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Hepato-protective Effect of an Ayurvedic Formulation Prak-20 in CCl_4 Induced Toxicity in Rats: Results of Three Studies

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ABSTRACT
Prak-20 is a proprietary herbo-mineral ayurvedic formulation routinely used for years in the clinical practice of the first author in the treatment of liver ailments. Prak-20 is a judicious combination of nineteen herbs and Mandoor Bhasma. Three experimental studies were carried to evaluate its hepato-protective properties. In the first experiment, Prak-20 was compared vis-à-vis Liv52 in CCl_4 challenged Wister rats. The second study was a single blinded study of Prak-20 in CCl_4 challenged Wister rats. The third experiment was conducted to note the effect of different doses of Prak-20 in CCl_4 challenged albino rats. Serum alkaline phosphatase (ALP), serum alanine transaminase (ALT), serum aspartate transaminase (AST), along with liver histology was done in all animals at the completion of the first two studies. There was no significant elevation of ALT, AST and ALP levels despite CCl_4 exposure in Prak-20 treated animals. Similarly, the histology studies revealed that all animals of the treatment group had regenerating hepatocytes and no necrotic and degenerative changes were noticed in any animal. In the third experiment, ALT and AST levels were studied after 24 and 48 hours of experiment. There was 54 and 37 percent decrease in ALT, AST levels in CCl_4 plus Prak-20 (1.8 gm/d/rat) treated animals, respectively, as compared to CCl_4 treated animals. Prak-20 treated animals also had minimum necrotic changes after seven days. Based on the findings of aforesaid experiments which were carried at three centers, it may be concluded that Prak-20 is a potent hepato-protective herbo-mineral ayurvedic formulation. Further studies are required to understand its mechanism of action.

Keywords: Ayurveda, hepato-protective, herbo-mineral formulation.

INTRODUCTION

Liver play a major role in detoxification and excretion of many endogenous and exogenous compounds. Any impairment to its function may lead to serious implications and even death. Management of liver diseases is still a challenge to the modern scientific community. [1] There are few conventional drugs that can stimulate liver function and offer hepato protection or help in the regeneration of hepatic cells. [2] Globally, many herbal preparations are used in the treatment of liver disorders. [3] Phytoconstituents remain to be a major contributor in the treatment of liver disorders. Formulations available as remedy for hepatic disorders are poly herbals without elucidating the efficacy of individual herb of the formulation.

Prak-20 is a herbo-mineral ayurvedic formulation containing nineteen herbs and Mandoor Bhasma. [4] The herbs used in Prak-20 is well described in the ayurvedic treatises mentioned under schedule Y of the Drug & Cosmetic Act. [5] The hepato-protective effect of Prak-20 has been observed in clinical practice of the first author for over two decades. This formulation was also found to have anti-fibrotic properties [6] and helped in fast recovery of acute viral hepatitis patients. [7] The herbs used in Prak-20 have the following properties viz. hepatoprotective, improves liver and kidney function, relives hepatomegaly and splenomegaly, jaundice, cholagogue, anti-nauseant, anti-spasmodic, dyspepsia, anti-pyretic, appetizer and carminative. In this article, we report the observations of three animal experiments which were carried at three centers to study the hepato-protective properties of Prak-20 against CCl_4 induced liver injury.

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MATERIALS AND METHODS

Composition of Prak-20

Prak-20 is a patent and proprietary ayurvedic medicine prepared by Bharat Bhaishajya Shala Private Limited, Dehradun, under Good Manufacturing Practices (GMP) certificate issued by Department of AYUSH provincial government of Uttarakhand. In house standard for raw material and process quality control have been developed to

bring reproducibility of Prak-20. The composition of Prak-20 is given in the Table 1.

Experimental Animals: Adult rats (Wister / Swiss albino) were kept in polypropylene cages with stainless lid with rice husk bedding. Individual animal was identified by specific marking and cages were identified with label pasted on cages with relevant information. Animals were housed at a temperature of 20 ± 2°C and relative humidity of 25 to 75 %. A 12:12 light: dark cycle was followed. All animals had free access to water and standard pelleted laboratory animal diet.

Ethical Clearance: Three experiments were done in rats to evaluate the hepatoprotective potential in Carbon tetrachloride (CCl_4) induced hepato toxicity. The first experiment was carried out at India Institute of Toxicology, Mumbai. The second experiment was carried out at Fredrick Institute of Plant Protection and Toxicology, Padappai and the third study was carried out at G. B. Pant Hospital, Delhi. Prior to the start of these experiments, clearance from the Institutional ethical board was obtained from the respective institutes.

Table 1: Composition of Prak-20

Common name	Scientific name	Proportion
Sunthi	*Zingiber officinale*	13.88 mg
Maricha	*Piper nigrum*	13.88 mg
Pippali	*Piper longum* (fruit)	13.88 mg
Haritaki	*Terminalia chebulia*	13.88 mg
Vibhitaki	*Termnallia bellirica*	13.88 mg
Amalaki	*Emblica officinalis*	13.88 mg
Chitraka	*Plumbago zeylanica*	13.88 mg
Musta	*Cyperus rotundus*	13.88 mg
Katuki	*Picrorrhiza kurroa*	13.88 mg
Devadaru	*Cedrus deodara*	13.88 mg
Vidanga	*Embellia ribes*	13.88 mg
Kulu/Kushta	*Saussuria lappa*	13.88 mg
Haridra	*Curcuma longa*	13.88 mg
Daruharidra	*Berberis aristata*	13.88 mg
Danti	*Baliospermum montanum*	13.88 mg
Indrayav	*Holarrhena antidysentrica* (seeds)	13.88 mg
Pippali mula	*Piper longum*	13.88 mg
Trivrit	*Ipomoea turpethum*	13.88 mg
Punarnava	*Boerhavia diffusa*	27.77 mg
Mandoor Bhasma	Ferric Oxide	250 mg

Experimental study I: The aim of this study was to evaluate the hepatoprotective potential of Prak-20 vis-a-vis Liv52 in CCl_4 challenged Wister rats.

A total number of 32 animals were randomly divided into 4 groups. Except for the control group all the animals of other groups received subcutaneous injection of 0.2 ml of CCl_4 with 0.2 ml of liquid paraffin twice a week. The treatment schedule is given below:

Group I: Normal control.
Group II: CCl_4.
Group III: CCl_4 plus oral administration of 30 mg Prak-20 per day for 13 weeks.
Group IV: CCl_4 plus oral administration of 30 mg Liv52 per day for 13 weeks.

The animals were observed for 13 weeks. Their body weight and food intake was taken weekly. All the animals were sacrificed at the end of the experiment and a detailed necropsy was performed with particular emphasis on Liver. The livers were taken out and weighed and volume estimated by the displacement method. Serum alkaline phosphatase (ALP), serum alanine transaminase (ALT), Aspartate transaminase (AST), was estimated by using Boehringer Knoll Autoanalyser System 4010. Histopathology evaluation of the liver of each animal was done after completion of the experiment.

Experimental study II: This was a blinded study of Prak-20 coded as A (dark colour) and B (light colour) in CCl_4 challenged Wister rats.

A total of 30 female Wister rats were used for this experiment and were divided into 6 groups. The animals received either liquid paraffin (10 ml/kg body weight) or 1 ml of 50 % (v/v) CCl_4 in 9 ml of liquid paraffin/kg body weight i.p. every 72 hrs from day 0 to day 15. The treatment schedule is given below:

Group I: Liquid paraffin from day 0 to day 15. From day 16 to day 30, the animals were given 10ml/kg body weight of distilled water daily by oral intubation.
Group II: CCl_4 from day 0 to day 15.
Group III: CCl_4 from day 0 to day 15. From day 16 to day 30, the animals were treated with Prak-20 (Code A) 30 mg/kg body weight by oral intubation.
Group IV: Liquid paraffin from day 0 to day 15. From day 16 to day 30, the animals were given 30 mg/kg body weight of Prak-20 (Code A) by oral intubation.
Group V: CCl_4 from day 0 to day 15. From day 16 to day 30, the animals were treated with Prak-20 (Code B) 30 mg/kg body weight by oral intubation.
Group VI: Liquid paraffin from day 0 to day 15. From day 16 to day 30, the animals were given 30 mg/kg body weight of Prak-20 (Code B) by oral intubation.

Animal were sacrificed 24 hrs after the last treatment. Prior to sacrifice, blood was collected and plasma was separated for estimation of ALT, AST and ALP using a semi-auto analyzer. The animals were then euthanized and the liver was collected for histopathological examination.

Experimental study III: The aim of this study was to evaluate the hepatoprotective effect of various doses of Prak-20 in CCl_4 challenged rats.

Seventy albino rats were used for this study. Twenty animals were used as normal control. The rest 50 rats were randomly divided into 5 groups. All these animals were challenged with one subcutaneous injection of 0.5 ml of Carbon tetrachloride (CCl_4) per kg body weight. The details of treatment schedule are mentioned below:

Group I: Normal control
Group II: CCl_4
Group III: CCl_4 + Prak-20 (40 mg/day/rat) for 7 days
Group IV: CCl_4 + Prak-20 (600 mg/day/rat) for 7 days
Group V: CCl_4 + Prak-20 (1.2 g/day/rat) for 7 days
Group VI: CCl_4 + Prak-20 (1.8 g/day/rat) for 7 days

Blood samples were collected from all animals after 24 hrs and 48 hrs after CCl_4 administration and were analyzed for ALT and AST. After 7 days the animals were sacrificed and the liver was collected for histopathological examination.

Statistical analysis: The mean ± SEM were calculated for each parameter. The statistical analysis of the results was carried out with a SPSS 10.0 program based on an Analysis of Variance (ANOVA) followed by the Dunnet's test.

RESULTS

Experiment I:

Table 2: Histology and Mean ± S.E. of various parameters studied in CCl_4 challenged rats treated with Prak-20

Groups	N	Body Wt. (gm)	Liver Weight		Liver volume (ml)	AKP (IU/ml)	ALT (IU/ml)	AST (IU/ml)	Histology
			Absolute (gm)	Relative (%)					
I	8	253.25 ± 9.4	8.27 ± 0.23	3.27 ± 0.09	8.5 ± 0.31	89.05 ± 6.46	10.93 ± 0.95	42.75 ± 1.12	Within normal limits
II	8	262.86 ± 12.14	12.96 ± 0.64*	4.83 ± 0.12*	13.0 ± 0.67*	151 ± 23.40	37.09 ± 5.13*	53.14 ± 5.51	Centro-lobular necrosis with fatty changes
III	8	234.86 ± 11.60	9.45 ± 0.65	4.0 ± 0.16*	9.29 ± 0.69	104.74 ± 20.0	14.42 ± 4.36	42.21 ± 2.28	Devoid of degeneration of hepatocytes in all animals
IV	8	241.33 ± 11.46	10.15 ± 0.44	4.22 ± 0.11*	10.17 ± 0.37	92.73 ± 15.46	18.0 ± 4.71	39.75 ± 2.26	Moderate to severe degeneration in 50% and mild to moderate regeneration in 50%

* Differs significantly [P<0.05] from the control group

Table 3: Effect of two coded Prak-20 formulation on liver enzymes (Mean ± S.E.) and liver histology in CCl_4 challenged rats

Groups	No.	Treatment	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Histology
1	6	Control	75.9 ± 2.5	111.6 ± 3.77	60.1 ± 2.45	Within normal limits
2	6	CCl_4	130.5 ± 3.72*	150.1 ± 4.95*	86.0 ± 2.86*	Diffused swelling of hepatocytes along with necrosis
3	6	CCl_4 + Prak-20 (Code A)	84.0 ± 3.19	119.0 ± 3.31	65.2 ± 2.78	Significant improvement in necrotic condition
4	6	Prak-20 (Code A)	72.1 ± 2.37	120.1 ± 4.09	58.1 ± 1.77	Within normal limits
5	6	CCl_4 + Prak-20 (Code B)	83.0 ± 3.19	118.0 ± 3.31	65.2 ± 2.74	Significant improvement in necrotic condition
6	6	Prak-20 (Code B)	73.2 ± 1.96	118.1 ± 2.58	57.1 ± 1.72	Within normal limits

* Differs significantly [P<0.05] from the control group

Table 4: Effect of different doses of Prak-20 on ALT / AST (Mean ± S.E.) and liver histology in CCl_4 challenged rats

Exp. Group	No.	After 24 hrs of CCl_4		After 48 hrs of CCl_4		Histology done after 7 days of CCl_4 exposure
		AST IU/L	ALT IU/L	AST IU/L	ALT IU/L	
Control	20	29 ± 0.58#	26 ± 0.93#	38 ± 1.24#	31 ± 0.71#	Normal limits
CCl_4	10	114 ± 2.76*	289 ± 6.49*	97 ± 1.38*	166 ± 1.71*	Necrosis + 10 Inflammation +10 Fatty Liver + 10
CCl_4+ Prak-20 (40mg/d/rat)	10	98 ± 1.79*#	166 ± 1.73*#	109 ± 1.63*#	225 ± 1.57*#	Necrosis + 9 Inflammation + 9
CCl_4+ Prak-20 (600mg/d/rat)	10	108 ± 1.46*	152 ± 2.06*#	114 ± 1.76*	148 ± 2.31*#	Necrosis + 5 Inflammation + 4 Fatty Liver + 9
CCl_4+ Prak-20 (1.2 g/d/rat)	10	99 ± 0.93*#	158 ± 1.31*#	58 ± 1.71*#	72 ± 47*#	Necrosis + 3 Inflammation + 2 Fatty Liver + 5
CCl_4+ Prak-20 (1.8 g/d/rat)	10	53 ± 1.17*#	50 ± 1.73*#	62 ± 1.45*#	70 ± 1.87*#	Necrosis + 1 Fatty Liver + 2

* Differs significantly [P<0.05] from the control group
# Differs significantly [P<0.05] from the CCl_4 treated group

All the animals were studied for the gain in body weight. However, no significant variation in body weight was observed in any group at the end of study. Two animals (25 %) in group IV and one animal each from group II and III died during the experiment. All these animals died after 7 weeks of treatment.

Size, shape color and consistency of liver were almost normal in the control group. The liver of animals in group II showed slight to moderate fatty changes. In group III animals no remarkable changes were noticed except the paleness of liver was slightly less as compared to the CCl_4 treated animals of group II. In group IV there was no remarkable changes and the livers were comparable to the livers of group II animals. Significant increase in absolute liver weight and liver volume was found in animals treated with only CCl_4. These changes though seen in animals treated with Prak-20 and Liv52, however, they differ significantly from that of the control group (Table 2).

Significant increase in ALP levels and marked increase of ALT and AST levels were found in animals treated with CCl_4. However, no significant changes in the level of these enzymes were seen in animals treated with Prak-20 or Liv52.

Histopathological studies revealed that there was no abnormality in animals of the control group. In group II: three animals had typical centrolobular necrosis but not of severe degree; centrolobular necrosis with fatty changes was seen in three animals. In one animal necrosis of hepatocytes was seen in group of cells scattered in the lobule. In group III animals all the slide/section studied were devoid of degenerative and necrotic changes. Regenerating hepatocytes were seen in considerable number in almost all the areas of section. However, degree of regeneration varied from slight to moderate in different animals. Architectural pattern of the liver appeared to return to normalcy in all the animals. In group IV: two animals showed degenerative changes, necrotic areas with slight to moderate regenerative hepatocytes. Severe type of necrosis with degenerative changes was noted in one animal. Least degenerative changes and more active regenerative changes were observed in three animals.

Experiment II

Identical results were obtained with both the coded A and B Prak-20 formulation. The ALT, AST and ALP in plasma were significantly elevated in animal treated with CCl_4. These enzymes reverted back to normal levels in animals treated with Prak-20 (Table 3). The histopathological studies indicated diffused swelling of the hepatocytes along with

necrosis in CCl_4 treated animals. Significant improvement in necrotic condition was noted in animals treated with Prak-20.

Experiment III

The ALT and AST levels were significantly elevated in animals at 24 and 48 hrs after CCl_4 exposure. However, these enzymes were much lower in animals treated with Prak-20 at a dose of 1.2 gm/day and higher (Table 4). The best effect was seen in animals treated with 1.8 g of Prak-20 per day. There were 54 and 83 percent reduction in ALT and AST levels after 12 hrs and 37 and 58 percent reduction of ALT and AST levels after 48 hrs, respectively, as compared to the animals that received only CCl_4. The histopathological study indicated that in a 10 point scale the animals treated with CCl_4 had the maximum necrosis, inflammation and fatty liver. The necrosis was much less in animals treated with Prak-20 in dose of 600 mg/day and above (Table 4).

DISCUSSION

Prak-20 depicts strong hepatoprotection against CCl_4 induced liver injuries in three consequent experimental studies. It brings significant reduction in liver enzymes towards normalcy. Similarly, it not only arrests necrosis and degeneration but also brings considerable regeneration of hepatocytes.

Prak-20 is prepared using parts of 19 herbs and Mandoor Bhasma (MB). Majority of the herbs have been studied individually for their medicinal values such as, *Picrorhiza kurroa* has shown choleretic hepatoprotective, neuroprotective and immunostimulant properties. [8-9] *Curcuma longa* is well known for its antioxidant, hepatoprotective, anti-inflammatory, anticarcinogenic, and antimicrobial properties, in addition it is also used in cardiovascular disease and gastrointestinal disorders. [10] *Emblica officinalis* is found effective against inflammation [11], cancer, age-related renal disease [12], hepatitis [13] and diabetes. [14] Fruits of *Embelia ribes* possess analgesic, antipyretic, antibacterial, antifertility and anthelminthic activities. It has also been reported to be useful in jaundice and as a hepatoprotective agent. [15] Root of *Piper nigrum* is used as cholagogue in obstruction of bile duct and gall bladder.

Many studies so far have been conducted on establishing the efficacy of individual medicinal plants and their properties. In brief, 19 herbs used in Prak-20 have hepatoprotective, immune-modulator, anti inflammatory, anti fibrotic, improves liver and kidney functions, cholagogue, anti nauseant, anti spasmodic, anti pyretic, appetizer and carminative properties. Mandoor Bhasma is a major constituent of Prak-20. It is a powerful haematenic and is valuable in the treatment of hemolytic jaundice and microlytic anemia. The main focus of action of MB in body is liver. It is useful in the treatment of *Pleeha vriddhi* (Spleen enlargement); *Yakrit vriddhi* (Liver enlargement); *Kamala*, (Jaundice); Shotha (Oedema); Pandu, Raktaksaya (Anemia). [4]

Ayurvedic Pharmacopeias is full of compound formulations which are largely used by Ayurvedic physicians for centuries in their respective clinical practice. Prak-20 is modified and standardized form of a classical Ayurvedic formulation and has been used by the first author for decades in his clinical practice. This was used for treatment of patients suffering from jaundice and hepatomegaly. However, the chemistry of the finished product, maximum dose tolerance, safety and mode of action were never known to the author. Therefore, the first study was designed to revalidate the stated efficacy of Prak-20 in liver disorders by studying its effect against CCl_4 induced liver damage in animal model. This study showed cent percent regeneration of hepatocytes in Prak-20 treated animals after CCl_4 challenge. The other two studies were carried at different centers using the same toxin also indicated a similar trend. Prak-20 has emerged as potent hepatoprotective, anti-fibrotic, detoxifying and anti inflammatory Ayurvedic formulation. [16] It has been licensed for commercial manufacturing and is available in the market as prescription.

A vast literature is available regarding the substances of plant, mineral and animal origin used in the preparations of Ayurvedic formulations. Ayurvedic pharmacopeia also carries description of numerous Ayurvedic formulations and its uses on human beings. And this practice started centuries back and still continues.

In the aforesaid studies, authors tried to make an intervention between the stated efficacies of a compound Ayurvedic formulation within the ambit of modern research methodology of evaluation of a formulation. The findings credibly establish that the structured Ayurvedic formulations used in the treatment of liver disorders have strong hepato-protection activities. This opens a platform to design and carry more interventional, experimental and clinical studies to understand and development of intrigue composite Ayurvedic formulations.

CONCLUSION

Prak-20 has shown potential hepatoprotective activity against the CCl_4 induced liver damage in animal model. The observed effect of Prak-20 could be because of the synergistic effect of the various herbs used along with MB. The clinical use of Prak-20 as a hepatoprotector is substantiated in these experimental studies. However, more experimental studies and clinical trials are required to know the exact potential of this formulation.

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