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RESEARCH ARTICLE

Design, fabrication and characterization of sesamol loaded polymeric nanoparticles: *In vivo* hepatoprotective potential in Wistar rats

Manish Kumar Gupta[1], Vipul Sansare[1*], Birendra Shrivastava[1], Santosh Jadhav[2]

[1] School of Pharmaceutical Sciences, Jaipur National University, Jaipur, India- 302017

[2] Department of Pharmaceutical Chemistry, SVPM's College of Pharmacy, Malegaon, Maharashtra, India-413115

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ABSTRACT

Objective(s): Liver diseases affect millions of people worldwide, which are difficult to treat with conventional drug delivery. Numerous drugs have been investigated for treatment of diseases associated with liver however correct drug delivery system need to be find for delivery of drugs. Sesamol is a well-recognized antioxidant phytoactive found in sesame oil has reported to scavenge hydroxyl radical. However unfavorable physicochemical properties limits its use as effective therapeutic agent. Thus present study was started with aim to fabricate sesamol loaded polymeric nanoparticles to minimize limitations associated with conventional delivery of sesamol.

Methods: Drug encapsulated nanoparticles were formulated using solvent evaporation ultrasonication technique. The selected technique was found to be effective for preparation of nano sized particles with good physicochemical properties. The formulated nanoparticles were evaluated with respect to physicochemical properties and in vivo hepatoprotective potential.

Results: The drug loaded nanoparticles revealed significantly better hepatoprotective activity with reduction of serum liver injury markers and proinflammatory cytokines compared to standard Liv-52.

Conclusions: Thus formulated nano sized particles based system could be promising alternative to deliver sesamol.

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INTRODUCTION

Sesamol (SM), [3, 4-methylenedioxyphenol] is well known phenolic antioxidant phytoactive extracted from sesame oil. It is hydrolysis product of sesamolin formed thermal oxidation [1]. Sesamol is well established antioxidant and hepatoprotective phytoactive. Various previously published reports showed antioxidant and hepatoprotective potentials of sesamol in both cell as well as animal models. In addition to this it shows hypolipidemic and anti-clastogenic activity as well as reduce oxidative stress [2]. Although sesamol exhibits varieties of pharmacological actions the use of this phytoactive is limited due to limitations associated with pharmacokinetic properties. It has good aqueous solubility as well as lipophilicity. However limited oral bioavailability and rapid elimination through conjugation are major hurdles which limits its use [3]. To enhance the sesamol bioavailability, the sustained drug delivery is advisable, which prolongs drug circulation in the body by controlling its release from system.

Colloidal nanocarrier based systems with particle size in the range of 10 to 400 nm are promising alternative to conventional drug delivery

* Corresponding Author Email: *avipulsansare@gmail.com*

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in the area of treating various disease conditions. The novel drug loaded nanocarrier offers various advantages like increased solubility, enhanced targeting potential at cellular level by minimizing off target distribution and minimize dose related side effects by reducing dose size [4]. Various nanocarrier based systems like liposomes [5, 6], niosomes [7, 8], proniosomes [9, 10], phytosomes [11, 12], ethosomes [13, 14], transfersomes [15, 16], lipid nanoparticles (NPs) [17, 18], nanostructured lipid carriers (NLCs) [19, 20], polymeric NPs [21, 22], gold NPs and silver NPs were widely investigated for efficient delivery phytoactives. Out of these various types, the polymeric NPs are widely used for delivery of phytoactives through various routes like topical, oral, pulmonary and nasal. The desirable properties of polymeric NPs like biocompatibility and biodegradability potentiate its use for drug delivery [23].

Hepatic injury induction in rats using carbon tetrachloride (CCl_4) is commonly employed animal model to assess hepatoprotective activity of phytoactive. CCl_4 forms trichloromethyl free radical, CCl3* on metabolism. Free radical mediate production of reactive oxygen species through cytochrome P450 oxygenase system. The reactive oxygen species causes lipid peroxidation and lastly hepatocellular damage [24]. CCl3* interacts with cellular molecules like nucleic acid, protein and lipid which eventually leads to lipid metabolism impairment and fatty degeneration i.e. steatosis.

Thus present work was initiated with aim to formulate SM encapsulated Poly (D, L-lactide-co-glycolide) (PLGA) NPs to improve hepatoprotective potential and minimize demerits associated with conventional delivery of SM. SM loaded NPs dispersion was prepared using solvent evaporation ultrasonication technique and evaluated for particle diameter, stability, phytoactive encapsulation efficiency, drug release behavior *in vitro* as well as *in vivo* hepatoprotective potential in animal model. The formulated NPs revealed significantly better hepatoprotective potential than standard LIV-52 with reduction of serum liver injury markers and proinflammatory cytokines.

MATERIAL AND METHODS

Material

SM and polyvinyl alcohol were purchased from Sigma-Aldrich, USA. PLGA was purchased from Evonik India, India. Acetone was obtained from SDFCL, Mumbai, India. Dialysis membrane (Mol. Wt.13-14 kD) was purchased from Himedia, Mumbai, India. All other solvents, reagents and chemicals were analytical grades and purchased locally.

Methods

Preparation of SM encapsulated polymeric NPs

SM encapsulated polymeric NPs were fabricated using solvent evaporation ultrasonication technique. Briefly, accurately weighed quantities of SM and PLGA according to desired ratio were dissolved in acetone to form organic phase. Polyvinyl alcohol was dissolved in distilled water to form 0.5 % W/V solution as aqueous phase. The polymeric solution was injected in aqueous stabilizer solution with stirring at 15000 RPM using high speed magnetic stirrer to evaporate organic solvent. After stirring, the dispersion was subjected to ultrasonication using probe sonicator (Sonic vibra cell, VCX500, Sonics and Materials Inc., USA). The sonication was conducted at 80 amplitude for 10 minutes using 10 second pulses. At last, the formulated nano-dispersion stirred for 2 hours in order to remove acetone.

Evaluation of SM loaded polymeric NPs

Phytoactive encapsulated polymeric NPs were evaluated with respect to particle size, zeta potential, encapsulation efficiency, drug release behavior and *in vivo* hepatoprotective potential.

Particle size and zeta potential

Particle size and zeta potential of SM loaded polymeric NPs based dispersion was assessed using Zetasizer, Nano-ZS (Malvern, United Kingdom). Briefly 0.5 mL of NPs based dispersion was taken in polystyrene cuvettes, suitably diluted with double distilled water and subjected to measurement.

Phytoactive encapsulation efficiency

The entrapment efficiency of fabricated drug loaded NPs was estimated indirectly using ultracentrifugation method. Briefly 0.1 mL of NPs dispersion was taken in centrifuge tubes and diluted to 1 mL using double distilled water. The resulting diluted dispersion centrifuged at 80000 rpm for 1 hour at 4^0C temperature in order to obtain sediment of drug loaded NPs. After centrifugation supernatant was carefully removed and analyzed for unentrapped drug content by UV spectrometry. Entrapment efficiency was calculated by following equation:

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$$\% \text{ EE} = \frac{\text{Total amount of SM added} - \text{amount of SM in supernatant}}{\text{Total amount of SM added}} \times 100$$

SM release behavior

SM release behavior of an SM loaded NPs was assessed using dialysis diffusion technique. Briefly NPs dispersion equivalent to 8 mg of SM was filled in dialysis bag (molecular weight: 12–14 kDa) and fixed in United States Pharmacopeia (USP) type II dissolution apparatus whose vessels were filled with 500 mL of dissolution medium i.e. phosphate buffer pH 6.8. The rotational speed of the paddle and temperature of the buffer were adjusted at 50 rpm and 37°C ± 0.5 °C, respectively. At predetermined time intervals the 2 mL of sample was withdrew and replace with equal volume of buffer to maintain sink condition throughout the experiment. The collected aliquots were filtered and analyzed by UV-Spectrophotometry using phosphate buffer pH 6.8 as blank.

In vivo hepatoprotective potential

Male Wistar rats of weight in the range of 150 to 200 gm were selected as test animals to evaluate hepatoprotective potential of SM loaded NPs. The animal study protocol was approved by the Institutional Animals Ethical Committee of Indira Institute of Pharmacy (Approval number: IIP/IAEC/08/2019-20). All animals were purchased from Global bioresearch solution Pvt. Ltd, Shirwal, India.

Rats were randomly divided into four groups, each having 6 rats. Group I was serve as vehicle control (VC) group and received olive oil at the dose of 1 mL/kg BW. Group II serve as positive control (PC) and received standard hepatotoxic drug CCl_4. Group III was marked as standard group and received Liv-52 at the dose of 1 mL/kg BW [25]. SM loaded NPs based dispersion at the dose of 8 mg/kg BW was administered in group IV animals.

Hepatic injury to the 18 experimental animals was induced by oral administration CCl_4 with dose of 4 mL/kg BW for 10 days. While administration, CCl_4 was mixed with same volume of olive oil). In group II, olive oil was administered at the dose of 1 mL/kg BW of for 10 days (no hepatic injury as well as treatment for group II). In standard group III, three days after hepatic injury induction, Liv-52 was administered with dose 1 mL/kg BW daily for four weeks. In test group IV three days after hepatic injury induction, SM loaded NPs based dispersion was injected with dose 8 mg/kg BW daily for four weeks [1].

On completion of treatment, rats were anesthetized using ether and blood sampling was carried out through retro-orbital plexus. Blood was centrifuged at 4000 rpm at 4°C for 20 minutes to separate serum and stored at –20°C for estimation of serum liver injury markers. At last, all animals were sacrificed by cervical dislocation to harvest their liver for estimation of oxidative stress parameters. Group II (PC) animals were sacrificed three days after hepatotoxicity induction i.e. on 13th day and animals from other groups were sacrificed on the 42th day, i.e. 1 day after four week treatment. After harvesting liver was homogenized with phosphate–buffered saline pH 7.4 (10%) at 4^0C. The post–mitochondrial supernatant (PMS) of liver was used to measure oxidative stress parameters. Rat liver homogenates was centrifuge in chilled phosphate buffer, pH 7.4 at 4°C to prepare PMS.

Assessment of liver injury markers

Assessment of liver injury markers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) is very important operation to measure hepatoprotective efficacy of SM loaded NPs. Diagnostic kits (Reckon diagnostic, India) were utilized to assess liver injury markers. The manufacturer protocol was used to perform the assays.

Estimation of antioxidant parameters

The lipid peroxidation (LPO), reduced glutathione (GSH) as well as superoxide dismutase (SOD) are stress parameters were also measured in PMS.

Estimation of LPO

The LPO in liver PMS were estimated using method described by Wills [26]. The malondialdehyde (MDA) level, a measure of LPO was measured as liver thiobarbituric acid reactive species. The LPO level in animals was assessed and denoted as nanomoles of MDA per milligram of protein. The molar extinction coefficient of the chromophore was 1.56×10^5 M^{-1} cm^{-1}.

Assessment of SOD

The method reported by Kono et al. was used for measurement of SOD activity in liver PMS [27]. SOD activity was measure and denoted in the form of SOD per milligram of protein (SOD units/mg Pr).

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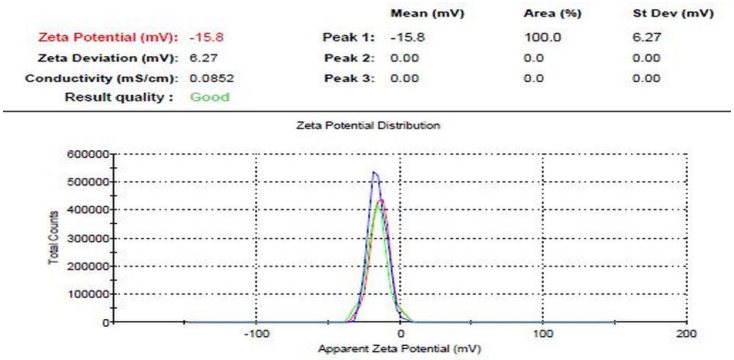

Fig. 1.

Assessment of GSH levels

The GSH estimation procedure reported by of Jollow et al. was used for estimation of GSH [28]. The GSH level was measured and denoted as nmoles of GSH per µg of protein (nmoles of GSH/ µg Pr).

Estimation of pro-inflammatory cytokine, tumor necrosis factor-α (TNF-α)

TNF-α levels in liver homogenates was measured using the ELISA kit (RayBiotech). The manufacturer guidelines were studied to perform measurements.

Statistical Analysis

The one way analysis of variance (ANOVA) and Tukey test was used for comparison of means various treatments. Differences between the means were considered statistically significant at $p < 0.05$. All experimental data expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Preparation and evaluation SM loaded PLGA NPs

SM encapsulated PLGA NPs were successfully prepared using solvent evaporation ultrasonication technique [29, 30]. The PLGA as biocompatible polymer was utilized to form polymeric matrix for encapsulation of SM. The formulation variables and process variables of SM loaded NPs were previously optimized in our laboratory. The formulated NPs revealed 200 ± 7.142 nm particle diameter with 0.05 ± 0.021 polydispersity index. The particle diameter of NPs govern its uptake in hepatic parenchymal cells. The numerous scientific research reports have reported that nanocarriers with particle diameter up to 200 nm can efficiently engulf by hepatic parenchymal cells and generate significant therapeutic effect [31, 32]. The SM loaded NPs revealed particle diameter close to 200 nm which confirm its suitability to reach at hepatic parenchymal cells. The zeta potential of optimized SM loaded NPs was found -15.8 ± 3.518 mV which confirms physical stability with minimum particle aggregation (Fig. 1) [33]. The entrapment efficiency of SM in polymeric NPs was found to be 73.81 ± 3.173%. Thus selected technique was found to be effective for preparation of NPs with better particle size and entrapment efficiency.

In vitro drug release

The percent cumulative drug release from polymeric NPs is important evaluation parameter governing bioavailability of the drug and therapeutic efficacy. The SM release profile showed biphasic behavior. In first 24 hour, it was observed to be a burst release of SM with 66.21± 2.13 % cumulative drug release followed by a slow release of SM up to 72 hours (Fig. 2). It has observed that drug particles closer to the surface of matrix are dissolved first, diffuse through polymer network and release form device for absorption [34]. The SM from solution releases with greater extent in 8 hours because of greater aqueous solubility of drug.

Estimation of Serum Liver Injury Markers

AST and ALT enzymes are release in plasma due to damage of structural integrity of hepatic cells. CCl_4 administration for ten days resulted in induction of hepatic cellular damage which eventually leads to release of AST and ALT in plasma. Elevation of AST and ALT levels due to administration of CCl_4 in experimental animals confirmed hepatic cellular damage. CCl_4 administration in experimental animals resulted in 2368.06% elevation in ALT level compared to

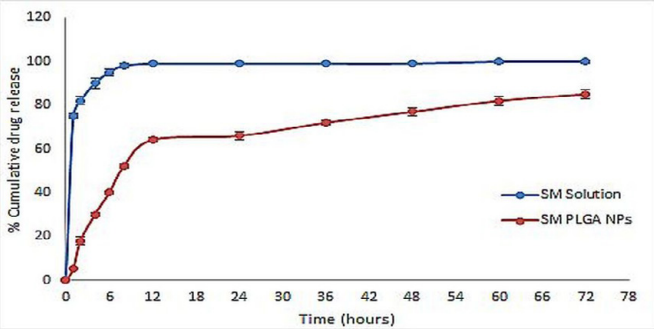

Fig. 2.

Table 1. % inhibition in ALT and AST levels in treatment groups with respect to CCl_4 group (n=6).

Group	% Inhibition of ALT versus CCl_4 group	% Inhibition of AST versus CCl_4 group
Group III	45.75 ± 2.78	73.45 ± 3.51
Group IV	56.17 ± 2.741	78.83 ± 2.58

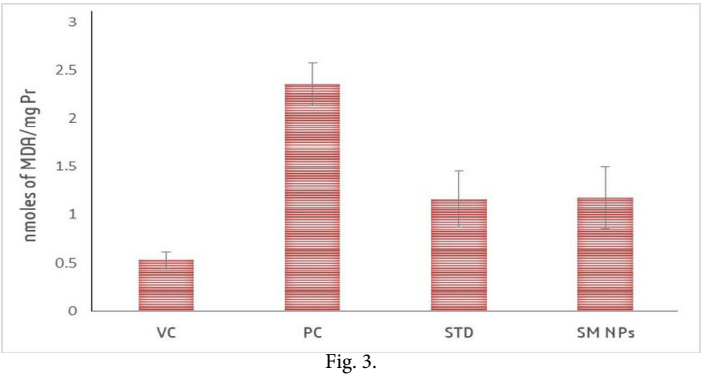

Fig. 3.

group I (VC). The elevated level of serum liver injury marker like ALT was sign of hepatotoxicity induction in rats. The four weeks treatment with SM loaded polymeric NPs at the dose of 8 mg/kg BW revealed reduction in elevated ALT levels by 56.17 ± 2.74 (Table 1). Liv-52 (Group III) administration in hepatotoxic rats resulted 45.75 ± 2.78% reduction in ALT level. The fabricated drug loaded NPs based dispersion was quite successful in significant reduction ($p < 0.05$) of elevated ALT level in experimental animals compared to Liv-52.

In similar way, AST level was elevated by 980.73% due to administration of CCl_4 compared to group I (VC). The standard hepatoprotective drug Liv-52 reduced elevated AST level by 73.45 ± 3.51. Whereas SM loaded NPs revealed significantly better ($p < 0.05$) results than Liv-52 with reduction of elevated AST by 78.83 ± 2.58. Sesamol has reported to exhibit anti-MMP-9 (matrix metallopeptidase 9) activity [35]. MMP-9 is potential enzyme involved in necrosis of hepatocytes. Hepatocytes necrosis results in release of ALT and AST from liver. Sesamol inhibits MMP-9 activity and protects liver against tissue necrosis, by reduction of ALT as well as AST.

Antioxidant parameters

LPO

LPO measured as MDA content in liver PMS. CCl_4 administration resulted in elevation in MDA level by 470%. CCl_4 administration for ten days resulted in induction of hepatic injury which leads to LPO and elevation MDA levels in liver. The four week treatment with SM encapsulated NPs resulted in reduction in MDA content by 51.25 ± 2.37%. The standard drug Liv-52 reduce elevated MDA content by 50.61 ± 2.71% on four week treatment. The SM loaded NPs showed significantly better results ($p < 0.05$) compared to standard drug (Fig. 3).

The significantly better results are shown by

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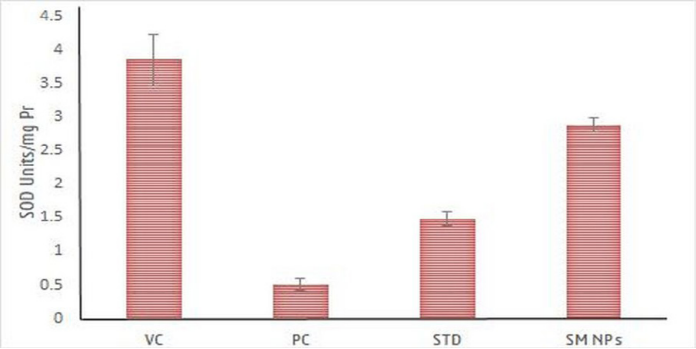

Fig. 4.

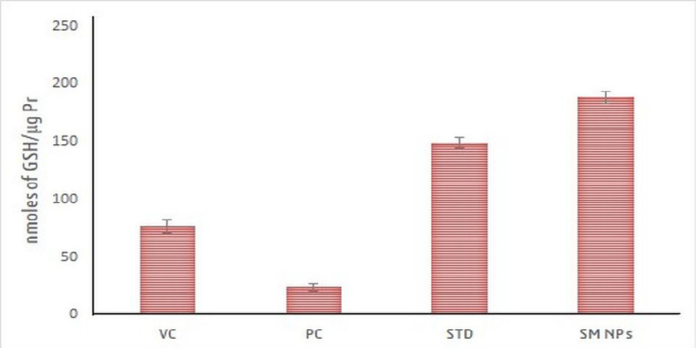

Fig. 5.

nanocarrier based formulation may be due to improvement in cellular permeation of SM on its loading in polymeric NPs. Drug loaded polymeric NPs stabilized with polyvinyl alcohol are expected to permeate across liver cells biomembrane resulting in significantly better reduction of MDA level as shown in the this study.

SOD

The positive control CCl_4 group revealed significantly low ($p < 0.05$) SOD level (0.5047 ± 0.0891 SOD units/ mg Pr) compared to VC group. Treatment with standard drug Liv-52 for four weeks showed almost 3 times increase (1.4903 ± 0.1079 SOD units/ mg Pr) in SOD level. Whereas SM NPs treatment showed 5 times increase in SOD level (2.891 ± 0.1034 SOD units/ mg Pr) (Fig. 4). Hepatocellular damage induction by CCl_4 administration is indicated by reduced SOD level. The severity of hepatocellular is govern by SOD level. The reduce SOD level due to hepatic injury could be attributed due to its inactivation by LPO and reactive oxygen species. Since four week treatment with SM loaded polymeric NPs significantly reduce the MDA content, hence, as assumed SOD was significantly ($p < 0.05$) elevated in SM NPs group.

GSH

The total GSH level in PC group (23.922 ± 3.39 nmole of GSH/ µg Pr) was significantly low ($p < 0.05$) by CCl_4 administration than VC group. The SM loaded NPs successfully increased total GSH level by 7.61 times on four weeks treatment. The Liv-52 showed comparable results with increase in total GSH level by 6.37 times however it was found to be less effective compare to SM loaded NPs (Fig. 5). The Tukey test showed significant difference ($p < 0.05$) between GSH levels showed by SM loaded NPs and standard Liv-52.

Proinflammatory cytokine TNF-α

The administration of CCl_4 resulted in significant elevation of proinflammatory cytokine TNF-α level (608.732 ± 10.62 TNF Pg/mL) in PC group. The elevation of TNF-α level was indication of hepatic injury induction. The four weeks treatment with SM loaded NPs reduced the

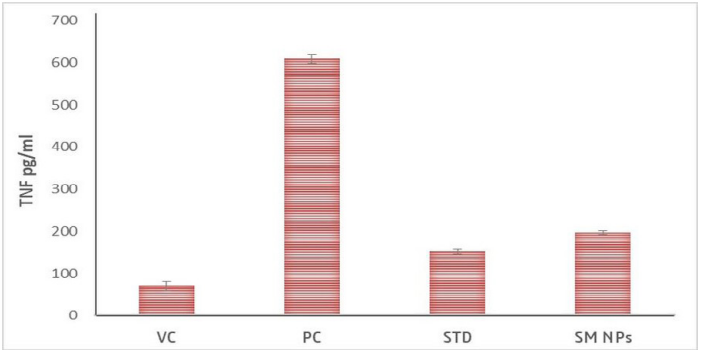

Fig. 6.

increased levels of TNF-α (197.15 ± 4.68 TNF Pg/mL) (Fig. 6). The Liv-52 treatment showed vast reduction of TNF-α level (152.61 ± 4.95 TNF Pg/mL). The results obtained with Liv-52 were far better than SM loaded NPs.

CONCLUSION

The present work was initiated to fabricate SM loaded polymeric NPs. The SM loaded polymeric nano sized particles were successfully formulated using solvent evaporation ultrasonication technique. The selected methods was found to be efficient for preparation of NPs with acceptable physicochemical properties and *in vitro* release behavior. CCl_4 was utilized to induce hepatic injury in experimental animals. The increased level of serum liver injury markers like AST and ALT in experimental animals confirmed induction of hepatic injury. The four weeks treatment with drug loaded NPs showed significantly better hepatoprotective potential than Liv-52 with reduction of elevated AST and ALT levels. In addition to this SM loaded NPs significantly reduce increased LPO and TNF-α compared to standard Liv-52. Thus PLGA NPs could be viable alternative for encapsulation of SM.

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CONFLICT OF INTEREST

Authors declare no potential conflict of interest.

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