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An experimental approach to development and evaluation of hepatoprotective polyherbal formulation

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The mainstream of this research is to explore the pharmacological assessment of polyharbal suspension (PHS) for its hepatic defensive potential. Hydro-alcoholic fruit extracts of *Solanum xanthocarpum* Schrad. and Wendl (SXHE), & *Trichosanthes dioica* Roxb (TXHE) and whole plant extract *Hedyotis corymbosa* (L.) (HCHE) were inspected for hepatoprotective activity against simvastatin induced hepatic toxicity. The trituration method was used for formulating polyharbal suspension by preparing different fractions containing SXHE, HCHE, and TXHE with 0.5% w/v carboxymethyl cellulose as a suspending agent with other excipients. Hence, therapeutically four effective formulations were assigned *viz*., F_{1E}, F_{2B}, $F_{3C,}$ and F_{4H} and among all the formulations, F_{4H} has exhibited impressive hepatoprotective effect by reducing the elevated hepatic biomarkers and restoring the antioxidant enzymes. These biochemical observations were supplemented by histopathological examination of the liver. It has been observed that many allopathic and plant-based formulations represented minimal or negligible *in vivo* activity because of poor bioavailability, so to overcome these limitations, the developed polyharbal suspensions were further optimized by adding trikatu in different ratios and assigned the name as $F_{1E\beta}$, $F_{2B\beta,}$ $F_{3C\beta,}$ and $F_{4H\beta}$**.** Among all the optimized suspensions containing 30 mg/100 mL of trikatu extract, $F_{4H\beta}$ possessed maximum effective hepatoprotective and antioxidant potential in comparison to Liv-52 and silymarin treated rodents. So, the outcome of the present research indicated that the selected medicinal plants and their formulation possess better hepatoprotective and antioxidant activities.

Keywords: Hepatoxicity, Superoxide dismutase, Trikatu, Polyharbal Formulation

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It is well known that the liver is the most integral organ performing multiple metabolic functions, detoxification of endogenous and exogenous xenobiotics, and is essential for the body energetics. The Global Burden of Disease study data in 2017 expressed 2.14 million human deaths due to liver-associated dysfunction[1]. The phytochemical compounds including silymarin, catechin, schisandrin, and many more illustrate liver protective activity. The concept of polyherbal is intrinsic to ayurveda and historically, the ayurvedic literature "Sarangdhar Samhita" highlighted the concept of synergism behind polyherbal formulations. These formulations have extensive curative province and minimal adverse effects[2,3]. *S. xanthocarpum, H. corymbosa,* and *T. dioica* have been used for ages in ayurveda as distinguished therapeutic agents[4]. Fruits and flowers of *S. xanthocarpum* as good carminative. The decoction of root castoff as febrifuge, expectorant, and diuretic[5]. As per the Charaka and Sushruta fruit and the entire plant, an extract is used for the treatment of dysuria, gonorrhea, ear infection, misperistalsis, diabetes, helminthic, spasm, tumour, cardiotonic, and hypertension[6]. *H. corymbosa* is widely utilized in current disorders including skin afflictions, hepatitis, and eye illnesses[7]. It has febrifugal, anthelmintic, diaphoretic, and stomachic properties[8]. It cures jaundice, different illnesses of the liver, viral fever, congestion, and other ailments like bronchitis, immune-potentiation, tumours, gastritis, burning sensation in palms of the hand, neural putrefaction, disturbed bile, and hepatopathy[9]. T. dioica is used in the ayurveda similarly by the common and natural folkloric specialists in different human distresses. In ayurveda, it is used alone or in combination with various herbs to treat hyperacidity. Nadkarni depicted its usage as anti-parasitic, antiperiodic, febrifuge, refrigerants, anti-coagulants, and blood-purifiers. A decoction of leaves mixed with Piper nigrum natural product powder is used to treat *inflammatory bowel* disease[10,11]. The plant is used as stomachic, antitumour and antidiabetic[12]. In ayurveda leaves and fruits of T. dioica used to treat alcohol misuse and jaundice[13-17]. *The polyherbal formulation under this research study contains plant ingredients of S. xanthocarpum, H. corymbosa, T. dioica.* Therefore, the present research was undertaken to

develop and evaluate polyherbal formulation from the hydro-alcoholic extracts of *S. xanthocarpum, H. corymbosa*, and *T. dioica* against simvastatin induced hepatotoxic model and compare these effects with standard marketed formulation Liv-52 and reference drug as silymarin.

Material and Methods

Plant materials

The plants' materials used for the preparation of a polyherbal suspension includes *S. xanthocarpum* (fruits), *H. corymbosa*, (whole plant), *T. dioica* (fruits) species collected and authenticated by Dr. Alok Lehari (Taxonomist) at National Botanical Research Institute, Lucknow, Uttar Pradesh, India. A receipt of the sample was preserved in our exploration research facility for future reference.

Experimental animals

Ethical rules were followed for taking care of and performing experiments on laboratory animals. Wistar rodents of either sex weighing between 150-200 g were obtained from the animal house of Shri Ram Murti Smarak College of Engineering and Technology, Bareilly, Uttar Pradesh, India. They were inspected for physical wellness before the study. The rodents were reserved in polypropylene cages for one week under ordinary research laboratory conditions in the animal house at temperature (22±2 °C), relative humidity (55±5%) and light (12 h light/dark cycles), and research protocols were approved by Institutional Animal Ethics Committee 715/C/02/CPCSEA.

Preparation of polyherbal suspensions

Air dried and crushed plant materials were extracted with a hydro-alcoholic solvent system (40:60) at room temperature with occasional shaking for 72 h until the solvent was evaporated under reduced pressure using a rotary evaporator. Polyherbal suspensions containing extracts showing significant hepatoprotective activity were prepared using the trituration method, using 0.5% w/v carboxymethyl cellulose (CMC) as a suspending agent and other excipients. Formulation F_{1E} was prepared by using 0.3 kg of hydro-alcoholic fruit extracts of *S. xanthocarpum (*SXHE) and 0.2 kg hydro-alcoholic whole plant extracts of *H. corymbosa* (HCHE). F_{2B} was prepared by using hydro-alcoholic extracts of 450 mg of HCHE and 50 mg of hydro-alcoholic extracts T. *dioica* (TXHE). F_{3C} was prepared by using 400 mg of hydro-alcoholic extracts of SXHE and 100 mg of TXHE while F_{4H} formulation was prepared by the combination of 350 mg of hydro-alcoholic extracts of SXHE, 75 mg of HCHE, and 75 mg of TXHE. Table 1 shows the formulation composition of polyherbal suspensions.

Assessment of antioxidant and liver defensive effect of F_{1E}, F_{2B}, $F_{3C,}$ and F_{4H} polyherbal suspensions in Sim-20 model

After acclimatization, the rodents were separated into eight different groups of six rodents each. Group-I, rodents served as normal control were received normal saline with standard diet once daily for 30 days. Group-II, rodents served as negative control were received Sim-20 (simvastatin 20 mg/kg, body weight) for 30 days. Test group (III-VI), rodents received polyherbal suspension F_{1E}, F_{2B}, F_{3C}, and F_{4H}, at 20 mg/kg, body weight, p.o., correspondingly for the same period of 30 days. Group-VII, rodents (I-positive control) received Liv-52 (1.0 mL/kg body weight) for 30 days. Group-VIII, rodents (II-positive control) received silymarin (Syl-20) at 20 mg/kg body weight, p.o., daily for the same period. Rodents were anesthetized and blood samples were withdrawn separately from each group for assessment of liver serum biomarkers on the 31st day. The liver was also isolated, flushed with saline, and set away at -80°C on the same day and was utilized for the estimation of antioxidative and histopathological analysis[18].

Optimization of different polyherbal suspensions with the addition of trikatu extract

Prepared polyherbal suspensions (F_{1E}, F_{2B}, F_{3C}, and F_{4H}) were optimized by the addition of 30 mg of trikatu in equal ratio, and the name of optimized polyherbal formulations was assigned as $F_{1E\beta}$, $F_{2B\beta,}$ $F_{3C\beta,}$ and $F_{4H\beta}$ with 0.5% w/v CMC respectively. Each 100 mL of suspension has the following proportions of extracts as given in Table 2.

Table 1 — Formulation composition of polyherbal suspensions.

Ingredients	F_{1E}	F_{2B}	F_{3C}	F_{4H}
S. xanthocarpum	300 mg	-	400 mg	350 mg
H. corymbosa	200 mg	450 mg	-	75 mg
T. dioica	-	50 mg	100 mg	75 mg
CMC (0.5% w/v)	500 mg	500 mg	500 mg	500 mg
Sucrose	10,000 mg	10,000 mg	10,000 mg	10,000 mg
Sorbitol	5000 mg	5000 mg	5000 mg	5000 mg
Methyl paraben	0.05%	0.05%	0.05%	0.05%
Propyl paraben	0.02%	0.02%	0.02%	0.02%
Lemon oil	0.1%	0.1%	0.1%	0.1%
Purified water q.s.	up to 100 mL	up to 100 mL	up to 100 mL	up to100 mL

Table 2 — Formulation composition of polyherbal suspensions

Ingredients	$F_{1E\beta}$	$F_{2B\beta}$	$F_{3C\beta}$	$F_{4H\beta}$
S. xanthocarpum	300 mg	-	400 mg	350 mg
H. corymbosa	200 mg	450 mg	-	75 mg
T. dioica	-	50 mg	100 mg	75 mg
P. longum	10 mg	10 mg	10 mg	10 mg
P. nigram	10 mg	10 mg	10 mg	10 mg
Z. officinale	10 mg	10 mg	10 mg	10 mg
CMC (0.5% w/v)	500 mg	500 mg	500 mg	500 mg
Sucrose	10,000 mg	10,000 mg	10,000 mg	10,000 mg
Sorbitol	5000 mg	5000 mg	5000 mg	5000 mg
Methyl paraben	0.05%	0.05%	0.05%	0.05%
Propyl paraben	0.02%	0.02%	0.02%	0.02%
Lemon oil	0.1%	0.1%	0.1%	0.1%
Purified water q.s.	up to 100 mL	up to 100 mL	up to 100 mL	up to 100 mL

Assessment of antioxidant and liver defensive impact of $F_{1E\beta}$, $F_{2B\beta}$, $F_{3C\beta}$, and $F_{4H\beta}$ polyherbal suspensions in Sim-20 model

After acclimatization, the rodents were separated into eight different groups of six rodents in each. Group-I, rodents served as normal control were received normal saline with standard diet once daily for 30 days. Group-II, rodents served as a negative control was dosed with Sim-20 (simvastatin 20 mg/kg, body weight) for the same period. Test group (III-VI), rodents received polyherbal suspension $F_{1E\beta}$, $F_{2B\beta}$, $F_{3C\beta}$, and $F_{4H\beta}$ at 15 mg/kg, body weight, p.o. correspondingly for 30 days. Group-VII and VIII (positive control) received silymarin (Syl-20) at 20 mg/kg body weight, p.o and Liv-52 at the dose of (1 mL/kg, body weight, p.o.) along with Sim-20 mg/kg body weight, correspondingly daily for 30 days. Rodents were anaesthetized, and blood samples were withdrawn separately to assess the liver serum biomarkers on the 31st day. Liver samples were isolated, flushed with saline, and set away at -80°C on the same day. They were utilized for the estimation of antioxidative and histopathological analysis[18].

Histopathology

For histological assessments, the hepatic tissues were fixed with 10% solution formalin, dehydrated in graded (50-100%) alcohol and embedded in paraffin. Fine hepatic sections (5 µM) were cut using a rotatory microtome (AMR 400, Amos Scientific, Australia) and stained with hematoxylin and eosin (H&E). The liver sections were examined for photo microscopic analysis (Nikon Eclipse E400 microscope with digital camera, United States of America).

Evaluation of polyherbal suspension

The poly-herbal suspensions were evaluated for their organoleptic and physicochemical properties. These evaluations were done for 5 months at 30 days of interval.

Organoleptic evaluation

Following physical parameters like color, odor, texture, and taste of the polyherbal suspensions were tested.

Physicochemical properties

Following parameters like flow rate, viscosity, sedimentation volume, redispersibility, and pH were studied.

Statistical analysis

All the values were represented as Mean ± S.E.M. All the studied groups were analyzed by one-way ANOVA followed by "Newman's keules" tests to assess significant differences. Graph Pad Prism 5.01 version software was used to assess the data.

Results and Discussion

It has been reported that cirrhosis causes 1.16 million mortality and hepatic cancer 788,000 deaths, making them the 11th and 16th most common causes of death each year. Combined, they represent 3.5% of all mortality around the world. The liver is the paramount organ that plays an essential role in preserving the biological steadiness of vertebrates. The hepatic functional spectrum embraces metabolism and disposition of xenobiotics, biomolecules, hemostasis mechanism, and immunomodulation, *etc*.[19].

Conventional or allopathic medications are used in the treatment of hepatic dysfunction are sometimes inadequate and can have serious adverse effects. So, there is a worldwide trend to go back to an alternative system of medicines.[19] In PHFs pharmacological standards cooperate uniquely to deliver the most extreme remedial viability with the least symptoms. There are two ways which based on the nature of interaction to interpret how synergism performs *viz*. pharmacodynamics and pharmacokinetics[20-22]. As far as pharmacokinetic synergism, the capacity of herbs to encourage the absorption, distribution, metabolism, and elimination of the other herbs is engaged. Then again, in pharmacodynamics synergism, researchers study the synergistic effect of the impact of dynamic constituents with comparable remedial action are focused on a comparative receptor or physiological framework[23]. Here, the blend of herbals simultaneously follows up on multiple targets to give a thorough relief. Owing to the additive or synergism

effect, polyherbal offers some great assistances which lacks in single component herbal preparation[24].

Better therapeutic impact can be accomplished with a solitary multicomponent preparation[25-26] polyherbal formulations reduce the daily intake of a particular constituent and produce desired therapeutic effects through a different mechanism of action. Several PHFs were prepared by the combination of *S. xanthocarpum, H. corymbosa,* and *T. dioica,* and the best formulations were selected based on their hepatoprotective activity shown on the pharmacological model used under investigation. Here, the blending of herbals may target multiple sites simultaneously and produce additive or synergistic therapeutic effects. The hepatoprotective efficacy of the polyherbal formulations was assessed against simvastatin induced hepatotoxicity. Ten different Polyherbal suspensions were prepared from SXHE and HCHE by combining these in different proportions and among those, the therapeutically most effective combination is F_{1E}. Similarly, ten different polyherbal suspensions were prepared from HCHE and TXHE by adding them in different proportions and among those, the therapeutically most effective combination is F_{2B}. Likewise, ten polyherbal suspensions were prepared by SXHE and TXHE from which F_{3C} is the therapeutically most effective combination. However, twelve polyherbal suspensions were prepared from the combination of all three selected plant extracts (SXHE, HCHE, and TXHE) in different proportions, and among those, F_{4H} is $_{\text{the}}$ therapeutically most effective combination against simvastatin induced hepatopathy.

Sometimes metabolism of medications by the enzymatic system leads to the production of highly active and poisonous compounds which yield hepatic injury, e.g., in the case of simvastatin, carbon tetrachloride, D-galactosamine, *etc*. A primary mechanism of simvastatin persuaded hepatotoxicity is that it causes apoptosis[27] by hindering the respiratory chain, depolarising the mitochondrial membrane, and releasing calcium ions. In our study, a substantial increase in hepatic serum markers like SGOT, SGPT, ALP, BLB, were observed after the administration of simvastatin (20 mg/kg, p.o). Sim-20 also affects the antioxidant system, by depletion of SOD, GSH, and CAT with an enlarged level of LPO[28]. The attenuated conditions of SOD and CAT were observed in the negative control (Sim-20) treated group as an indication of hepatic damage. The rodents treated with F_{4H} PHF at 20 mg/kg, b.w., elevated level of these antioxidant enzymes, which shows the best antioxidant action as compared to other PHF ($F_{3C,}$ $F_{2B,}$ F_{1E}) as well as standard drugs (Liv-52 and Syl-20).

Glutathione (GSH) is the major antioxidative tripeptide that is present within the cellular structure and plays an essential role in the detoxification process, participates in the biotransformation of various nutrients, and controls cellular homeostasis[29]. Various mechanisms were associated with the hepatic GSH depletion, perhaps caused by oxidative stress, enlarged lipid peroxidation, and inhibiting the biosynthesis of GSH. All these mechanisms are accountable for the decrease of GSH[30]. The enhanced hepatic GSH level was shown in F_{4H} PHF treated group at 20, mg/kg., dose level might be either due to regeneration of GSH or their *de novo* synthesis of GSH. Reduced LPO levels to stop the formation of unwarranted free radicals would be the major concerned in any antioxidant therapy. The animals treated with F_{4H} PHF greatly reduced the level of LPO compared to other PHF (F3C, F2B, F1E) and standard drugs (Liv-52 and Syl-20). Based on the evaluated biochemical parameters, histopathological study, and pharmacological activity it is found that the polyherbal formulation F_{4H} shown to be the best in terms of hepatoprotective and antioxidant effect, which is greater than the commercial formulation Liv-52 and reference standard silymarin treated group in Sim-20 hepatotoxicity models.

The consequence of hepatoprotective and antioxidant outcomes of F_{1E}, F_{2B}, $F_{3C,}$ and F_{4H} polyherbal suspensions in Sim-20 model

Sim-20 treated animals showed a noteworthy upsurge in hepatic markers (SGOT, SGPT, ALP, bilirubin) and decreased TP levels. The animals treated with F_{1E}, F_{2B}, $F_{3C,}$ and F_{4H} polyherbal suspensions demonstrated a noteworthy decrease in hepatic markers and increased TP levels. Hepatic damage initiated by simvastatin caused an increase in hepatic markers as SGOT by 154.81%, SGPT by 92.93%, ALP by 139.84%, and BLB by 695.8% however, decreased the TP levels by 47.72%, as equated with normal control (Group-I). The % decreased in liver serum marker in F_{1E} treated group at the dose of 50 mg/kg, body weight, as SGOT 52.54%, SGPT 31.88%, ALP 52.2%, BLB 10.47%, however, there was an increase in TP 52.17% as juxtaposed with Sim-20 treated animals. The animals treated with F_{2B} polyherbal suspension showed a percentage decrement in hepatic markers like SGOT 42.89%,

SGPT 16.76%, ALP 43.99%, and BLB 17.27%, however, decrease in TP by 30.43% as compared with group-II animals. F_{3C} and F_{4H} suspension showed a percentage decrease in SGOT by 17.36% & 54.88%, SGPT by 10.64% & 34.25%, ALP by 9.2% & 54.47%, BLB by 10.47% & 64.92%, while increased in TP by 13.04% and 60.43% correspondingly as compared with Sim-20 treated animals. While Group-VII animals treated with Liv-52 showed a percentage decrease in hepatic serum markers at the proportion of (1.0 mL/kg, body weight) and Group-VIII rodents dosed with silymarin (20 mg/kg, body weight) exhibited SGOT 55.54% & 58.80%, SGPT 35.93%, & 39.98%, ALP 55.15% & 57.91%, BLB 78.53% & 85.34%, however, increased in TP level by 65.21% & 78.26% respectively as compared with the animal treated with Sim-20. Among these polyherbal suspensions, the maximum liver protective effect was seen in the F_{4H} formulation which is nearly equivalent to the rodents treated with Liv-52 and Syl-20 treated group. The results are tabulated in Table 3.

The consequence of F_{1E}, F_{2B}, $F_{3C,}$ and F_{4H} polyherbal suspensions on hepatic antioxidant parameters in the Sim-20 model

Hepatic cytochromes P_{450} (CYPs) are the drugs metabolizing enzymes and comprise a supergroup of monooxygenases[28]. Statins produce a noteworthy quantity of ROS that causes lipid peroxidation, prompting a decline in the mitochondrial layer potential and encouraging hepatotoxicity. The highest amount of ROS is formed by simvastatin[31]. Simvastatin is primarily metabolized by CYP3A4 enzymes. All of these effects are dose- and time-dependent[29]. Simvastatin produced oxidative stress by producing ROS, redox-condition changes, mitochondrial damage, aldehyde production, and plasma membrane breakdown[32]. During hepatic injury, Kupffer cells produce ROS and cytokines, both of which can lead to excessive hepatic damage[33,34]. Hepatotoxicity induced by simvastatin was characterized by elevated levels of hepatic serum indicators like ALP, SGOT, SGPT, and BLB, however, it diminished the degree of total proteins (TP) level which was normally located in the cell cytoplasm. All these hepatic markers are released into systemic circulation during hepatic damage[35]. Bilirubin activity was considered to be one of the best indicators of hepatic impairment[36]. The abnormal upsurge in the levels of serum BLB specifies hepatobiliary ailment and causes the unsettling severe influence of hepatocellular function[37]. The outcomes depicted in Fig. 1 demonstrated the noteworthy percentage increase in the level of LPO in Sim-20 (Group-II) inebriated rodents as 125% juxtaposed with normal control (Group-I). Rodents dosed with F_{1E}, F_{2B}, $F_{3C,}$ and F_{4H} polyherbal suspensions at the doses of 50 mg/kg, body weight, noteworthy reduced the level of LPO by 47.88%, 42.06%, 33.59%, and 50.74% respectively. Sim-20 treated animals showed a noteworthy percentage reduction in antioxidant parameters like GSH, SOD, and CAT as 47.5%, 41.66%, and 42.42% correspondingly as equated to Group-I (normal control). These antioxidant effects had a noteworthy increase in rodents treated with polyherbal suspensions F_{1E}, F_{2B}, $F_{3C,}$ and F_{4H} treated groups. The % increase showed by F_{3C}, F_{2B}, F_{1E} and F_{4H} polyherbal suspensions in SOD as 12%, 31.5%, 53.12%, 78.12%, CAT 14.28%, 38.09%, 42.85%, 57.14% while in GSH 15.78%, 36.84%, 47.36%, 68.42% correspondingly as equated with the Sim-20 treated rodents. However, percentage upsurge in GSH, SOD and CAT treated with Liv-52 at the portion of (1.0 mL/kg, body weight) and silymarin (20 mg/kg, body weight) showed SOD 59.37% & 65.6%, CAT & 47.61%, 52.38, GSH 52.01% & 52.6% correspondingly which is juxtapose to Sim-20 treated animals. Out of these polyherbal suspensions, F_{4H} showed the greatest antioxidant potential, which

Table 3 — Effect of polyherbal suspension F_{1E}, F_{2B}, $F_{3C,}$ and F_{4H} on hepatic serum markers and total protein, in Sim-20 model

Group	Treatment	SGOT	SGPT	ALP	BLB	TP
Group-I	Control	60.2 ± 2.1	53.8 ± 1.3	63.5 ± 2.2	0.24 ± 0.01	4.4 ± 0.03
Group-II	Sim-20	153.4 ± 3.5†	103.8 ± 2.9†	152.3 ± 1.8†	1.91 ± 0.02†	2.3 ± 0.02†
Group-III	F_{3C}+Sim20	127.24 ± 2.1^{c}	92.7 ± 2.2^{b}	138.2 ± 2.4^{c}	1.72 ± 0.03^{c}	2.6 ± 0.06^{c}
Group-IV	F_{2B}+Sim20	87.6 ± 2.2^{c}	86.4 ± 2.4^{c}	85.3 ± 2.1^{c}	1.58 ± 0.01^{c}	3.0 ± 0.01^{c}
Group-V	F_{1E}+Sim20	72.8 ± 2.1^{c}	71.5 ± 2.3^{c}	72.8 ± 3.1^{c}	0.89 ± 0.06^{c}	3.5 ± 0.04^{c}
Group-VI	F_{4H}+Sim20	69.2 ± 1.8^{c}	68.24 ± 2.1^{c}	69.34 ± 1.9^{c}	0.67 ± 0.04^{c}	3.69± 0.06^{c}
Group-VII	Liv-52+Sim20	68.2 ± 2.8^{c}	66.5 ± 2.8^{c}	68.3 ± 3.4^{c}	0.41 ± 0.04^{c}	3.8 ± 0.03^{c}
Group-VIII	SYL-20+Sim20	63.2 ± 2.6^{c}	62.3 ± 2.1^{c}	64.1 ± 1.2^{c}	0.28 ± 0.07^{c}	4.1 ± 0.04^{c}

All the values are expressed as mean ± S.E.M & n=6; n: non-significant; *P* values: †<0.001 related with rodents of the control group (Group I); *P* values: ^{a}<0.05, ^{b}<0.01, ^{c}<0.001 related with rodents of toxic group (Group II)

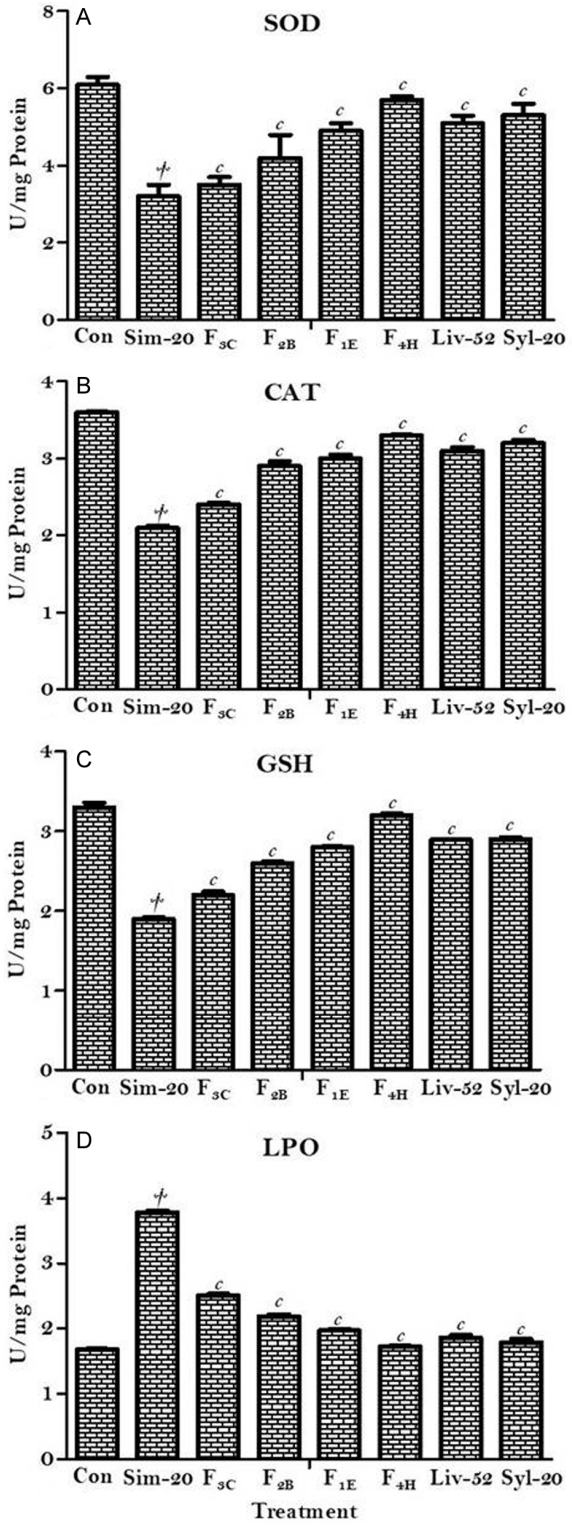

Fig. 1 — Effect of F_{1E}, F_{2B}, F_{3C}, and F_{4H} polyherbal suspensions on *in vivo* hepatic antioxidant parameters against simvastatin induced hepatotoxicity. [All the values are expressed as mean ± S.E.M & n=6; n: non-significant; *P* values: †<0.001 related with rodents of the control group (Group I); *P* values: ^{a}<0.05, ^{b}<0.01, ^{c}<0.001 related with rodents of the toxic group (Group II)]

is greater than that of Liv-52 and Syl-20-treated animals.

Histopathological study reveals the hepatoprotective effect of F_{1E}, F_{2B}, F_{3C}, and F_{4H} polyherbal suspensions

The liver fragment of the control group of animals showed the typical hepatic cells with the central vein. Liver fragment of simvastatin (20 mg/kg, p.o.) treated rodents showed fatty changes (shown by arrow 2), putrefaction, CV blockage (arrow 1), ballooning degeneration (arrow 5, 6), and the loss of cell membrane (arrow 3, 4).Liver fragments of animals treated with simvastatin (20 mg/kg) + PHS F_{3C}), demonstrated the less fatty changes with negligible provocative conditions, loss of cell membrane (arrow 1, 3), less invasion of the leucocytes (arrow 2). The liver segment of rodents treated with [(Sim-20 mg/kg, p.o.) + F_{2B})], demonstrated the recovery of hepatocyte around CV (arrow 1) with less loss of cell membrane.A liver segment of animals treated with [(Sim-20 mg/kg, p.o.) + F_{1E})] demonstrated the well brought out the focal vein, hepatic cells with protected cell plasm. A liver segment of animals treated with [(Sim-20mg/kg, p.o.) + F_{1E}] demonstrated the well brought out the focal vein, hepatic cells with preserved cell plasm.A liver segment of animals treated Sim-20 and Liv-52 demonstrated the well brought out the focal vein, a hepatic cell with very much safeguarded cell plasm (Fig. 2). A liver segment of rodents treated with Sim-20 and Syl-20 demonstrated the well brought focal vein, a hepatic cell with very much safeguarded cytoplasm.

As per bioavailability of the phytoconstituents, it has been found that due to poor pharmacokinetic and pharmacodynamic properties it would have not been possible to attain the desired therapeutic efficacy. Many allopathic and plant-based formulations despite their imposing *in vitro* observations demonstrate trace or negligible *in vivo* activity because of poor lipid dissolvability, inappropriate molecular size, poor absorption, and bioavailability.

To overcome these limitations, the therapeutic efficacy of developed polyherbal suspensions (F1E, F2B, F3C, and F4H), is further optimized by the addition of trikatu in equal ratio as a bioavailability modifier. Trikatu is an Ayurvedic formulation mentioned in ayurveda that is used for the treatment of several ailments[38]. Trikatu Churna (TC) is an important polyherbal ayurvedic formulation of Piper nigrum, Piper longum, and Zingiber officinale in equal proportion[39,40]. Since ancient times, it has been

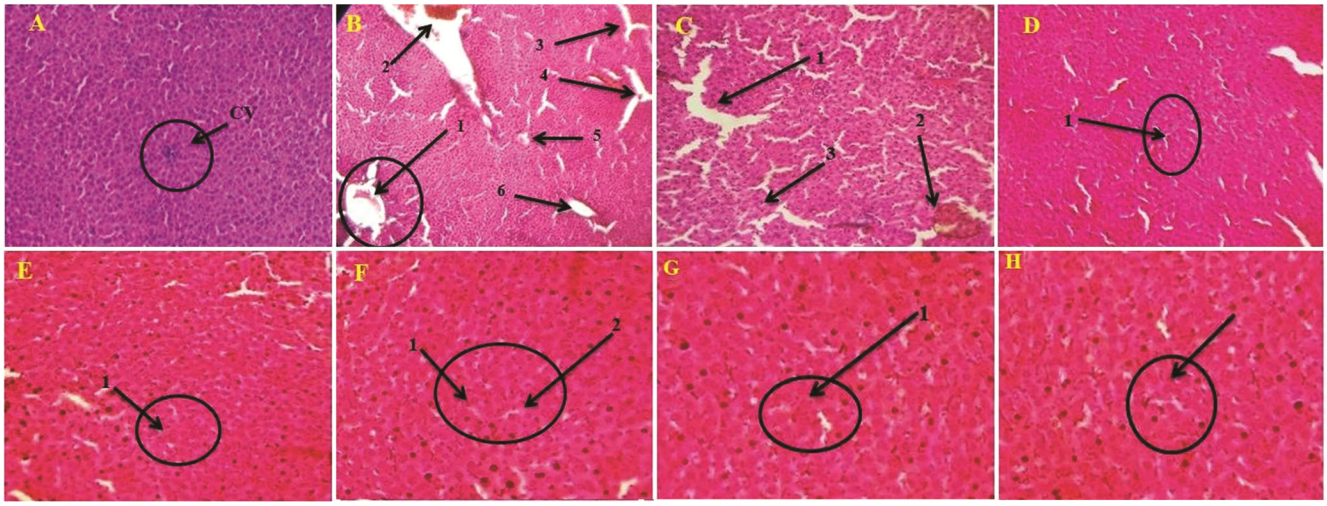

Fig. 2 — Photomicrograph of rat liver. (A) Control Group; (B) Toxic group (Sim-20); (C) Sim-20mg/kg + polyherbal suspension F_{3C}; (D) Sim-20 mg/kg + polyherbal suspension F_{2B}; (E) Sim-20 mg/kg + polyherbal suspension F_{1E}; (F) Sim-20 mg/kg + polyherbal suspension F_{4H}; (G) Sim-20 mg/kg, + Liv-52; and (H)Sim-20 + Syl-20. (10×, H & E stain).

recommended for digestive impairment[41], respiratory disorders[42], throat diseases, skin diseases, sinusitis, anti-inflammatory[43], cough, and filariasis, antioxidant[44], antihyperlipidemic[45], anti-anorectic[46], antitumour[47], hepatoprotective[48], antimicrobial[49], anthelmintic[50], immunomodulatory[51], antiarthritic[52], and anti-inflammatory[53] activities. Piperine is an amide alkaloid and is the major constituent of *Piper longum* Linn. *and Piper nigrum* Linn. that enhances the bioavailability of drugs and nutrients by inhibiting the cytochrome P_{450}-dependent metabolism of drugs[54].

Due to its bio-enhancing property piperine is used in most herbal formulations to enhance the bioavailability of therapeutically important phytoconstituents. Gingerol is the chief phytoconstituent of the Zinger (*Zingiber officinale* Rosc.) which better absorbs other substances including drugs by regulating gastrointestinal functions[54]. Thus, piperine and gingerol act as bioavailability enhancers, and these molecules do not show any hepatoprotective activity. However, when piperine and gingerol are blended with other drugs, they increase the therapeutic effect of the drug molecule in several ways. As per bioavailability of the phytoconstituents, it has been found that due to poor pharmacokinetic and pharmacodynamic properties it would have not been possible to attain the desired therapeutic efficacy. Many allopathic and plant-based formulations despite their imposing *in vitro* observations demonstrate trace or negligible *in vivo* activity because of poor lipid dissolvability, inappropriate molecular size, poor absorption, and bioavailability.

According to ayurveda's Bhaisajyaratnawali, trikatu is a compound herbal remedy made up of three bitter herbs that are combined in equal parts. This outstanding combination is made from dried *Piper nigrum* (Maricha), *Piper longum* (Peepli), and *Zingiber officinale* (Sunthi) rhizomes. In numerous herbal preparations, trikatu is utilized to increase bioavailability. The biomarker for *P. longum* and *P. nigrum* is piperine. In addition to increasing the medication's transmembrane bioavailability, piperine also potentiates the drug molecule through conformational interactions, reduces HCl production, and improves blood flow to the gastrointestinal tract (GIT)[55]. inhibition of gastric emptying time, intestinal motility, and gastrointestinal transit[56,57] alterations to GIT Permeability of epithelial cell membranes[58,59].

Thermogenic qualities and bioenergetics[60]. preventing the first pass drug metabolizing enzymes are metabolized and inhibited. Gamma-glutamyl transpeptidase activity stimulation, which improves amino acid uptake[61]. Trikatu is also included in a number of ayurvedic remedies in an effort to correct the imbalances in the "tridoshas- vatta, pitta, and kapha". It elevates the Pitta while calming the overactive Vata and Kapha. It has a bitter (katu) flavor, a hot (ushna) potency, a light (laghu) and dry (ruksha) quality, and a therapeutic impact on the digestive system (amapachaka). Modern pharmacological investigations have also shown that trikatu, combined with other phytoconstituents and synthetic medications, can increase their bioavailability, assisting in achieving therapeutic

objectives. In addition to its well-known health advantages, trikatu has the potential to have immunomodulatory, antiviral, expectorant, carminative, hypolipidemic, hypoglycemic, antiemetic, and anti-inflammatory effects. Piperine is an amide alkaloid and is the major constituent of *Piper longum* Linn. *and Piper nigrum* Linn. that enhances the bioavailability of drugs and nutrients by inhibiting the cytochrome P_{450}-dependent metabolism of drugs. Piperine has been used in the management of tuberculosis in humans due to its bio-enhancing properties. The bioavailability of rifampin is enhanced by nearly 60-65% in the presence of amide alkaloid piperine, so the dose is reduced from 450 to 200 mg. This literature tempted the author to incorporate trikatu extract in different proportions to develop polyherbal suspensions (F_{1E}, F_{2B}, $F_{3C,}$ and F_{4H}) and study its therapeutic effect.

The consequence of hepatoprotective and antioxidant outcomes of optimized polyherbal suspensions ($F_{1E\beta}$, $F_{2B\beta,}$ $F_{3C\beta,}$ and $F_{4H\beta}$) in Sim-20 model

Rodents that were intoxicated with Sim-20 showed a noteworthy upsurge in the level of hepatic serum markers i.e., SGOT, SGPT, ALP, BLB while lessened the level of TP. Polyherbal suspensions $F_{1E\beta}$, $F_{2B\beta,}$ $F_{3C\beta,}$ and $F_{4H\beta}$ significantly lessening the hepatic markers and upsurge the TP levels. Rodents dosed with simvastatin (Group-II) significant rise in the hepatic markers SGOT by 154.81%, SGPT by 92.93%, ALP by 139.84%, BLB by 695.8%, and reduced the level of TP by 47.72% as equated with normal control (Group-I). Group-V rodents that were dosed with $F_{1E\beta}$ PHS exhibited a noteworthy upsurge in hepatic serum markers as SGOT 53.19%, SGPT 33.14%, ALP 54.82%, BLB 59.68%, however lessening the TP levels by 60.43% as juxtaposed to Group-II rodents. Group-IV albinos which were treated with $F_{2B\beta}$ PHS reflect noteworthy reduced level of SGOT by 46.8%, SGPT by 19.46%, ALP by 46.21 %, BLB by 28.79%, however, increased the level of TP by 53.04% as related with Group-II rodents. Rodents treated with $F_{3C\beta}$, PHS i.e., Group-III, meaningfully reduced the levels of the hepatic marker as SGOT by 20.20%, SGPT by 13.04%, ALP by 14.51%, BLB by 16.75%, however, augmented the TP level by 16.95%. Albinos were dosed with $F_{4H\beta}$ PHS i.e., Group-VI rodents, showed significant deduction in the level of hepatic serum markers as SGOT by 54.01%, SGPT by 65.14%, ALP by 58.62%, BLB by 86.31%, however, augmented the TP level by 85.21% as Group-II animals. Percentage decrease in hepatic serum markers appeared by Liv-52 [(Group-VII) at the dose of (1.0 mL/kg, body weight)] and silymarin [(20 mg/kg, body weight) (Group-VIII)] as SGOT by 55.54% & 58.80%; SGPT by 35.93% & 39.98%; ALP by 55.15% & 57.91%; BLB by 78.53% & 85.34% correspondingly, however, increased the level of TP by 65.21% & 78.26% correspondingly as juxtaposed with Sim-20 treated group (Group II). Among these polyherbal suspensions, $F_{4H\beta}$ showed the best pharmacological effect which is practically higher than the reference standard drugs i.e., Liv-52 and Syl-20. The results are indicated in Table 4.

The consequence of $F_{1E\beta}$, $F_{2B\beta}$, $F_{3C\beta}$, and $F_{4H\beta}$ optimized polyherbal suspensions on hepatic antioxidant markers in the Sim-20 model

The antioxidant outcomes were clearly shown in Fig.3. Here the rodents intoxicated with Sim-20 (Group-II) revealed a 125% increase in the LPO level compared to the (Group-I) normal control. Albinos which were dosed with $F_{1E\beta}$, $F_{2B\beta,}$ $F_{3C\beta,}$ and $F_{4H\beta}$ PHS (25 mg/kg, p.o.,) noteworthy reduced the level of LPO by 50.52%, 43.65%, 34.65%, and 54.76% correspondingly. Rodents that were intoxicated with Sim-20, noteworthy lessened the SOD, CAT, and GSH levels in comparison with Group-I (normal control) rodents. Those albinos who were inebriated

Table 4 — Outcomes of optimized polyherbal suspension $F_{1E\beta}$, $F_{2B\beta,}$ $F_{3C\beta,}$ and $F_{4H\beta}$ on hepatic serum markers in Sim-20 model

Groups	Treatment	SGOT	SGPT	ALP	BLB	TP
Group-I	Control	60.2 ± 2.1	53.8 ± 1.3	63.5 ± 2.2	0.24 ± 0.01	4.4 ± 0.03
Group-II	Sim-20	153.4±3.5†	103.8 ±2.9†	152.3 ±1.8†	1.91 ± 0.02†	2.3± 0.02†
Group-III	$F_{3C\beta}$	122.4±2.3^{c}	90.3±2.0^{c}	130.2±2.3^{c}	1.59± 0.03^{c}	2.69±0.07^{c}
Group-IV	$F_{2B\beta}$	81.6 ± 2.7^{c}	83.6 ± 2.4^{c}	81.6 ± 2.1^{c}	1.36 ± 0.01^{c}	3.52± 0.01^{c}
Group-V	$F_{1E\beta}$	71.8±2.1^{c}	69.4±2.6^{c}	68.8±2.2^{c}	0.77±0.05^{c}	3.69±0.02^{c}
Group-VI	$F_{4H\beta}$	70.54±1.9^{c}	67.62±2.7^{c}	63.02±1.8^{c}	0.26±0.04^{c}	4.26±0.03^{c}
Group-VII	Sim-20+Liv-52	68.2±2.8^{c}	66.5±2.8^{c}	68.3±3.4^{c}	0.41±0.04^{c}	3.8±0.03^{c}
Group-VIII	Sim-20+SYL-20	63.2 ± 2.6^{c}	62.3 ±2.1^{c}	64.1 ± 1.2^{c}	0.28 ± 0.07^{c}	4.1 ± 0.04^{c}

All the values are expressed as mean ± S.E.M & n=6; n: non-significant; *P* values: †<0.001 related with rodents of the control group (Group I); *P* values: ^{a}<0.05, ^{b}<0.01, ^{c}<0.001 related with rodents of a toxic group (Group II)

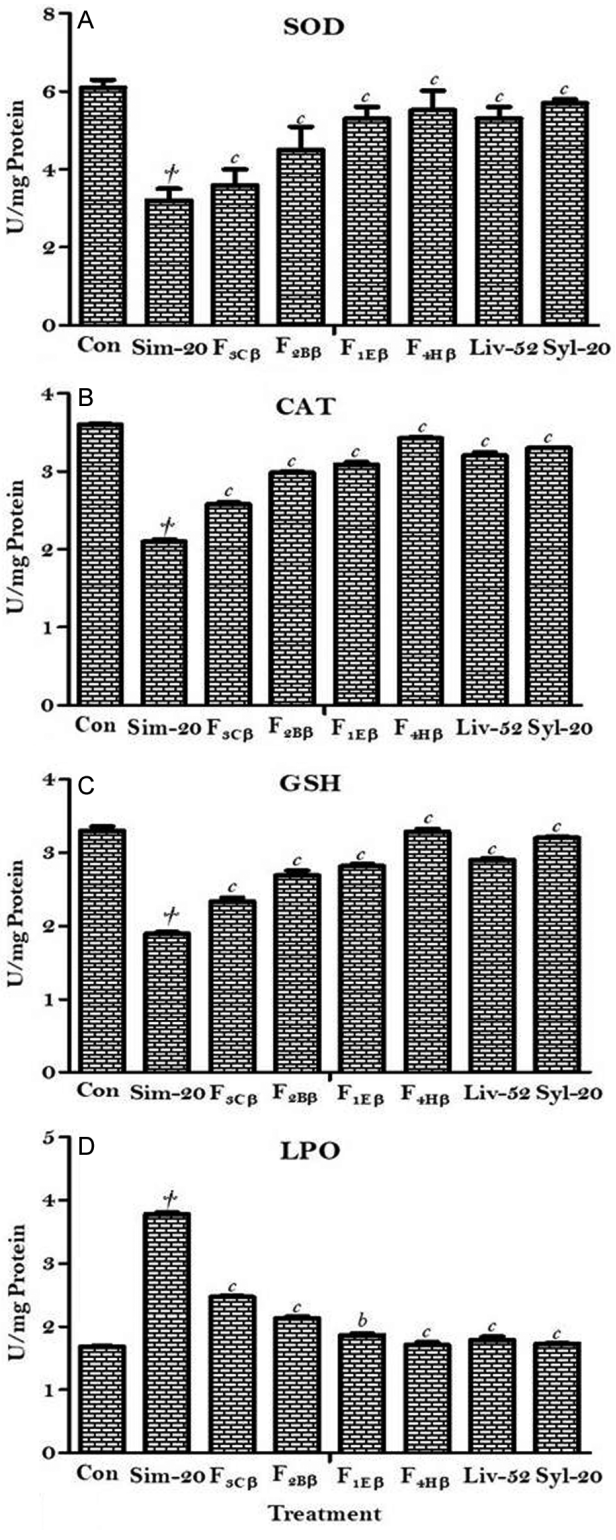

Fig. 3 —optimized polyherbal suspension $F_{1E\beta}$, $F_{2B\beta}$, $F_{3C\beta}$, and $F_{4H\beta}$ on *in vivo* hepatic antioxidant parameters against simvastatin induced hepatotoxicity. [All the values are expressed as mean ± S.E.M & n=6; n: non-significant; *P* values: †<0.001 related with rodents of the control group (Group I); *P* values: ^{a}<0.05, ^{b}<0.01, ^{c}<0.001 related with rodents of toxic group (Group II)]

Sim-20, knowingly reduced the antioxidant parameters as GSH by 47.5%, SOD by 41.66%, and CAT by 42.42%, juxtaposed with Group-I animals. These antioxidant enzymes had meaningfully increased in Group-III, Group-IV, Group-V, and Group VI albinos after the treatment with polyherbal suspension $F_{1E\beta}$, $F_{2B\beta}$, $F_{3C\beta}$, and $F_{4H\beta}$. The percentage augmentation showed by $F_{3C\beta}$, $F_{2B\beta}$, $F_{1E\beta}$, and $F_{4H\beta}$ PHS in SOD as 12.5%, 40.62%, 65.62%, 72.5% correspondingly, as equated with the Sim-20 dosed albinos. The percentage increment showed in CAT, by $F_{3C\beta}$, $F_{2B\beta}$, $F_{1E\beta}$, and $F_{4H\beta}$ PHS as CAT 22.85%, 41.9%, 47.14%, 62.85% correspondingly, as compared with the Group-II albinos. The percentage rise is shown in GSH by $F_{3C\beta}$, $F_{2B\beta}$, $F_{1E\beta}$, and $F_{4H\beta}$ PHS as 23.15%, 41.57%, 50.52%, 73.15% congruently as juxtaposed to Group-II animals. However, the rodents treated with Liv-52 i.e., Group-VII and silymarin i.e., Group-VIII revealed a percentage increment in the level of antioxidant enzymes as SOD by 65.6% & 78.12%, CAT by 52.38% & 57.14%, GSH by 52.6% & 68.42% correspondingly as juxtaposed to Group-II albinos. Among all these PHS, $F_{4H\beta}$ showed the most extreme hepatoprotective & antioxidant effect which is practically higher than the reference standard drugs i.e., Liv-52 and Syl-20.

Histopathological study reveals the hepatoprotective effect of $F_{1E\beta}$, $F_{2B\beta}$, $F_{3C\beta}$, and $F_{4H\beta}$ polyherbal suspensions

The liver segment of the control group of animals (Group-I) shown the typical hepatic cells with well-protected cytoplasm. The liver portion of Sim-20 mg/kg, p.o., dosed rodents serves as (Group-II) shown the massive fatty changes (arrow 2), putrefaction, (arrow 1), CV clog (arrow 3, 5 and 6), and the loss of cell boundaries (arrow 4, 5) and focal vein blockage (arrow 7). The liver segment of animals treated with Sim-20+ $F_{3C\beta}$ showed less fatty changes with negligible fiery conditions, loss of cell membrane (arrow 1 and 3), less infiltration of the leucocytes (arrow 4 and 5). Liver segments of animals treated with Sim-20+ $F_{2B\beta}$ showed the recovery of hepatocyte around CV (arrow 1) with less loss of cell membrane (arrow 3). The hepatic segment of animals treated with Sim-20+ $F_{1E\beta}$ showed the well brought CV, the hepatic cell with preserved cytoplasm. The hepatic segment of animals charged with Sim-20+$F_{4H\beta}$ showed the well brought CV, the hepatic cell with preserved cytoplasm. The liver segment of animals treated with Sim-20+Liv-52 showed the well-defined CV, the hepatic cell with protected cytoplasm. The

liver segment of the animal treated with Sim-20+Syl-20 showed the well brought focal vein, the hepatic cell with less infiltration of the leucocytes (arrow 1 and 2).

From the experimental study, it is proved that trikatu has the tremendous potential to increase the bioavailability of therapeutically important constituents responsible for hepatoprotective activity at reduced dose level as shown in Table 5. & Table 6. The incorporation of trikatu extract (30 mg/100 mL of polyherbal formulation) not only reduces the dose, toxicity of the drugs but is also important from an economical point of view. The hepatoprotective and antioxidant effect produced by the PHSs containing trikatu extract (30 mg/100 mL) was found to be optimum.

Table 5 — Organoleptic tests of optimized polyherbal suspension $F_{4H\beta}$ from 0 to 5th month

Month	Name of PHSs	Organoleptic parameters			
		Colour	Odour	Taste	Texture
0	$F_{4H\beta}$	Slightly brownish	Characteristics	Acrid	Suspension
1st	$F_{4H\beta}$	Slightly brownish	Characteristics	Acrid	Suspension
2nd	$F_{4H\beta}$	Slightly brownish	Characteristics	Acrid	Suspension
3rd	$F_{4H\beta}$	Slightly brownish	Characteristics	Acrid	Suspension
4th	$F_{4H\beta}$	Slightly brownish	Characteristics	Acrid	Suspension
5th	$F_{4H\beta}$	Slightly brownish	Characteristics	Acrid	Suspension

Evaluation of best optimized Polyherbal Formulations

Among all these polyherbal suspensions ($F_{1E\beta}$, $F_{2B\beta}$, $F_{3C\beta}$, and $F_{4H\beta}$), $F_{4H\beta}$ polyherbal suspension showed the best liver protective and antioxidant activity and this result was further supported by biological as well as histopathological study (Fig. 3 & Fig. 4). Therefore, our best polyherbal suspension i.e. $F_{4H\beta}$ was evaluated for organoleptic and stability studies. Organoleptic parameters like colour, odour, texture, and taste were evaluated for $F_{4H\beta}$ (Table 5). Using standard procedure stability studies like flow rate, viscosity, sedimentation volume, redispersibility, and pH were analyzed (Table 6).

Table 6 — Results of stability studies of polyherbal suspension $F_{4H\beta}$ from 0 to 5th month

Months	Polyherbal suspension	Stabilities parameters				
		Re-dispersibility	pH	Sedimentation volume (mL)	Flow rate (5mL/sec)	Viscosity (cp)
0	$F_{4H\beta}$	Uniform	6.61	0.24	5.3	54.3
1st	$F_{4H\beta}$	Uniform	6.62	0.22	5.1	54.6
2nd	$F_{4H\beta}$	Uniform	6.64	0.23	5.4	54.8
3rd	$F_{4H\beta}$	Uniform	6.62	0.22	5.1	54.9
4th	$F_{4H\beta}$	Uniform	6.65	0.25	5.6	52.9
5th	$F_{4H\beta}$	Uniform	6.63	0.26	5.8	52.2

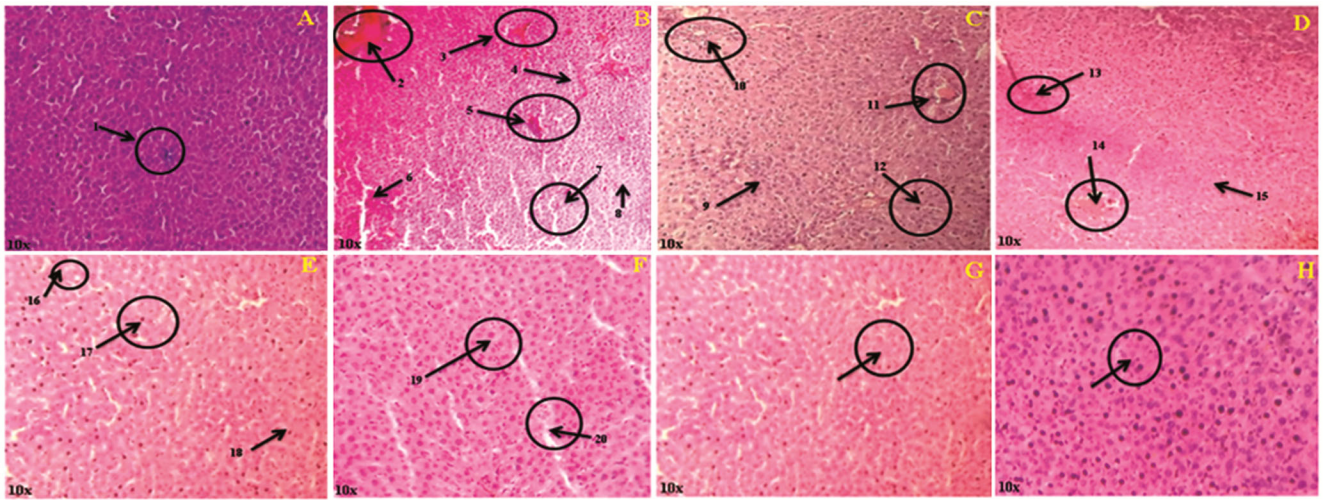

Fig. 4 — Photomicrograph of rat liver. (A) Control Group; (B) Toxic group (Sim-20); (C). Sim-20mg/kg + polyherbal suspension $F_{3C\beta}$; (D). Sim-20mg/kg + polyherbal suspension $F_{2B\beta}$; (E). Sim-20mg/kg + polyherbal suspension $F_{1E\beta}$; (F). Sim-20mg/kg+ $F_{4H\beta}$; (G). Sim-20+Liv-52; (H). Sim-20 + Syl-20 (10× & 45×, H & E stain).

Conclusion

Among all the optimized suspensions containing 30 mg/100 mL of trikatu extract, $F_{4H\beta}$ possessed even more effective hepatoprotective and antioxidant activity as compared to Liv-52 and Silymarin treated groups. This profound efficacy was further supported and powerfully reinforced by the histological evidence. So, the final outcome of the present research clearly indicated that among all the optimized polyharbal suspensons, $F_{4H\beta}$ possessed even more effective hepatoprotective and antioxidant activity as compared to Liv-52 and silymarin treated groups, possess better hepatoprotective and antioxidant activities and support the folkloric claims scientifically for *S. xanthocarpum, H. corymbosa,* and *T. dioica* plant species.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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