokines, polyherbal syrup

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1. INTRODUCTION:

The metabolic activation of CC14 to short-lived reactive intermediates is closely associated with its hepatotoxic action. Cytochrome P450, the hepatic mixed-function oxidase system's terminal oxidase, catalyzes the reductive de-halogenation of CC14 [1]. Through spin trapping experiments, the presence of free radicals during CC14 metabolism has been demonstrated [2].

Reactive intermediates either bind covalently to biological components or cause enhanced lipid peroxidation through the oxygen-mediated interaction of free radical intermediates, which damages unsaturated fatty acids, are the two possible causes of CCl4-induced cell damage. This leads to the destruction of lipids, especially unsaturated phospholipids, which harms the plasma membrane and intracellular membranes[3]. The partial pressure of oxygen in tissues influences the course of CCl4-induced hepatotoxicity to some extent. Low partial pressure leads to covalent metabolite binding and the predominant formation of CC13* and CHC12* radicals [4].

This leads to steatosis, or fatty liver, and primarily affects the metabolism of lipids (increased synthesis, decreased transport out of the hepatocyte). On the other hand, high oxygen partial pressure causes a shift in CC14 metabolism that leads to the production of the CC13-OO* radical and lipid peroxidation, effectively causing the cell to go from steatosis to apoptosis [5]. Since the dawn of humankind, plants have been utilized to treat and cure various illnesses. Using plants, plant extracts, or pure chemicals that have been separated from natural products to treat illnesses is known as phytotherapy. Diseases like diabetes, jaundice, heart problems, heavy metal toxicity, congested abdomens and pelvis cavities, scarlet fever, and others have all been treated with plants. Only 1 to 2% of the 250,000–500,000 species of terrestrial plants are thought to have undergone any meaningful research. Even though there are more synthetic drugs on the market today than natural ones, many synthetic drugs still have natural sources as their source originated from both plants and animals [6].

Standardized plant extracts are starting to be accepted by modern science; thus, efforts should be made to do accurate research on these therapeutic plants, which may also be utilized to treat liver disorders. Numerous plants have been studied for their potential hepato-therapeutic properties, and the extracts from these plants have shown curative effect. The plant may contain structurally distinct compounds that interact synergistically. Because of the complexity of these medications and their biological changes, a safety assessment is required [7].

Many traditional remedies employing herbal drugs continue to make a major contribution to health in terms of prevention and treatment of many diseases [8].

Tridax procumbens L. sometimes referred to as *T. procumbens*, is an Asian Ayurvedic plant. It is a member of the Asteraceae family. Folk medicine has long utilized to treat wounds and skin conditions and to prevent blood clotting. Its properties include anticoagulant[8], antileishmanial, antioxidant[9], immunomodulatory, anticancer [10], insecticidal, antiseptic, cardiovascular, anthelmintic, and antibacterial[11]. It is consumed as a beverage to cure liver disorders, diarrhea, dysentery, and bronchial catarrh. The plant is known to include flavones, glycosides, beta-sitosterol, luteolin, glucotureoline, and quercetin, according to earlier studies[12].

Betel vine, or *Piper betel* (L), is a member of the Piperaceae family. Moreover, betel leaf—a recognized bioactive compound contains several phytochemicals and nutritional elements (BAC). The antibacterial, antidiabetic, antiulcer, antioxidant, analgesic,[13], anticancer, antimutagenic. The properties of betel leaf essential oil (BLEO) include larvicidal, anti-filarial, tyrosinase, and insecticidal properties [14]. Moreover, betel leaves showed actions against bacteria, nociception, anthelmintics, allergies, cancer, hypercholesterolemia, antidiabetic, and platelets[15].

Worldwide grown, chicory (*Cichorium intybus* L.) is a perennial herb belonging to the Asteraceae family's Cichorium genus. Chicory has primarily been utilized in animal feed up to this point. Still, it has also been employed in the food business on a few occasions for teas, salads, tea blends, coffee supplements, and as a source of inulin. Apart from the previously mentioned advantages, *C. intybus* L. exhibits a wide range of biological activities, such as antidiabetic, antioxidant, hepatoprotective, anti-inflammatory, and tumor-inhibitory action[16]. Additionally, the plant has blood-cleansing, laxative, detoxifying, energizing, and antioxidant properties[17].

Various people refer to *Moringa oleifera* Lam. as "the miracle plant" because of its various health advantages for people. Nearly all necessary growth factors, amino acids, vitamins, minerals, proteins, and metals, including iron, potassium, and zinc, are found in the leaves of the moringa plant. In addition to these, plant leaves can now be utilized to make a variety of medications and nutritional supplements [18]. Research has indicated that it may be effective in preventing or treating inflammation, diabetes, cancer, obesity, renal disease, and liver-related disorders[19].

The perennial plant *Chrysanthemum indicum* L. (*C. indicum* L.), which belongs to the Compositae family, has been utilized in traditional medicine. From this plant, more than 190 chemical components, including terpenoids, flavonoids, phenylpropanoids, and phenolic acids, have been identified till date. Several subsequent investigations have demonstrated the multi-pharmacological activity of extracts or monomeric compounds derived from *C. indicum* L., including anti-oxidation, anti-inflammatory, anti-tumor, immunological modulation, and hepatoprotective properties[20-21].

Hepatoprotective activities have been reported for each of the chosen plant extracts [22]. However, the literature survey shows that no sufficient scientific data have been observed on the pharmacological evaluation of these plants in combined form. So, the objective of the study is

to study the Protective effect of Polyherbal syrup containing five extracts, including *Tridax procumbens Linn, Piper betal, Cichorium intybus, Moringa olifera* and *Chrysanthemum indicum*, against CCl_4-induced hepatotoxicity.

2. Materials and Methods:

2.1. Plant material and preparation of extract

Fresh leaves of *Tridax procumbens* Linn. (family: Asteraceae) *Piper betal* (family: Piperaceae), *Cichorium intybus* (family: Asteraceae), *Moringa olifera*(family: Moringaceae), Chrysanthum indicum (family: Compositae) were collected from Mahatma Phule Krishi Vidyapeeth's medicinal and aromatic herb garden in Rahuri, Maharashtra. It was then authenticated by Dr. Dinesh L Shirodkar, Sr. Botanist, Botanical Survey of India (BSI), Pune (BSI/WRC/Tech./2013/462). The dried plant material were coarsely powdered (passed through sieve no. 40) and immersed in a stopper cup for maceration with 70% v/v ethanol in water for 3 days separately. These extracts of plants were then evaporated at 45°C and then dried. The dried extracts were stored in airtight container for further use.

2.2. Chemicals and apparatus

CCL4 (Himedia Laboratories, Mumbai), GSH, DTNB, TBA (Sigma-Aldrich, USA) were used for the study. DMEM, FBS, antibiotics and other chemicals required for cell culture were the products of Gibco, USA. MTT dye and TBARS estimation kit were purchased from Himedia, India. Biochemical kits for assaying ALT, AST and ALP were purchased from Accurex Biomedical Pvt. Ltd., India. Kits for assaying albumin, total bilirubin, and total protein were purchased from Agappae Dignostics, India. All other solvents, chemicals, enzymes and reagents used in the analysis were of analytical grade. Apparatus such as the metabolic cage (Dolphin, Mumbai), the biochemistry analyzer (Benspera Clinical Chemistry Analyser C-61), UV-spectrophotometer (Jasco model V-630) and centrifuge (Remi) were used in the study.

2.3. Animals

Healthy adult male Wistar rats (Rattus norvegicus) weighing 150–200 g were acquired from SCITESLA Private Limited Mumbai, animal house. Rats were housed in polypropylene cages lined with husk in standard environmental conditions (temperature 25±2° C; relative humidity 55±10%; and 12:12 light: dark cycle). The rats were fed on a standard pellet diet(Amrut rat and mice feed, Sangli, India) *ad libitum* and had free access to water. The animals were acclimatized to the laboratory conditions for at least one week prior to the experiment. The experimental protocol was approved by Institutional Animal Ethical Committee(SCI/RD/KAL/SYP/23-24/05) of SCITESLA Private Limited Mumbai, India and studies were carried out accordance with current CPCSEA regulations.

2.4. Preparation of Polyherbal Syrup

Polyherbal formulations was prepared from five extracts including *Tridax procumbens* Linn, *Piper betal, Cichorium intybus, Moringa olifera* and *Chrysanthum indicum* for the treatment of liver disorders. The polyherbal syrup was prepared by following procedure: a) Syrup base was prepared by dissolving 66.7gm sucrose in 80ml deionized water in a water bath. b) 4000 mg of natural extract in 20ml of deionized water to prepare solution of 200mg/ml. This natural extract solution was added to 80ml of syrup base, to make final volume of 100ml.c)Same procedure was followed for all the natural extract samples. After preparing syrup of all 5 natural extract samples, they all were mixed in equal proportions. The final concentration was of the polyherbal syrup was 40mg/ml.

2.5 Evaluation of Syrup: Organoleptic properties(color, odor, taste and appearance) and physicochemical properties (pH, specific gravity, viscosity, turbidity and homogeneity)were assessed for the polyherbal formulation. Testing for stability of the syrup was conducted at 4°C and 47°C. Every physicochemical parameter was in the assessed three times, 24 hours, 48 hours, and 72 hours apart, to look for any changes. Abbes refractometer was used to calculate the refractive index [23-24].

Stability testing of the prepared herbal syrup was performed on keeping the samples at accelerated temperature conditions. The final syrup was taken in culture tubes and was kept at accelerated temperature at 4°C, Room temperature and at 47°C respectively. The samples were tested for all the physicochemical parameters, turbidity and homogeneity over the interval of 24hrs, 36 hrs and 72hrs to observe any changes [25-26].

2.6Acute Toxicity Study:

This study employed the same methodology as the Organization for Economic Co-operation and Development guideline no. 425 (Acute oral toxicity 2008) to investigate the acute oral toxicity of polyherbal syrup. The rats were fasted overnight before receiving a single dose of polyherbal syrup (2000 mg/kg, p.o., suspended in 0.5% w/v CMC). Following the administration of polyherbal syrup, anomalies or indicators of toxicity were noted at 0, 30, 60, 120, 180, and 240 minutes, as well as once a day for the following 14 days. The number of rats that survived was recorded at the end of the study period [27-28].

2.7: CCL_4 Induced Hepatotoxicity:

Animals were randomly divided to 6 different groups, as follow (n=6, each group):

- Normal Control (N.C.) Group: administered normal saline (0.9% NaCl, p.o.) daily throughout the experiment.
- Toxic Control (T.C.) Group: administered normal saline (0.9% NaCl) daily throughout the experiment + CCl_4 (50% CCl_4/olive oil; 2 mL/kg,i.p.)
- Standard Control Group: administered Silymarin (100 mg/kg body weight, p.o.) + CCl_4 (50% CCl_4/olive oil; 2 mL/kg,i.p.)
- Polyherbal Syrup 1 Group: administered Polyherbal syrup (50 mg/kg body weight, p.o.) + CCl_4 (50% CCl_4/olive oil; 2 mL/kg, i.p.)

- Polyherbal Syrup 2 Group: administered Polyherbal syrup (100 mg/kg body weight, p.o.) + CCl_4 (50% CCl_4/olive oil; 2 mL/kg, i.p.)
- Polyherbal Syrup 3 Group: administered Polyherbal syrup (200 mg/kg body weight, p.o.) + CCl_4 (50% CCl_4/olive oil; 2 mL/kg, i.p.)

All the rats administered above treatment once daily for 8 weeks along with CCl_4 (50% CCl4/olive oil; 2 mL/kg,i.p.) 3 times a week for 8 weeks to induce the liver fibrosis [29].

2.8 Biochemical Estimation for Liver Function

After the completion of 8-weeks treatment, the blood samples from all the groups were withdrawn from the retro-orbital plexus under mild isoflurane anaesthesia. The blood samples were collected in the micro centrifuge tube (Eppendorf, Germany) and allowed to stand for 30 minutes, and centrifuged at 6000 RPM for 15 minutes at 2-8 ^{0}C (NEAUATION, India).The serum was separated and transferred in labelled micro centrifuge tubes and used for estimation of liver enzymes, AST, ALT, ALP, TNF-alpha and IL-6 as per the procedure given by manufacturer ELISA kit (Krishgen, India).After the blood withdrawal, the animals were sacrificed; liver was isolated, cleaned with ice cold saline and blotted out free of blood, further the liver was subjected to biochemical analysisand half portion was used for histopathological studies.

2.9: Homogenization of liver samples:

The remaining half portion of liver was placed in phosphate buffer solution and homogenized (10 %w/v), after the homogenation, the homogenates samples were centrifuged at 4000 RPM for 15 minutes at 4 ^{0}C.The supernatant was separated and transferred to labelled 15 ml tubes (Himedia, India). The supernatant was used for estimation of and SOD, CAT, GSH and LPO in liver homogenates as per respective assays [30-32].

2.10: Histopathological analysis:

Rat livers were dehydrated in graded alcohol and then embedded in paraffin after being preserved in a 10% neutral buffered formalin solution. Subsequently, 3-4 micron thick paraffin sections were taken and mounted onto glass slides. Haematoxylin and eosin were used as a counter stain on these sections to aid in light microscopic examination. A light microscope, namely an Olympus CX-21 type, was used for the examination, and the Olympus Camera DP21 (U-TV0.5XC-3) that came with it was used for taking pictures.[33].

2.11: Statistical analysis:

All parameters were expressed as a mean ±standard error of mean (SEM). Statistical difference was tested by using one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test. A difference in the mean p value <0.05 was considered as statistically significant [34].

3. Results:

3.1 Evaluation of Syrup:

The organoleptic characteristics of the prepared syrup formulations, including color, odor, and taste were assessed and it complies quality as per official standards.

3.2 Preliminary phytochemical screening:

Polyherbal syrup containing extracts of *Chrysanthum indicum, Cichorium intybus, Piper betal, Moringa olifera*, and *Tridax procumbens* Linn showed the presence of amino acids, sugars, phenolic compounds, alkaloids, flavonoids, and tannins in addition to steroids and terpenoids.

3.3 Acute Toxicity Study:

Oral administration of polyherbal syrup at 2000 mg/kg, p.o. did not elicit any behavioral signs or symptoms of toxicity up to 14 days. Hence, the median lethal dose (LD_{50}) of the polyherbal syrup was then greater than 2000 mg/kg body weight. Therefore doses 50,100 and 200 mg/ kg b.wt. were chosen for further experimentation.

3.4 Effect of Polyherbal syrup on hepatic markers in CCl_4-induced liver damage in rats:

The administration of CCl_4 resulted in significant hepatocellular damage in rats as evident from a significant elevation in serum levels ofAST, ALT and ALP (Table1) and oxidant parameters like GSH and MDA and pro-inflammatory cytokines TNF-α and IL-6(Table 2& 3) release from blood leukocytes indicative of hepatic injury in control group. Oral administration of polyherbal syrup at50, 100 and 200 mg/kg and standard drug Silymarin reversed the hepatotoxicity significantly ($p<0.01$)by decrease in serum levels of AST, ALT and ALP, oxidant parameters (like GSH and MDA) and pro-inflammatory cytokine TNF-α release from blood leukocytes indicative of hepatoprotection in a dose dependent manner when compared with control.

3.5Effect of Polyherbal syrup on oxidative stress markers in CCl_4-induced liver damage in rats:

In the present study, the toxic control rats exhibited significantly increased in MDA formation(an index of lipid peroxidation process) when compared to normal control group.Co-treatment with polyherbal syrup at50, 100 and 200 mg/kg and standard drug Silymarin significantly attenuated the MDA formation in dose depended manner when compared to toxic control group. The prevention of increased in the MDA formation were found to be significantly higher ($p<0.001$) in 200 mg/kgdose levels along with standard silymarin treatment (Table 2)

Glutathione (GSH) is non-enzymatic antioxidant found to protect the tissues from oxidative stress and subsequently disease condition. The GSH was measured in terms of non-protein thiol contents. The experimental findings demonstrated that NPSH were found to reduced significantly ($p<0.0001$) when compared to normal control group. Co-treatment with polyherbal syrup at50, 100 and 200 mg/kg and standard drug Silymarin significantly restored the GSH levels at 100, 200 mg/kg in dose depended manner when compared to toxic control group.(Table 2)

Superoxide dismutase (SOD) is endogenous antioxidant enzyme, found to increase during stress to scavenge the formed free radicals in the tissues due to oxidative stress. In the present study, we observed that, afterCCl4

administration, the SOD activity was found to declined significantly (p<0.0001) in toxic control group compared to normal group. Further, pre-treatment with polyherbal syrup at50, 100 and 200 mg/kg and standard drug Silymarin improved significantly (p<0.0001) the decreased SOD activity at 100, 200 mg/kg respectively.(Table 2)

In the present study, we estimated the pro-inflammatory cytokines mainly TNF-α and IL 6 to understand the inflammation induced by CCL_4 is mediated through immunomodulate inflammation through cytokines. Results findings demonstrated that, the both the cytokines participated in the inflammatory response produced by administration of CCL_4 in the rats. The observation suggested that both cytokines' levels were found to be increased significantly (p<0.0001) in toxic control group when compared to normal control group. Further, co-administration with polyherbal syrup at50, 100 and 200 mg/kg and standard drug Silymarin significantly attenuated the both cytokines' levels in dose dependent manner. Moreover, standard silymarin at 100 mg/kg attenuated the both cytokines level more significantly when compared to toxic control group.(Table.3)

3.6: Histopathological analysis:

Microscopical examinations of the liver sections of CCl_4 treated toxic control rats revealed intense centrilobular necrosis, steatosis and often swelling of the hepatic cytoplasm. Administration of polyherbal syrup at 50, 100 and 200 mg/kg and standard drug Silymarin for 8 weeks continuously exhibited a significant improvement of the hepatocellular architecture when compared to CCl_4 treated toxic control group, as evident from a considerable reduction in necrosis and fatty change as shown in figure (Figure 1).

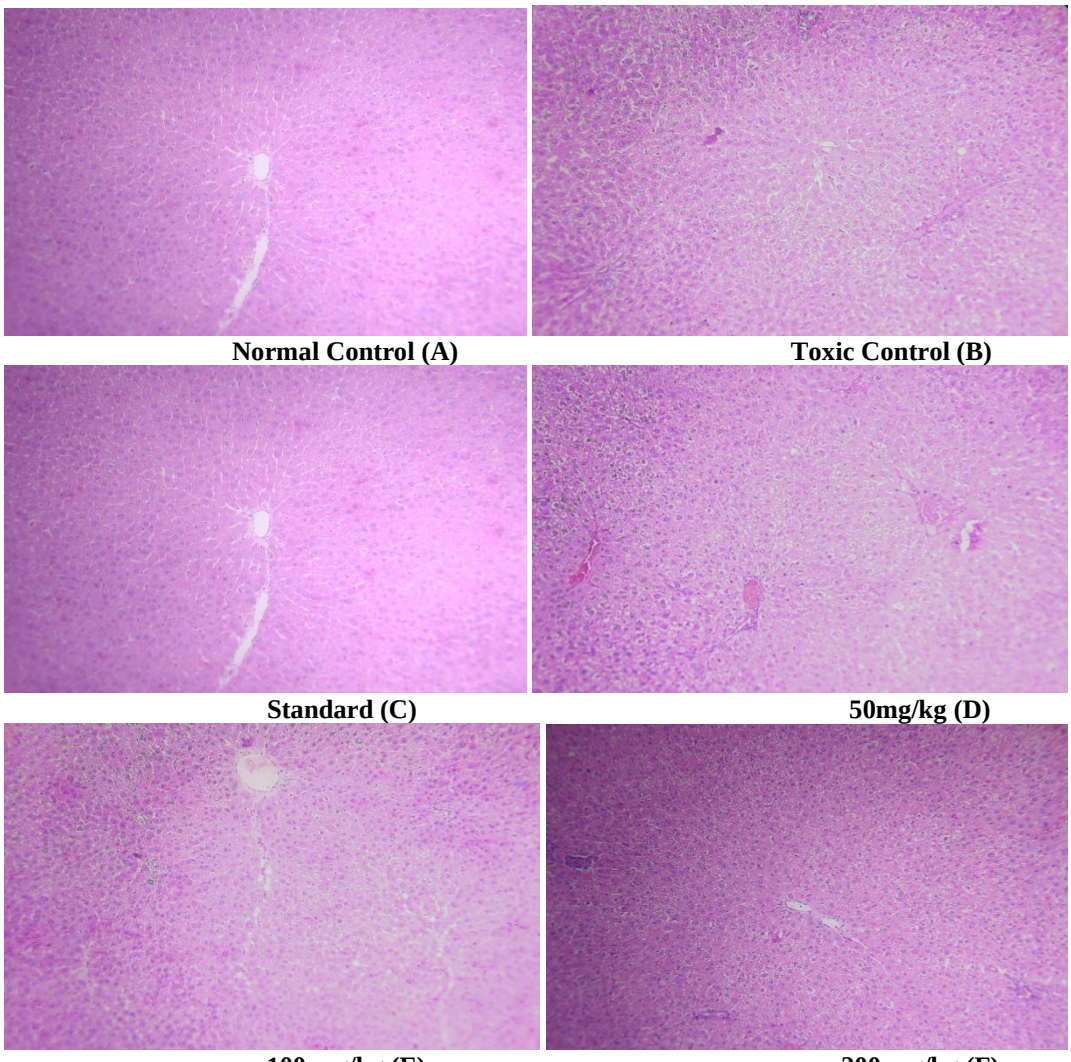

Figure1: Effect of polyherbal syrup on histopathological changes in rat liver (A) Normal control; (B) CCL_4 control; (C) Silymarine + CCL_4 treatment; (D) polyherbal syrup 50mg/kg + CCL_4- treatment; (E) polyherbal syrup 100 mg/kg + CCL_4 treatment; (F) polyherbal syrup 200 mg/kg + CCL_4 treatment illustrating reduced severity of lesions respectively at 40X microscopic examination.

Table 1: Effects of Polyherbal syrup formulation on CCL_4 induced hepatotoxicity in rats

Treatment and groups	AST (IU/mL)	ALT (IU/mL)	ALP (IU/mL)
Normal Control	121±12.73	92±8.49	144±24.04
Toxic Control	913±96.17###	703.5±17.68###	328.5±13.44
Silymarin (100 mg/kg)	126.5±10.61***	115±8.49***	475.5±48.79
Polyherbal Syrup (50 mg/kg)	611±41.01	515.5±20.5	468.5±136.47
Polyherbal Syrup (100 mg/kg)	373±39.60**	257±9.90***	347.5±31.82
Polyherbal Syrup (200 mg/kg)	156.5±21.92***	164.5±7.78***	470±31.11

Values are Mean ± SD, n=6, ###P<0.0001 when compared to normal control groups; **P<0.001 and ***P<0.0001 when compared to toxic control, ns-non significant compared to vehicle control: One way ANOVA followed by Bonferroni post hoc test

Table 2: Effects of Polyherbal syrup formulation on Lipid peroxidation and antioxidant parameters inCCL_4 induced hepatotoxicity in rats

Treatment and groups	nM of MDA/gm of tissue	NPSH contents/gm of tissue	SOD activity IU/mg of proteins	CAT activity (µM of H2O2/ gm of tissue)
Normal Control	0.096±0.069	16.20±1.16	39.33±11.11	15.43±21.40
Toxic Control	0.318±0.442###	19.27±1.45	13.1±4.37###	13.23±7.61###
Silymarin (100 mg/kg)	0.026±0.0073***	20.60±3.62	37.00±6.18	25.10±21.03*
Polyherbal Syrup (50 mg/kg)	0.215±0.252***	19.17±4.49	15.11±4.31***	1.63±0.38
Polyherbal Syrup (100 mg/kg)	0.026±0.022***	17.63±2.03	34.2±5.89***	36.07±40.98*
Polyherbal Syrup (200 mg/kg)	0.062±0.0073***	17.43±4.06	36.7±7.12***	5.64±5.74**

Values are Mean ± SD, n=6, ###P<0.0001 when compared to normal control groups; **P<0.001 and ***P<0.0001 when compared to toxic control, ns-non significant compared to vehicle control: One way ANOVA followed by Bonferroni post hoc test

Table 3: Effects of Polyherbal syrup formulation on Proinflammatory cytokines in CCL_4 induced hepatotoxicity in rats

Treatment and groups	TNF-α levels (pg/ml)	IL-6 levels (pg/ml)
Normal Control	112.78±4.849	152.04±50.32
Toxic Control	627.77±19.88###	833.98±176.1###
Silymarin (100 mg/kg)	149.12±10.18***	189.10±44.03***
Test Syrup (50 mg/kg)	583.88±42.67	556.76±35.64
Test Syrup (100 mg/kg)	423.42±37.82	448.54±50.32*
Test Syrup (200 mg/kg)	153.75± 23.52***	251.37±23.06***

Values are Mean ± SD, n=6, ###P<0.0001 when compared to normal control groups; **P<0.001 and ***P<0.0001 when compared to vehicle control, ns-non significant compared to vehicle control: One way ANOVA followed by Bonferroni post hoc test

Discussion:

The liver is responsible for the biotransformation of drugs and chemicals. In this context, high concentrations of toxic chemicals and their metabolites exposed to the liver, may generate oxidative stress and inflammation and ultimately result in liver injury, considered one of the major worldwide health problems and its prevention and treatment are still limited. CCl_4 is one of the major constituents of environmental pollutants, and causes xenobiotic-induced liver diseases. In the recent past, an increasing usage of phytochemicals as radical scavenges against oxidative stress and inflammation. In the field of environmental toxicology, plant-based natural products quench free radicals by increasing the level of cellular antioxidant enzymes and anti-inflammatory cytokines [35].

In the present study, the Protective effect of polyherbal formulation, against CCl_4-induced liver injury was investigated. Oxidative stress results in free radical generation by various biochemical procedures that damage proteins, DNA, and lipids [36]. CCl_4-induced oxidative stress cause hepatocyte necrosis and inflammation [37]. Antioxidant, fights free radicals which are produced via toxins and xenobiotics.

Preliminary phytochemical investigation of different extracts was carried out to obtain the information about presence of various phytoconstituents and methanolic extract found to contain carbohydrates, flavonoids, phenolic compounds and tannins. Alkaloids, flavonoids and saponins known to possess hepatoprotective activity [38].

Acute toxicity studies of polyherbal syrup at the dose of 2000mg/kg showed no toxic symptoms or death in any of the animals up to14 days and till the end of the study.

Thus, the drug was considered to be safe. CCl_4, as a conventional toxin, is mediated by the production of free radicals ($CCl_3^{\cdot}$ and $CCl_3O_2^{\cdot}$); metabolites of CCl_4. These reactive radicals lead to lipid peroxidation and cell membrane damage results establishment of oxidative

stress that eventually causes the release of intracellular enzymes including ALT and AST, considered markers of hepatotoxicity [39-40]. Elevated cytoplasmic enzymes such as AST, ALT, and ALP are reviewed as a sign of hepatic dysfunction and damage were observed in CCl_4 groups [41]. Polyherbal syrup treatment effectively protected rats against CCl_4-induced liver damage by reducing elevated serum level of ALT, AST, and ALP (Table 1). Biological systems aim to protect themselves against oxidative injury e.g., GSH, and SOD. GSH is one of the key protectors and is considered the main detoxifying and antioxidant molecule produced by cells. It makes conjugates with foreign compounds (CCl_3•) to eliminate the toxic material. SOD plays an important role in the exclusion of free radicals derived from the metabolism of xenobiotics in the liver. CCl_4-induced oxidative stress was accompanied by reduced levels of GSH, SOD and CAT suggesting that these free radicals lead to an impaired antioxidant defense system. The depletion of GSH could be related to reducing the synthesis in the injured hepatocytes. In addition, GSH stores may be rapidly consumed for scavenging free radicals produced by CCl_4, especially when liver necrosis initiates. Therefore, the abnormal level of these oxidant stress markers indicates the extent of the cell damage observed in CCl_4 group, while polyherbal syrup markedly improved the levels of CAT, SOD, and GSH near to normal (Table. 3). ROS are known to attack polyunsaturated fatty acids in cell membranes. ROS produced by CCl_4, causes lipid peroxidation in the cell membrane, and MDA is a biomarker of lipid peroxidation. Our results showed that polyherbal syrup markedly inhibited the increased level of MDA in CCl_4-induced rats, indicating that the hepatoprotective effect of polyherbal syrup may be associated with the inhibition of lipid peroxidation, which leads to increase cellular antioxidant and maintenance of hepatocyte membrane integrity[42]. Hepatic damage was further confirmed by histopathological examination which showed degenerative changes and massive necrosis in CCl_4-rats. Polyherbal syrup decreased hepatic necrosis maintained cellular shape, and minimized inflammation and vacuolation. The protective effects of polyherbal syrup were observed in dose-dependent manner, with the prophylactic benefits being significantly more powerful with a higher dose (200 mg/kg)) than lower doses.

Conclusion:

The present studies confirm the liver protective action of the polyherbal syrup containing extracts of *Tridax procumbens Linn, Piper betal, Cichorium intybus, Moringa olifera and Chrysanthum indicum* against experimentally induced liver damage in rats, which was comparable to that of a standard hepatoprotective drug silymarin. Onthe basis of the current investigation, it was noted that the polyherbal syrup actedin a similar fashion to silymarin (standard drug) and itis suggested that these results provide pharmacological evidence for the plants being used as hepatoprotective agent in folklore medicine.

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Conflict of interest: No conflicts of interest are disclosed by the authors.

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Authors' contributions: K.A.: writing the manuscript, experimentation, and results analysis; R.P.: Supervision: V.D., V.P., P.C.: formal analysis; PM: formal editing and analysis

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