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HEPATOPROTECTIVE ACTIVITY OF A STANDARDIZED POLYHERBAL LIVER FORMULATION

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Keywords:

Achillea millefolium, *Cichorium intybus*, *Picrorhiza kurroa*, *Capparis spinosa*, LivPro, Liver, Formulation, Standardization, Hepatoprotective

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ABSTRACT: The present study compiled the formulation and standardization of polyherbal liver syrup named "LivPro" followed by in-vivo and in-vitro hepatoprotective activity in CCl_4 intoxicated rat. LivPro was formulated by using the lyophilized ethanolic extract of *A. millefolium*, *C. spinosa*, *C. intybus*, and *P. kurroa*. Further, the standardization of LivPro was performed following the guidelines of WHO and Indian Pharmacopoeia. Wistar albino rats were treated with vehicle, silymarin, and LivPro (5, 10 and 15 ml/kg) continuously for 14 days. CCl_4 was administered on every alternate day for a week. Results postulated that LivPro had pH 5.04, specific gravity 0.8269; viscosity 78.01 poise, relative density 1.271 gm/ml, and refractive index 1.432. LivPro showed a rich quantity of flavonoid (480 mg/gm), total phenol (396 mg/gm) and alkaloid (103 mg/gm). The concentration of lead and arsenic in the tested formulation was found to be within the limit. The total plate count and viable aerobic microbial count in the formulation were 480, and 415 CFU/gm and the yeast and mold count was less than 100 CFU/gm. Pre and post-treatment of LivPro showed extremely significant ($P<0.001$) decrease in thiopental induced sleeping and 76.19% hepatoprotection in bromsulphthalein uptake test. Treatment with LivPro (10 and 15 ml/kg) resulted in significant normalization ($P<0.01$ to 0.001) of biochemical parameters except albumin. LivPro showed significant elevation ($P<0.001$) of glutathione and reduction ($P<0.001$) in lipid peroxidase enzymes in liver tissue. LivPro (15 ml/kg) treatment showed complete recovery of hepatocytes with reduced vaculation, clear cell lining as well as the absence of multinucleated giant cell.

INTRODUCTION: Drugs are rarely administered as pure chemical substance alone and are almost always given as formulated preparations or medicines. These can vary from a relatively simple solution to complex drug delivery systems through the use of appropriate additives or excipients in the formulations.

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The principal objective of dosage form design is to achieve a predictable therapeutic response of a drug in a formulation with reproducible quality. To ensure formulation quality, numerous features *viz.* chemical and physical stability, uniformity of dose, and acceptability to the users, including both prescriber and patient, are required.

Quality of herbal medicine directly affects their safety and efficacy. Method developments for quality control of polyherbal formulations are far more complex than chemical drugs due to the presence of a number of phytoconstituents from a wide range of the chemical class. Co-presence of several components, active as well as inert also

concert to provide a wide range of activity like therapeutic, prophylactic, rejuvenating and protective.

So to enjoy the domino effect of polyherbal formulation, we must have to overcome the standardization and quality control difficulties to identify the actual ingredients responsible for therapeutic effect as well as adverse effects. Resolving complicated technical issues towards the improvement of herbal medicines, quality control is essentially needed to decrease adverse events attributable to poor quality.

The liver plays a central role in transforming, clearing chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents, when taken in overdoses and occasionally when introduced within therapeutic ranges, may cause injury to the liver. More than 900 drugs have been implicated in causing liver injury [1,] and it is one of the most common reasons for a drug to be withdrawn from the market. Several mechanisms are responsible for either inducing hepatic injury or worsening the damage process. Many chemicals damage mitochondria, an intracellular organelle that produces energy. Its dysfunction releases an excessive amount of oxidants which in turn injures hepatic cells. Injury to hepatocytes and bile duct cells lead to accumulation of bile acid inside the liver [2].

In spite of the tremendous advances made in allopathic medicine, no effective hepatoprotective medicine is available. However, only a small proportion of hepatoprotective plants, as well as formulations used in traditional medicine, are pharmacologically evaluated for their safety and efficacy. Keeping the focus on above-mentioned shortcoming, we aimed to develop a polyherbal liver formulation which will fulfill the standards of safety, quality, and efficacy parameters. The plants selected for the present study were *A. millefolium* flowers, *C. spinosa* stems, *C. intybus* seeds and *P. kurroa* rhizomes well reputed in Indian system of medicine and widely used as hepatoprotective herbals from the ancient time

MATERIAL AND METHOD:

Plant Material and Chemicals: The flowers of *Achillea millefolium* L. (Ref. no. NISCAIR/ RHMD/Consult/-2012-13/2019/98), seeds of *Cichorium intybus* L. (Ref. no. NISCAIR/ RHMD/Consult/-2009-10/1243/47) and rhizomes of *Picrorhiza kurroa* Royle ex Benth (Ref. no. NISCAIR/RHMD/Consult/-2009-10/1233/37) were purchased from the Khari Baoli, New Delhi, India and identified by Dr. H. B. Singh, Scientist F and Head, Raw Material Herbarium and Museum, NISCAIR, New Delhi, India. Whereas, the stems of *Capparis spinosa* L. were procured from Medicinal and Aromatic Plants Project, Department of Agriculture Botany, Mahatma Phule Krishi Vidyapeeth, Ahmednagar, Maharashtra, India and taxonomically identified by Dr. Suresh Dhodake, Officer Incharge, Medicinal and Aromatic Plants Project, MPKV, Ahmednagar, Maharashtra, India (Ref. no. MAPP/certificate/27/2012). Bromo-sulphathalein was procured from HiMedia Lab. Ltd., Mumbai and thiopental were obtained as a gift sample from Neon Lab. Ltd., Mumbai. Other chemicals used were of analytical grade and were procured locally. Biochemical kits were obtained from Aspen Diagnostic Pvt. Ltd., India.

Preparation of Lyophilized Extracts: Coarse powder (1 kg) of each plant material was defatted individually with a sufficient quantity of petroleum ether (40-60°C) with the aid of Soxhlet apparatus for 24 hr. The defatted crude cakes were then extracted separately with ethanol for 48 h by cold maceration and then filtered through medical gauze. The respective filtrates were collected, concentrated near dryness using rotary vacuum evaporator, then lyophilized and used for the preparation of polyherbal liver syrup, LivPro.

Preparation of LivPro Syrup:

1. **Step 1:** The sugar base was prepared by adding 660 gm of sugar in 1000 ml of de-ionized heated water upto 90°C followed by mixing with a mechanical stirrer.

2. **Step 2:** Sugar base was mixed sequentially with sodium EDTA, citric acid, sodium methylparaben, and sodium propylparaben and filtered through nylon cloth (30 mof).

3. **Step 3:** Filtered sugar base was further mixed with sodium benzoate, bronopol, and then finally with the thiourea.

4. **Step 4:** The measured quantity of xanthan gum was added into the glycerin. The resulting mixture was kept aside and occasionally stirred to make it lump free and achieve a homogenous solution.

5. **Step 5:** Accurately weighed lyophilized extract were added in a measured quantity of sorbitol and mixed with a mechanical stirrer and passed through the nylon cloth (30 mof).

6. **Step 6:** Formulation was prepared by mixing glycerin containing xanthan gum with sorbitol containing extracts. This was finally mixed with the sugar base (prepared in step 3) and stirred with a mechanical stirrer (200 rpm/min). The final volume of the formulation was made with a sugar base.

Standardization of LivPro Syrup: The standardization of LivPro was performed following the guidelines of WHO and Indian Pharmacopoeia. Standardization parameters include physical determinations such as color, odor, taste, pH, specific gravity, viscosity, weight per milliliter, refractive index, total solid, alcohol content, presence/absence of reducing/non reducing sugar and quantitative estimation of alkaloid, flavonoid, saponin and total phenol. Limit tests for arsenic and heavy metal in LivPro syrup were performed. Microbial tests like total plate count, total viable aerobic count, presence/absence of *Salmonella* species, yeast, and mold count were also performed.

Assessment of hepatoprotective activity of LivPro syrup:

1. **Test animals:** Laboratory bred Wistar albino rats of either sex weighing between 150-250 gm were maintained under standard laboratory conditions at 25 ± 2°C, relative humidity 50 ± 15% and photoperiod of 12 hr-dark and light. Commercial pellet diet (Hindustan Lever, India) and water were provided *ad-libitum*. Animals were allowed to free access to water and food during the experiment, but no water and food were allowed before and after 1 hr of dosing. To avoid diurnal variation, all the experiments were carried out at the same time of day *i.e.* between 10:00 am to 05:00 pm. Ethical Committee approval was obtained from the Institutional Animal Ethical Committee of Radharaman College of Pharmacy, Bhopal before carrying out the experiments (IAEC/RCP/2012/01).

2. **Treatment Protocol:** The doses of LivPro were calculated precisely match with the human doses employed according to the manufacturer's instructions of available herbal liver syrups in the market. The average recommended a human dose of 15-30 ml/day was converted to that of the rat by standard conversion table [3]. A common minimum dose of 5 ml/kg of LivPro was selected as that of human dose with two higher doses of 10 and 15 ml/kg/day.

Animals were randomly divided into 6 groups with 12 rats in each. Group I and III-VI were treated as vehicle control, positive control (silymarin, p.o.) and three doses of LivPro (5, 10 and 15 ml/kg, p.o.) continuously for 14 days. On the 7th day, all the groups, including group II (CCl_4 control) were treated with CCl_4 (1 ml/kg), 2 hr after drug treatment and afterward on alternate days for a week.

On the 14th day, 2 hr after drug treatment, 6 animals from each group were used to determine thiopental induced sleeping time and remaining 6 animals from each group were used to access biochemical analysis of serum for marker enzymes, bromosulfophthalein uptake test, free radical scavenging ability of the liver and histopathological study of the liver. Vehicle control group animals were treated with normal saline (0.2 ml/100 gm). Standard drug silymarin was prepared freshly in 1% gum acacia in normal saline. LivPro was administered as it is as per the required quantity. All the treatments were given by orogastric intubation. The treatment plan was as follows:

- **Group I:** Vehicle control group: normal saline for fourteen days
- **Group II:** CCl_4 control group (1 ml/kg) on the seventh day afterward alternate days for one week
- **Group III:** Silymarin (20 mg/kg) + CCl_4 (1 ml/kg)

- **Group IV:** LivPro (5 ml/kg) + CCl_4 (1 ml/kg)
- **Group V:** LivPro (10 ml/kg) + CCl_4 (1 ml/kg)
- **Group VI:** LivPro (15 ml/kg) + CCl_4 (1 ml/kg)

Body weights of all the animals were recorded on 1st, 7th, and the 14th day before sacrifice. On the 14th day, 2 hr after drug treatment six animals of each group were given thiopental sodium (40 mg/kg) intraperitoneally and the effects of the drug on CCl_4 induced prolongation of thiopental sodium sleeping time were studied [4]. Remaining six animals of each group were anesthetized by light ether anesthesia, and blood was withdrawn by an intracardiac puncture.

Blood was allowed to coagulate for 30 min at room temperature, and serum was separated by centrifugation at 3000 rpm for 5 min. The serum was used to estimate serum SGOT, SGPT, ALP, cholesterol, HDL, LDL, triglyceride, total and direct bilirubin, protein and albumin as per the method described in diagnostic kits (Aspen Diagnostic Pvt. Ltd., India).

The liver, kidney, spleen, and heart were harvested, washed in normal saline, blotted in filter paper, and weighed. Each liver was cut into three parts. From part one, three slices of 60 mg were weighted and used for BSP uptake. Percentage hepatoprotection was calculated with the method described by Ranjan and Subramanyan [5]. The second part of the liver was used for estimation glutathione, lipid peroxidase and superoxide dismutase [6, 7, 8]. The third part of the liver was preserved in 10% formalin solution for histopathological assessment of liver damage. Hematoxylin and eosin staining of liver tissues and permanent tissue slides were prepared by following the method described by Nanji *et al.* [9].

Statistical Analysis: The results were expressed in term of Mean ± SEM. Experimental data of various physical and biochemical parameters were analyzed using one way ANOVA followed by Turkey-Kramer multiple comparisons using InStat-3 graph pad version. Differences between compared groups were considered significant at $P<0.05$.

RESULTS: Results postulated that the developed liver syrup LivPro was dark brown colored, bitter, and had a characteristic bitter odor. The results obtained from the pH study of the formulation indicated that the formulation made with simple syrup had a pH value of 5.04. The specific gravity of LivPro was found to be 0.8269; viscosity was 78.01 poise. The relative density and refractive index of the sample were found to be 1.271 gm/ml and 1.432, respectively. The estimation of total solid content was found to be 57.20% whereas, the alcohol content was found to be nil in formulated syrup.

A qualitative investigation of LivPro revealed the presence of reducing sugar as it showed a positive test for Fehling's and Benedict's test. Quantitative estimation of phytoconstituents indicated that LivPro has a rich quantity of flavonoid (480 mg/gm) and total phenol (396 mg/gm) whereas the alkaloid was found to be 103 mg/gm. The finding showed that saponin was absent in formulated syrup.

The concentration of lead and arsenic in the tested formulation was found to be within limit as one gm of the syrup complies with the 20 ppm of lead standard solution and five gram of syrup complies with the 10 ppm of the standard arsenic solution. The microbial limit tests in the formulation were found to be within the limit as per WHO guideline [10].

The total plate count and viable aerobic microbial count in the formulation were 480 and 415 CFU/gm, respectively. In our findings, the yeast and mould count was less than 100 CFU/gm.

Effect of LivPro on body weight and relative organs weight: Administration of CCl_4 on seventh day afterward alternate days for a week to the groups except vehicle control resulted in drastic decrease in body weights of negative control (5.44%), silymarin treated (2.97%) and LivPro 5 ml/kg (3.23%). Whereas, LivPro (10 and 15 ml/kg) showed dose-dependent gain in body weight by 2.21 and 4.33% in comparison to the CCl_4 treated group **Table 1**.

Results revealed that rats administered with CCl_4 alone showed an increase in relative liver weight when compared with the normal control group.

Treatment with silymarin and different doses of LivPro (5, 10 and 15 ml/kg) continuously for 14 days resulted in a highly significant decrease (P<0.001) of the relative liver weight. Whereas, the LivPro has a non-significant effect on relative kidney, spleen, and heart weights in comparison to the CCl_4 treated rats **Table 1**.

Thiopental Induced Sleeping Time: LivPro showed dose-dependent hepatoprotection of 34.14, 59.14, and 73.17% at 5, 10, and 15 ml/kg doses **Table 1**.

Bromsulphthalein Uptake by Liver Slices: LivPro at 15 ml/kg dose showed 28.00, 54.03 and 76.19% of hepatoprotection of liver at 10, 20 and 30 min respectively whereas, the livers of silymarin treated animals showed 29.60, 56.45 and 79.36% hepatoprotection at 10, 20 and 30 min respectively as shown in **Table 1**.

TABLE 1: EFFECT OF LivPro ON BODY WEIGHT AND THIOPENTAL INDUCED SLEEPING TIME OF CCl_4 TREATED RATS

S. no.	Treatment (mg/kg, p.o.)	% change in body weight		Thiopental induced sleeping time		% Hepatoprotection in BSP uptake (min)			Liver weight in g/100 g of body weight
		7th day	14th day	Sleeping time (min)	% Hepato-protection	10	20	30	
1	Vehicle control	3.45	6.04	49.26 ± 2.75	-	-	-	-	3.25 ± 0.13
2	CCl_4 (1)	2.85	-5.44	213.32 ± 7.01	-	-	-	-	4.47 ± 0.07
3	Silymarin (20 mg/kg)	3.62	-2.97	61.38 ± 3.54***	92.68	29.60	56.45	79.36	3.48 ± 0.17***
4	LivPro (5)	1.14	-3.23	157.19 ± 8.17***	34.14	17.60	32.25	56.34	3.56 ± 0.15***
5	LivPro (10)	1.74	2.21	116.28 ± 4.60***	59.14	24.80	50.00	72.22	3.49 ± 0.08***
6	LivPro (15)	1.86	4.33	93.14 ± 3.81***	73.17	28.00	54.03	76.19	3.38 ± 0.14***

The results are expressed as Mean ± SEM. One way ANOVA followed by Turkey's multiple comparison tests. ***P<0.001 when compared to the negative control group.

Effect of LivPro on Serum Biochemical Parameters: Results of the biochemical parameters revealed that the levels of marker enzymes SGOT, SGPT, ALP, cholesterol, triglyceride, LDL and total and direct bilirubin in plasma increased severely whereas the level of HDL, protein, and albumin decreased considerably in the negative control group. LivPro treatment significantly decreased (P<0.001 and 0.05) SGOT, SGPT, ALP, cholesterol, triglyceride, LDL and total and direct bilirubin at 10 and 15 mg/kg doses in comparison to the negative control group. Pre and post-treatment of LivPro extreme significantly (P<0.001) normalized serum HDL and protein at 10 and 15 ml/kg doses whereas, LivPro at 15 ml/kg dose resulted in a significant increase (P<0.05) in serum albumin **Table 2**.

TABLE 2: HEPATOPROTECTIVE EFFECT OF LivPro ON SERUM BIOCHEMICAL PARAMETERS OF CCl_4 TREATED RATS

S. no.	Treatment (ml/kg, p.o.)	SGOT IU/l	SGPT IU/l	ALP IU/l	Cholesterol mg/dl	Triglycerides mg/dl	HDL mg/dl	LDL mg/dl	Bilirubin mg/dl		Protein gm/dl	Albumin mg/dl
									Total	Direct		
1	Vehicle control	37.96 ± 2.67	41.14 ± 2.62	48.99 ± 2.11	105.02 ± 6.31	122.59 ±7.12	46.44 ± 3.37	34.06 ± 2.37	0.44 ± 0.04	0.22 ± 0.03	8.00 ± 0.37	4.98 ± 0.52
2	CCl_4 (1)	86.36 ± 4.99	81.88 ± 3.75	130.64 ± 7.94	220.68 ±12.99	228.82 ± 5.53	39.41 ± 2.72	135.50 ± 6.76	0.92 ± 0.05	0.56 ± 0.05	5.11 ± 0.31	3.66 ± 0.19
3	Silymarin (20 mg/kg)	43.29 ± 2.12***	45.70 ± 3.57***	50.96 ± 6.69***	133.13 ± 9.95***	169.40 ± 6.68***	57.90 ± 2.05***	41.35 ± 2.04***	0.46 ± 0.10***	0.21 ± 0.03***	8.03 ± 0.33***	4.20 ± 0.11 ns
4	LivPro (5)	85.15 ± 3.23ns	61.97 ± 5.58*	101.31 ± 3.46**	164.01 ± 7.70***	201.98 ± 6.47	50.72 ± 2.86ns	72.89 ± 4.81***	0.49 ± 0.04***	0.24 ± 0.03***	8.12 ± 0.19***	4.01 ± 0.11ns
5	LivPro (10)	63.59 ± 3.10***	60.91 ± 3.87**	92.63 ± 3.15***	152.11 ± 5.58***	195.14 ± 8.98*	59.24 ± 2.22***	53.74 ± 2.29***	0.45 ± 0.05***	0.23 ± 0.01***	8.25 ± 0.15***	4.54 ± 0.12ns
6	LivPro (15)	57.08 ± 3.57***	56.87 ± 3.39**	73.65 ± 2.02***	141.40 ± 5.81***	194.61 ± 6.32*	61.26 ± 3.16***	41.21 ± 3.21***	0.45 ± 0.02***	0.23 ± 0.09***	8.31 ± 0.13***	4.86 ± 0.11*

The results are expressed as Mean ± SEM. One way ANOVA followed by Turkey's multiple comparison tests. *P<0.05, **P<0.01, ***P<0.001 and ns = not significant when compared to the negative control group.

Estimation of Free Radical Scavenging Ability of Liver: Treatment with LivPro (10 and 15 ml/kg) showed a highly significant increase (P<0.001) in glutathione level. LivPro 5, 10 and 15 ml/kg dose levels extremely significantly brought down (P<0.001) the liver antioxidant enzymes level of lipid peroxidase. However, LivPro has a non-significant effect on superoxide dismutase enzyme **Table 3**.

TABLE 3: EFFECT OF LivPro ON FREE RADICAL SCAVENGING ABILITY OF CCl_4 TREATED RAT-LIVER

S. no.	Treatment (ml/kg, p.o.)	Glutathione µg/gm of liver	Lipid peroxidase nmol/gm of protein	Superoxide dismutase Unit/mg of protein
1	Vehicle control	22.93 ± 1.91	4.76 ± 0.77	6.20 ± 0.91
2	CCl_4 (1)	7.74 ± 0.78	28.15 ± 1.93	3.59 ± 0.62
3	Silymarin (20 mg/kg)	$20.35 \pm 1.49^{***}$	$6.11 \pm 0.69^{***}$	5.96 ± 0.84^{ns}
4	LivPro (5)	10.59 ± 0.67^{ns}	$17.90 \pm 1.13^{***}$	3.46 ± 0.61^{ns}
5	LivPro (10)	$15.27 \pm 1.03^{***}$	$12.48 \pm 1.08^{***}$	4.05 ± 0.66^{ns}
6	LivPro (15)	$18.97 \pm 1.42^{***}$	$9.18 \pm 1.01^{***}$	5.53 ± 0.78^{ns}

The results are expressed as Mean ± SEM. One way ANOVA followed by Turkey's multiple comparison tests. ***P<0.001 and ns = not significant when compared to the negative control group.

Histopathological Studies: Histopathological observations and photomicrographs of liver slides were shown in **Fig. 1 a-f**. Section of vehicle control liver tissue showing normal hepatic cells with well-preserved cytoplasm, prominent nucleus, and visible central veins. CCl_4 intoxicated liver tissue showed neutrophilic infiltration, sinusoidal dilatation, zonal necrosis, extensive diffuse vacuolar degeneration engorged with blood and microvesicular fatty changes in hepatocytes. Silymarin treated liver tissue showed normal hepatocytes, portal vein, portal artery, and the hepatic duct of portal triad. LivPro (5 ml/kg) pretreatment showed reduced inflammation, degenerative changes, steatosis, and mild sinusoidal dilation. LivPro (10 ml/kg) treatment showed a prominent nucleus and reduced infiltration. LivPro (15 ml/kg) treatment showed complete recovery of hepatocytes with reduced vacuolations, normal hepatocytes, clear cell lining as well as the absence of multinucleated giant cell.

DISCUSSION: Non-availability of pharmacopoeial standards for plant-derived medicines is the major drawback over-shadowing the time tested healing properties of plant-based medicines. This can only be countered by the process of standardization of phytopharmaceuticals evaluating their performances, limitations, optimal dosage, contraindications, and applications [11].

Inconsistent and variable biological effects of plant-derived medicines are, perhaps, the main discouraging issues for researchers in the field of natural products. Phytopharmaceuticals can only be considered as a rational drug if standardized for reproducible efficacy and safety, and their pharmaceutical quality is approved. Pharmacological, toxicological, and clinical study data of herbal drugs needs to be well documented to obtain reproductive results. WHO, taking cognizance of this problem and has published guidelines to ensure the reliability and repeatability of herbal medicines.

Usually, the undesirable biological and photochemical variability of plant materials is due to different growth, harvest, drying, and storage conditions. Therefore, cultivation of plants under standardized conditions is desirable. The polarity of the solvent, mode of extraction, and instability of the constituents may influence the composition of extracts and thus must be kept constant [12].

The WHO has issued some guidelines for quality assessment of crude plant material, plant preparations, and finished product along with safety assessment and shelf life of herbal medicines [13]. The steps in standardization and quality control include determining the identity of the plants from classical texts, equating it to scientific botanical identity and ensuring that correctly identified raw material is supplied and used.

Looking into these inconsistencies of herbal formulations we have aimed at developing standardized polyherbal liver syrup using four well-known plants *viz A. millefolium*, *C. spinosa*, *C. intybus*, and *P. kurroa* having potential hepatoprotective effects.

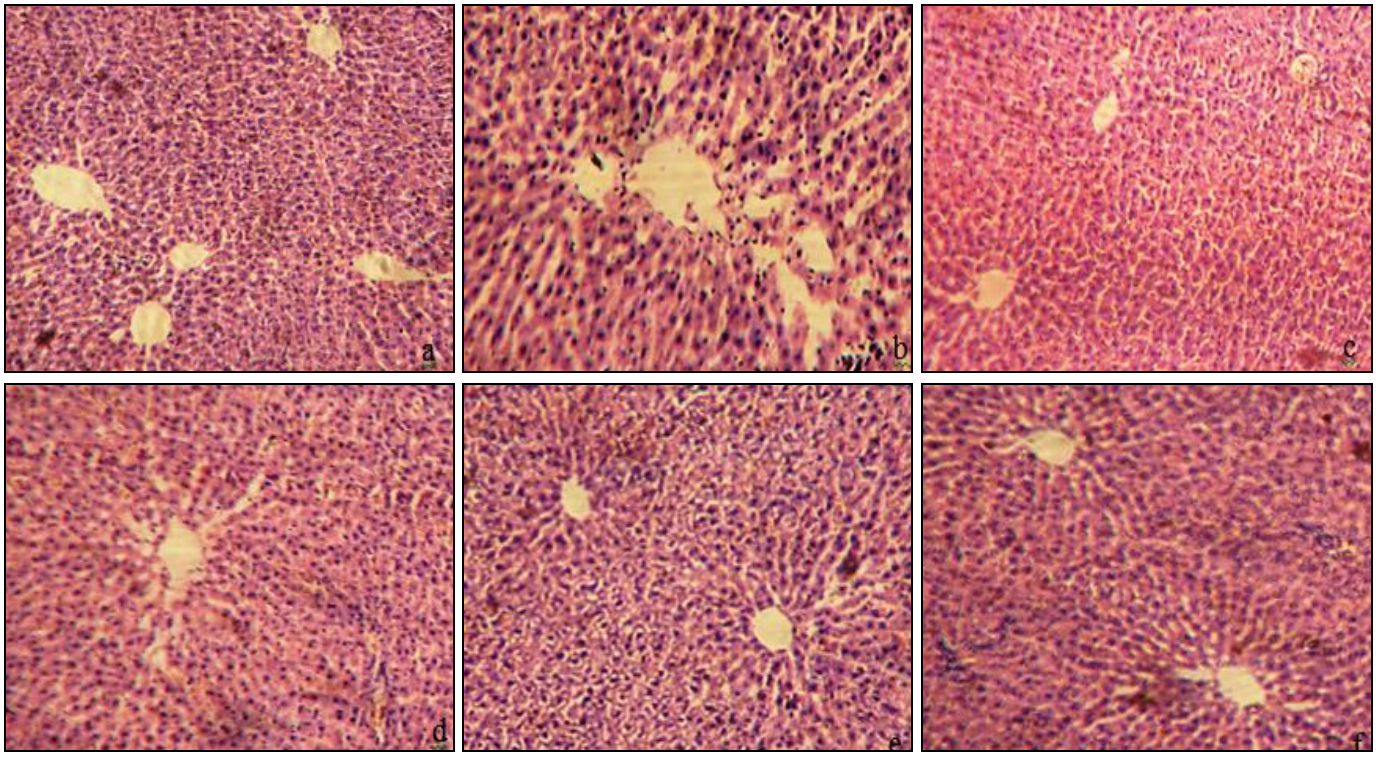

FIG. 1: ASSESSMENT OF CCl_4 INDUCED HEPATOTOXICITY BY HISTOPATHOLOGY IN HAEMATOXYLIN-EOSIN STAINED LIVER SECTIONS. (a: Control group showed normal hepatic cell with well-preserved cytoplasm; b: CCl_4 intoxicated liver tissue showed necrosis with inflammation; c: Silymarin treated animal showed focal necrosis with slightly altered hepatocytes; d: LivPro (5 ml/kg) showed inflammation, degenerative changes, steatosis, and mild sinusoidal dilation; e: LivPro (10 ml/kg) showed prominent nucleus and reduced infiltration; f: LivPro (15 ml/kg) showed complete recovery of hepatocytes with reduced vacuolations, normal hepatocytes, and absence of multinucleated giant cell (10×)

Hepatic disorders are a common problem in the world, especially in developing countries. However, there are no effective drugs which can regulate and restore the functional integrity of liver. In recent years, scientists have carried out market research on traditional medicine in an attempt to develop new drugs and their formulations for preserving the normal hepatic physiological mechanisms. Compounds that can either decrease the necrotic damage to hepatocytes via enhanced defense mechanisms against toxic insult or improve the repair of damaged hepatocytes are considered potentially useful in the treatment of human hepatic disorders [14].

Hepatoprotective potential of the standardized formulation has been assessed on CCl_4 induced acute liver damage in rats. CCl_4, a well-known toxicant for producing hepatic chemical injury in animals, has been used as an experimental model to test the potential hepatoprotective activity of drugs by several investigators [15]. Presently, it is hypothesized that herbal drugs inhibit the CYP2E1 enzyme activity in hepatic microsomes *in vivo* induced by CCl_4 and nitrosamines [16].

The formulated polyherbal liver syrup has been formulated and standardized following WHO and ISM guidelines. Formulation compiled all the standards and microbial and heavy metals found were within limit. Quantitative estimation of liver syrup showed the rich presence of flavonoid, phenol, and alkaloid. The toxic effect of CCl_4 is an example of free radical disease, due to its conversion into highly reactive toxic free radical, which attacks the membranes of smooth and rough endoplasmic reticulum and results in hepatic damage.

It is evident from the results that the animals received the CCl_4 alone showed a marked decrease in body weight and increase in relative liver weight whereas the animals treated with CCl_4 along with LivPro showed dose-dependent normalization in body weight and relative liver weight. These findings suggested that LivPro treatment significantly neutralized the toxic effect of CCl_4 and showed good recovery in the general health of the treated animals.

The ability of LivPro to reduce the prolongation of thiopental induced sleeping time in CCl_4 poisoned rats is indicative of its hepatoprotective potential. It has been established that since the barbiturates are metabolized almost exclusively in the liver, the sleeping time after a given dose of thiopental is a measure of hepatic metabolism ability [17]. In case of any liver damage, like CCl_4 intoxication, the sleeping time after a given dose of the barbiturate is prolonged, because the amount of the barbiturate metabolized per unit time is less. BSP uptake forms a sensitive test for the functional integrity of liver. LivPro at all doses has significantly improved the capacity of the damaged liver to take up BSP, showing its enhanced capacity to excrete the dye from the blood. Simultaneous treatment of LivPro with CCl_4 exhibited less damage to the hepatic cells, as compared to the rats treated with CCl_4 alone.

CCl_4 treatment significantly increased the serum enzyme levels, namely SGOT, SGPT, and ALP indicating chemical induced hepatocellular toxicity, which is very sensitive markers employed in the diagnosis of liver diseases. When the hepatocellular plasma membrane is damaged, these enzymes are quantified to assess the type and extent of liver injury [18]. Liver injury due to toxins can result in defective excretion of bile reflected as an increased level of ALP in serum [19].

The pre and post treatment of polyherbal formulation LivPro significantly restored these liver enzyme parameters. This postulated that the LivPro can preserve the structural integrity of the cell as well as the repair of hepatic tissue damage caused by CCl_4. This also indicates curative efficacy of active ingredients (luteolin, cichotyboside, and picroliv) of constituent herbs of LivPro syrup *viz. A. millefolium, C. intybus* and *P. kurroa* that are scientifically well proven to reduce SGPT and SGOT levels, increasing liver ATPase activity thus having a protective effect on liver [20, 21, 22]. Metabolic or chemical liver toxicity results in the fatty liver. Fat metabolism in the affected liver was leading to hypercholesterolemia and increased levels of serum triglycerides and LDL [23]. At the 14^{th} day, the levels of serum cholesterol, triglycerides and LDL were found to be significantly increased in CCl_4 intoxicated rats, which probably may be mediated by chemical toxicity caused by the CCl_4. However, the values of these biochemical parameters were recorded to get normalized in LivPro treated group. This may be attributed to the individual constituents of LivPro namely *A. millefolium, C. spinosa* and *P. kurroa* which are scientifically well established to possess hypocholesterolemic activity [24, 25, 26].

In hepatopathy, bilirubin comes into the blood stream in conformity with the extent of liver damage [27]. Chemical agents producing sufficient injury to hepatic parenchyma causes elevation if bilirubin content in plasma [28]. Bilirubin is one of the most useful clinical indicators of the severity of necrosis, and its accumulation is a measure of binding, conjugation, and excretory capacity of the hepatocyte. LivPro at all doses significantly restored the altered total and direct bilirubin levels, which were comparable with the efficacy shown by silymarin and also directly, indicated the effectiveness of the herbal formulation on the functional status of the liver.

Hypoalbuminemia deemed as a useful index as evident by the occurrence of CCl_4 intoxicated rats when compared to control group. Samudram *et al.* [29] also reported a significant decrease in serum proteins in CCl_4 induced hepatic damage in rats. The values get normalized in LivPro treated group. This may be due to the anabolic activity of *A. millefolium, C. spinosa* and *C. intybus,* which lead to an increase in total serum proteins.

Hepatoprotective activity is usually associated with antioxidant activity since CCl_4 causes free radical-mediated damage [30]. Earlier studies regarding the mechanism of CCl_4 induced hepatotoxicity have shown that glutathione plays a key role in detoxifying the reactive, toxic metabolites of CCl_4 and liver necrosis begins when the GSH stores are markedly in depleted state [31, 32].

An elevated level of MDA in negative control group reflects an enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals [33]. The enzymatic antioxidant defense systems are the natural protector against lipid peroxidation. SOD enzyme is an important scavenger of superoxide ion and hydrogen peroxide, prevents the generation of

hydroxyl radical and protects the cellular constituents from oxidative damage [34]. After LivPro administration, the above changes were reversed as compared with CCl_4 treatment. These findings showed that LivPro can scavenge reactive free radicals and could lead to a decrease in the severity of oxidative damage in the liver.

CONCLUSION: The results indicate the protective and curative effect of LivPro on CCl_4 induced hepatotoxicity, which may be mediated through enhanced antioxidant defiance status, anti-inflammatory and immune modulating properties of different constituents such as flavonoids, terpenoids, saponins and steroids present in the LivPro. The findings of this study suggest that LivPro can be used as a safe and effective alternative chemopreventive agent in the management of liver disorders.

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CONFLICT OF INTEREST: Nil

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