Study of the Hepatoprotective Activity of Polyherbal Formulation on Alcohol Induced Hepatotoxicity

Shah Zalak1*, Paranjape Archana1, Soni Hardik2, Mandal Snigdha Das1, Patel Janki1

1Department of Pharmacology, Parul Institute of Pharmacy and Research, Parul University, Vadodara-391760, Gujarat, India
2Vasu Research Centre, A Division of Vasu Healthcare Pvt. Ltd. 896/A, G.I.D.C, Makarpura, Vadodara-390010, Gujarat, India.

ABSTRACT

Many traditional systems of medicines employ herbal drugs for the hepatoprotection. Aim of the study was designed to evaluate the hepatoprotective potential of polyherbal formulation against alcohol induced hepatotoxicity in wistar albino rats. Group I animals were treated with 1% CMC for 18 days. Group II, III and IV animals were treated with 1% CMC, polyherbal formulation 180mg/kg/day and silymarin 100mg/kg/day respectively for 18 days and then orally administration with ethanol 3.76 g/kg/day simultaneously for 18 days. After 24 hours of last dosing, the blood was obtained through retro-orbital plexus under light anaesthesia and the animals were sacrificed. Hepatoprotective potential was assessed by various biochemical parameters such as AST, ALT, ALP, LDH, bilirubin, cholesterol, TG and thiopentone sodium induced sleep time. Group III rats showed significant (p<0.01) decrease in AST, ALT, ALP, LDH, bilirubin, cholesterol, TG and thiopentone sodium induced sleep time. Group IV rats showed significant (p<0.01) decrease in TP levels as compared to group II rats. Hepatoprotective potential of polyherbal formulation 180mg/kg/day was comparable to that of standard drug silymarin 100mg/kg/day. Results of the study were well supported by histopathological observations. This study confirms that polyherbal formulation possesses hepatoprotective potential comparable to that of standard drug silymarin as it exhibited comparable protective potential against PCM induced hepatotoxicity in albino rats.

Keywords: Polyherbal formulation, Hepatoprotective potential, Alcohol, Hepatotoxicity, Silymarin

INTRODUCTION:

The liver is the largest glandular organ in the body and performs multiple critical functions to keep the body free from toxins and harmful substances. The liver synthesizes, concentrates, and secretes bile acids and excretes other toxicants, such as bilirubin. It also detoxifies a variety of drugs and xenobiotics and secretes bile that has an important role in digestion. Chronic liver disease represents the fourth leading cause of death among all races and sexes in the 45-54 year age group. It is well known that free radicals cause cell damage through mechanisms of covalent binding and lipid peroxidation with subsequent tissue injury. Antioxidant agents of natural origin have attracted special interest because they can protect human body from free radicals. Alcohol is the most abused substance worldwide and a significant source of liver injury. Long term alcohol consumption induces oxidative stress in the liver due to imbalance between prooxidant and antioxidant system. The major toxic metabolites of ethanol are acetaldehyde and free radicals. Three pathologica...
widespread use, there is a lack of scientific evidence for its safety and efficacy. Thus, present investigation was undertaken to investigate the hepatoprotective activity of polyherbal formulation on alcohol induced hepatotoxicity in rats. The formulation contains many herbal plants with antihepatotoxic actions which are complementary to each other. Polyherbal formulation contains Eclipta alba, Andrographis paniculata, Triphla churna (formulation), Phyllanthus niruri, Boerhavia diffusa and Tinospora cordifolia.

Table 1: Composition of Polyherbal formulation.

| Ingredients (Extract of) | Family       | Part Used                  | Ingredients (Powder of) | Family       | Part Used                  |
|--------------------------|--------------|----------------------------|--------------------------|--------------|----------------------------|
| Eclipta alba             | Asteraceae   | Leaves, root, aerial, seed and stem | Eclipta alba             | Asteraceae   | Leaves, root, aerial, seed and stem |
| Andrographis paniculata  | Acanthaceae  | Aerial                      | Phyllanthus niruri       | Phyllanthaceae | Leaves, root, aerial, seed and stem |
|                          |              |                             | Boerhavia diffusa       | Nyctaginaceae | Root                       |
|                          |              |                             | Tinospora cordifolia     | Menispermaceae | Stem                       |

Objectives:
The objectives of the present investigations were

1. To carry out acute toxicity study of polyherbal formulation in rats.
2. To investigate hepatoprotective effect of polyherbal formulation against alcohol induced hepatotoxicity in rats.

MATERIALS AND METHODS:

Drugs and Diagnostic Kits
Silymarin (Zydus) and Thiopentone sodium were purchased from local Market, Baroda. Polyherbal formulation was obtained from Vasu Research Center, Vadodara, Gujarat. Aspartate amino transferase (AST), Alanine Amino transferase (ALT), Alkaline phosphatase (ALP), Total Protein (TP), Total Bilirubin (TBL), Direct Bilirubin (DBL) and Lactate Dehydrogenase (LDH), Triglyceride (TG) kits were purchased from Span Diagnostics, Surat, Gujarat, India. All other chemicals and reagents were purchased from analytical reagent quality.

Animals
Albino wistar rats of either sex weighing between 200-220g procured from Jay Research Foundation, Vapi and flair lab, Surat were used in the study. The animals were grouped in poly propylene cages with not more than six animals per cage. The animals were maintained under standard laboratory conditions at an ambient temperature of 23±2°C having 50±5% relative humidity with 12 hours light and dark cycle. The rats were acclimatised to laboratory conditions for 10 days before the commencement of experiment. The use and care of the animals in the experiment protocol has been approved by the Institutional Animal Ethics Committee (IAEC -Regd. No. 516/01/A/CPCSEA) following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Acute toxicity studies
Acute toxicity study was determined as per OECD guidelines 423. Acute toxicity study for polyherbal formulation was performed by using female albino rat. The animals were fasted overnight prior to the experiment and maintained under standard conditions. The tablet of polyherbal formulation in the form of suspension prepared in purified water was administered orally in increasing dose and found safe up to a dose of 2000 mg/kg.

Alcohol induced hepatotoxicity
Experimental Protocol:
Rats were divided into four groups, each group containing of six rats.
1) Group I (Normal control): received 1% CMC only, orally.
2) Group II (+ve control): received 20% ethanol (3.76 g/kg/day, p.o) daily for eighteen days
3) Group III: received 20% ethanol (3.76 g/kg/day, p.o.) and Polyherbal formulation (180mg/kg, p.o) simultaneously for eighteen days.
4) Group IV: received 20% ethanol (3.76 g/kg/day, p.o.) and Silymarin (100mg/kg body, p.o) simultaneously for eighteen days.

Biochemical determination
Serum alanine transaminase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), Total bilirubin (TBL), Direct bilirubin (DBL), Total cholesterol (TC), Lactate Dehydrogenase (LDH), Total Protein (TP) and Triglyceride (TG) were estimated using standard kits from Span Diagnostic Ltd Surat, India. All the enzymatic estimations were assessed as per standard kit methods using UV spectrophotometer and the standard kit methods were obtained in detail from the leaflets provided in the commercial kits.

Histopathological Parameters
Slices of liver were stored in 10% buffered neutral formalin solution. The tissues were mounted by embedding in paraffin wax in the laboratory and sections of the size of 6mm were cut. The sections were stained with eosin and haemotoxylin dyes. The slides were observed under light microscope and photomicrographs were captured by using camera. These were observed for fibrosis, fatty infiltration, centrlobular necrosis and lymphocyte infiltration.

Statistical Analysis
Results were expressed as mean ± standard error of mean, (n=6). Statistical analysis was performed with one-way analysis of variance ANOVA followed by Tukey’s multiple comparison tests using Graph Pad Prism-5 software. p<0.05 was considered to be statistically significant.

RESULTS
Acute toxicity Studies
The polyherbal formulation did not show any sign and symptoms of toxicity and mortality up to 2000 mg/kg dose.

Biochemical Parameter

Effect of polyherbal formulation on different liver specific variable in control and experimental groups of animals are shown in Table 2. Alcohol treatment significantly (p<0.01) increased the relative liver weight to 4.34±0.095/100g b.wt as compared to the normal control group I with that of 2.53±0.535/100g b.wt. Administration of polyherbal formulation 180mg/kg/day significantly (p<0.05) reduced the relative liver weight to 2.73±0.120 b.wt. as compared to that of alcohol treatment with that of 4.34±0.095/100g b.wt Polyherbal formulation treated group was more close to standard silymarin treated group. PCM treated group II rats showed increased serum AST (161.08±2.32U/L, P<0.01) ALT(120.46±4.41U/L, P<0.01) ALP(124.51±7.23U/L, P<0.01) and LDH(170.44±3.70U/L, P<0.01) as compared to normal control group I rats (80.43±0.08U/L, 67.08±0.80U/L, 26.01±1.12U/L, 62.82±0.90U/L) respectively. The polyherbal formulation treated group III significantly decreased AST(114.34±3.28U/L), ALT(91.67±0.91U/L), ALP(91.67±0.91U/L) and LDH(143.29±1.30U/L) as compared to group II. Silymarin 100mg/kg/day significantly (P<0.01) declines the elevated levels of AST, ALT, ALP and LDH to the levels of 93.53±1.03U/L, 64.58±4.78U/L and 67.76±2.10U/L respectively as compared to alcohol treated group II.

Alcohol treated group II significantly (P<0.01) decreased serum TP(3.34±0.12 g/dl, P<0.01) while increased TBL(138.18±0.06 mg/100ml, P<0.01), DBL(0.92±0.05 mg/100ml, P<0.01) and cholesterol(173.43±1.02 mg/dl, P<0.01) as compared to control group 5.81±0.05 g/dl,0.49±0.01 mg/100ml, 0.24±0.01 mg/100ml and 103.22±2.20 mg/dl respectively. The polyherbal formulation treated group III significantly increased TP(4.38±0.60), while decreased TBL(0.81±0.02), DBL(0.36±0.01), cholesterol(117.70±1.18) as compared to group IV silymarin 100mg/kg/day significantly increased TP(4.94±0.01 g/dl, P<0.01), while decreased TBL(0.73±0.02 mg/100ml, P<0.01), DBL(0.29±0.015 mg/100ml, P<0.01) and cholesterol(102.08±0.73 mg/dl, P<0.01) as compared to PCM treated group.

Alcohol treated group II showed significant (P<0.01) increase in serum Tg(180.7±1.60 U/L) as compared to control group (75.97±1.70U/L). The polyherbal formulation treated group III significantly decreased (102.02±2.31 U/L) as compared to group IV silymarin(90.62±1.04 U/L) both compared to PCM treated group.

| Treatment groups and liver specific variables | Normal control | Hepatotoxic control: 1% CMC 1 ml/kg b.wt. | Polyherbal formulation 180mg/kg/day + ethan 3.76 g/kg/day, p.o. | Silymarin 100 mg/kg b.wt. + ethan(3.76 g/kg/day, p.o.) |
|------------------------------|-----------------|---------------------------------------|-------------------------------------------------|--------------------------------------------------|
| AST (U/L)                     | 80.43±1.50      | 161.08±2.32#                          | 114.34±3.28**                                   | 93.53±1.03**                                    |
| ALT (U/L)                     | 87.08±0.08      | 120.46±4.41#                          | 84.40±1.59**                                   | 64.58±4.78**                                    |
| ALP (U/L)                     | 60.82±1.12      | 124.51±7.23#                          | 91.67±0.91**                                   | 67.76±2.10**                                    |
| LDH (U/L)                     | 62.82±0.90      | 170.44±3.70#                          | 143.29±1.30**                                  | 124.48±1.30**                                   |
| TP (g/dl)                     | 5.81±0.05       | 3.34±0.12#                            | 4.38±0.06**                                   | 4.94±0.09**                                    |
| TBL(mg/100ml)                 | 0.49±0.01       | 1.38±0.06#                            | 0.81±0.02**                                   | 0.73±0.02**                                    |
| Cholesterol (mg/dl)           | 103.43±2.20     | 173.43±1.02#                          | 117.78±1.18**                                  | 102.08±0.73**                                   |
| TG (U/L)                      | 75.97±1.70      | 180.7±1.60#                           | 102.02±2.31**                                  | 90.62±1.04**                                   |
| Initial b.wt.(g)              | 180±5.99        | 163.8±5.19#                           | 180±4.04**                                    | 193.2±1.62**                                   |
| Final b.wt.(g)                | 205±1.92        | 157.5±9.09#                           | 190.02±4.09**                                  | 229.2±6.68**                                   |

All the values are expressed as mean±SEM(n=6), where * indicates p<0.01 as compared with respective control group I; ** indicates p<0.05, *** indicates p<0.01 as compared with respective group II.

| Group | Thioptentone sodium induced sleeping time | Liver wt.(g) | Relative liver wt. (Liver wt./100gb wt.) |
|-------|------------------------------------------|--------------|----------------------------------------|
| Group I | 202.50±4.96 | 76.67±4.94 | 6.09±0.32 |
| Group II | 53.33±4.22* | 243.33±7.77* | 8.64±0.62* |
| Group III | 163.57±5.2** | 32.50±8.83** | 6.98±0.59** |
| Group IV | 179.17±9.4*** | 120.63±6.4*** | 6.08±0.40*** |

All the values are expressed as mean±SEM(n=6), where * indicates p<0.01 as compared with respective control group I; **=p<0.001 and ***=p<0.005 as compared with respective group II.

DISCUSSION:

Liver is one of the vital organ of animal body and plays a central role in transforming and clearing the chemicals, but it is susceptible to the toxicity from these agents.13 Liver diseases which are still a global health problem may be classified as acute or chronic hepatitis, hepatosis, and cirrhosis. Unfortunately, treatments of choice for liver diseases are controversial because the conventional or synthetic drugs for the treatment of these diseases are insufficient and sometimes cause serious side effects.14,15 Since ancient times, mankind has made use of plants in the treatment of various ailments because their toxicity factors appear to have lower side effects.16 Many currently available drugs were derived either directly or indirectly from medicinal plants. Recent interest in natural therapies and alternative medicines has made researchers pay attention to the traditional herbal medicine.17 In this regard the present study was carried out to find the hepatoprotective potential of polyherbal formulation on alcohol induced hepatotoxicity.
Liver can be injured by many chemicals and drugs. In the present study ethanol was selected as a hepatotoxicant to induce liver damage, since it is clinically relevant. Ethanol produces a constellation of dose related deleterious effects in the liver. In chronic alcoholics, hepatomegaly occurs due to accumulation of lipids and proteins in hepatocytes, with an impaired protein secretion by hepatocytes. Water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells, resulting in increased total liver mass and volume as observed in present study. This alcohol induced increase in total wet-liver weight was prevented by treatment with polyherbal formulation, thus indicating a hepatoprotective effect.

The hepatoprotective activity of the polyherbal formulation was monitored by estimating serum transaminases, serum alkaline phosphatase and bilirubin which are indicators of the functional state of liver. The increase in the levels of serum bilirubin reflected the degree of jaundice, while increase in hepatic enzymes indicate cellular leakage and loss of functional integrity of cell membrane. It has been found that polyherbal formulation effectively prevents ethanol induced biochemical changes of liver toxicity.
CONCLUSION:

In conclusion, the present study has demonstrated that polyherbal formulation possesses hepatoprotective effect as it exhibited protective effect against alcohol induced hepatotoxicity in wistar rats demonstrated by significant decrease in AST, ALT, ALP, LDH, cholesterol, bilirubin, TG and increase in TP concentration and prevention of alcohol induced histopathological changes in liver.

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CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

REFERENCES:

1. Hartmut J, Gregory JG, Arthur IC, Jack AH, Dominique P, John JL. Mechanisms of hepatotoxicity. Toxicology Science 2002; 65:166–76.
2. JL. Kurian, Plants that heals, oriental writer publishing house, Pune, 2007; 1,53.
3. Joseph T, Dipiro, et al. Pharmacotherapy – A Pathophysiological Approach, 6th Edn, McGraw – Hill Medical Publishing Division PP 729.
4. Osawa BH, Kavakshi S, Namiki M. In Kuroda Y, Shanakal DM, Pharmacy and Research, Parul University and Chairman of Vasu Research Center, Vadodara for providing necessary facilities to carry out this research work.
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