International Journal of Medicine and Pharmaceutical Research
CODEN (USA): IJCPNH | ISSN: 2321-2624
Journal Home Page: www.pharmaresearchlibrary.com/ijmpr

Preparation and evaluation of polyherbal formulation for its hepatoprotective activity against ethanol mediated paracetamol induced intoxication rat model

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A B S T R A C T

Objective: The present work was executed to evaluate the hepatoprotective potency of a polyherbal preparation. The objective of this study is to induce experimental hepatotoxicity using ethanol mediated paracetamol in normal Albino wistar rats and study the hepatoprotective activity of polyherbal formulation by comparison of changes in levels of liver parameters between normal and hepatic intoxication rats. **Methods:** The effect of methanol extract of poly herbal preparation containing Pods of *Moringa oleifera,* Fruits of *Piper Longum* and Seeds of *Hordeum Vulgare* was investigated in ethanol mediated paracetamol induced intoxication rat model. **Results:** Ethanol mediated Paracetamol administration caused severe hepatic damage in rats as evidenced by elevated serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin levels and cholesterol. The selected hepatic parameters after the oral administration of 150 and 300 mg/kg b. w of poly herbal formulation was significantly lower the blood glucose levels. **Conclusions:** The display of synergy or antagonism by the composite herbal extracts in ameliorating liver toxicity depended on the type and number of individual herbal extract used in constituting the experimental herbal formulations.

A R T I C L E I N F O

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A R T I C L E H I S T O R Y: Received 25 Nov 2020, **Accepted** 31 Jan 2021, **Available Online** 10 February 2021

Citation: *A. Prathyusha, et al.* Preparation and evaluation of polyherbal formulation for its hepatoprotective activity against ethanol mediated paracetamol induced intoxication rat model. *Int. J. Med. Pharm. Res., 2021, 9(1): 28-33.*

CONTENTS

1. Introduction

Liver is the largest not only in organ size but also in functions. Liver has more than 500 separate functions including synthesis, secretes, excretes, stores, generates, metabolises, protects and detoxicates various substances, etc. Any type of injury (due to systemic drugs, food preservatives, agrochemicals and addiction to alcohol) or

impairments of its functions may lead to many complications in one's health. There is a no rational therapy available for liver disorder, and it is a still challenge to modern medicine[1]. Many environmental and therapeutic agent produce hepatic injury when inhaled, ingested or administered parentally. The therapeutic agents damaging the liver are in general, not true hepatotoxins but cause injury by sensitization reactions[2].

Hepatic injury can be life threatening when the entirely or most of the liver is exposed to any hepatotoxin, including Carbon tetrachloride. It is used to study hepatotoxic potential because it is life threatening when an entire liver or most of the liver is exposed to carbon tetrachloride; this requires metabolic activation, particularly by liver cytochrome P-450 enzyme, to form reactive toxic metabolites that in turn cause liver injury in experimental animals and humans[2-4].

The location of liver is defined mainly by the biotransformation of carbon tetrachloride, which is cytochrome P-450-dependent. Free radical initiates the process of lipid peroxidation, which generally leads to the inhibition of enzyme activity [4-7].

The present study was conducted on Preparation and evaluation of polyherbal formulation for its hepatoprotective activity against ethanol mediated paracetamol induced intoxication rat model.

2. Material and Methods

Chemical and drugs

Hepatic biomarkers estimate kits such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin (TBR) were procured from Erba diagnostics from local suppliers, in Guntur, India. Nitrobluetetrazolium (NBT) purchased from Sisco research labs Pvt Ltd, Mumbai and 2-deoxy-D-ribose and 2, 2-diphenyl-1-picrylhyrazil (DPPH) procured from Sigma chemicals. We received a gift sample of Silymarin from Micro labs, Bangalore, India. The remaining chemicals and reagents which were used in this study were of analytical grade.

Experimental animals

Mahaveer enterprises, Hyderabad, India, supplied Albino rats Wistar strain weighing 180 to 250 g. These rats were kept in our animal house at the desired temperature of (23±2)^{o}C with a humidity of 50-60 % was maintained. The darkness and light cycle of 12 hours: 12 hours was also considered. They were fed sumptuously with pellet diet and water at regular intervals. The research lab has approval from CPCSEA, Govt. of India (Regd No. HCOP/IAEC/PR-2/2018) and the conducted study was in accordance with the Institutional Animal Ethics Committee (Hindu College of Pharmacy- Guntur)

Preparation of poly herbal tablets

All the factual extracts and excipients were passed at the hand of British Standard Sieves (BSS) #120 more above mentioned to use. The prescribed quantities of Pods of *Moringa oleifera (50g),* fruits of *Piper Longum (50g)* and seeds of *Hordeum Vulgare (50g)* were weighed accurately via an electronic offset and dissolved in 1 ml of isopropyl liquor and mixed by all of 10g of glucose and 4 g of microcrystalline cellulose. The heap was dried at 500^{0}C for 30 minutes.

The agglomeration was passed again over BSS # 40 to earn granules, which were weighed. The granules were easily lubricated by the whole of magnesium stearate (3% w/w) and purified talc (1% w/w) and characterized for the fines, biggest slice of the cake density and extricate of repose. Poly herbal tablets containing three bioactive extracts (each 50 mg) were skilled. Round shaped tablets, each weighting 500 mg were compressed by a six station-tableting machine.

Screening of* hepatoprotective activity of Poly Herbal Formulation*:

Albino Wister rats are divided into 5 groups each group containing 6 rats. Group A was treated with 1% CMC suspension, Group B was maintained with ethanol 3.7 g/kg for 7days and on day 8th PCM 2 g/kg was administered. Group C, D and E maintained with 3.7 g/kg for 7 days followed by administration of 2 g/kg PCM on 8th day and 8th day onwards Group C, D and E received different doses of polyherbal syrup and silarin-140 mg tablet till 14th day respectively. The treatment protocol was summarized and given below.

Group A - Normal control, 1 % CMC suspension orally, 1ml/kg once daily for 7 days

Group B - Ethanol 3.7 g/kg for 7 days and on 8th day Paracetamol 2g/kg followed by 1ml/kg of 1 % CMC once daily p.o from 8th day to 14th day.

Group C - Ethanol 3.7 g/kg for 7 days and on 8th day Paracetamol 2g/kg followed by 150 mg/kg dose of polyherbal tablet once daily p.o from 8th day to 14th day.

Group D - Ethanol 3.7 g/kg for 7 days and on 8th day Paracetamol 2g/kg followed by 300 mg/kg dose of polyherbal tablet once daily p.o from 8th day to 14th day.

Group F - Ethanol 3.7 g/kg for 7 days and on 8th day Paracetamol 2g/kg followed by 140 mg/kg dose of Silarin once daily p.o from 8th day to 14th day.

Statistical analysis:

Results were expressed as Mean ± SEM, (n=6). Statistical analysis was performed with one way analysis of variance (1 way ANOVA) followed by Bonferroni's multiple comparison tests using Graph Pad Prism-5 software. P value less than 0.05 was considered to be statistically significant. $^{###}$=P<0.001 when Group B compared with Group A and *=P<0.05, **=P<0.01, ***=P<0.001 and ns = not significant when rest of Groups compared with Group B.

3. Results and Discussion

Effect of poly herbal formulation on the biochemical parameters

Alanine aminotransferase levels (ALT or SGPT):

The SGPT levels animals treated with polyherbal formulation at a dose of 150 mg/kg and 300 mg/kg produced 82.62% and 93.51% protection respectively against ethanol mediated paracetamol intoxication. Whereas the standard silarin at a dose of 140 mg/kg produced 100.32% protection against ethanol mediated paracetamol intoxication. The results were compiled in table no.1, 2&3 and graphically represented in histogram no.1.

Aspartate aminotransferase levels (AST or SGOT):

The SGOT levels animals treated with polyherbal formulation at a dose of 150 mg/kg and 300 mg/kg produced 88.28% and 96.79% protection respectively against ethanol mediated paracetamol intoxication. Whereas the standard silarin at a dose of 140 mg/kg produced 99.91% protection against ethanol mediated paracetamol intoxication. The results were compiled in table no.1, 2&3 and graphically represented in histogram no.1.

Alkaline phosphatase levels (ALP):

The ALP levels animals treated with polyherbal formulation at a dose of 150 mg/kg and 300 mg/kg produced 86.58% and 101.12% protection respectively against ethanol mediated paracetamol intoxication. Whereas the standard silarin at a dose of 140 mg/kg produced 99.41% protection against ethanol mediated paracetamol intoxication. The results were compiled in table no.1, 2&3 and graphically represented in histogram no.1.

Total bilirubin:

The total bilirubin levels animals treated with polyherbal formulation at a dose of 150 mg/kg and 300 mg/kg produced 78.19% and 92.72% protection respectively against ethanol mediated paracetamol intoxication. Whereas the standard silarin at a dose of 140 mg/kg produced 95.64% protection against ethanol mediated paracetamol intoxication. The results were compiled in table no.1, 2&3 and graphically represented in histogram no.1.

Cholesterol levels:

The cholesterol levels animals treated with polyherbal formulation at a dose of 150 mg/kg and 300 mg/kg produced 57.08% and 92.69% protection respectively against ethanol mediated paracetamol intoxication. Whereas the standard silarin at a dose of 140 mg/kg produced 90.61% protection against ethanol mediated paracetamol intoxication. The results were compiled in table no.1, 2&3 and graphically represented in histogram no.1.

Histopathological studies:

Histopathologically, the non-PCM-intoxicated liver pretreated with 1% CMC (normal) shows normal lobular architecture and normal hepatic cells with well preserved cytoplasm and well defined sinusoids line and nucleus around the perivenular area (Figure.no.1, Group:A). The section of ethanol mediated paracetamol intoxicated liver demonstrates infiltration of lymphocytes, the presence of steatosis, haemorrhage and extensive coagulative necrosis of the perivenular, and midzonal region with periportal sparing (Figure.no.1, Group: B). Coagulative-type necrosis of hepatocytes in ethanol mediated paracetamol induced liver toxicity is present predominantly in the perivenular zone (zone 3). These pathological changes were found to be lesser as the dose of polyherbal formulation increased indicating the extract ability to reverse the ethanol mediated paracetamol induced intoxication (Figures.no.1, Group: C, D & E). Table.no.4 shows the histopathological scoring of the liver tissues pretreated with the respective test solution. Interestingly, the presence of marked steatosis, necrosis, inflammation, and hemorrhage following treatment with ethanol mediated paracetamol (shown by the negative control group) was reduced remarkably when pretreated with the polyherbal formulation or silarin.

Discussion

Historical literatures reveals that knowledge recording liver diseases existed since Brahmic period as this was mentioned in Ayurvedic text books Sushruta samhita written in fourth and fifth centuries B.C. even the treatment in the Indian ancient pharmacopoeia mentioned precise treatment for the two types including dietary modification, medicinal plants remedies and minerals [8-12]. More ever, the researchers conducted over the lost several decades as shown plant and plant-based therapies have a potential to manage and treat liver disorders[29] and its complications[13-16]. Although biomedical science has unraveled substantially the pathologically processes involved in causing/fostering diabetes and has designed therapeutic agents with a range of action to fight hyperglycemia, the efficacy of these therapeutic agents is compromised in several ways[17-19]. Individual agents or herbs act only on part of the pathogenic process and only to a partial extent. This may be the reason that even after so much advancement in understanding the disease process and availability of a wide range of therapeutic agents, the disease is still progressing[20, 21]. For testing hepatoprotective potential of plants, CCL_4, paracetamol and ethanol induced hepatotoxicity in rodents is considered to be good preliminary screening models and is widely used.

In the present study, the polyherbal preparation has shown significant hepatoprotective action by two doses (150, 300 mg/kg b.w) and proved to be effective in ethanol mediated induced hepatic damage in animals. It is very difficult to mention which of the extract was more responsible for this favorable response. According to Ayurvedic texts, a combination of herbs or herbal extracts are substances are used to get the enhanced desired action and eliminate unwanted side effects.

Table No.1: Basal levels of selected liver biochemical parameters and physical parameters in all group rats; on day 0 (before treatment) in curative study of ethanol mediated paracetamol induced hepatotoxicity

Group/ Treatment	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	T.BIL (mg/dl)	CHOL (mg/dl)
Group A: Vehicle control (1% Sodium CMC suspension)	43.39±0.98	83.43±0.79	124.84±0.93	0.41±0.01	87.52±1.64
Group B: Toxicant control (Treated with Ethanol 3.7 g/kg + PCM (2 g/kg)	43.66±0.81	83.51±0.54	122.89±0.93	0.43±0.01	82.06±2.18
Group C Treated with Ethanol 3.7 g/kg + PCM (2 g/kg) + PHF (150 mg/kg)	42.64±0.88	82.87±0.91	124.44±0.99	0.42±0.01	89.15±2.72
Group D Treated with Ethanol 3.7 g/kg + PCM (2 g/kg) + PHF (300 mg/kg)	42.16±0.84	83.27±0.98	124.42±0.95	0.43±0.02	84.16±3.01
Group E Treated with Ethanol 3.7 g/kg + PCM (2 g/kg) + Silarin (140 mg/kg)	42.86±0.90	82.59±1.05	124.20±0.92	0.42±0.01	91.57±2.43

Table No.2: Influence of Polyherbal formulation on selected liver biochemical parameters and physical parameters in all group rats; on day 15th in curative study of ethanol mediated paracetamol induced hepatotoxicity

Group/ Treatment	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	T.BIL (mg/dl)	CHOL (mg/dl)
Group A: Vehicle control (1% Sodium CMC suspension)	44.72±0.89	84.69±0.73	125.80±0.93	0.42±0.01	86.69±0.73
Group B: Toxicant control (Treated with Ethanol 3.7 g/kg + PCM (2 g/kg)	322.50±1.51 ###	369.10±1.52 ###	408.67±0.96 ###	3.17±0.03 ###	199.32±3.09 ###
Group C Treated with Ethanol 3.7 g/kg + PCM (2 g/kg) + PHF (150 mg/kg)	93.02±2.86 ***	118.01±1.96 ***	163.74±2.15 ***	1.02±0.08 ***	135.04±3.63 ***
Group D Treated with Ethanol 3.7 g/kg + PCM (2 g/kg) + PHF (300 mg/kg)	62.75±0.91 ***	93.81±1.21 ***	122.68±1.06 ***	0.62±0.01 ***	94.92±2.57 ***
Group E Treated with Ethanol 3.7 g/kg + PCM (2 g/kg) + Silarin (140 mg/kg)	43.82±1.53 ***	84.85±1.62 ***	127.45±1.60 ***	0.54±0.01 ***	97.26±3.71 ***

Data represented as Mean values of six rats ± S.E.M; Where, # indicates P <0.05, ##indicates P<0.01 and ###indicates P<0.001 as compared Group B with normal control group (Group A); *indicates P<0.05, **indicates P<0.01 and ***indicates P<0.001 as compared rest of groups with Group B treated with alcohol + Paracetamol. Note: PHF: Polyherbal formulation.

Table No.3: Percentage protection of Polyherbal formulation and Silarin against ethanol mediated paracetamol induced hepatotoxicity.

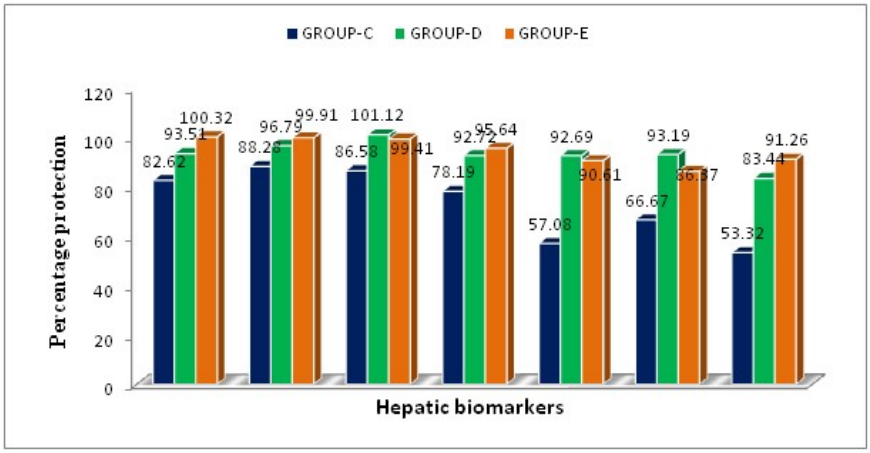
Group/ Treatment	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	T.BIL (mg/dl)	CHOL (mg/dl)
Group C Treated with Ethanol 3.7 g/kg + PCM (2 g/kg) + PHF (150 mg/kg)	82.62	88.28	86.58	78.19	57.08
Group D Treated with Ethanol 3.7 g/kg + PCM (2 g/kg) + PHF (300 mg/kg)	93.51	96.79	101.12	92.72	92.69
Group E Treated with Ethanol 3.7 g/kg + PCM (2 g/kg) + Silarin (140 mg/kg)	100.32	99.91	99.41	95.64	90.61

Table No.4: Histopathological evaluation of the effect of polyherbal formulation and silarin against ethanol mediated paracetamol induced hepatotoxicity in rats.

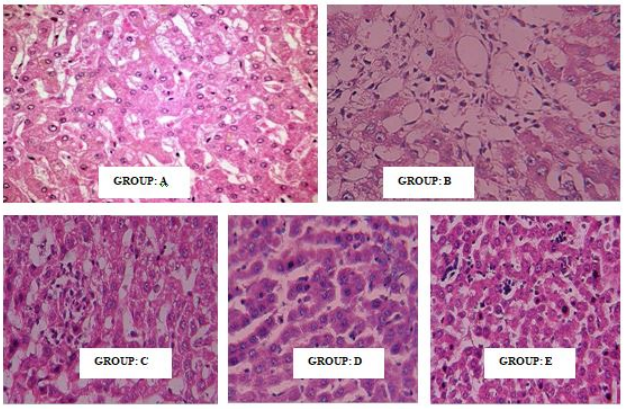
Group/ Treatment	Steatosis	Necrosis	Inflammation	Haemorrhage
Group A: Vehicle control (1% Sodium CMC suspension)	--	--	--	--

Group B: Toxicant control (Treated with Ethanol 3.7 g/kg + PCM (2 g/kg)	++	+++	+++	++
Group C Treated with Ethanol 3.7 g/kg + PCM (2 g/kg) + PHF (150 mg/kg)	+	++	++	+
Group D Treated with Ethanol 3.7 g/kg + PCM (2 g/kg) + PHF (300 mg/kg)	-	+	-	-
Group E Treated with Ethanol 3.7 g/kg + PCM (2 g/kg) + Silarin (140 mg/kg)	-	+	+	-

The severity of various features of hepatic injury was evaluated based on those following scoring schemes: −: normal, +: mild effect, ++: moderate effect, +++: severe effect.



Histogram.No.1: Percentage protection of Polyherbal formulation and Silarin against ethanol mediated paracetamol induced hepatotoxicity.



Figures.no.1: *A photomicrograph of hepatic tissue sections.*

4. CONCLUSION

Thus, our study findings demonstrate the antidiabetic effect of the polyherbal formulation at the dose levels of 150 and 300 mg/kg. The hepatoprotective potential of the polyherbal formulation is comparable with that of Silymarin, which is evidenced by decreased levels of selected hepatic parameters, An overview of the current results showed that the capacities of the herbal formulations to exert hepatoprotective activity in the animal models.

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