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

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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, liver weight(wt.) and relative liver wt. levels while significant ($p<0.01$) increase 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

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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.[1] It also detoxifies a variety of drugs and xenobiotics and secreates bile that has an important role in digestion.[2] Chronic liver disease represents the fourth leading cause of death among all races and sexes in the 45-54 year age group.[3] 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.[4] Alcohol is the most abused substance worldwide and a significant source of liver injury.[5] Long term alcohol consumption induces oxidative stress in the liver due to imbalance between prooxidant and antioxidant system.[6] The major toxic metabolites of ethanol are acetaldehyde and free radicals.[7] Three pathologically life-threatening liver diseases induced alone by alcohol abuse are fatty liver (steatosis), hepatitis and cirrhosis.[8] Oxidative stress is a key step in the pathogenesis of tissue injury by ethanol generating reactive oxygen species(ROS) in many tissues and decreasing in the endogenous antioxidants.[9] Numerous medicinal plants and their formulations are used for liver disorders in ethno medical practices as well as in traditional systems of medicine in India.[10] Today, it is estimated that about 80% of the world population relies on botanical preparations as medicine to meet their health needs.[11] In the traditional system of Indian medicine, plant formulation and combined extracts of plants are used as a drug of choice rather than individually. Various herbal formulations are well known for their hepatoprotective effects.[12] Realizing the fact, this research was undertaken to evaluate the hepatoprotective activity of the polyherbal formulation against alcohol induced hepatic damage in wistar albino rats. One of such polyherbal formulation manufactured by Vasu Healthcare Ltd. is composed of five medicinal palnts. Despite of its

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
			Triphla churna	-	formulation
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 used were of analytical reagent quality.

Animals

Albino wistar rats of either sex weighing between 200-220gm 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, centrilobular 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 2000mg/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±o.535/100g b.wt. Administration of polyherbal formulation 180mg/kg/day significantly (p<0.05) reduced the relative liver weight to 2.73±0.120g b.wt. as as compared to that of alcohol treatment with that of 4.34±0.095/100g b.wt Polyherbl 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±4.37U/L, P<0.01) as compared to normal control group I rats (80.43±1.50U/L, 87.08±0.8U/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(84.40±1.59U/L), ALP (91.67±0.91U/L) 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 U/L, 78 67.76±2.10 U/L and 124.48±1.30 U/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(1.38±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±0.06), while decreased TBL(0.81±0.02), DBL(0.36±0.01), cholesterol(117.78±1.18) as compared to group IV silymarin 100mg/kg/day significantly increased TP(4.94±0.09 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±1.16U/L) as compared to control group (75.97±1.70U/L). The polyherbal formulation treated group III significantly decreased (102.02±2.31U/L) as compared to group IV silymarin(90.62±1.04 U/L) both compared to PCM treated group.

Table 2: Effect of Polyherbal formulation on different biochemical parameter in alcohol induced hepatotoxicity.

Treatment groups and liver specific variables	I Normal control: 1% CMC 1 ml/kg b.wt.	II Hepatotoxic control: 1% CMC 1 ml/kg b.wt + ethanol (3.76g/kg/day, p.o.)	III Polyherbal formulation 180mg/kg/day + ethanol (3.76 g/kg/day, p.o.)	IV Silymarin 100 mg/kg b.wt.+ ethanol(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±4.37#	143.29±1.03**	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**
DBL(mg/100ml)	0.24±0.01	0.92±0.05#	0.36±0.01**	0.29±0.015**
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±1.16#	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±10.92	157±5.90#	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; ns indicates p>0.05, **p,<0.01 as compared with respective group II.

Tabel 3: Effect of Polyherbal formulation on thiopentone induced sleeping time and weight of liver.

Group	Thiopentone sodium induced sleeping time		Liver wt.(g)	Relative liver wt. (Liver wt./100gb.wt.)
	Onset (s)	Duration (Min)		
Group I	202.50±4.96	76.67±4.94	6.09±0.32	3.12±0.08
Group II	53.33±4.22a	243.33±4.77 a	8.64±0.62 a	5.52±0.51 a
Group III	163.5±7.25***	132.50±8.83***	6.98±0.59***	3.57±0.27***
Group IV	179.17±9.44***	120.63±6.64***	6.08±0.40*	2.54±0.092*

All the values are expressed as mean±SEM(n=6), where ap<0.001 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.

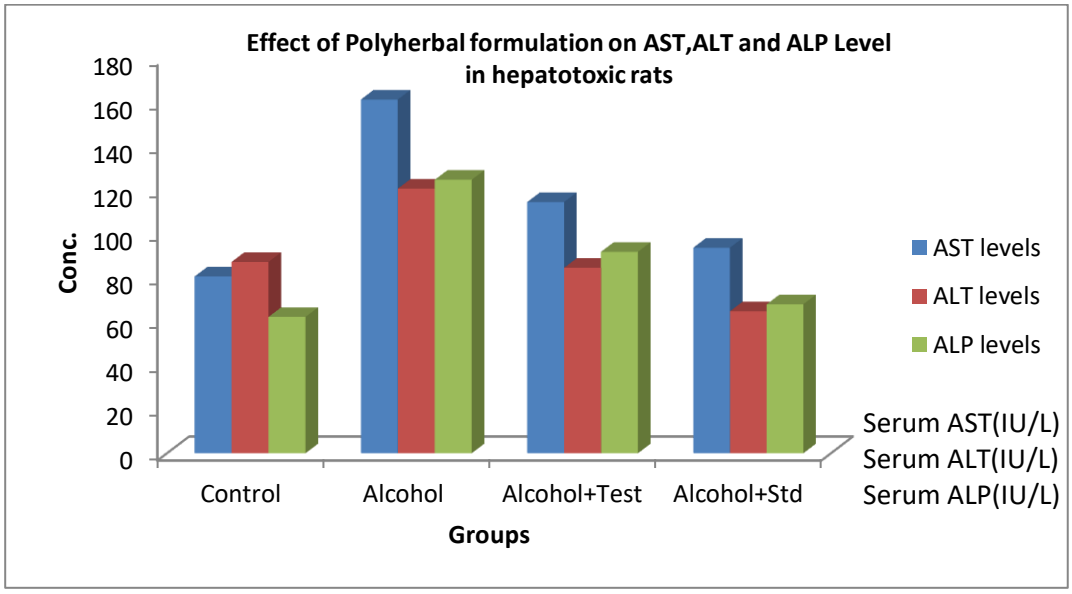

Fig. 1: Effect of Polyherbal formulation on serum aspartate amino transferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) level in hepatotoxic rats

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.[18] In chronic alcoholics, hepatomegaly occurs due to accumulation of lipids and proteins in hepatocytes.[19] with an impaired protein secretion by hepatocytes.[20] Water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells, resulting in increased total liver mass and volume[21] 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.[22] 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.[23] It has been found that polyherbal formulation effectively prevents ethanol induced biochemical changes of liver toxicity.

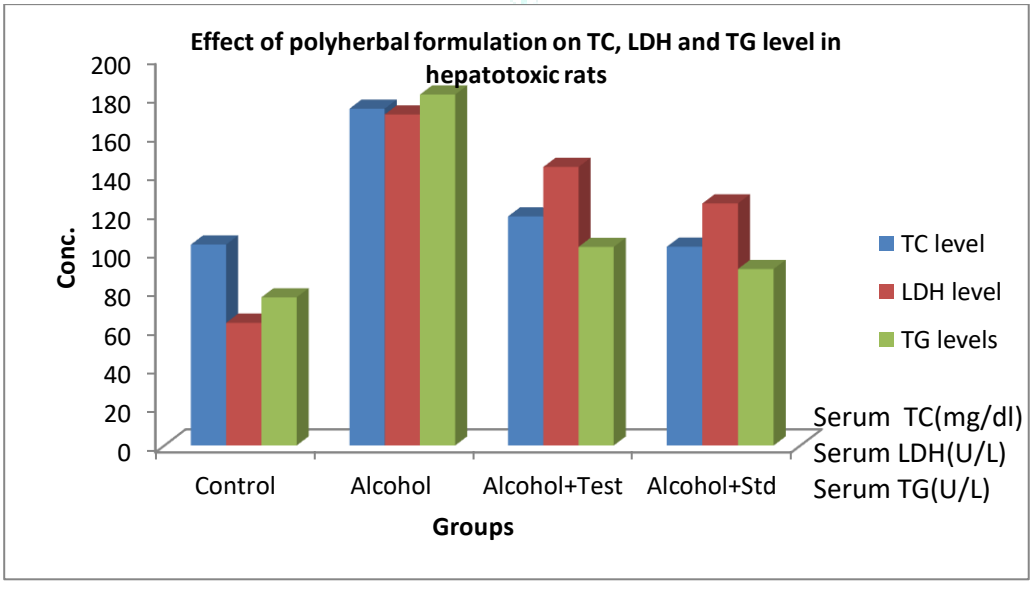

Fig. 2: Effect of Polyherbal formulation on serum total cholesterol(TC), lactate dehydrogenase(LDH) and triglycerides(TG) level in hepatotoxic rats.

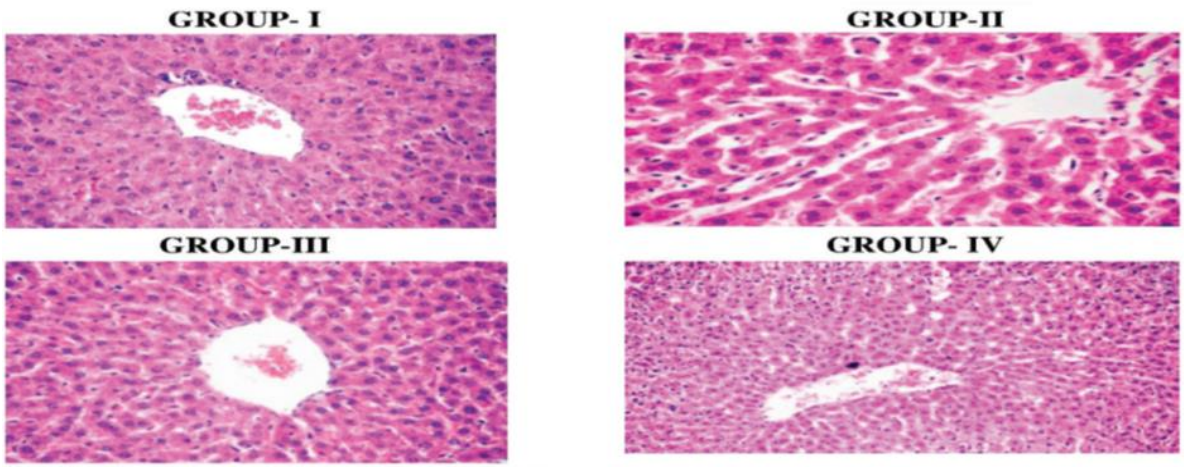

Fig 3: Effect of Polyherbal formulation on histopathological changes induced by alcohol in rats. (1) Group I: control group,(2) Group II: Animals treated with ethanol (3.76 g/kg/day, p.o.) (3)Group III: Animals treated with Polyherbal formulation (180mg/kg) (4)Group IV: Animals treated with Silymarin (100mg/kg)

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.

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