IJPSR (2021), Volume 12, Issue 8 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 28 August 2020; received in revised form, 03 February 2021; accepted, 19 May 2021; published 01 August 2021

HEPATOPROTECTIVE EFFECTS OF POLYHERBAL FORMULATION AGAINST CARBON TETRACHLORIDE-INDUCED HEPATIC INJURY IN ALBINO RATS

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Keywords:

Bauhinia variegata, Oxalis corniculata and Pterocarpus marsupium, Carbon tetrachloride

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ABSTRACT: Objective: To investigate the hepatoprotective activity of polyherbal formulation composed of extracts of *Bauhinia variegata*, *Oxalis corniculata*, and *Pterocarpus marsupium*. **Methods:** The hepatoprotective activity of the formulation and extracts were assessed on the basis of histopathological changes in the liver and estimation of SGOT, SGPT, SALP, serum bilirubin of carbon tetrachloride-induced hepatotoxic rats. **Results:** The result of the present studies strongly indicates that the hepatoprotective property of polyherbal formulation is evidenced by less damaged hepatocytes cells. **Conclusion:** Polyherbal formulation composed of aqueous extracts of *Bauhinia variegata, Oxalis corniculata*, and *Pterocarpus marsupium* revealed significant hepatoprotective profile as compared to standard formulation Liv-52.

INTRODUCTION: The liver plays a vital role in the metabolism and elimination of various exogenous and endogenous compounds. As a result of its continuous involvement, it is susceptible to toxic injuries caused by certain agents, and any damage to hepatic cells disturbs body metabolism. In recent times a lot of interest has been generated to find out a natural remedy for hepatic disorders caused by toxins like alcohol and hepatitis virus [1]. To ensure the survival of an individual and maintain the function of the liver, the conventional treatment focus on symptom management and liver transplantation in severe cases of liver disease [2]. There are no drugs currently in use to increase the detoxification power of the organ.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(8).4465-75

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(8).4465-75

Therefore, Testing and the use of botanical hepatoprotective agents are substantially increasing. So it would be highly imperative to demonstrate the effectiveness of the plant extracts in the presence of chemical-induced hepatotoxicity. CCl_4, a potent hepatotoxic agent, is the most widely used criterion for evaluating the hepatoprotective activity of plant extracts [3, 4]. CCl_4 is a potent hepatotoxin, when administrated, which leads to necrosis in liver tissue, ultimately leads to liver injury. CCl_4 induced liver injury depends on the metabolism of toxic substances by the liver NAPDH-cytochrome system results in the formation of highly reactive intermediate trichloromethyl free radicals, which binds to cell protein covalently, which leads to membrane lipid peroxidation and finally cell necrosis [5].

Oxalis corniculata: *Oxalis corniculata* Linn is an annual herb commonly known as Indian horrel belonging to the family Oxalidaceae. Oxalis means greek oxys -acid, sharp, sour, referring to the taste of the leaves and stem. Corniculata means horn-like appendages.

The plant is an herb, the branchlets creeping and rooting at the nodes [6]. Carl Linnaeus first described the species from material from the Mediterranean region [7]. Puliyarai (Oxalis corniculata) has the origin in South Europe and Hawaii. This herb is also found in tropical regions of Canada and North America as well as in India, Pakistan, Afghanistan, China, Indonesia, and Taiwan [8].

Oxalis corniculata have a wide range of phytochemical constituents isolated from the plant like flavanoids, tannins, phytosterols, phenol, glycosides, fatty acids galacto glycerolipid, and volatile oil. The leaves contain flavonoids, iso beta –D-glucopyrunoside. It is a rich source of essential fatty acids like palmitic acid, oleic, linoleic, linolenic, and stearic acids [9]. It possesses various important pharmacological activities like anti-oxidant, anti-cancer, anthelmintic, anti-inflammatory, antimicrobial, astringent, diuretic, febrifuge, cardio-relaxant and stomachic properties, anti-hypertensive, hypoglycemic, antihypertensive, anti-psychotic, nervous system stimulant, anthelmintic, antidiuretic, emmenagogue, depurative, lithon-triptic, & have chronotropic and inotropic effect [10-15].

Pterocarpus marsupium: *Pterocarpus marsupium* Roxb, is a deciduous tree that commonly grows in Sri Lanka and India [16]. Pterocarpus Marsupium belonged to the family Fabaceae, known as Indian kino tree and Malabar tree [17]. *P. marsupium* is a very rich source of flavonoids and polyphenolic compounds. All the active phytoconstituents of P. marsupium were thermostable. It contains pterostilbene (45%), alkaloids (0.4%), tannins (5%) and protein. The primary phytoconstituents were liquiritigenin, isoliquiritigenin, pterostilbene, pterosupin, epicatechin, catechin, kinotannic acid, kinoin, kino red, β-eudesmol, carsupin, marsupial, marsupinol, pentosan, p-hydroxy-benzaldehyde [18]. P. marsupium has been traditionally used in the treatment of leucoderma, elephantiasis, diarrhea, cough, discoloration of hair, rectalgia, headache, inflammations, as an antipyretic, anti-helminthic, aphrodisiac, mental aberrations, and ulcer. It is nontoxic and useful in jaundice, wounds, diabetes, stomachache, and ulcer. The bark is used for the treatment of stomachache, cholera, dysentery, urinary complaints, tongue diseases, and toothache. The heartwood and bark of Pterocarpus marsupium are known for their anti-diabetic activity [19].

Bauhinia variegata: *Bauhinia variegata* Linn. (Leguminosae), commonly known as 'Kachnar', is a medium-sized deciduous tree widely distributed in most tropical countries, including rocky hills of Circars, Deccan, and carnatic regions of South India [20]. The stem bark of B. variegata is composed of kaempferol-3-glucoside, lupeol, [5, 7] dihydroxy and [5, 7] dimethoxy flavanone-4-O-α-L rhamnopyrosyl-β-D-glycopyranosides, and beta-sitosterol [21]. There are a number of phytochemicals isolated from the various part of kanchnar such as flavonoids, kaempferol, ombuin, hesperidine, triterpenecaffeate, flavonol glycosides, flavanone, stigmasteroles, octacosanol, Phenanthraquinone, protein, calcium and phosphorous, volatile oil, germacrene D, quercetin, rutin, etc. [22]. Kanchnar is well described in ancient Indian science of life known as Ayurveda, and its Stem bark & flowers are used as medicine in various formulations [23]. It has chelation action, anticarcinogenic activity, anti-diabetic action, haemagglutinating activity, hypo-lipidaemic activity, haematinic activity, immune-modulatory activity, antimicrobial activity, nephro-protective activity, neural activity, antioxidant effects, anti-tumor activity, and antiulcer activity [22].

Almost all the parts find uses inside the traditional medicine against various ailments like leprosy, piles, asthma, ulcer, liver complaints, and snakebite. It also finds its uses in the treatment of skin diseases, wound healing, obesity, stomatitis, dyspepsia, flatulence and is used as tonic, astringent, and laxative as well [24]. There are hardly any proven remedies for the prevalent liver disorders among people. No drug has been developed in the modem system of medicine that may stimulate the liver function, protect it from damage, or help in the regeneration of hepatic cells. The only drugs, which are available for the treatment of liver disorders, are corticosteroids and immuno- suppressive agents, but their use is accompanied by serious side effects. There is an ever-increasing need for an agent, which could protect the liver against damages [1].

As a result, alternative treatments based on natural plant products and herbal mixtures belonging to the realm of polyherbal formulation, complementary and alternative medicine (CAM) are becoming increasingly popular. The medicinal plants *viz. Pterocarpus marsupium*, *Oxalis corniculata*, and

Bauhinia variegata utilized as traditional medicine as a liver tonic and above medicinal plants have been reported to contain some active constituents to have hepatoprotective property. Hence, considering all the above aspects, the present study is designed to investigate the above-mentioned medicinal plants utilized traditionally in the development of hepatoprotective polyherbal formulation by using various models with quality control profiles.

MATERIALS AND METHODS:

Collection and authentication of plant materials: All three plant materials, viz Bauhinia variegata, Pterocarpus marsupium, and Oxalis corniculata were collected from Pragati Pharma Belgaum. The specimens of the above plants were deposited in the herbarium of SSVPS science college, Dhule (KLERLSCI-1439). After authentication plants were dried at room temperature until they become free from moisture. The shade-dried plant material (bark of *Bauhinia variegata*, leaves of *Oxalis corniculata*, and heartwood of *Pterocarpus marsupium*) were powdered and passed through sieve #40. All three plants (bark of *Bauhinia variegata*, leaves of *Oxalis corniculata*, and heartwood of *Pterocarpus marsupium*) were subjected to successive continuous hot soxhlet extraction with Petroleum ether, chloroform, and alcohol.

After the effective extraction, solvents were distilled off, the extracts were then concentrated on a water bath, and the extract obtained with each solvent was weighed. Acute toxicity study was carried out according to OECD guidelines, and $1/10^{th}$ of this lethal dose was taken as an effective dose (therapeutic dose) for subsequent hepatoprotective activity.

Preparation of Herbal Formulation: Polyherbal preparation was made of bio-active aqueous extracts of *Bauhinia variegata*, *Pterocarpus marsupium,* and *Oxalis corniculata* in the form of syrup. The formulation of all bio-active extracts was prepared by applying probability for that all the bioactive extracts were mixed in proportion by keeping in consideration the individual lethal dose value obtained previously. As per the probability applied and calculated LD_{50} cut-off value, the formulation was developed [25].

TABLE 1: DOSE SELECTION AND FINALIZING LD_{50} CUT OFF VALUE OF COMBINE BIOACTIVE EXTRACTS

Formulation	Bioactive aqueous Extract of	LD50 cut off mg/kg b.w.	Therapeutic Dose
Formulation A	*Oxalis corniculata*, *Bauhinia variegata* and *Pterocarpus marsupium* (1:1:0.4)	2000 mg/kg	200 mg/kg b.w
Formulation B	*Oxalis corniculata* and *Pterocarpus marsupium* (1:0.4)	3000 mg/kg	300 mg/kg b.w
Formulation C	*Oxalis corniculata* and *Bauhinia variegate* (1:1)	5000 mg/kg	500 mg/kg b.w
Formulation D	*Bauhinia variegata* and *Pterocarpus marsupium* (1:0.4)	2000 mg/kg	200 mg/kg b.w

TABLE 2: GENERAL FORMULA FOR POLYHERBAL FORMULATION:

S. no.	Formulation A		Formulation B		Formulation C		Formulation D	
	Ingredies	Quantity	Ingredients	Quantity	Ingredients	Quantity	Ingredients	Quantity
1	*Oxalis corniculata* *Bauhinia variegata* *Pterocarpus marsupium*	1.35 g	*Oxalis corniculata* *Pterocarpus marsupium*	2.025g	*Oxalis corniculata* *Bauhinia variegata*	3.37g	*Bauhinia variegata* *Pterocarpus marsupium*	2.025g
2	Refined sugar	45%	Refined sugar	45%	Refined sugar	45%	Refined sugar	45%
3	Sodium methyl paraben	0.18 %	Sodium methyl paraben	0.18 %	Sodium methyl paraben	0.18 %	Sodium methyl paraben	0.18 %
4	Caramel Coloring agent	0.005%	Caramel Coloring agent	0.005%	Caramel Coloring agent	0.005%	Caramel Coloring agent	0.005%
5	Flavor Vanilla	0.04%	Flavor Vanilla	0.04%	Flavor Vanilla	0.04%	Flavor Vanilla	0.04%
6	Distilled water	qs 100ml	Distilled water	qs 100ml	Distilled water	qs 100ml	Distilled water	qs 100ml

Procedure: A syrup base consisting of 45% w/v sugar was prepared and cooled to about 50-55 °C, and preservatives were added, and the said herbal extract were weighed and mixed well. Addition of sweeteners, color and flavor was done. It was then filtered and the final volume of the syrup was made up with distilled water and mixed well.

Evaluation of Hepatoprotective Activity:

Experiment Animals: Female Albino Wistar strain rats weighing between 120-150 gm were used for the hepatoprotective model. The animals were bred in an animal house, Sri. Venkateshwara Enterprises, Bangalore. (CPCSEA Reg. No. 276) Rats were kept in polypropylene cages and were allowed free access to food and water. The rats were housed in a group of six at 25 °C and were exposed to 12 h of darkness and light each. The bedding materials of cages were changed every day.

Determination of LD_{50} Study [26]: The acute oral toxicity study was carried out as per the guidelines by Organization for Economic Co-operation and Development (OECD), received draft guidelines 423, received from the committee for the purpose of control and supervision of experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. It was carried out by administering large doses of extracts from 300 mg/Kg to 5000 mg/Kg. The substance is administered orally to a group of experimental animals (consisting of 3 rats) at one of the defined doses. The dose at which half the population of animals was found to be dead within the first 24 h is considered as LD_{50}, and $1/10^{th}$ dose of the lethal dose is the therapeutic dose for pharmacological activity.

Screening Method: Evaluation of hepatoprotective activity by using parameters likes estimation of SGPT, SGOT, and estimation of SALP/ALP, serum bilirubin, and histopathological study on polyherbal formulations was performed. The method, according to Handa S.S. and Anupam Sharma, has been used in this study. Rats were divided into seven groups of six animals each (n=6). The above-developed formulation (A, B, C, and D) were administered on a group of 6 female albino Wistar rats, weighing about 120-150g; for recording enzymatic levels and histopathology during the evaluation, animals were administered with carbon tetrachloride (2 ml/kg i.p.) to induce hepatotoxicity. A marked increase in the serum level of SGOT, SGPT, SALP, and Serum Bilirubin was taken as an indication of hepatotoxicity.

Group 1: Served as Control and received a single daily dose of 1 ml/kg i.p. of sucrose solution for 4 days along with 1 ml/kg s. c. of olive oil on 2nd and 3rd days.

Group 2: Also received a single daily dose of 1 ml/kg p. aqueous sucrose solution for 4 days with 2 ml/kg of Carbon tetrachloride by subcutaneous route dissolved in an equal volume of olive oil on 2nd and 3rd days.

Group 3: Received standard drug Liv-52 as a single daily dose of 5 ml/kg of the oral route for 4 days with 2 ml/kg of carbon tetrachloride by subcutaneous route on 2^{nd} and 3^{rd} days.

Group 4, 5, 6, and 7 received a single daily dose of formulation A, B, C, and D by oral route for 4 days, respectively, with 2 ml/kg of carbon tetrachloride by subcutaneous route on 2nd and 3^{rd} days [27, 28].

TABLE 3: SCHEDULE FOR CARBON TETRACHLORIDE MODEL

S. no.	Group	Days				
		1	2	3	4	5
1	1-Control	SS	SS, OO	SS, OO	SS	Animals were
2	2-Carbon Tetrachloride	SS	SS, CCl_4	SS, CCl_4	SS	sacrificed
3	3- Std drug Liv-52	SD	SD, CCl_4	SD, CCl_4	SD	under light
4	4- formulation A	TS	TS, CCl_4	TS, CCl_4	TS	anesthetic ether
5	5- formulation B	TS	TS, CCl_4	TS, CCl_4	TS	
6	6- formulation C	TS	TS, CCl_4	TS, CCl_4	TS	
7	7- formulation D	TS	TS, CCl_4	TS, CCl_4	TS	

All the rats in all the groups were sacrificed on the 5th day under light anesthetic ether. Blood from each rat was collected through cardiac puncture under ether anesthesia for a biochemical investigation like SGOT, SGPT, SALP, and serum bilirubin estimation. Blood was allowed to coagulate at 37 °C for 30 min, and the serum was separated by centrifugation at 2500 rpm for 10 min.

The liver of all the experimental animals was removed and processed immediately for histological investigation.

Histological Investigation: The liver from each animal was removed after dissection. The liver lobes were fixed for 48 h in 10% formalin and were embedded in paraffin. Subsequently, 5 sections of livers were stained with haematoxylin and eosin. These sections were observed under a light microscope for histological changes and compounds to normal liver physiology.

Biochemical Investigation:

Estimation of Serum Glutamate Pyruvate Transaminase (SGPT): SGPT or ALT is located in the cytosol of the liver cell. During liver cell inflammation, they are released into circulation due to increased permeability of cell membrane break down of liver cells. Hence, determination of SGPT as an index of the extent of liver damage. A diagnostic reagent kit was used for the determination of SGPT, also called as "Alanine amino transaminase" (ALT) activity by method of Reitman and Frankel [29].

Estimation of SGOT: SGOT (AST) is located on the cytosol of liver cells. In addition, it is also found in the mitochondria. It is also found in many tissues such as the heart, liver, skeletal muscle, and kidney, which rich source of SGOT in that order, liver are being the second richest source of SGOT, the importance of SGOT levels in hepatic damage of hepatic cells leads to increased levels of SGOT in blood serum. SGOT Kit is based on Reitman and Frankel's method [29]. SGOT catalyzes the transfer of the amino group of L-aspartate (ASP) to α-ketoglutarate of the (α -KG), resulting in the formation of oxaloacetate (OAA) and L-glutamate (L-Glu). The oxaloacetate so formed is allowed to react with 2, 4-DNPH to form 2, 4 dinitrophenyl hydrazone derivative, which is brown colored in alkaline medium. The hydrazone derivative of oxaloacetate, similar to pyruvate, is considerably more chromogenic than that of α -KG. The final colour developed does not obey Beer's law.

Estimation of SALP/ALP: SALP Kit is based on Kind and King Method [30]. Alkaline phosphatase (ALP) at an alkaline pH hydrolyses di-sodium Phenylphosphate to form phenol. The Phenol formed reacts with 4 - Aminoantipyrine in the presence of Potassium ferricyanide, as an oxidizing agent, to form a red-colored complex. The intensity of the colour formed is directly proportional to the activity of ALP present in the sample. Serum Bilirubin kit is based on Jendrassik and Grof's method [31].

Bilirubin reacts with diazotized Sulfanilic acid to form a coloured compound. The unconjugated bilirubin couples with the Sulfanilic acid in the presence of caffeine - benzoate accelerator. The intensity of the colour formed is directly proportional to the amount of bilirubin present in the sample.

Statistical Analysis: Results of the biochemical estimation were expressed as mean ± S.D. for determination of significant intergroup difference, each parameter was analyzed separately, and one-way analysis of Variance (ANOVA) was carried out [32]. The calculated F ratio has been tabulated along with the critical value of F ratio. Dunnet's test was used for individual comparisons [33, 34].

RESULTS AND DISCUSSION: To assess the hepatoprotective activity of polyherbal formulation of *Bauhinia variegata*, *Oxalis corniculata*, and *Pterocarpus marsupium*, carbon tetrachloride-induced hepatotoxicity was produced in female albino rats, and parameters like enzyme study (SGOT, SGPT, SALP, and Serum bilirubin) and histopathological studies were carried out and the extent of regenerative changes were observed.

In acute toxicity studies, no mortality and no change in general behavior were observed in the animals treated with all the extracts of *Oxalis corniculata*, *Bauhinia variegata*, and *Pterocarpus marsupium* up to a dose which is tabulated in **Table 4**:

TABLE 4: LD_{50} OF *OXALIS CORNICULATA, BAUHINIA VARIEGATA* AND *PTEROCARPUS MARSUPIUM*

S. no	Bioactive (Aqueous) extracts of	LD50 Cut off mg/kg B.W	Therapeutic Dose
1	*Oxalis corniculata*	5000mg/kg	500mg/kg
2	*Bauhinia variegata*	5000mg/kg	500mg/kg
3	*Pterocarpus Marsupium*	2000mg/kg	200mg/kg

The dose at which half the population of animals was found to be dead within first 24 h is considered as LD50 and 1/10th dose of the lethal dose is therapeutic dose for pharmacological activity. The petroleum ether (40-60 °C), chloroform, Alcohol and aqueous extracts were tested for hepatoprotective activity, and the aqueous extract of all the plants had better hepatoprotective potential and thus it is called bioactive. Hence polyherbal formulation was developed using bioactive aqueous extracts of *Bauhinia variegata*, *Oxalis corniculata,* and *Pterocarpus marsupium*. The result of hepatoprotective activity of polyherbal formulation before stability study showed that formulation A is composed of *Oxalis corniculata*, *Bauhinia variegata*, and *Pterocarpus marsupium* (1:1:0.4) possess better hepatoprotective potential as compared to formulation B, C, and D as shown in **Table 5 to 8**.

TABLE 5: SHOWING ENZYMATIC SGPT LEVEL (IU/ L) OF POLYHERBAL FORMULATION

S. no.	Control	CCl_4	Standard	Formulation A	Formulation B	Formulation C	Formulation D
1	36	142	43	62	77	72	68
2	38	149	42	63	81	80	74
3	41	200	49	55	75	83	70
4	54	156	59	60	72	79	79
5	52	190	60	51	77	67	66
6	43	213	64	53	69	73	63
Mean	44	175	52	57.33	75.17	75.67	70.0
SD	7.403	29.73	9.411	5.007	4.215	5.989	5.762
SE	3.002	12.14	3.842	2.044	1.721	2.445	2.352
F ratio				71.89			
P value	-	-	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

TABLE 6: SHOWING ENZYMATIC SGOT LEVEL (IU/ L) OF POLYHERBAL FORMULATION

S. no.	Control	CCl_4	Standard	Formulation A	Formulation B	Formulation C	Formulation D
1	37	130	43	39	53	52	70
2	60	132	49	58	63	53	66
3	49	149	52	48	63	64	52
4	42	143	53	52	61	60	69
5	53	150	40	41	65	63	58
6	55	149	43	59	62	59	57
Mean	49.33	142.2	46.67	49.50	61.17	58.50	62.00
SD	8.548	9.020	5.391	8.408	4.215	5.010	7.348
SE	3.490	3.683	2.201	3.433	1.721	2.045	3.000
F ratio				135.8			
P-value	-	-	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

TABLE 7: SHOWING ENZYMATIC SALP LEVEL (IU/ L) OF POLYHERBAL FORMULATION

S. no.	Control	CCl_4	Standard	Formulation A	Formulation B	Formulation C	Formulation D
1	27	79	26	30	49	40	51
2	24	86	24	28	48	38	48
3	23	97	27	34	60	42	60
4	22	88	32	28	52	30	52
5	21	77	30	31	62	38	62
6	28	78	34	33	55	31	55
Mean	24.17	84.17	28.83	30.67	54.33	36.50	54.67
SD	2.787	7.731	3.817	2.503	5.750	4.889	5.428
SE	1.138	3.156	1.558	1.022	2.348	1.996	2.216
F ratio				107.6			
P-value	-	-	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

TABLE 8: SHOWING SERUM BILIRUBIN LEVEL (IU/ L) OF POLYHERBAL FORMULATION

S. no	Control	CCl_4	Standard	ulation A	Formulation B	Formulation C	Formulation D
1	2.22	8.30	4.30	3.20	6.00	5.18	7.00
2	2.01	11.30	5.18	5.50	6.00	6.20	7.50
3	2.22	12.80	4.90	6.20	6.20	5.60	6.00

4	2.24	13.40	5.20	5.30	5.60	6.40	6.20
5	2.25	12.60	4.50	3.50	6.40	6.00	5.40
6	2.01	9.40	5.60	6.40	5.40	5.40	7.00
Mean	2.158	11.30	4.947	5.017	3.933	5.797	6.517
SD	0.1155	2.047	0.4828	1.359	0.3724	0.4784	0.7808
SE	0.04715	0.8359	0.1971	0.5546	0.1520	0.1953	0.3188
F ratio				43.59			
P value	-	-	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

Formulation A has reduced the increased SGOT levels from 142.2 to 49.50 IU/L and SGPT levels from 175.0 IU/L to 57.33 IU/L, SALP level from 84.17 IU/L to 30.67 IU/L, and serum bilirubin level 11.30 IU/L to 5.01 IU/L. The accelerated stability at RT to 40 °C studies was aimed at establishing the physical, chemical, and biological stability of all 4 formulations. **Tables 9** to **12** showed no significant change in the stability profile of the formulations compared to the physical study of formulations before the stability study.

TABLE 9: ACCELERATED STABILITY STUDY OF FORMULATION A

Test	Observation						
	Initial	I Month		II Month		III Month	
		RT	40 ºC	RT	40 ºC	RT	40 ºC
Nature	Clear liquid	Clear liquid	Clear liquid	Clear liquid	Clear liquid	Clear liquid	Clear liquid
Colour	Brown	Brown	Brown	Brown	Brown	Brown	Brown
Odour	Charac-teristic	Charac-teristic	Charac-teristic	Charac-teristic	Charac-teristic	Charac-teristic	Charac-teristic
Taste	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet
Clarity	Clear	Clear	Clear	Clear	Clear	Clear	Clear
Viscosity (cps)	2.15	2.17	2.35	2.20	2.58	2.22	2.84
pH	7.2	7.2	7.2	7.2	7.2	7.2	7.2
Specific gravity	1.11	1.11	1.13	1.11	1.15	1.11	1.17

TABLE 10: ACCELERATED STABILITY STUDY OF FORMULATION B

Test	Observation						
	Initial	I Month		II Month		III Month	
		RT	40 ºC	RT	40 ºC	RT	40 ºC
Nature	Clear liquid	Clear liquid	Clear liquid	Clear liquid	Clear liquid	Clear liquid	Clear liquid
Colour	Brown	Brown	Brown	Brown	Brown	Brown	Brown
Odour	Charac-teristic	Charac-teristic	Charac-teristic	Charac-teristic	Charac-teristic	Charac-teristic	Charac-teristic
Taste	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet
Clarity	Clear	Clear	Clear	Clear	Clear	Clear	Clear
Viscosity(cps)	2.16	2.18	2.35	2.22	2.60	2.24	2.89
pH	6.9	6.8	6.8	6.8	6.8	6.8	6.8
Specificgravity	1.10	1.10	1.12	1.10	1.14	1.10	1.16

TABLE 11: ACCELERATED STABILITY STUDY OF FORMULATION C

Test	Observation						
	Initial	I Month		II Month		III Month	
		RT	40 ºC	RT	40 ºC	RT	40 ºC
Nature	Clear liquid	Clear liquid	Clear liquid	Clear liquid	Clear liquid	Clear liquid	Clear liquid
Colour	Brown	Brown	Brown	Brown	Brown	Brown	Brown
Odour	Charac-teristic	Charac-teristic	Charac-teristic	Charac-teristic	Charac-teristic	Charac-teristic	Charac-teristic
Taste	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet
Clarity	Clear	Clear	Clear	Clear	Clear	Clear	Clear
Viscosity(cps)	2.15	2.16	2.36	2.21	2.57	2.21	2.85
pH	6.8	6.8	6.8	6.8	6.8	6.8	6.8
Specificgravity	1.11	1.11	1.13	1.11	1.15	1.11	1.17

TABLE 12: ACCELERATED STABILITY STUDY OF FORMULATION D

Test	Observation						
	Initial	I Month		II Month		III Month	
		RT	40 ºC	RT	40 ºC	RT	40 ºC
Nature	Clear liquid	Clear liquid	Clear liquid	Clear liquid	Clear liquid	Clear liquid	Clear liquid
Colour	Brown	Brown	Brown	Brown	Brown	Brown	Brown
Odour	Charac-teristic	Charac-teristic	Charac-teristic	Charac-teristic	Charac-teristic	Charac teristic	Charac teristic
Taste	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet
Clarity	Clear	Clear	Clear	Clear	Clear	Clear	Clear
Viscosity(cps)	2.16	2.17	2.31	2.19	2.64	2.22	2.90
pH	7.1	7.1	7.1	7.1	7.1	7.1	7.1
Specific Gravity	1.10	1.10	1.12	1.10	1.13	1.10	1.16

The result of hepatoprotective activity of polyherbal formulation after stability study showed that formulation A is still having better hepatoprotective potential as compared to standard as shown in table No. 13 to 16. Formulation A has reduced the increased SGOT levels from 292.7 to 89.00 IU/L and SGPT levels from 263.0 IU/L to 74.50 IU/L, SALP level from 144.5 IU/L to 49.17 IU/L, and serum bilirubin level 10.83 IU/L to 4.450 IU/L.

TABLE 13: SHOWING ENZYMATIC SGPT LEVEL (IU/ L) AFTER STABILITY STUDY

S. no.	Control	CCl_4	Standard	Formulation A	Formulation B	Formulation C	Formulation D
1	36	212	67	70	73	74	71
2	38	225	78	79	75	79	79
3	41	250	73	80	79	81	72
4	54	321	77	78	76	83	74
5	52	309	67	68	105	109	97
6	43	261	63	72	85	87	68
Mean	44	263	70.83	74.50	82.17	85.50	76.83
SD	7.403	44.04	6.080	5.128	11.94	12.29	10.53
SE	3.022	17.98	2.482	2.094	4.875	5.018	4.301
F ratio				91.74			
P value	-	-	P<0.001	P<0.05	P<0.05	P<0.05	P<0.05

P<0.01 is significant

TABLE 14: SHOWING ENZYMATIC SGOT LEVEL (IU/ L) AFTER STABILITY STUDY

S. no.	Control	CCl_4	Standard	Formulation A	Formulation B	Formulation C	Formulation D
1	37	218	71	78	81	78	72
2	60	336	101	93	98	108	112
3	49	291	93	98	107	110	108
4	42	273	81	79	81	89	83
5	53	309	91	93	95	97	111
6	55	329	87	93	108	117	113
Mean	49.33	292.7	87.33	89	95	99.83	99.83
SD	8.548	43.43	10.39	8.367	11.95	14.61	17.72
SE	3.490	17.73	4.240	3.416	4.879	5.963	7.236
F ratio				95.18			
P value	-	-	P<0.001	P<0.001	P<0.001	P<0.001	P<0.05

P<0.01 is significant

TABLE 15: SHOWING ENZYMATIC SALP LEVEL (IU/ L) AFTER STABILITY STUDY

S. no.	Control	CCl_4	Standard	Formulation A	Formulation B	Formulation C	Formulation D
1	27	161	43	41	49	52	51
2	24	149	51	58	61	53	59
3	23	142	49	48	48	50	51
4	22	128	49	47	49	47	51
5	21	124	50	51	53	42	48
6	28	163	49	50	52	52	57
Mean	24.17	144.5	48.50	49.17	52	49.33	52.83
SD	2.787	16.33	2.811	5.565	4.817	4.179	4.215

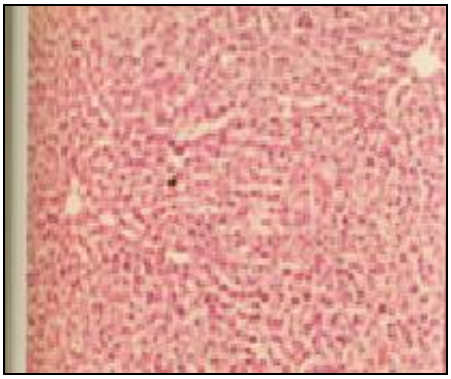
SE	1.138	6.667	1.147	2.272	1.966	1.706	1.721
F ratio				167.6			
P value	-	-	P<0.001	P<0.001	P<0.001	P<0.001	P<0.05

P<0.01 is significant

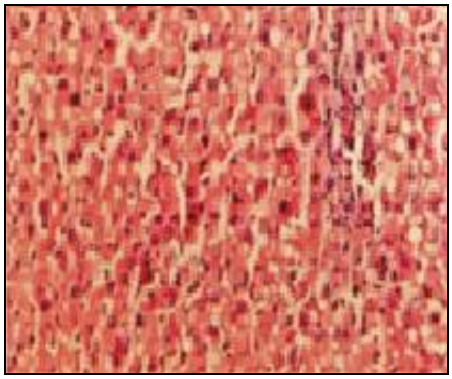
TABLE 16: SHOWING SERUM BILIRUBIN LEVEL (IU/ L) AFTER STABILITY STUDY

S. no.	Control	CCl_4	Standard Liv-52	Formulation A	Formulation B	Formulation C	Formulation D
1	2.2	8.0	3.0	3.0	5.0	4.0	6.0
2	2.0	11.0	5.0	4.5	6.0	6.200	7.5
3	2.2	12	5.0	6.0	6.2	5.0	7.0
4	2.2	13	3.0	4.3	4.0	6.0	7.0
5	2.2	12	4.5	3.5	6.0	6.0	5.0
6	2.0	9	3.0	5.4	6.0	6.0	6.0
Mean	2.158	10.83	3.917	4.450	5.533	5.5	6.417
SD	0.1155	1.941	1.021	1.126	0.8641	0.8367	0.9174
SE	0.04715	0.7923	0.4167	0.4595	0.3528	0.3416	0.3745
F ratio				36.81			
P value	-	-	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

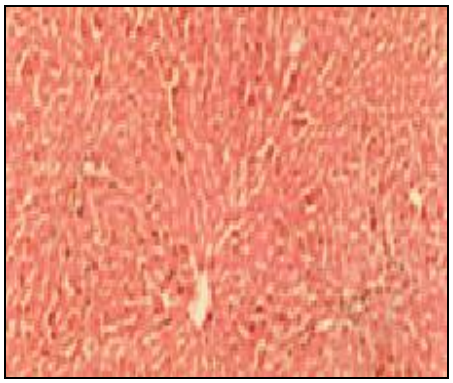
P<0.01 is significant



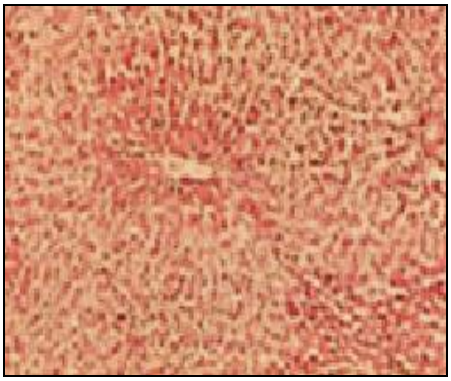
A. NORMAL LIVER



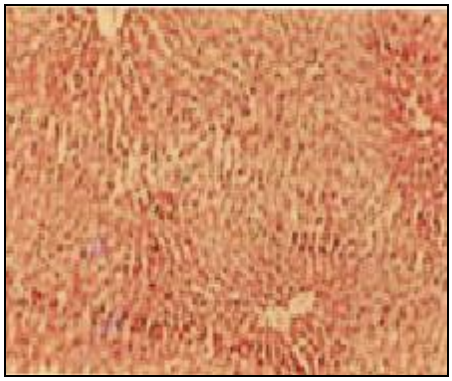
B. CARBON TETRACHLORIDE



C. LIV 52



D. FORMULATION B



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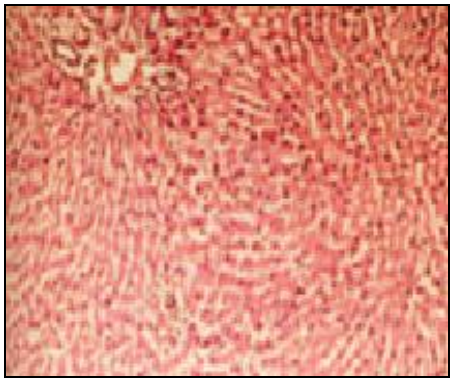
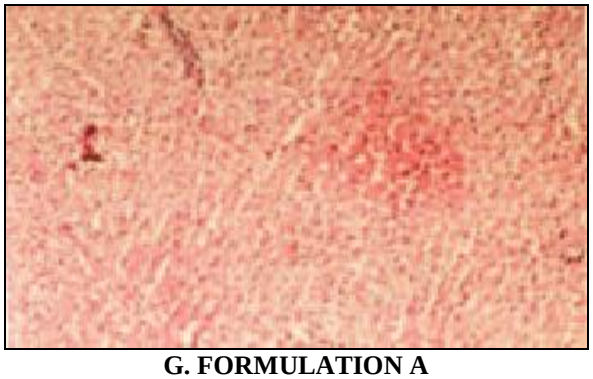


F. FORMULATION D



G. FORMULATION A

The microscopic histopathological evaluation of livers from the control group, CCl_4 treated group, Formulation A, Formulation B, Formulation C, Formulation D group, and standard drug Liv-52 have supported the hepatoprotective activity. Histopathological liver sections of the different groups showed the following observations. The control group, when observed under 100 × H.E of magnification, showed normal hepatocytes, sinusoids, Kupffer cells, and architecture within normal limits.

The CCl_4 treated group when observed under 100×H.E of magnification hepatocellular necrosis with fatty change. The standard Liv-52 group, when observed under 400 × H.E of magnification, showed normal hepatocytes and normal architecture. Amongst all formulations, Formulation A, when observed under 100×H.E of magnification, showed normal hepatocytes and normal architecture as compared to Formulation B, C, and D. Hence proved to be a good hepatoprotective **Plate 1**.

Plate 1: Photo showing histopathology study of Polyherbal formulation composed of *Oxalis corniculata*, *Bauhinia variegata,* and *Pterocarpus marsupium*. Histology of hepatic tissue of different treatment groups against CCl_4 induced hepatic toxicity. (a) Control group with normal histological features, (b) toxicant CCl_4 group necrotic areas and vacuole formation, (c) showing almost normal histology after treatment with standard Liv-52 drug. (d) (e) (f) (g) The normal architecture of hepatic tissue in animals treated with Formulation A Formulation B Formulation C and Formulation D of Polyherbal formulation composed of *Oxalis corniculata*, *Bauhinia variegata* and *Pterocarpus marsupium*.

ACKNOWLEDGEMENT: The authors are grateful to the Sandip Institute of Pharmaceutical Sciences, Sandip Foundation Nashik for the kind provision of research laboratories to conduct these research activities.

CONFLICTS OF INTEREST: There are no conflicts of interest.

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How to cite this article:
Jadhav AG, Dhikale RS: Hepatoprotective effects of polyherbal formulation against carbon tetrachloride-induced hepatic injury in albino rats. Int J Pharm Sci & Res 2021; 12(8): 4465-75. doi: 10.13040/IJPSR.0975-8232.12(8).4465-75.