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Research Article

TOXICITY STUDIES OF A DEVELOPED HEPATOPROTECTIVE POLYHERBAL FORMULATION IN EXPERIMENTAL RATS

MONIKA SACHDEVA*, MEENAKSHI BAJPAI, B.K.RAZDAN

Uttarakhand Technical University, Suddhowala, Dehradun. Email: monikapharm10@yahoo.co.in

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ABSTRACT

Objective: In the present study acute, sub acute and sub chronic toxicity studies were performed on scientifically developed hepatoprotective polyherbal formulation (PF). PF consists of dried methanolic extracts of dried rhizome of Curcuma longa, dried leaves of Murraya koenigii, Nyctanthes arbortristis, and Occimum sanctum.

Methods: In acute toxicity study, PF was administered once orally at doses ranging from 250mg/kg -5000 mg/kg. Body weight and food consumption was noted for a period of 14 days. Animals were also observed daily for any behavioral or other toxic changes. In sub acute toxicity study animals were administered drugs in dose range of 500-2000 mg/kg p.o. for 28 days. Sub chronic toxicity study was also performed. Drug in dose range of 250-1000 mg/kg was administered once daily for 90 days. At the end of the study blood was withdrawn for hematology and biochemical estimations. Animals were then sacrificed and liver, kidney, heart and brain were dissected out which were observed for any gross morphological changes. Weight of organs was also noted.

Results: The results showed no evidence of any changes in body weight and food intake, hematological parameters, liver and kidney function test when compared with control. The organs did not show any evidence of gross morphological changes.

Conclusion: It is concluded that PF, at a dose of 1000 mg/kg, is safe for long term treatment of hepatic disorders

Keywords: polyherbal formulation, hepatoprotective, acute, sub acute, sub chronic toxicity

INTRODUCTION

Medicinal plants play a key role in the human health care .About 80% of the world population rely on traditional medicine which is based on plants.[1] Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. These drugs are either single plant extracts or fractions or mixtures of extracts from different plants. .These plant extracts are standardized for their safety and efficacy [2] Liver has a major role in regulation of physiological processes. Liver diseases are among the most serious ailment. The use of natural remedies for the treatment of liver diseases has a long history, starting with the ayurvedic treatment and extending to other systems of medicines. In spite of the availability of more than 300 preparations used in Indian system of medicine for the treatment of liver diseases only a small portion of hepatoprotective plants as well as formulations are pharmacologically evaluated for their efficacy. The 21st century has seen a paradigm shift towards evaluation of herbal products.[3]

A Polyherbal formulation was developed for its hepatoprotective activity (PF).It consists of dried methanolic extracts of dried rhizome of Curcuma longa, dried leaves of Murraya koenigii, Nyctanthes arbortristis, and Occimum sanctum. All the ingredients of herbal origin are used in traditional medicines to treat hepatic disorders, diabetes etc.

Curcuma longa which consists of curcumin is known to possess antioxidant, hepatoprotective, antimicrobial, anti-inflammatory effects.[4-6] Murraya koenigii consists of carbazole alkaloid is used for its antibacterial, analgesic, hepatoprotective, ant diabetic, hypolipidemic activities.[7-8] Nyctanthes arbotristis which contains flavanol glycosides is used for its antipyretic, hepatoprotective, antimicrobial and immunomodulating properties[9,10]. Occimum sanctum consists of mainly eugenol and is also known as Queen of Herbs. Its extracts are used in ayurvedic remedies for common cold, headache, stomach disorders, heart disease, and hepatic disorders etc [11, 12].

In addition to beneficial effects observed, it is also important to generate toxicological information of the test drug for ensuring safety on use, especially for longer periods. [13]

In the present study, acute, sub acute and sub chronic toxicity studies of the developed polyherbal formulation was investigated to assess its safety and tolerability for long term treatment of hepatic disorders.

MATERIALS AND METHODS

Extraction and Standardization.

The plant material was procured from a market in Old Delhi and authenticated at NISCAIR, New Delhi.

The quality of plant material was established as per monographs in Quality standards of Indian medicinal plants [14], Indian Pharmacopoeia and Ayurvedic Pharmacopoeia of India. Plant material was dried and coarsely powdered. Weighed quantity of the drugs was extracted separately in soxhlet with methanol.

The extracts were concentrated to dryness in a rotary vacuum evaporator and dried in lyophilizer under reduced pressure. The extracts were further standardized using TLC.

Experimental animals

Wistar albino rats with body weight 110-150 gm of either sex were used. They were kept in departmental animal house at 26 ±2 degree Celsius and relative humidity 44-56% with light and dark cycle of 12 hour in polypropylene cages. Animals were provided with standard rodent pallet diet and water ad libitum. All the procedures were reviewed and approved by IAEC.

Hepatoprotective activity of polyherbal formulation was carried by D-galactosamine induced hepatotoxicity in rats (400mg/kg i.p.). PF was found to have significant hepatoprotective activity at a dose level of 250 mg/kg.

Acute toxicity studies

A preliminary study was conducted to assess acute oral toxicity of PF which was administered as a suspension in 0.5% carboxymethylcellulose (CMC)

Albino rats of either sex were randomly distributed to four different groups with 6 animals in each group. The animals were fasted overnight and the drug was administered orally at dose of 250, 2000 & 5000 mg/kg of body weight as a suspension in 0.5% CMC. The animals were observed for mortality (twice daily) and clinical signs for first 30 min. 1hr., 2hr., 6hr., after dosing and thereafter once a day for continuous 14 days. Body weight and food intake was also noted at 0day.1 week and 2nd week

Group I Control, 0.5%CMC

Group II PF 250 mg/kg

Group III PF 2000 mg/kg

Group IV PF 5000 mg/kg

This study was carried out in accordance with OECD guidelines for testing of chemicals, Toxicity guideline no. 420, acute oral toxicity, fixed dose method [15]

Sub Acute toxicity studies

Wistar albino rats of either sex of 125-150 gm. were used and assigned to different groups, with six animals in each group.

Group I Control 0.5%CMC

Group II PF 500mg/kg

Group III PF 1000 mg/kg

Group IV PF 2000 mg/kg

Each group received the respective treatment p.o. once daily for 28 days. The rats were observed daily for mortality and clinical signs. Body weight and food intake of treated rats were recorded at initiation of the study and at I, II, III, & IV week This study was carried out in accordance with OECD guidelines for testing of chemicals, Toxicity guideline no. 407, repeated dose 28 day oral toxicity study in rodents.[16]

Sub chronic toxicity studies

A 90 day sub chronic oral toxicity study was conducted in accordance with OECD guidelines for testing of chemicals, Toxicity guideline no. 408,repeated dose 90 day oral toxicity study in rodents[17]

Wistar albino rats of either sex of 125-150 gm. were used and assigned to different groups with six animals in each group.

Group 1 Control 0.5%CMC

Group II PF 250 mg/kg

Group III PF 500 mg/kg

Group IV PF 1000mg/kg

Each group received the respective treatment p.o. once daily for 90 days. All the animals were observed twice daily for mortality and any behavioral changes. Body weight was noted on the day of commencement of the study and thereafter on 30, 60, and 90 days. Blood was withdrawn at the end of study i.e. 12 weeks. .Hematological parameters like hemoglobin (Hb) Red blood cell (RBC) count, Total leukocyte count (TLC), Differential leukocyte count (DLC) [18] was estimated using fully automatic blood cell counter. Serum was separated from blood by centrifugation and biochemical parameters like serum Aspartate Aminotransferase (AST), serum Alanine Aminotransferase (ALT) [19] Blood urea and serum creatinine [20] were estimated.

Animals were then sacrificed and liver heart, kidney and brain were dissected out. The organs were weighed and were observed for any gross changes.

Statistical analysis

Data is presented as the mean + S.E.M .Results were analyzed using one way ANOVA followed by Dunnett's t test.

RESULTS

Acute oral toxicity studies

Acute oral toxicity studies were carried out for PF up to a dose level of 5000mg/kg. The animals survived through out the experimental period and did not show any sign of toxic symptoms or any kind of behavioral changes immediately after dosing and during the period of 14 days. The findings of the study did not reveal any major adverse effect on the body weight through out the treatment period.

Sub acute toxicity studies

During sub acute toxicity studies PF treated groups did not show any significant changes in body weight, food intake at weekly intervals. Values of hematological parameters were found to be comparable with the control group

Sub chronic toxicity studies

Sub chronic toxicity studies were carried out with in a dose range of 250-1000 mg/kg.The drug was administered once daily for90 days.

Effect of PF on mortality

Treatment with PF produced no mortality, behavioral changes or toxic effects in the animals through out the period of 90 days.

Effect of PF on body weight

During the experimental period, all the animals from all the treated dose groups exhibited comparable body weight gain (17%-18%) with that of control group. (Table: 1)

Table1: It shows Body weights of rats after 90 days of administration of developed polyherbal Formulation

GROUPS	INITIAL(gm)	AFTER90 DAYS(gm)	% gain in body wt.
I	138.4±6.78	170.7±5.43	18.8
II	142.1±5.96	180.3±3.14	17.2
III	134.2±7.85	171.7±5.67	18.1
IV	141.5±5.90	173.4±4.48	18.2

Values are mean+S.E of 6 animals. One way ANOVA is used. Group I –control, group-II- PF, 250mg/kg, GroupIII- PF 500mg/kg, GroupIV-1000mg/kg

Effect of PF on hematological parameters

Estimation of hematological parameters rendered no significant changes as compared to control group. (Table: 2)

Table2: It shows Hematological findings after 90 days of administration of developed polyherbal formulation

GROUPS	Hb %	RBC mm³	WBCm³	DLC				
				N%	E%	B%	L%	M%
I	13.6±2.4	8.35±4.46	9.6±3.11	26.6±2.67	1.2±4.65	--	71.3±2.87	2.3±4.5
II	14.1±3.29	7.63±5.34	10.2±2.57	24.3±1.46	0.98±2.67	--	72.4±2.32	3.1±5.10
III	12.2±2.90	9.02±4.98	11.1±4.83	22.4±2.09	1.9±3.93	--	74.2±1.07	1.9±2.30
IV	11.6±3.01	8.91±6.76	10.0±4.02	25.6±3.23	1.5±5.44	--	71.5±3.56	3.21±3.53

Values are mean+S.E of 6 animals. One way ANOVA is used. Group I –control, group-II- PF, 500mg/kg, GroupIII- PF 1000 mg/kg, GroupIV-2000mg/kg N-neutrophils;E- eosinophils; B-basophils; L-lymphocytes; M- monocytes

Effect of PF on biochemical parameters

Repeated oral dose for 90 days did not cause significant changes in hepatic transaminase i.e. AST & ALT levels. Rena function was also evaluated by estimation of blood urea and serum creatinine levels. No significant changes were noted in treated groups as compared to control. (Table: 3)

Table 3: It shows Effect of 90 days administration of developed Polyherbal formulation in rats.

GROUPS	AST(IU/L)	ALT(IU/L)	BLOOD UREA (mg %)	SERUM CREATININE (mg %)
I	23.4±1.26	57.4±3.93	18.0±1.64	1.14±2.98
II	22.5±2.47	69.3±3.07	17.1±4.75	0.95±2.40
III	26.4±1.98	61.4±2.42	16.9±4.89	0.98±3.04
IV	23.8±2.26	60.9±3.28	18.2±3.46	1.02±1.69

Values are mean+S.E of 6 animals. One way ANOVA is used. Group I –control, group-II- PF, 250mg/kg, GroupIII- PF 500mg/kg, GroupIV-1000mg/kg

Effect of PF on organ weight and gross morphology

Treatment with developed PF did not induce any remarkable gross morphological changes in treated groups. Weight of isolated organs such as liver, kidney, heart and brain did not differ significantly from the control animals. (Table: 4)

Table 4: It shows Organ weight after 90 days of administration of developed polyherbal formulation in rats

GROUPS	LIVER(gm)	KIDNEY(gm)	HEART(gm)	BRAIN(gm)
I	7.98±2.87	1.49±1.98	0.64±2.88	1.24±3.76
II	8.24±3.68	1.23±3.76	0.62±1.47	1.15±4.13
III	8.36±4.09	1.42±3.34	0.59±3.57	1.31±3.61
IV	8.12±5.82	1.31±1.58	0.60±4.09	1.21±3.09

Values are mean+S.E of 6 animals. One way ANOVA is used. Group I –control, group-II- PF, 250mg/kg, GroupIII- PF 500mg/kg, GroupIV-1000mg/kg

DISCUSSION

Liver, one of the essential organs involved in metabolism and detoxification, is frequently challenged with numerous toxic assaults. Various herbal preparations have been shown to benefit in treating liver disease.

Herbal preparations, in spite of being safe drugs need to be authenticated by various toxicity studies before being introduced for consumption.

PF has been developed for treating liver disease. This formulation consists of ingredients of herbal origin which have been used as liver protective agents.

In the present study, acute oral administration of PF to rats at a dose level of up to 5000mg/kg did not cause any mortality or toxic symptoms.

Sub acute and Sub chronic i.e. repeated dose oral toxicity studies are carried to assess the safety of a drug which is to be used for a prolonged period of time. Treatment with PF orally daily for 90 days up to a dose level of 1000 mg/kg did not cause any mortality, behavioral changes or any toxic symptoms.

Guidelines of toxicity studies lay emphasis on reporting changes in body weight.[21] 10% decrease in body weight on chronic exposure has been fixed as an acceptable limit.[22] In this study, all the animals showed normal body weight gain till the end of study which shows that treatment with PF did not effect the normal health status of animals.

Hematopoietic system is one of the targets for toxic compounds and is an important index of physiological and pathological states [23] Treatment with PF had no significant effect on hematological parameters.

Treatment with PF in rats did not alter the hepatic and renal function as identified from the hepatic enzymes AST &ALT and renal markers such as serum creatine and blood urea [24, 25] which shows that the developed PF maintains the integrity of liver and kidney.

Another advantage of sub chronic toxicity testing is the information which could be gathered on specific organ toxicity on repeated administration. In this study, any of the treated animals with PF did not reveal any gross morphological changes in any of the organs.

Weight of the isolated organs of treated animals was also found to be comparable to control group.

CONCLUSION

So these studies suggest that the developed polyherbal formulation which was developed for its hepatoprotective activity at the dose level of 250mg/kg p.o. once daily is safe even at a very high dose of 1000mg/kg for long term treatment of hepatic disorders.

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