HIGHER THERAPEUTIC BENEFITS OF AQUEOUS EXTRACT OF TRIPHALA LOADED SOLID LIPID NANOPARTICLE IN TETRACYCLINE INDUCED HEPATOTOXICITY IN ANIMAL MODEL

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Abstract

The current study is focused on the in-vivo therapeutic effect of hepatoprotective activity of aqueous extract of triphala loaded solid lipid nanoparticle (SLNs) in Tetracycline induced hepatotoxicity in mice. Albino mice of either sex (35-40 gm) were selected and treated preclinically for 14 days and designated into various treatment groups including Normal, Toxic, standard and formulation treatment groups.The biochemical markers like (AST, ALT, ALP and serum bilirubin) were evaluated by using Erba diagnostic kit. The *in vivo* antioxidant activity was determined by estimating the tissue levels of glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation (LPO). The triphala/ bioactive & their formulation (100 and 50mg/kg) produced significant effect by decreasing the activity or level of AST, ALT, ALP and serum bilirubin and while it significantly increased the levels of tissue GSH, SOD and CAT in a dose-dependent manner. The in-vivo histopathology of liver was also carried out.Overall, the current research significantly concluded that aqueous extract of triphala/bioactive & their formulation possesses hepatoprotective activity against tetracycline induced hepatotoxicity.

Keywords: *Silymarin; Triphala; Solid lipid nanoparticle; Antioxidant activity, Hepatoprotective activity, tetracycline.*

1. Introduction

Liver is the largest organ inside your body. It is also one of the most important. The liver has many jobs, including changing food into energy and cleaning alcohol and poisons from the blood. The liver also makes bile, a yellowish-green liquid that helps with digestion [1]. Liver has extremelydedicated tissues which control a numerous high volume biochemical reactions like synthesis and breakdown of various small and complex molecules [2]. There are many kinds of liver diseases. Viruses cause some of them, like hepatitis A, hepatitis B and hepatitis C. Others can be the result of drugs, poisons or drinking too much alcohol. If the liver forms scar tissue because of an illness, it's called cirrhosis. Jaundice, or yellowing of the skin, can be one sign of liver

disease.Various kinds of conventional drugs, especially potent drugs devoid of pinpoint potential production in clinical platform and exhibits adverse effects. Potentialdrug delivery of drug into the target cells may improve the activity of the drug at the target site [3]. Hepatic-targeted drug delivery system (HTDDS) can be attained using a diversity of nano drug delivery systems such as nanoparticles, microspheres,niosomes, liposomes, phytosomes, polymer conjugates and recombinant chylomicrons, which are dynamicallyengrossed by the liver [4]. Nanoparticles possess promising drug delivery system of controlled and targeted drug release with usefulness in the drug delivery arena became evident with the fact that they can be designed to spontaneously absorb biologically active molecules through formation of salt bonds, hydrogen bonds, or hydrophobic interactions [5]. Solid lipid nanoparticles (SLNs) are the contemporary submicron sized lipid emulsions phase where liquid lipid (oil) has been replaced by solid or lipid. The various technique are bound with the synthesis and production of SLNswhich include ultrasound, solvent emulsification or evaporation, high shear homogenization, high pressure homogenization, and micro emulsion method [6]. Hepatic diseases involve any physiochemical, biological and morphological changes in normal liver functioning. Liver disease is any disturbance of liver function that causes illness. The liver is responsible for many critical functions within the body and should it become diseased or injured, the loss of those functions can cause significant damage to the body [7]. Liver disease is also referred to as hepatic disease. Liver disease is a broad term that covers all the potential problems that may occur to cause the liver to fail to perform its designated functions. Usually, more than 75% or three quarters of liver tissue needs to be affected before decrease in function occurs [8]. Globally the prevalence of hepatotoxicity is 17.9%, in which India constitutes 5-6 % overall global incidence [9]. Drugs are an important cause of liver injury. More than 900 drugs, toxins, and herbs have been reported to cause liver injury, and drugs account for 20-40% of all instances of fulminant hepatic failure [10]. Approximately 75% of the idiosyncratic drug reactions result in liver transplantation or death. Drug induced hepatic injury is the most common reason cited for withdrawal of an approved drug. Physicians must be vigilant in identifying drug-related liver injury because early detection can decrease the severity of hepatotoxicity if the drug is discontinued [11]. The manifestations of drug-induced hepatotoxicity are highly variable, ranging from asymptomatic elevation of liver enzymes to fulminant hepatic failure. Tetracycline antibiotics are bacteriostatic agents with a broad spectrum of antimicrobial activity [12].Excessive use and overdoses of tetracycline exhibited serious hepatic dysfunction in rats [13] and humans [14]. This dysfunction of the liver resulted in the disturbance of nitrogen metabolism, jaundice and other signs of hepatocellular damage. Triphala extract powder is a globally recognized and used as Ayurvedic formulation of Indian system of medicine [15]. The Triphala and its extract are easily manufactured and available in the global market as a dietary supplement [16]. It consists of the dried fruits of three plants, *Emblicaofficinalis*Gaertn., (Indian gooseberry, Amalaki, Ma-kham-pom), *Terminaliachebula* Retz. (Chebulicmyrobalan, Haritaki, Sa-mor-Thai) and *Terminaliabellerica* (Gaertn.) Roxb. (Bellericmyrobalan, Vibhitaka, Sa-mor-Phe-phek) [17-18]. Triphala is rich in active ingredients like tannins, carbohydrates, saponins, ellagic acid, sorbitol and ascorbic acid [19].It is a therapeutic agent for treatment of a variety of conditions such as headache, dyspepsia, constipation, liverconditions, fatigue,

infections and assimilation, and is alsoreported to possess many biological activities includingantidiabetic, antimutagenic, antimicrobial, radioprotective, hypocholesterolaemic, antiviral,immunomodulatory, and anticancer. Triphala helps in improving digestion, assimilation and liver functions, and reducing lipid peroxidation, blood sugar and serum cholesterol [20].With the foundation of Ayurveda and nanotechnology based drug delivery system, the current research is focused on the higher therapeutic benefits of aqueous Triphala extract loaded SLNs for the estimation of enhanced hepatoprotective activity against tetracycline induced hepatotoxicity animal model.

2. Materials and methods

2.1 Plant materials

The fruits of *Emblica officinalis, Terminalia chebula* and *Terminalia belerica* were collected from Sagar, MP (local vendor). The plant was identified, confirmed and authenticated from department of botany, Dr. Harisingh Gour Central University, Sagar (M.P.). A herbarium has been deposited in the Botany Department. A Voucher Specimen no for *Emblica officinalis* (Bot./Her/1208), *Terminaliachebula* (Bot./Her/940) and *Terminaliabelerica* (Bot./Her/1810). The fruits of triphala were shade dried at room temperature and powdered through grinder to make coarse powder.

2.2 Chemical reagents

Liver function tests were evaluated using an enzymatic kit (Erba diagnostic kit.). Tetracycline were purchased from Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

2.3 Extraction Procedures

200gm coarse powder of triphala fruits were packed in soxhlet apparatus and defatted with petroleum ether (60-80ºC), to ensure complete defatting. After defatting, the marc was dried at room temperature and extracted with distilled water by hot maceration technique then filtered and evaporate the filtrate and dried and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts.

3 Qualitative phytochemical analysis of plant extract

Thetriphala fruits extract obtained were subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate [21, 22]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein, amino acid and tannins etc.

3.1 Determination of λ_{max} of gallic acid

The gallic acid (10 mg) was accurately weighed and dissolved in 100 ml of PBS (pH 7.4). Then, 1 ml of this stock solution was pipetted out into a 10 ml volumetric flask and volume was made up to the mark with PBS (pH 7.4). The resulting solution was scanned between 200-400 nm using Cintra 10 GB UV-visible spectrophotometer. The λ_{max} was found to be 223 nm. A graph of concentration Vs absorbance was plotted

3.2 Preparation of solid lipid nanoparticles

The solid lipid nanoparticles were prepared by solvent injection method as reported by *Garudet al 2012* [23]. Tristearin, soya lecithin and drugs (10mg) were taken into different ratio and were dissolved in minimum quantity of absolute alcohol and heated about 70 ^{0}C in a beaker. In another beaker, tween 80 (0.5 % v/v) was dissolved in phosphate saline buffer (pH 7.4) solution and heated at the same temperature as in organic phase. Then this organic phase i.e. alcoholic solution containing lipid mixture and drug was added to preheated aqueous solution at the same temperature (about 70^0 C) at constant stirring. The preformed lipid suspension was then sonicated by using probe sonicator to form solid lipid nanoparticles (SLNs).

3.3 Optimization of formulation and process variables

For optimization of lipids ratio, the SLN formulations were prepared with varying ratio of two lipids i.e. tristearin and soya lecithin in the different ratios (viz. 1:0.5, 1:1, 1.5:1, 1:2 %w/w) keeping other parameters constant. Optimization was done on the basis of average particle size and poly dispersity index (PDI) of SLNs, which were determined using Zetasizer DTS ver 4.10 (Malvern Instrument, UK). For optimization of drug: lipid ratio formulation S_3 was selected and various formulations containing different drug lipid ratio (5.0:100, 10.0:100, 15:100, 20:100, 25:100 w/w) were prepared keeping other parameters constant. Optimization of drug lipid ratio was done on the basis of two parameters average particle size and drug entrapment.

4 Characterizations of Solid Lipid Nanoparticles

4.1 Particle size determination

The average particle size and size distribution of the solid lipid nanoparticles were determined by photon correlation spectroscopy using a Zetasizer DTS ver 4.10 (Malvern Instrument, UK). The samples of solid lipid nanoparticle dispersions were diluted to 1:9 v/v with deionized water. The particles size and size distribution were represented by average (diameter) of the Gaussion distribution function in the logarithmic axis mode.

4.2 Surface charge measurement

The surface charge of solid lipid nanoparticle was determined by measurement of zeta potential (ε) of the lipid nanoparticles calculated according to Helmholtz-Smoluchowsky from their electrophoretic mobility. For measurement of zeta potential, Zetasizer DTS ver 4.10 (Malvern Instrument, UK) was used. The field strength was

20 V/cm on a large bore measures cell. Samples were diluted with double distilled water adjusted to a conductivity of 50 μS/cm with a solution of 0.9% NaCl.

4.3 Entrapment efficiency

Bio-active entrapment in solid lipid nanoparticles was determined by using sephadexminicolumn. To prepare minicolumn, first sephadex G-50 was allowed to swell in 0.9% NaCl aqueous solution for 24 hr. and then the hydrated gel was filled in to the barrel of 2 ml disposable syringe plugged with filter pad. The barrels were centrifuged at 2000 rpm for 2 minutes to remove excess of saline solution to form the sephadex separating column.To separate free drug from SLN formulation 0.2 ml of SLN dispersion was applied drop wise on the top of the sephadex column and then centrifuged at 2000 rpm for 2 min. to expel and remove void volume containing SLN in to the centrifuged tubes. This eluted SLN dispersion was collected and lysed by disrupting with 0.01% Triton-X100 and then the amount of entrapped drug was analyzed using spectrophotometric method.

4.4 *In-vitro*drug release

The drug release of gallic acid, AET(aq. extract of triphala) loaded SLN was performed in PBS (pH 7.4) using dialysis membrane (molecular weight cut off point 1 KD). The dialysis membrane retains nanoparticles and allows the free drug into the dissolution media. One ml of pure SLN suspension free of any unentrapped drug was taken into a dialysis bag and placed in beaker containing 50 ml of PBS (pH 7.4). The beaker was placed over a magnetic stirrer and temperature was maintained at 37 ± 1^0C throughout the study. The samples were withdrawn at definite time intervals and replaced with the same volume of PBS (pH 7.4). The withdrawn samples were analyzed for drug content by spectrophotometer at λmax 223, 227, 230 nm against blank.

5. Experimental animals

Albino mice weighing 35-40 gm was procured from animal house of PBRI, Bhopal, India.Animals were further randomly divided into various treatment groups and kept in propylene cage with sterile husk as bedding. Animals were housed in relative humidity of ~50-55 % at 22 ± 3 ℃ and 12:12 light and dark cycle. Animals were fed with standard pellets (Golden feeds, New Delhi, India) and water *ad libitum*. All animal experiments were approved by Instutional Animal Ethics Committee (IAEC) of PBRI, Bhopal (Reg No. - **1283/c/09/CPCSEA)** and Sagar Institute of Pharmaceutical Sciences (SIPS), Sagar (**SIPS/EC/2013/34**) according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India.

5.1 Determination of acute toxicity (LD50)

Acute oral toxicity was performed as per OECD 423 guidelines [24]. The procedure was followed by using OECD-423 (Acute Toxic Class Method). The acute toxic class method is a step wise procedure with three animals of a single sex per step. Depending on the mortality or moribund status of the animals and the average

two to three steps may be necessary to allow judgment on the acute toxicity of the test substance. Healthy female rats (each set of three rats) were used for this experiment. The method used to defined doses (2000, 300, 50, 5 mg/kg body weight, Up-and-Down Procedure). The starting dose level of aqueous extract of triphala (AET) was 2000 mg/kg body weight p.o as most of the crude extracts posses LD 50 value more than 200 mg/kg p.o. Dose volume was administered 0.2ml per 100gm body weight to overnight fasted rats with were *ad libidum*. Food was withheld for a further 3-4 hours after administration of AET and observed for signs for toxicity.

5.2*In vivo* hepatoprotective activity

5.2.1 Tetracycline induced hepatotoxicity

Mice either sex weighing between 35-40 gm were divided in seven groups of each containing six rats [26].

Group I- Normal control (given normal saline 2ml/kg, p.o.),

Group II- Tetracycline (20mg/100kg b.w., p.o.) for 14 days,

Group III- Silymarin (25mg/kg, p.o.) served as standard and simultaneously administered tetracycline (20mg/100kg b.w., p.o.) for 14 days,

Group IV- TPL (100mg/kg p.o.) and simultaneously administered tetracycline (20mg/100kg b.w., p.o.) for 14 days,

Group V- TPL-SLNs (50mg/kg p.o.) and simultaneously administered tetracycline (20mg/100kg b.w., p.o.) for 14 days,

Group VI- GA (50mg/kg p.o.) and simultaneously administered tetracycline (20mg/100kg b.w., p.o.) for 14 days,

Group VII- GA-SLNs (25mg/kg p.o.) and simultaneously administered tetracycline (20mg/100kg b.w., p.o.) for 14 days.

5.3 Biochemical analysis

After 1 hour of last dose of toxicant rats were sacrificed by cervical decapitation. Blood samples were collected by retro-orbital puncture & allowed to clot. Serum was separated by centrifuging at 3000 rpm for 15 min. and biochemical parameters like (AST, ALT, ALP and serum bilirubin) was estimated by using Erba diagnostic kit.After blood collection liver were excised and washed with normal saline. One part of liver tissue were collected and preserved in 10% formalin solution for histopathological studies [25]. Another part of liver homogenized with 0.1M Tris buffer and phosphate buffer (pH7.4) then centrifuge for 10min. at 3000 rpm. Supernatant was taken & used for determination of oxidative stress enzymes like (SOD, CAT, GSH) and LPO (Lipid peroxidation).

5.4 Histopathological studies

After sacrifice of animals' liver was excised and kept in 10% Buffered Formalin. 50 µ thick section of liver was cut using spensor microtome. Liver sections were stained with haematoxylin and eosine, and observed under microscope at 40 x magnification.

5.5 Statistical analysis

The data obtained from animal experiments are expressed as mean ± SEM (standard error of mean). For statistical analysis data were subjected to analysis of variance (ANOVA) followed by Dunnet's test. $P<0.01$ was considered as level of significance [26].

6. Results&Discussion

6.1 Extraction and Yield

The crude drug extracts was obtained post hot maceration technique, the plant extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from sample using water as solvents are depicted in the **Table 1.**

Table 1 Percentage yield of Triphala

S.No.	***Solvent***	***Ext. time (hrs)***	***Color &consistency***	***% yield***
1.	Distilled water	24	Yellowish brown semisolid mass	13.78%

6.2 Preliminary phytochemical screening

Preliminary phytochemical screening of triphala aqueous extract discovered the presence of numerous components such as Phenolic compound, tannins, flavonoids, saponins, carbohydrates, protein and amino acids were the most prominent ones and the results are summarized in **Table 2.**

Table 2 Result of phytochemical screening of aqueous extract of triphala

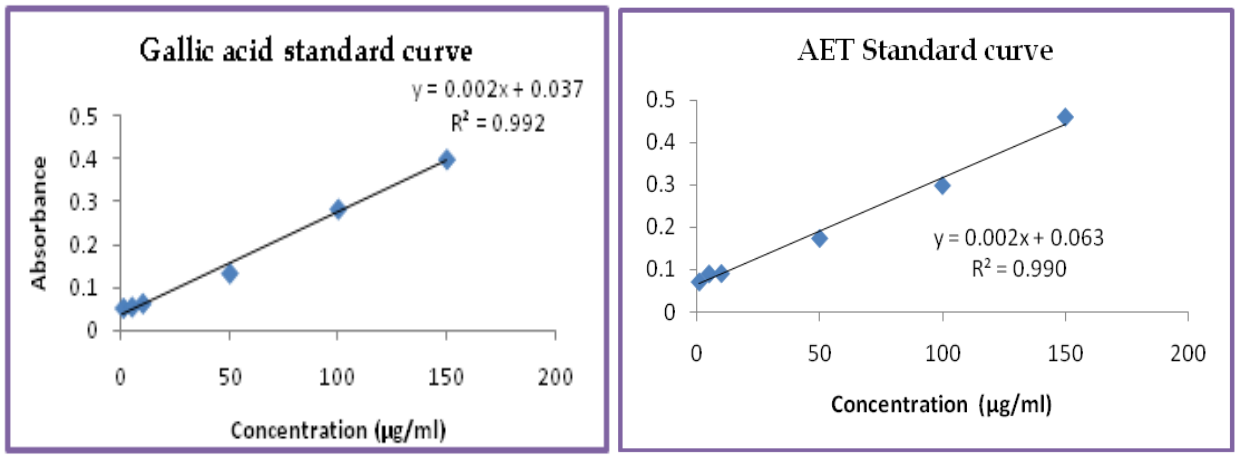
S. No.	*Constituents*	*Aqueous extract*
1.	**Tests for Alkaloids:**	
	Mayer's test	+ve
	Dragendorff's test	-ve
	Wagner's test	-ve
2.	**Tests for Carbohydrates:**	
	Molisch's test	-ve
	Fehling's test	+ve
	Benedict's test	+ve
3.	**Tests for Glycoside:**	
	Modified Borntrager's test	+ve
	Legal's test	-ve
4.	**Tests for Phytosterols and Triterpenoids:**	
	Liebermann's test	-ve
	Salkowaski test	-ve
5.	**Tests for Protein and Amino acids:**	
	Ninhydrin test	+ve
	Biuret test	+ve
6.	**Tests for Phenolic and Tannins:**	
	Ferric chloride test	+ve
	Gelatin test	+ve

S. No.	*Constituents*	*Aqueous extract*
	Lead acetate test	
7.	**Tests for Flavonoids:** Shinoda Test	+ve
8.	**Tests for Saponins:** Foam test	+ve
	Haemolysis test	+ve

Key: ***+ve:- Positive; -ve:- Negative,***

6.3 Solubility Profile & UV Spectrophotometry evaluation

Solubility of gallic acidwas freely soluble in methanol, soluble in ethanol, sparingly soluble in PBS (pH 7.4) and insoluble in distill water.λ_{max} of gallic acid and AET was found to be 223 and 232 nm by using U.V. spectrophotometer in linearity range 5-150µg/ml**Fig.1.** Partition coefficient of gallic acid and AET was found to be1.1 and 0.97 respectively.



(A) (B)

Fig.1 (A) Calibration curve of gallic acid at 223 nm, (B) Calibration curve of ATE at 232 nm in PBS pH 7.4

6.4 Formulation Optimization

The initial step in the study was to optimize the lipid ratio (tristearin/soya lecithin) of SLN formulation by varying the proportion of tristearin and soya lecithin and other variables were kept constant. It was observed that the size of preparation was decreased as the concentration of tristearin increases and that of soya lecithin decreases. This may be due to the decrease in surfactant action of lecithin at lower concentration. The optimized particles size was found to be 284.35 ±9.2 (nm) with the tristearin/soya lecithin ratio **Table 3**. Bioactive (ATPL/GA)/lipid ratio was optimizing on the basis of average particle size and percent drug entrapment. As the bioactive (ATPL/GA) concentration increases from 2.5 mg to 10 mg the particle size increased from 306.47 ±6.35 nm to 451.25 ±9.43 nm; 308.57 ±6.75 nm to 424 ±9.93 nm; 312 ± 7.89 nm to 449 ± 10.54 nm; respectively while percent drug entrapment increases exponentially and after that it was found to be constant as the amount of drug increased. It may be due to the saturation of lipids with the drug molecules. On the basis of result, ratio 10: 100 w/w of drug to total lipid content was found to be optimum and taken for further studies **Table 4 & 5.** The result of particle size, zeta potential, PDI, % drug entrapment of optimized formulation of ATPL/gallic acid SLN was given in **Table 6**. *In vitro* drug release of optimized formulation of ATPL/gallic acid SLN was found after 48 hr 98.64±5.34 and 91.06±3.85 respectively **Table 7.**

Table 3 Lipid/lecithin ratios, particle size and PDI

F. code	*Lipid /Lecithin ratio (% wt)*	*Particle Size (nm)*	*PDI*
A	1:1	284.35 ±9.2	0.24 ±0.01
B	1:0.75	332.27 ±10.48	0.29 ±0.03
C	1:0.5	386.61±4.74	0.28 ±0.034
D	1:0.25	429.42 ±12.5	0.38 ±0.041

Table 4 ATPL/lipid ratios, particle size and percentage drug entrapment

F. code	*Drug/Lipid (% wt.)*	*Particle Size (nm)*	*Drug Entrapment (%)*
AI	2.5:97.5	308.57 ±6.75	31.51 ±2.8
AII	5:95	350. ±12.11	35.87 ±1.65

AIII	7.5:92.5	402 ±9.54	37.22±2.39
AIV	10:90	424 ±9.93	27 ±2.45

Table 5 GA/lipid ratios, particle size and percentage drug entrapment

F. code	*Drug/Lipid (% wt.)*	*Particle Size (nm)*	*Drug Entrapment (%)*
AI	2.5:97.5	312 ± 7.89	34.17 ± 2.9
AII	5:95	362 ±12.94	39.34 ± 2.01
AIII	7.5:92.5	417 ± 10.01	40.12 ± 2.45
AIV	10:90	449 ± 10.54	30.51 ± 2.51

Table 6 Particle Size, Zeta Potential, PDI, % Drug Entrapment

Formulation	Particle Size (nm)	Zeta Potential (mV)	Polydispersity Index (PDI)	% Drug Entrapped
ATPL-SLN	299 ± 2.9	-ve6.8	0.221	35.87±1.65
Gallic acid SLN	387.13±3.4	-ve8.9	0.234	39.34 ±2.01

Table 7*In vitro* drug release study of optimized SLNs

S. No.	*Time (hr)*	*% Drug Release*	
		GA-SLN	*ATPL-SLN*
1	0.5	6.04±0.24	8.36±0.29
2	1	12.57±0.78	15.12±1.04

3	2	20.86±1.24	25.24±1.46
4	4	31.74±1.83	37.22±1.99
5	8	49.92±2.58	55.31±2.73
6	24	78.33±3.22	83.19±5.01
7	48	91.06±3.85	98.64±5.34

6.5 Acute toxicity studies

Acute toxicity studies revealed that aqueous extract of triphala fruits was safe at all doses when administered orally to rats, up to a dose of 2000 mg/kg. No mortality was observed during the 14 days of the observation period. Hence three doses50 and 100mg/kg were selected in the present study. Tetracycline is a well-known antibiotic that induces NASH in human and rodents. The major effect seems to be inhibition of transport of lipid out of the hepatocyte, which can be detected within 30 min of dosing in experimental animals. This effect may well be due to the inhibition of protein synthesis caused by tetracycline which will inhibit the production of the apolipoprotein complex involved in transport of the very low density lipoprotein (VLDL) out of the hepatocyte .

6.6 Serum Biomarker estimation

By Estimating the concentration of serum marker enzymes, like AST, ALT, ALP and total bilirubin, make assessment of liver function. In liver damage the level of serum marker enzymes elevated. The protective effects of triphala/bioactive & their formulation against hepatotoxicity were investigated in mice and it was found that there was significant increase ($P<0.01$) in all serum marker enzymes of liver i.e. AST, ALT, ALP and total bilirubin in tetracycline alone group as compared to control group. These effect of tetracycline were reversed extremely significant ($P<0.01$) using standard antihepatotoxic drug (Silymarin), thus indicated the potent hepatoprotective nature of silymarin. Further, there was decrease in the elevated serum levels of aforementioned liver enzymes significantly ($P<0.01$) by triphala/ bioactive & their formulation. The order of hepatoprotective effect was: VII > V > VI > IV respectively **(Table 8)**. Further activity of oxidative stress enzymes i.e. SOD, GSH and CAT by tetracycline alone group was significantly decreased ($P<0.01$) as compared to control group. Standard antihepatotoxic drug (silymarin) significantly ($P<0.01$) reversed the effect of tetracycline. Further, there was increase the levels of aforementioned oxidative stress enzymes significantly ($P<0.01$) by triphala/bioactive & their formulation. The order of potency was:VII > V > VI > IV respectively **(Table 9**). Also elevation of MDA levels induced by tetracycline was also significantly ($P<0.01$) decreased by triphala/bioactive & their formulation. The order of protection was:VII > V > VI > IV respectively **(Table 9).**

Table 8 Effect of triphala/bioactive &their formulation on biochemical parameters against tetracycline induced hepatotoxicity in mice

Group	*Treatment*	*Dose*	*ALT (IU/L)*	*AST (IU/L)*	*ALP (IU/L)*	*Total Bilirubin (mg/dl)*
1	Normal control	2ml/kg,p.o	21.03± 0.510	51.66±0.9128	11.26±0.55	0.21±0.009
2	Toxicant control	20mg/kg,p.o.	73.26± 3.083##	119.83±4.393##	62.16± 2.329##	0.57± 3.02##
3	Standard (SLY)	25mg/kg,p.o	47.65± 0.689**	86.77±1.762**	24.03±0.706**	0.28± 0.005**
4	TPL+ TC	100mg/kg, p.o	49.53±1.275**	92.72±4.461**	43.28±0.747**	0.38±0.014**
5	TPL-SLNs + TC	50mg/kg,p.o	48.21 ±2.63**	89.70±4.055**	36.34±1.692**	0.32±0.013**
6	GA + TC	50mg/kg,p.o	50.18± 1.203**	90.65±5.607**	40.44±2.314**	0.37±0.020**
7	GA-SLNs+ TC	25mg/kg,p.o	48.01± 0.707**	87.36±3.413**	33.50±1.096**	0.30±0.005**

Values are significant difference when compared with control group, P<0.01;** Values are significant difference when compared with model group, P<0.01; * Values are significant difference when compared with model group, P<0.05; Data are mean ± SEM; where n=6 mice, in each group.

Where: TC: Tetracycline, **SLY**: Silymarin, **TPL**: Triphala Extract, **TPL-SLNs**: Solid lipid nanoparticles of Triphala, **GA**: Gallic acid, GA-**SLNs**: Solid lipid nanoparticles of Gallic acid

Table 9 Effect of triphala/bioactive & their formulation fruits SOD, LPO, CAT & GSH against tetracycline induced hepatotoxicity in mice

Group	*Treatment*	*Dose*	*CAT (u/mg)*	*SOD (u/mg)*	*GSH (mM/mg)*	*LPO (nM/mg)*
1	Normal control	2ml/kg,p.o	32.66±0.507	1.59±0.016	18.74±0.16	2.39±0.085
2	Toxicant control	20mg/kg,p.o.	13.43±0.39$^{\#\#}$	0.19±0.019$^{\#\#}$	5.06±0.120$^{\#\#}$	6.73±0.132$^{\#\#}$
3	Standard (SLY)	25mg/kg,p.o	33.38±0.55**	1.61±0.016**	18.27±0.138**	2.67±0.122**
4	TPL+ TC	100mg/kg, p.o	21.47±0.586**	0.90±0.033**	5.97±0.252*	3.45±0.197**
5	TPL-SLNs + TC	50mg/kg,p.o	25.24±0.764**	0.96±0.037**	7.17±0.323**	3.01±0.203**
6	GA + TC	50mg/kg,p.o	22.33±0.823**	0.92±0.017**	6.56±0.273**	3.08±0.204**
7	GA-SLNs+ TC	25mg/kg,p.o	27.76±0.959**	0.97±0.024**	8.73±0.273**	2.83±0.113**

$^{\#\#}$ Values are significant difference when compared with control group, P<0.01;** Values are significant difference when compared with model group, P<0.01; * Values are significant difference when compared with model group, P<0.05; Data are mean ± SEM; where n=6 rat, in each group.

6.9 Histopathology Profile

Histopathology profile of the control animals showed normal hepatic architecture with distinct hepatic cells well preserved cytoplasm sinusoidal spaces and central vein. Disarrangement of normal hepatic cells with intense centrilobular necrosis was observed in tetracycline intoxicated liver. Moderate accumulation of fatty lobules and cellular necrosis were observed in the animal treated with crude Triphala & Gallic acid. However SLNs of triphala & gallic acid exhibit a significant liver protection against tetracycline induced liver toxicity, as evidenced by the presence of normal hepatic cords and well defined cytoplasm and absence of necrosis.

Histopathology results further evidence that solid lipid nanoparticles can be passively targeted to liver to deliver hepatoprotective agents or other drugs to the liver. Histopathological studies conclude that these SLNs resided in the liver and released triphala & gallic acid for a longer period of time, which resulted in higher therapeutic benefit **Fig. 3.**

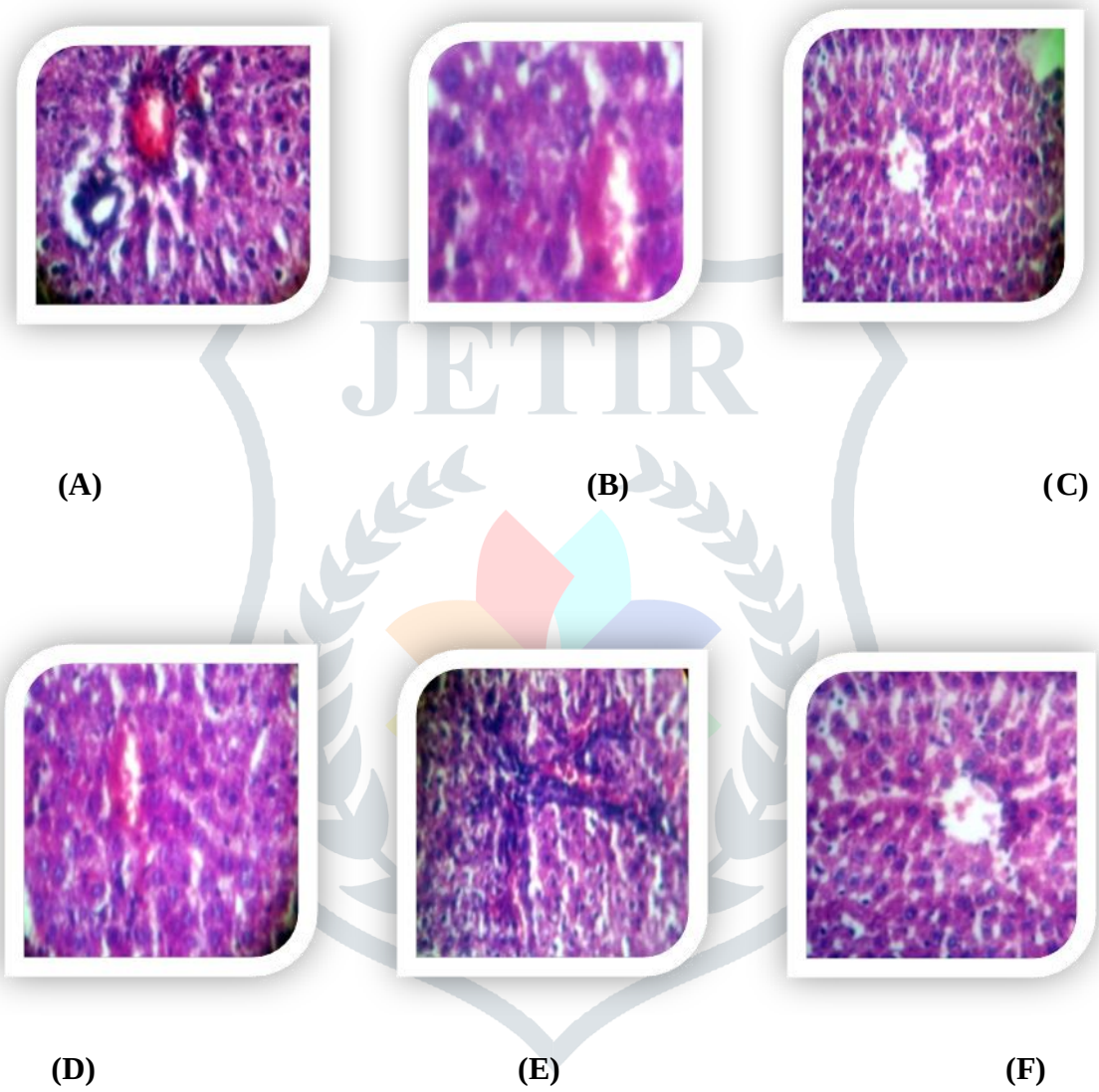


Fig. 3 Histopathology of the liver in Tetracycline induced hepatotoxocity studies (A)TOX (20mg/100kg b.w.) treated showed that liver cell necrosis & inflammation. **(B)** Silymarin (25mg/kg) treated showed that normal architecture of liver similar to control. **(C)** TPL (100mg/kg) treated showed that minimal inflammation with moderate portal triaditis and their lobular architecture was normal. **(D)** TPL-SLNs(50mg/kg) treated showed that absence of necrosis & their lobular architecture was normal. **(E)** GA (50mg/kg) treated showed that minimal inflammation & their lobular architecture was normal. **(F)** GA-SLNs (25mg/kg) treated showed that absence of necrosis & inflammation. Normal lobular architecture of liver similar to control & standard (silymarin).

7. Conclusion

Many significant approaches have been developed for the therapy of liver diseases. Both systems (herbal and nanocarriers) exhibit a higher specificity in terms of delivering the drug load to the site of action. Solid Lipid nanoparticles showed great potential for selective drug delivery to targeting cells. A large number of formulations have been prepared till date for liver targeting by polymeric nanocarriers as targeting ligands. To date, very few delivery systems are marketed as liver targeted drug delivery system. In future, liver-targeted drug delivery system will be available to serve the mankind.

8. Conflict of Interest

The Authors Claim no conflict of Interest

9. References

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