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Pharmacological Screening of Polyherbal Formulation for Hepato Protective Activity against Carbon Tetrachloride (CCl_4) Induced Hepatotoxicity on Albino Rat

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ABSTRACT

The present study was designed to evaluate hepatoprotective activity of *polyherbal formulation* (*Andrographis paniculata, Boerhaavia diffusa, Eclipta alba, Picrorhiza kurroa***)** in Wistar albino rats using carbon tetrachloride (CCl_4) induced hepatic damaged experimental animals. Carbon tetrachloride (CCl_4) (1ml/kg, i.p) was administered a single dose to induce hepatotoxicity. Polyherbal formulation (200 mg/kg, p.o, 400 mg/kg, p.o,) and liv-52 syrup (50ml/kg, p.o.) were administered once daily for 8 days. The degree of Hepatoprotection was measured using morphological parameters of changing in color and weight of liver is determined. Biochemical parameters in Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Serum Alkaline Phosphatase (SALP), bilirubin and plasma protein and the histopathological parameters of histological changes in the liver architecture like architecture of hepatic lobules, swelling of liver cell, fatty changes, focal necrosis, inflammatory cell infiltration around portal areas, Kupffer cell hyperplasia *etc.* and the functional parameters of Pentobarbitone sleeping time was used as a functional parameter. PHF (Poly Herbal Formulation) pretreatment showed normal morphological parameters signs and significant effect of serum enzymes, total protein and bilirubin levels. Highly significantly protect the hepatic damage and morphological and histopathological changes and less Pentobarbitone sleeping time were observed in all the three polyherbal formulations treated rats. The result of this study strongly indicated that the polyherbal formulation has got a hepatoprotective action against carbon tetrachloride (CCl_4) induced hepatic damage in experimental animals.

1. INTRODUCTION

The liver is a vital organ having a wide range of functions including detoxification, protein synthesis and production of biochemical necessary for digestion. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision and reproduction [1]. Liver is the key organ to maintenance, performance and regulating homeostasis in the body. But liver is continuously and variedly exposed to exogenous substances like environmental toxins, drugs and alcohol which can eventually lead to various liver disorders, generally presenting as distinct patterns of diseases such as hepatocellular, Cholestatic (obstructive), or mixed type of liver disorders [2].

Almost all types of liver injuries may lead to hepatic failure and ultimately death. Thus liver diseases are one of the most fatal diseases in the world today [3]. Till date available modern drugs have not been able to come up with a satisfactory answer for liver disorders because of high cost and additional adverse effects. It is, therefore, necessary to search for alternative drugs for the treatment of liver diseases to replace the currently used drugs of doubtful efficacy and safety.

In the absence of reliable modern hepatoprotective drugs, in allopathic medical practices, herbs play a role in the management of various liver disorders. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices and in traditional system of medicine in India. The different plants in the herbal mixture will have different modes of action for curing the disease and in the combined form may sometimes exhibit synergistic activity (enhanced activity than of the individual herbs). Components of the plants, which are not active themselves, can act to improve the stability, solubility and bioavailability or half-life of the active compounds. Hence a particular active principle in the pure form may have only a fraction of the pharmacological activity that it has in its plant matrix, which again highlights the importance in using the plant as a whole or a mixture of plants for treating diseases.

A greater deal of research has been carried out to evaluate scientific basis for the claimed Hepatoprotective activity of herbal agents as single agent or in formulation. The plant herbal formulation under study contains plant ingredients like ethanolic extract of *Andrographis paniculata, Boerhaavia diffusa, Eclipta Alba, Picrorhiza kurroa.*

The form of extract whether hydro alcohol or ethanol and contact in dose are based on the

traditional knowledge and reports present on these plants. Formulations are developed based on Ayurvedic principles where plants are included for antioxidant activity, hepatoprotective activity, bioavailability enhancement and specific activity in modulation of different liver disease conditions as many of these herbal ingredients are known to have liver modifying activity. *Andrographis paniculata* is reported to antioxidant and hypoglycemic [4], *Boerhavia diffusa* is known for its antioxidant activity[5], decoction of the *Phyllanthus amarus* is known traditionally for its effect in jaundice [6], *Boerhaavia diffusa* is found to be protective in carbon tetra chloride induced liver damage [7], *Eclipta alba* is reported to hepatoprotective activity [8, 9]. We have undertaken this study to evaluate the efficacy of these formulations in experimental animals in which acute hepatotoxicity was induced by carbon tetrachloride.

2. MATERIALS AND METHODS

2.1 Plants collection and Preparation of plant extract

The fresh leaves of *Andrographis paniculata*, root of *Boerhavia diffusa*, whole plant of *Eclipta alba* and rhizome part of *Picrorhiza kurroa* were collected from the botanical garden-aziznagar, R.R.Dist. All the plant materials were identified and authenticated in the department of botany, Osmania College. The plant's voucher specimen number: PARC/2013/2026-2030 was deposited in the center herbarium.

All the medicinal plants and plants part were subjected to surface sterilization using ethanol and then dried in shade. All the dried plants were subjected to size reduction to a coarse powder by using dry grinder and passed through sieve (20 mesh). All the medicinal plant powdered samples (100 g) were defatted by treating with pet-ether and then extracted with ethanol by using Soxhlet apparatus. The solvent was removed under vacuum to get the solid mass. The residue was weighed and stored in air and waterproof containers, kept in refrigerator at 4°C. From this stock, fresh preparation was made whenever required.

2.2 Preliminary Phytochemical Analysis: All the different extracts were then subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents[10].

2.3 Animals

Healthy Swiss albino rats (200-250g) of either sex were taken for acute toxicity study and Wistar albino rats (200-250g) were taken to assess Hepatoprotective activity. All the

experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) of Pharmacy College, (No.1516/PO/a/11/CPCSEA). Animals were housed in polypropylene cages, maintained under standardized condition (12h light/dark cycle, 24°C, 35 to 60% humidity) and provided free access to standard pellets diet and purified drinking water *ad libitum.* The animals were deprived of food for 24h before experimentation but allowed free access to water throughout.

2.4 Acute toxicity studies

Acute toxicity study was performed according to Organization for Economic Co-operative and development guidelines No. 423 [11]. *Wistar albino* rats of either sex were divided into six groups with six animals each. Formulations - III was administered orally as single doses to rats at different dose levels of 50, 250, 500, 1000, 1500, and 2000 mg/kg b.w. Animals were observed individually during the first 30 minutes and periodically during 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total 14 days.

2.5 Drugs:

I) Standard Drug: *LIV 52* -SYRUP:

LIV 52 introduced in 1955, is herbal syrup for liver protection from Himalayan Company India. Since then it is recognized worldwide by health professionals. In India, it is a standard drug prescribed by the medical fraternity to treat any liver related diseases. Each 1ml syrup contains. *Capparis spinosa* 32mg, *Cichorium intybus* 32mg, *Mandur bhasma* 32mg, *Solanum nigrum* 32mg, *Terminalia arjuna* 32mg, *Cassia occidentalis* 16mg, *Achillea millefolium* 16mg, *Tamarix gallica* 16mg.

II) Test Drug: *Poly Herbal Formulation.*

The extracts of the plant's parts of *Andrographis paniculata*, *Boerhaavia diffusa*, *Eclipta Alba* and *Picrorhiza kurroa* were employed to prepare formulation in different proportions as 1:1:1:1 (Same proportion to prepare Herbal formulation).

Ingredients % w/w	Formulation
Andrographis paniculata (Ethanol Extract)	1.3 g
Boerhaavia diffusa (Ethanol Extract)	1.0 g
Eclipta alba (Ethanol Extract)	1.28 g
Picrorhiza kurroa (Ethanol Extract)	1.3g
Sorbitol	5.0 g
Sucrose	12 g
Carboxymethyl cellulose (CMC)	2.0 g
Olive Oil	1 g
Distilled Water	400 ml

2.6 Evaluation of Hepatoprotective Activity:

i) Carbon Tetrachloride (CCl4) Induced Hepatotoxicity:

Liver injury was induced by administration of Carbon tetrachloride (CCl_4) (1ml/kg, p.o) mixed with Liquid Paraffin (5 fold dilution)**.** Herbal formulation was administered to rats continuously for three days. On day fourth the rats were administered with herbal formulation followed by administration of toxicant after two hours. Again herbal formulation was administered on the fifth day, the rats were sacrificed to collect the blood and liver samples for blood analysis [12]. The experimental animals were divided into five groups, each group comprising six animals.

Group I: Normal Control Group,

Group II: CCl_4 Control Group,

Group III: Herbal formulation (100mg/kg, *p.o.*) + CCl_4 (1ml/kg, *p.o.*) Group,

Group 1V: Herbal formulation (200mg/kg, p.o.) + CCl_4 (1ml/kg, p.o.) Group,

Group V: liv-52 syrup (50ml/kg, p.o.) + CCl_4 (1ml/kg, p.o.) Group.

Biochemical Estimations:

At the end of the experimental period, the animals were killed by cervical dislocation. Blood was collected in the glass tubes from orbital sinus to obtain hemolysis free clear serum for the

analysis for the estimation of (SGOT) Serum Glutamate Oxaloacetate Transaminases and (SGPT) Serum Glutamate Pyruvate Transaminases, Bilirubin and Triglycerides[13,14]. All the animals were sacrificed by decapitation and livers were quickly excised freed from any adhering tissues, washed and perfused with chilled normal saline, minced and homogenized in ice bath using Potter-S-homogenizer (B. Braun, MelsungenAG, Germany, 1100 rpm for 2 min) in chilled 10mM Tris–HCl buffer (pH 7.4) to obtain 10% liver homogenate for the estimation of (GSH) glutathione[15,16], (LP) lipid peroxidation and estimation of Albumin was used standard kit (Bayer Diagnostic Ltd., Gujarat, India).

Histopathological Investigation:

The liver tissues were excised out, washed with the cold saline, fixed in 10% buffered formalin for 12 hours and processed and stained with hematoxylin and eosin dye for photomicroscopic observations.

2.7 Statistical Analysis:

The results were expressed as mean ± SEM. The data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. A value of $P< 0.05$ was considered as statistically significant.

3. RESULTS:

3.1 Preliminary Phytochemical Screening:

The preliminary phytochemical studies were performed for testing different phytochemical constituents present in Polyherbal formulation. The observations showed the presence of alkaloids, flavonoids, steroids, tannins and phenolic, which were found to be more in methanolic extract.

3.2 Effect Of PHF On CCl_4 Induced Liver Toxicity In Rats.

Table 1: Effect of Polyherbal formulation on serum SGOT, SGPT, Albumin, bilirubin in CCl_4 induced liver toxicity in rats.

GROUPS	SGOT (U/I)	SGPT (U/I)	Albumin(mg/dl)	bilirubin (g/dl)
Control	94.36±7.55	103.67±6.53	0.34±0.04	3.35±0.20
CCl_4 1ml/kg, p.o	1328.04±126.38[a]	811.89±34.84[a]	1.12±0.09[a]	2.35±0.10[a]
Herbal formulation 200mg/kg, p.o	852.96±61.54[*]	601.46±27.38[**]	0.79±0.05[**]	2.57±0.13[***]
Herbal formulation 400mg/kg, p.o	757.12±60.44[***]	523.91±31.50[***]	0.75±0.05[*]	2.70±0.11[**]
Std (liv-52 syrup)(50ml/kg, i.p.)	630.59±50.29[***]	470.47±22.49[***]	0.65±0.04[***]	2.89±0.20[***]

Values are mean ± SEM (n=6) two way ANOVA. Where, ** represents highly significant at $p< 0.001$, All p values are compared with toxicant.

3.3 Histopathological Study of Liver:

The histological profile of the hepatic tissue of the placebo control animals showed a normal lobular architecture. Normal hepatocytes were arranged in single cell cords radiating away from a central vein (A). Group II rats, treated with distilled water and CCl_4, showed disturbed liver architecture, exhibiting central lobular necrosis with tiny vacuoles, and fatty infiltrations (B). Group III and IV animals, pretreated with Livshis and Silymarin respectively, and subsequently treated with CCl_4 retained normal hepatic tissue architecture, so received significant protection from CCl_4-induced hepatic damage (C and D). Group V animals, treated with Liv-52 syrup alone, did not show any significant hepatic tissue architectural changes (E).

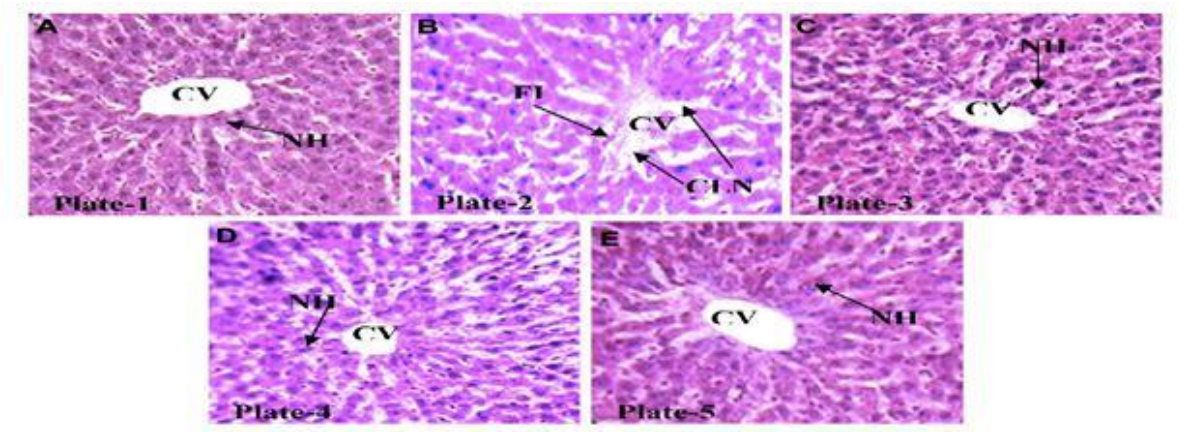

Figure.1: Effect of PHF on histopathological changes in male albino rat liver.

(A) Placebo control; (B) Pretreatment (distilled water) & CCl_4 treatment; (C) Pretreatment (PHF) & CCl_4 treatment; (D) Pretreatment (PHF) & CCl_4 treatment ;(E) Control & Liv-52. Hematoxylin and eosin stain, original magnification 400_. CLN: central lobular necrosis; CV: central vein; FI: fatty infiltration; NH: normal hepatocyte.

4. DISCUSSION

The study was taken up with an aim to determine the efficacy of marketed hepatoprotective agents. The present work dealt with the comparative study of selling Polyherbal hepatoprotective formulations. The results of carbon tetrachloride induced hepatotoxicity are shown. Carbon tetrachloride intoxication in normal rats significantly elevated the serum levels of SGOT, SGPT, Total Bilirubin, whereas there was a significant decrease in level of albumin that indicated acute hepatocellular damage and biliary obstruction.

In the histopathological examination of liver, sections explain histopathological changes such as central lobular necrosis, seen as the formation of tiny vacuoles, and FI were observed in hepatic tissue in Group II rats. Liver injury disturbs the hepatocyte transport function, resulting in the leakage of plasma membrane, thereby causing increased TB and increased enzyme levels in serum. Total bilirubin, SGOT, and SGPT enzymes are normally excreted in bile by the liver. In the presence of hepatotoxin, the liver bile excretion process becomes defective, resulting in increased enzyme levels in the serum.

This formulation was able to control this necrotic change that was comparable to that of Liv-52 treated group. Thus, the biochemical observations correlate well with the histopathology results of the liver samples.

5. CONCLUSION

In this study, the herbal formulation (400mg/kg, p.o) produced excellent hepatoprotective activity on Wister albino rats. The dose levels selected were 100 mg/kg,p.o,200mg/kg,p.o and 400mg/kg, p.o. and histopathological examination of the liver sections of the rats treated with toxicant showed necrosis and the fatty material changes. Thus it was concluded that the herbal formulation 400 mg/kg, p.o exhibited significant dose dependent hepatoprotective activity.

Biochemical analysis and histopathological studies revealed that in animals of group 2, carbon tetrachloride caused prominent centrilobular fatty change with prominent and enlarged central vein. There was a significant periportal inflammation. Necrosis was also observed indicating liver damage and inflammation of hepatocytes. Recoveries of hepatocytes were seen in sections of liver treated with liv-52 but there was a significant recovery shown by the liver sections treated with the polyherbal formulation. Central vein appeared clearly with disappearance of necrosis there by indicating a potent anti-hepatotoxic activity. The histopathological studies also revealed that the rats treated with polyherbal formulation had almost normal architecture of hepatocytes indicating significant recovery as compared to standard drug liv-52.

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7. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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