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COMPARATIVE HEPATOPROTECTIVE ACTIVITY OF AQUEOUS EXTRACT OF TRIPHALA AND SOLID LIPID NANOPARTICLE IN ISONIAZID-RIFAMPIN AND TETRACYCLINE INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

The aim of the present study was to investigate the effect of hepatoprotective activity of aqueous extract of triphala and solid lipid nanoparticle in isoniazid-rifampin and tetracycline induced hepatotoxicity in rats/mices. Wistar Albino rats and mice of either sex weighing between (200-250 and 35-40 gm) were divided in seven groups of each containing six rats/mices and treated for 21 days. Group I and II served as normal and toxic control, Group III were treated with Silymarin (25 mg/kg), and Group IV to VII were treated with aq. extract of triphala (100mg/kg), solid lipid nanoparticles of triphala (50mg/kg), gallic acid (50mg/kg) and solid lipid nanoparticles of gallic acid respectively. The biochemical markers like (AST, ALT, ALP and serum bilirubin) were estimated. The *in vivo* antioxidant activity was determined by estimating the tissue levels of glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation (LPO) and histopathology of liver was also carried out. 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. From the present study it can be concluded that aqueous extract of triphala/bioactive & their formulation possesses hepatoprotective activity against isoniazid-rifampin and tetracycline induced hepatotoxicity.

Keywords: Hepatoprotective, *In-vivo* antioxidant activity, Silymarin, Triphala, Solid lipid nanoparticle, Isoniazid-rifampin, Tetracycline.

1. INTRODUCTION

The liver is a vital organ present in vertebrates which perform many functions including detoxification, protein synthesis and production of various bio-chemicals which are very important for digestion [1]. Liver has highly specialized tissues which regulate a wide variety of high volume biochemical reactions like synthesis and breakdown of various small and complex molecules [2]. Many serious liver disorders affect millions of people worldwide, which are very difficult to treat effectively after many efforts. A large number of drugs, especially potent drugs may not be effective enough *in vivo* or may show high adverse effects. Enhanced delivery of drug into the target cells may improve the activity of the drug at the target site [3]. Therefore, it is important to understand the morphology of liver before explaining the targeting of the drug to liver. Similarly, before designing a novel drug delivery system, it is important to understand the molecular scale of the target tissues. Hepatic-targeted drug delivery system (HTDDS) can be achieved using a variety of vehicles such as microspheres, emulsions, liposomes, nanoparticles, albumin, lipoproteins, polymer conjugates and recombinant chylomicrons, which are actively absorbed by the liver [4]. Nanoparticles represent a promising drug delivery system of controlled and targeted drug release. They are specially designed to release the drug in the vicinity of target tissue. Solid lipid nanoparticles (SLNs) are the new generation of submicron sized lipid emulsions where liquid lipid (oil) has been substituted by solid lipid. The different technologies available for the fabrication of SLN are high shear homogenization, ultrasound, high pressure homogenization, solvent emulsification/evaporation and microemulsion method [5, 6]. Isoniazid (INH) and rifampicin (RIF) are the two major regimens currently used for the treatment of tuberculosis for a period of 4 to 6 months [7], which may induce hepatotoxicity [8]. The incidence of hepatic dysfunction is more, when INH and RIF are used in combination [9]. In India the prevalence of

hepatotoxicity is 11.6%, when compared to western countries where it is 4.3% [10]. Anti tubercular drug induced hepatotoxicity ranges from non specific elevation of transaminases to fulminant of liver failure [11]. The liver dysfunction is due to the synergistic effect of INH and RIF [12]. Hydrazine (HYZ) a metabolite of INH is converted to toxic compound by CYP450, which leads to hepatotoxicity. RIF, aggravates hepatotoxicity by inducing CYP450, as a result more toxic metabolites are generated from hydrazine [13]. In addition HYZ depletes the reserved glutathione (GSH) level in the liver, resulting in oxidative stress and cell death [14, 15]. Oxidative stress is one of the major attributing mechanisms for anti tubercular drug induced hepatotoxicity and liver damage [16]. Tetracycline antibiotics are bacteriostatic agents with a broad spectrum of antimicrobial activity. Large doses of tetracycline have been shown to induce hepatic dysfunction in rats [17] and humans [18]. This dysfunction of the liver resulted in the disturbance of nitrogen metabolism, jaundice and other signs of hepatocellular damage. Triphala powder is a world widely used herbal formulation of Indian system of medicine. It is easily available in the global market as a dietary supplement. It is a powdered mixture of shade dried fruit pulp of three important myrobalans, that is, *Emblica officinalis* Gaertn, *Terminalia bellerica* Roxb and *Terminalia chebula* Retz in equal proportion. It is considered as an important rejuvenating formulation in Ayurvedic system of medicine [19]. Triphala is rich in active ingredients like tannins, carbohydrates, saponins, ellagic acid, sorbitol and ascorbic acid. A large number of medicinal properties are attributed by triphala such as anti-aging, antimutagenic, anticancerous, anti-inflammatory, antibacterial, antiviral, antioxidant, antianemic, antidiabetic, antiparasitic, antidiarrhoeal, cardioprotective, hepatoprotective, hypo cholesterolaemic, radioprotective, colon cleanser and gas distentioner. Triphala helps in improving digestion, assimilation and liver functions, and reducing lipid peroxidation, blood sugar and serum cholesterol [20]. The present work is aimed at studying the hepatoprotective activity of aqueous extract of triphala/bioactive & their formulation in isoniazid-rifampin and tetracycline induced hepatotoxicity.

2. MATERIAL AND METHODS

2.1. Plant material

The fruits of *Emblica officinalis, Terminalia chebula* and *Terminalia belerica* were collected from local vendor from Sagar (M.P.). The plant was identified, confirmed and authenticated from department of botany, Dr. H.S. 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), *Terminalia chebula* (Bot./Her/940) and *Terminalia belerica* (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.). Isoniazid, rifampin and tetracycline were purchased from Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade.

2.3. Extraction Procedure

200gm coarse powder of triphala fruits were packed in soxhlet apparatus and defatted with petroleum ether (60-80°C), 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.

2.4. Qualitative phytochemical analysis of plant extract

The triphala 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 ect.

2.5. Determination of λ_{max} of gallic acid

The gallic acid (10 mg) was accurately weighed and dissolved in 100ml of PBS (pH 7.4). Then, 1ml of this stock solution was pipette 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

2.6. Preparation of solid lipid nanoparticles

The solid lipid nanoparticles were prepared by solvent injection method as reported by *Garud et 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°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°C) at constant stirring. The preformed lipid suspension was then sonicated by using probe sonicator to form solid lipid nanoparticles (SLNs).

2.7. 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.

2.8. Characterization of SLN

2.8.1. 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.

2.8.2. 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.

2.8.3. Surface morphology (SEM)

Surface morphology was determined using Scanning electron microscope. The samples for SEM were prepared by lightly sprinkling the SLN powder on a double adhesive tape, which was stuck on an aluminum stub. The stubs were then coated with gold to a thickness of about 300 Å using a sputter coater. All samples were examined under a scanning electron microscope, Carl Zeiss NTS GmbH (SUPRA 40VP, Oberkochen, Germany) at an acceleration voltage of 2.0 kV, and photomicrographs were taken at suitable magnification.

2.8.4. Entrapment efficiency

Bio-active entrapment in solid lipid nanoparticles was determined by using sephadex minicolumn. 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.

2.8.5. 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°C 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.

2.9. Experimental animals

Albino wistar rats weighing 200-250 gm and albino mice 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°C 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.

2.10. 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 libitum*. Food was withheld for a further 3-4 hours after administration of AET and observed for signs for toxicity.

2.11. *In vivo* hepatoprotective activity

2.11.1. Rifampicin + isoniazid induced hepatotoxicity

Wistar Rats either sex weighing between 200-250 g were divided in seven groups of each containing six rats [25].

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

Group II- [Rifampicin and isoniazid (100 + 50mg/kg, p.o.)] at every 72 h for 21 days,

Group III- Silymarin (25mg/kg, p.o.) served as standard for 21 days and simultaneously administered RIF+INH (100+50mg/kg p.o.) every 72 h,

Group IV – TPL (100mg/kg p.o.) for 21 days and simultaneously administered RIF+INH (100+50mg/kg p.o.) every 72 h,

Group V– TPL-SLNs (50mg/kg p.o.) for 21 days and simultaneously administered RIF+INH (100+50mg/kg p.o.) every 72 h,

Group VI – GA (50mg/kg p.o.) for 21 days and simultaneously administered RIF+INH (100+50mg/kg p.o.) every 72 h,

Group VII- GA-SLNs (25mg/kg p.o.) for 21 days and simultaneously administered RIF+INH (100+50mg/kg p.o.) every 72 h.

2.11.2. 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.

2.12. 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. 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).

2.13. Histopathological studies

After sacrifice of animals (Rat and mice)' 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.

2.14. 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.

3. RESULTS & DISCUSSION

The crude extracts so obtained after the hot maceration technique, 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

Solvent	Ext. time (hrs)	Colour and consistency	% yield
Distilled water	24	Yellowish brown semisolid mass	13.78%

Preliminary phytochemical screening of triphala aqueous extract revealed the presence of various 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

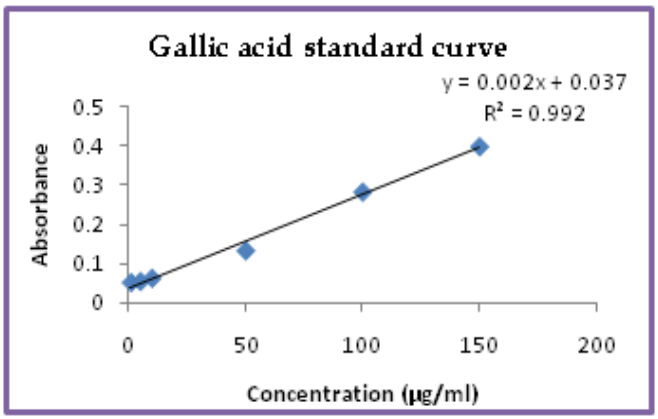
Constituents	Aqueous extract
Tests for Alkaloids:	
Mayer's test	+ve
Dragendorff's test	-ve
Wagner's test	-ve
Tests for Carbohydrates:	
Molisch's test	-ve
Fehling's test	+ve
Benedict's test	+ve
Tests for Glycoside:	
Modified Borntrager's test	+ve
Legal's test	-ve
Tests for Phytosterols and Triterpenoids:	
Liebermann's test	-ve
Salkowaski test	-ve
Tests for Protein and Amino acids:	
Ninhydrin test	+ve
Biuret test	+ve
Tests for Phenolic and Tannins:	
Ferric chloride test	+ve
Gelatin test	+ve
Lead acetate test	+ve
Tests for Flavonoids:	
Shinoda Test	+ve
Tests for Saponins:	
Foam test	+ve
Haemolysis test	+ve

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

Solubility of gallic acid was 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.

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.



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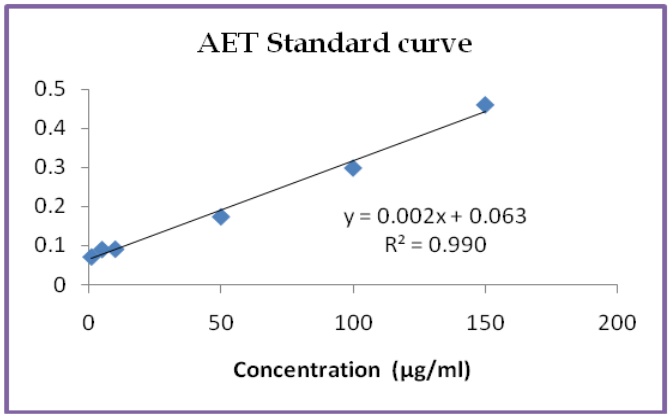


(B)

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

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

SEM images of GA-SLN, ATPL-SLN also was found to showing smooth surfaces of nanoparticles. While due to slight heat treatment before SEM (in which the lipids get melted) the spherical structure of SLNs was disturbed Fig. 2.

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 doses 50 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

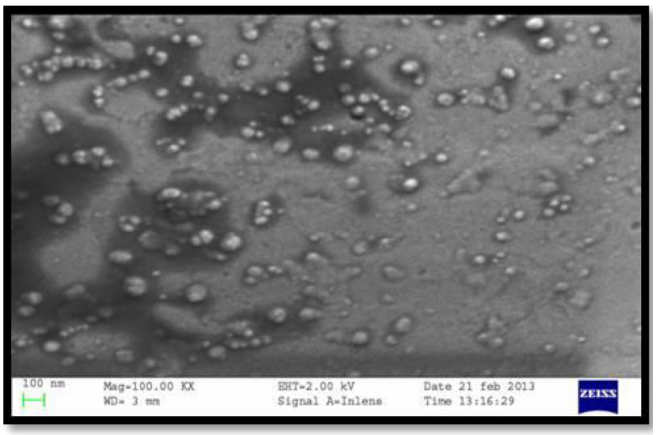
involved in transport of the very low density lipoprotein (VLDL) out of the hepatocyte . By Estimating the concentration of serum marker enzymes, like AST, ALT, ALP and total bilirubin, make assessment of liver function. In liver damage the level of serum marker enzymes elevated. The protective effects 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 6: Particle Size, Zeta Potential, PDI, % Drug Entrapment

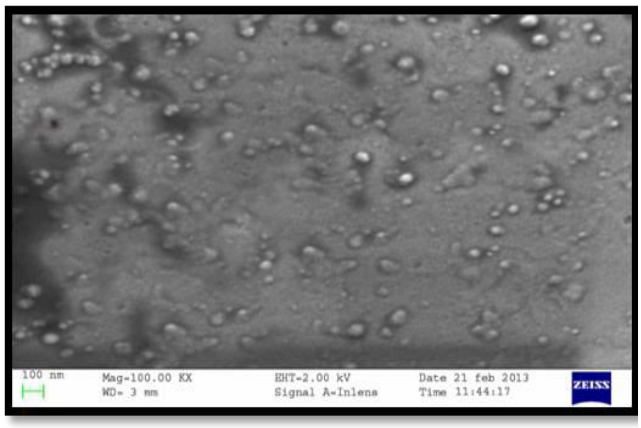
Formulation	Particle Size (nm)	Zeta Potential (mV)	Polydispersity Index (PDI)	% Drug Entrapped
ATPL-SLN	299 ± 2.9	-ve6.8	0.221	35.87±1.65
Gallic acid SLN	387.13±3.4	-ve8.9	0.234	39.34 ±2.01

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

Time (hr)	% Drug Release	
	GA-SLN	ATPL-SLN
0.5	6.04±0.24	8.36±0.29
1	12.57±0.78	15.12±1.04
2	20.86±1.24	25.24±1.46
4	31.74±1.83	37.22±1.99
8	49.92±2.58	55.31±2.73
24	78.33±3.22	83.19±5.01
48	91.06±3.85	98.64±5.34



(A)



(B)

Fig.2: (A) Scanning Electron Microscopic (SEM) image of GA-SLNs (B) (SEM) image of ATPL-SLNs

The protective effects of triphala/bioactive & their formulation against hepatotoxicity were investigated in rat 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 RIF+INH alone group as compared to control group. These effect of RIF+INH 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 10). Further activity of oxidative stress enzymes *i.e.* SOD, GSH and CAT by RIF+INH 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 RIF+INH. 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 11). Antitubercular drugs mediated oxidative damage is generally attributed to the formation of free radicals, which act as stimulator of lipid peroxidation (increases MDA levels) and source for destruction and damage to the cell membrane. Also elevation of MDA levels induced by RIF+INH was also significantly (P<0.01) decreased by Triphala/Bioactive & their formulation. The order of protection was: VII > V > VI > IV respectively (Table 11).

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.*

Table 10: Effect of triphala/bioactive & their formulation on biochemical parameters against rifampicin+isoniazid induced hepatotoxicity in rats

Group	Treatment	Dose	ALT (IU/L)	AST (IU/L)	ALP(IU/L)	Total Bilirubin (mg/dl)
1	Normal control	2ml/kg, p.o	45.5±0.457	40.66±0.181	11.29±0.529	0.20±0.008
2	Toxicant control (RIF+INH)	20mg/kg,p.o.	67.86±1.854$^{\#\#}$	103.05±2.661$^{\#\#}$	60.18±1.283$^{\#\#}$	0.42±0.016$^{\#\#}$
3	Standard (SLY)	25mg/kg,p.o	48.72± 1.092**	74.89±0.800**	24.11±0.713**	0.29±0.006**
4	TPL+ RIF+INH	100mg/kg,p.o	56.99±2.623**	94.67±2.664*	41.82±3.617**	0.36±0.015*
5	TPL-SLNs + RIF+INH	50mg/kg,p.o	54.01± 1.09**	82.35±2.335**	35.62±1.192**	0.32±0.011**
6	GA + RIF+INH	50mg/kg,p.o	55.65± 1.727**	84.27± 2.338**	38.48±2.989**	0.36±0.013*
7	GA-SLNs+RIF+INH	25mg/kg,p.o	52.29 ± 0.979**	79.24± 1.933*	33.27± 1.19**	0.31± 0.016**

*$^{\#\#}$ 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.*

*Where: **RIF+INH**: Rifampicin+Isoniazid, **SLY**: Silymarin, **TPL**: Triphala extract, **TPL-SLNs**: Solid lipid nanoparticles of triphala, **GA**: Gallic acid, GA-**SLNs**: Solid lipid nanoparticles of gallic acid*

Table 11: Effect of triphala/bioactive & their formulation on SOD, LPO, CAT & GSH against rifampicin+isoniazid induced hepatotoxicity in rats

Group	Treatment	Dose	CAT (u/mg)	SOD (u/mg)	GSH (mM/mg)	LPO (nM/mg)
1	Normal control	2ml/kg, p.o	32.57±0.436	1.51±0.158	18.62±0.120	2.39±0.106
2	Toxicant control RIF+INH	20mg/kg,p.o.	13.39±0.355$^{\#\#}$	0.19±0.095$^{\#\#}$	5.83±0.124$^{\#\#}$	6.99±0.042$^{\#\#}$
3	Standard (SLY)	25mg/kg,p.o	33.23±0.491**	1.67±0.159**	18.09±0.068**	2.78±0.024**
4	TPL+ RIF+INH	100mg/kg,p.o	21.43±0.567**	0.87±0.029**	6.51±0.285*	3.84±0.029**
5	TPL-SLNs + RIF+INH	50mg/kg,p.o	25.15±0.821**	0.98±0.056**	7.18±0.244**	3.21±0.016**
6	GA + RIF+INH	50mg/kg,p.o	22.32±0.738**	0.95±0.043**	6.89±0.220**	3.78±0.009**
7	GA-SLNs+ RIF+INH	25mg/kg,p.o	27.68±0.926**	1.03±0.050**	7.43±0.211**	2.81±0.087**

*$^{\#\#}$ 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.*

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 rifampicin & isoniazid/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 RIF+INH/TC 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 & 4.

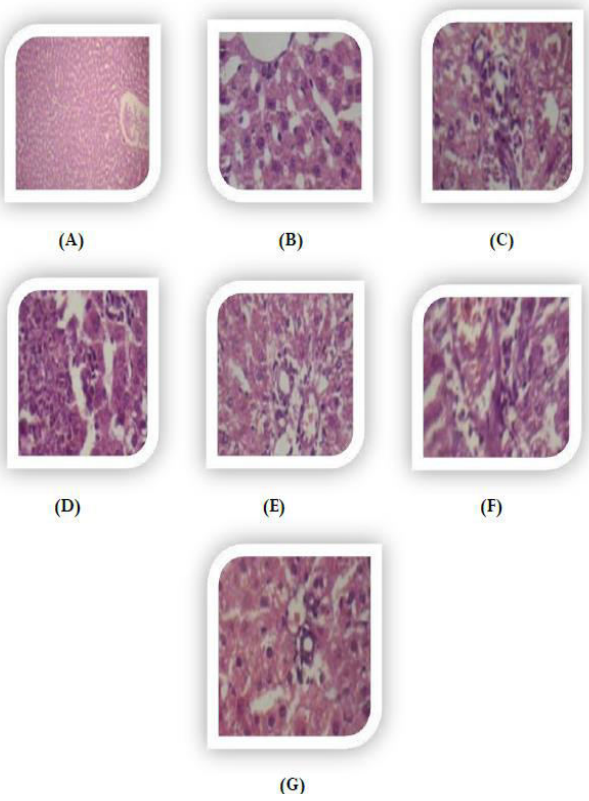


Fig. 3: Histopathology of the liver in Rifampicin+ Isoniazid induced hepatotoxocity studies

(A) *Normal control group showed normal hepatic architecture* ***(B)*** *RIF+INH (100+50mg/kg) treated showed normal architecture of liver completely lost & dialated central vein with inflammed sinusoids.* ***(C)*** *Silymarin(25mg/kg) treated showed normal lobular architecture of liver similar to control.* ***(D)*** *TPL(100mg/kg) treated showed that the nuclei were not very clear as in normal hepatocytes but as compared to toxin group.,the no. of hepatocytes with normal nucleus were much more.* ***(E)*** *TPL-SLNs (50mg/kg) treated showed that normal arrangement of hepatocytes around the central vein, absence of necrosis, fatty vacuoles were observed.* ***(F)*** *GA (50mg/kg) treated showed that lesser hepatocellular damage and sinusoidal dilation, slight dilation in central vein was observed.* ***(G)*** *GA-SLNs (25mg/kg) treated showed that absence of necrosis, normal arrangement of hepatocytes around the centralvein.normal architecture of liver similar to control & silymarin.*

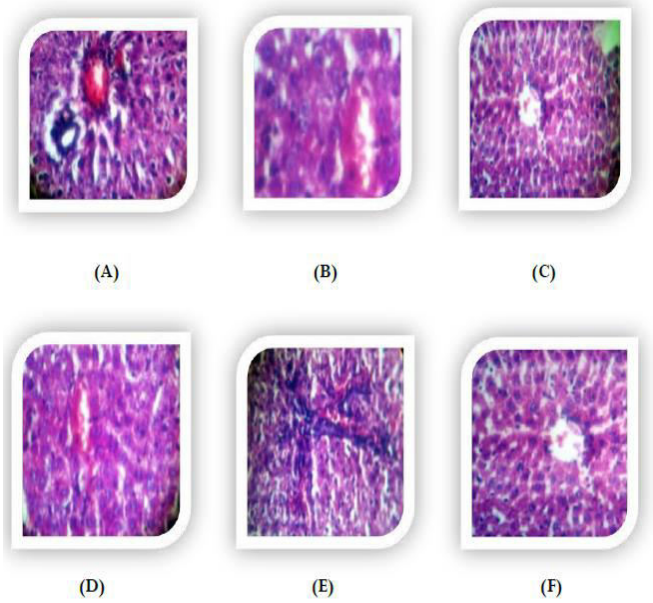


Fig. 4: Histopathology of the liver in Tetracycline induced hepatotoxocity studies

(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).*

4. 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. Nanocarriers show 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.

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