IJPSR (2017), Volume 8, Issue 11 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 26 March, 2017; received in revised form, 11 August, 2017; accepted, 10 September, 2017; published 01 November, 2017

FABRICATION, CHARACTERIZATION AND PHARMACOLOGICAL ACTIVITY OF USNIC ACID LOADED NANOPARTICLES

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Keywords:

Usnic Acid, Nanoprecipitation, PLGA, Tween 80, Sonication, Cytotoxicity

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ABSTRACT: Usnic acid is lichen metabolite. Lichen species are the source of usnic acid. It has Antitumor and Anti-proliferation activity. Usnic acid is poorly water soluble drug. So its dissolution and bioavailability is poor. To enhance dissolution and bioavailability of usnic acid nanotechnology has been adopted. So in this study usnic acid powder was formulated as a nanoparticle with the help of PLGA polymer. Nano-precipitation with sonication method was adopted to formulate usnic acid loaded nanoparticles. Obtained nanoparticles were undergone to characterization and evaluation studies. SEM study revealed that particles are in the range of nanometer level and it is in spherical shape. FTIR and DSC studies have shown that no interaction between drug and polymer. *In vitro* drug release was maximum for optimized formulation. Optimized formulation also exhibited more antitumor activity than free usnic acid. Kinetic study revealed that the drug release is zero order. It concludes nano-precipitation with sonication is effective method for preparation of usnic acid loaded nanoparticles.

INTRODUCTION: Usnic acid (2, 6 - diacetyl -7, 9-dihydroxy-8, 9h-dimethyl-1, 3(2H, 9Bh)-dibenzo-furandione) is a liche n metabolite [1, 2]. Usnic acid is a yellow pigment and occurs in two enantiomeric form is found in lichens [3, 4, 5]. Most of literature survey has shown usnic acid exhibit antitumour and anti-proliferation activity. Kumar and muller has found out usnic acid has cytotoxic activity against human keratinocyte cell culture [6] and lot of researches have been carried out study on usnic acid to reveal the cytotoxic activity [7].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(11).4758-66

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (11).4758-66

In addition to the antitumour activity, usnic acid has been shown to exhibit antiviral, antiprotozoal, antimicrobial, anti-inflammatory and analgesic activities [8, 9, 10]. Since usnic acid has hydrophobic nature it has poor dissolution. Nanopaticles are good platform to increase solubility and bioavailability of hydrophobic drug [11]. So nanotechnology was adopted to bring up the good dissolution and better bi-availability.

Nanoparticles are referred as nanosized colloidal particle ranging in size from 10 - 1000 nm in which drug is encapsulated by polymers. These small size nanoparticles can be easily absorbed into RES and other system of body [12]. Among various types of nanomaterials, polymeric nanoparticles have the advantages of relatively high biocompatibility, stability, and flexibility of conjugating ligands on their surface for targeted drug delivery [13, 14].

The objective of this current project was to develop the usnic acid loaded nanoparticles with PLGA polymer and evaluate physico chemical and pharmacological activities.

MATERIALS AND METHODS:

Materials: Usnic acid was procured from sigma Aldrich. Tween 80, Poly Ethylene Glycol, Choloroform, Acetone were purchased S. D. fine Chem Ltd., Mumbai. Solvents Methanol (HPLC) grade, HPLC water (lichrosolv) were purchased from Merck, India. Other reagents were purchased from local supplier.

Method of Preparation: All batches of formulations were prepared by nano precipitation method by sonication method [15, 16]. The drug and polymer as mentioned in the **Table 1** were dissolved in acetone (6 ml) and chloroform (4ml) respectively 0.4 ml of Tween 80 was added into drug solution and then organic phases were mixed by using vortex mixer. The organic phase was added dropwise (0.5ml/min) into aqueous phase (10 ml phosphate buffer of pH 7.4) containing 0.06 g PEG. Then it was sonicated using probe sonicator and the organic phase was removed by evaporation under reduced pressure.

TABLE 1: COMPOSITIONS OF USNIC ACID LOADED NANOPARTICLES

S. no.	Batch code	Amount of Drug in mg	Amount of Polymer in mg	Drug : Polymer
1	G_1	40	2	1:0.05
2	G_2	40	4	1:0.1
3	G_3	40	8	1:0.2
4	G_1S	40	2	1:0.05
5	G_2S	40	4	1:0.1
6	G_3S	40	8	1:0.2

G –formulations not consist of surfactant, GS – formulations consist of 0.4ml Tween 80

Characterization and Evaluation Studies:

Morphology: The usnic acid loaded nanoparticles were subjected to scanning Electron Microscope (SEM) for determining its size and shape. The nanoparticle size and shape were to be characterized and photographed [17].

FTIR Study: The spectra of the samples were recorded using an IR spectrophotometer. KBr pellets were prepared by gently mixing sample with KBr. Fourier transform infrared spectrum were recorded in the range of 400 - 4000 cm^{-1}.

Differential Scanning Calorimetry (DSC) Analysis: DSC experiments were carried out to determine the possible interactions between drug and polymers. Therefore in the present study DSC analysis were carried out on samples of pure drug and formulated nanoparticles [18, 19]. Weighed quantity of pure drug and formulation were taken in aluminum pans. The pans were sealed with aluminum caps. The samples were heated at 20 °C/min up to 300 °C. The location of the thermal transition and surface area of the peaks were obtained from the thermo grams.

Entrapment Efficiency: [20, 21] The nanosuspension with known amount of drug incorporated was centrifuged at 15,000 rpm for 15 minutes. The supernatant was separated and 1ml of supernatant was suitably diluted with phosphate buffer (pH 7.4) and the absorbance was measured spectro-photomertically at 290 nm. The drug entrapment efficiency was calculated by the following equation.

Entrapment Efficiency =
Amount of drug added - Amount of free drug in supernatant liquid × 100 / Amount of drug added

In vitro Drug Release Studies:

By UV Spectrophotomertic Method: [22] The *in vitro* drug release studies were performed using the dialysis bag diffusion technique. The release studies of usnic acid from nanoparticles were performed in phosphate buffer (pH 7.4). Total quantity of prepared formulations ware placed in a cellulose dialysis bag and tied at both ends. The dialysis bag was immersed in the receptor compartment containing 50 ml of phosphate buffer (pH 7.4) at 37 °C under magnetic stirring. An aliquot of receptor media (1ml) was withdrawn at regular intervals upto 24hr and the same volume was replaced by fresh dissolution medium (phosphate buffer pH 7.4) and analyzed spectro-photomertically at 290 mm. The percentage of drug released at various time intervals was calculated from calibration curve.

***In vitro* Drug Release for the Optimized Formulation (G_2S) by HPLC Method:** [23]

Procedure: The total quantity of optimized formulation was placed in the dialysis bag and bag immersed in the receptor compartment containing 50 ml phosphate buffer (pH 7.4) at 37 °C under magnetic stirring. The samples were withdrawn at the time intervals of 6, 12, 18 and 24 hrs with replacement of equal volume of dissolution medium. The withdrawn samples were filtered using sartorious membrance filter (0.45µ).

Sample Analysis: The filtered samples were suitably diluted and analyzed by HPLC method using Kromosil C8 column (250 x 4.6 mm internal diameter 5µm particle size) and a mobile phase composed of methanol / 20 mM phosphate buffer solution pH 7.4 (70:30 v/v). Sample aliquots (20µl) were injected and eluted with the mobile phase at a flow rate of 1ml/min. The usnic acid peak was verified at a wavelength of 280 nm. The amount of drug released at various time intervals was determined from the standard calibration curve.

Cytotoxic Evaluation of Usnic Acid and Usnic Acid Loaded Nanoparticles: In this present study usnic acid isolated from lichens and usnic acid loaded nanoparticles were evaluated for their cytotoxic activity [24, 25].

Induction of Cancer using DAL: Dalton's Ascites Lymphoma (DAL) was supplied by Amla cancer research center, Trissur, Kerala, India. The cells were maintained *in vivo* in swiss albino mice by intraperitoneal transplantation. While transforming the tumour cells to the grouped animal the DAL cells were aspirate from peritoneal cavity of the mice using saline.

The cell count was done and further dilutions were made, So that total cells should be 1x 10^6 cells/ml/mouse. This volume was given intraperitoneally and the tumour was allowed to grow in the mice for minimum of seven days before starting the study.

Treatment Protocol: Swiss albino mice were divided into 5 group of six each. The animal belonging to groups 2 - 5 were injected with DLA cells (1 x 10^6 cells/ml/mouse). Intraperitoneally, While the remaining group 1 served as the normal control group. After the inoculation, the groups were treated with compounds as given below.

Evaluation of Clinical Parameter:

Cancer Cell Count: The fluid (0.1 ml) from the peritoneal cavity of each mouse was withdrawn by sterile syringe and diluted with 0.8 ml of ice cold normal saline or sterile Phosphate Buffer Solution (PBS) and 0.1 ml of tryphan blue (0.1 mg/ml) and total number of the living cells were counted using heamocytometer.

Derived Parameters:

Body Weight: All the mice were weighed, from the day 1 to day 15 of the study. Average increase in body weight on the 15th day was determined.

Percentage Increase in Life Span (ILS): % ILS was calculated by the following formula:

$$\% \text{ ILS} = \frac{\text{Life span of the treated group}}{\text{Life span of normal control group}} - 1 \times 100$$

RESULT AND DISCUSSION:

Preparation of Nanoparticles: Usnic acid loaded nanoparticles were prepared by nano-precipitation method with sonication technique using bio-degradable polymers namely PLGA. This method was based on the interfacial deposition of polymer followed by diffusion of a semi-polar and miscible solvent in the aqueous medium in the presence of surfactant. Usnic acid is hydrophobic drug and for this type of drug nanoprecipitation method is ideal method for preparing nanoparticles [26, 27].

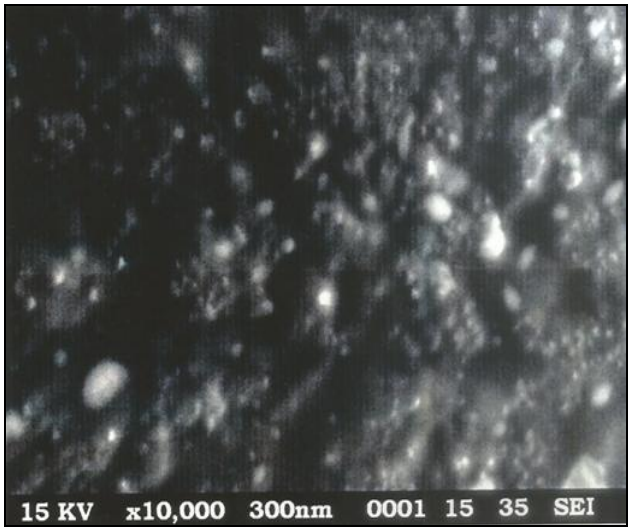

FIG. 1: SEM PHOTOGRAPH OF USNIC ACID LOADED NANOPARTICLES

Evaluation of Prepared Nanoparticles:

Morphology: The morphology of nanoparticles was examined by SEM. SEM images revealed that all the nanoparticles prepared displayed good morphology with spherical structure and smooth surface. Average size of optimized nanoparticle range is 200 nm to 300 nm respectively [28].

Drug - Polymer Interaction Study: FTIR spectrum of drug - PLGA optimized formulation (**Fig. 2**) showed peaks such as C-O stretching (1190.79 cm^{-1}), C = O stretching (1631.6979 cm^{-1}, 1692.35 cm^{-1}) and C-H stretching 2929.58 cm^{-1}. The absorption bands observed for the formulation of drug and polymer indicated were closed to the absorption bands of pure Usnic acid (**Fig. 2**). Therefore the interaction between usnic acid and polymer was negligible.

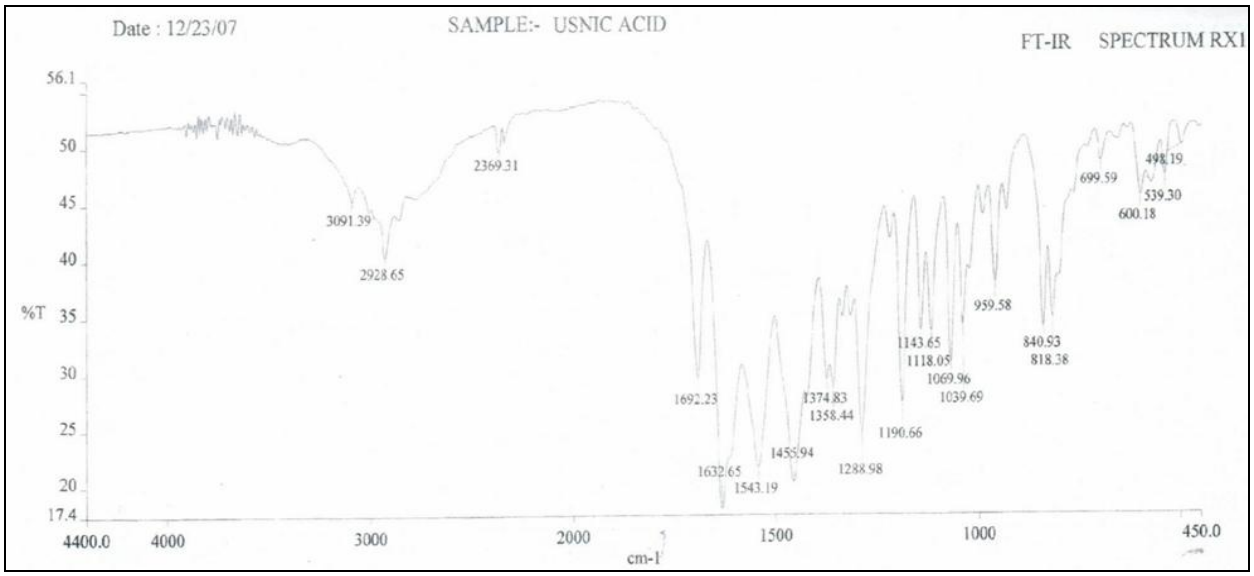

FIG. 2: FTIR SPECTRA OF USNIC ACID

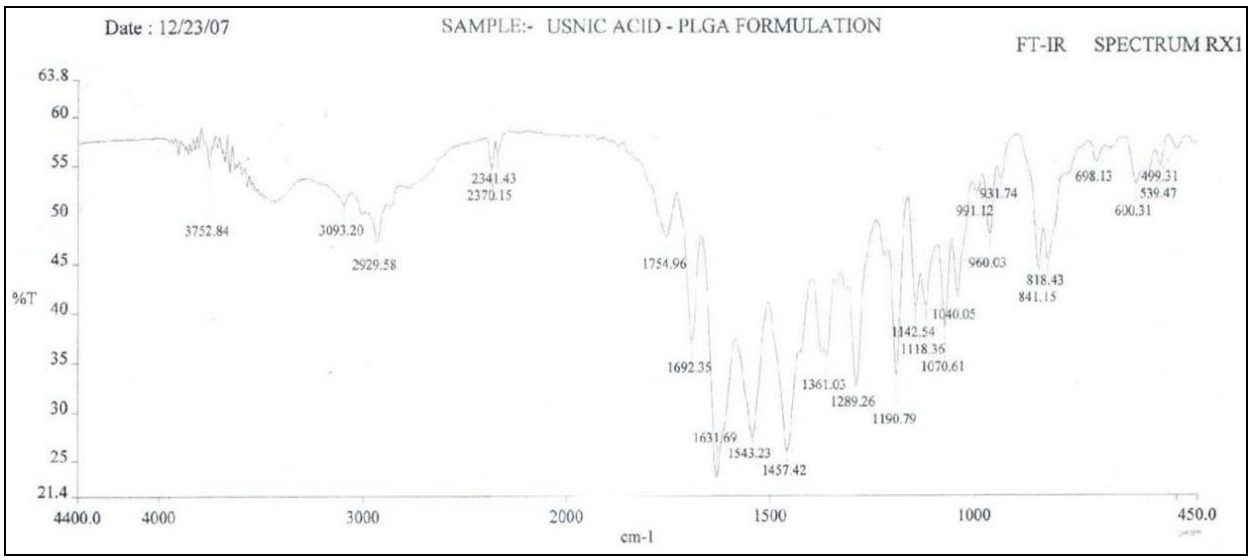

FIG. 3: FTIR SPECTRA OF USNIC ACID AND PLGA NANOPARTICLES

DSC Analysis: DSC thermo grams of free usnic acid, PLGA and usnic acid loaded nanoparticles were depicted in **Fig. 4** and **5**. DSC curve obtained for usnic acid showed exothermic peaks at 144.94 °C, 163.93 °C, 200.01 °C and 277.7 °C. DSC curves of PLGA and usnic loaded nanoparticles showed exothermic peaks at 45.80 °C, 67.83 °C, 169.64 °C, 178.3 °C and 61.82 °C, 111.56 °C and 153.28 °C respectively. It was observed that pure usnic acid showed an exothermic peak of melting at 200.01 °C and there was no peak obtained at this temperature for unsic acid loaded nanoparticles.

It confirmed that usnic acid could be in an amorphous or solid - solution state in the polymer matrix [29]. Thermograms of drug - polymer loaded nanoparticles showed that there was no melting peak of polymer (PLGA) observed and it confirmed that polymer also in amorphous state [30]. Hence DSC analysis results revealed that there was only physical interaction between drug and polymer, but there is no change in therapeutic action of drug.

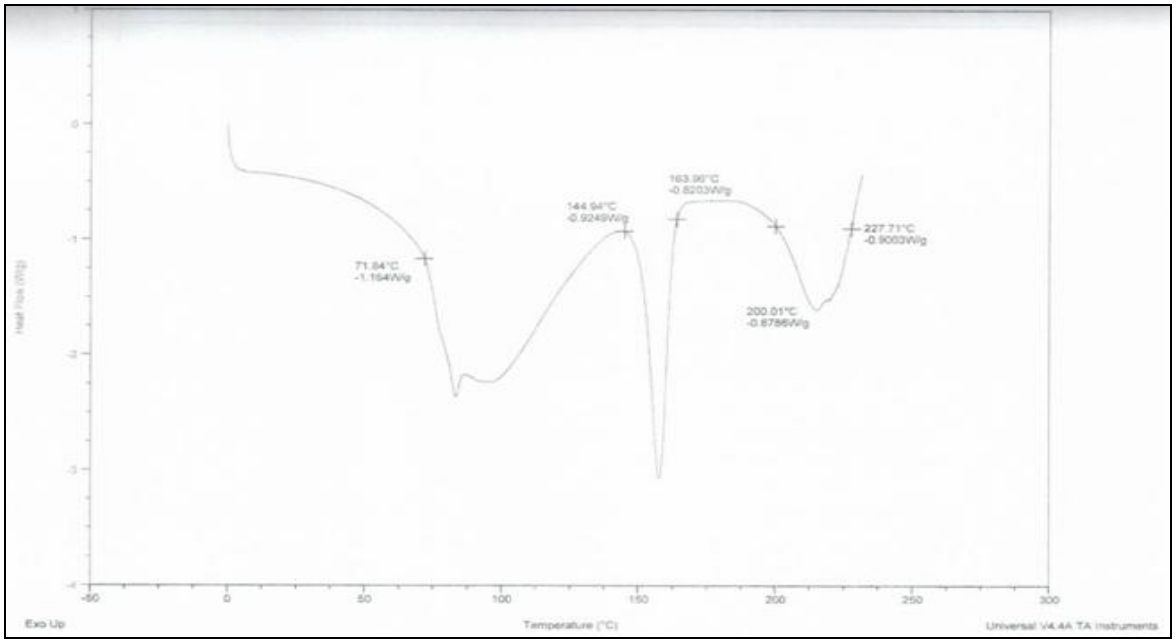

FIG. 4: DSC CURVE OF USNIC ACID

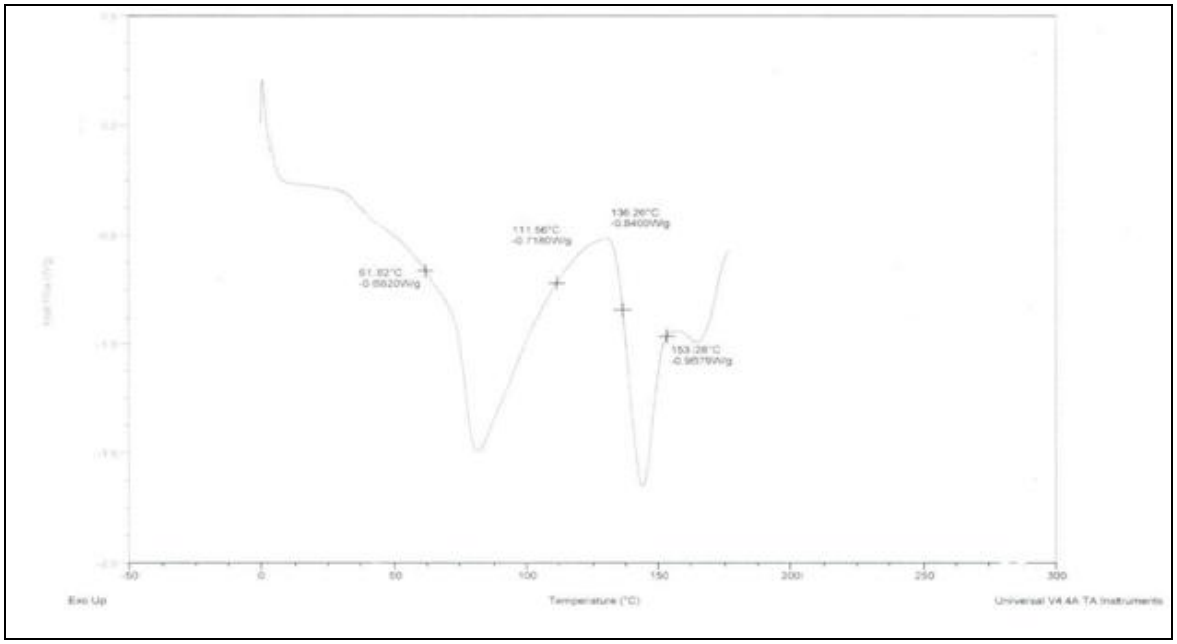

FIG. 5: DSC CURVE OF USNIC ACID LOADED NANOPARTICLES

Entrapment Efficiency: The entrapment efficiency of usnic acid with polymer PLGA in different ratio was determined and tabulated in **Table 2**. The entrapment efficiency of usnic acid loaded nanoparticles prepared by nanoprecipitation method with sonication was in the range of 74.95 to 95.27%. It showed that sonication technique increased the entrapment of drug with polymer by decreasing particle size and increasing the surface area between drug and polymer. The results obtained also showed that increased entrapment efficiency of drug with increased concentration of polymer.

TABLE 2: ENTRAPMENT EFFICIENCY OF DRUG WITH POLYMERS

S. no	Formulation code	Amount of drug in supernatant	Percentage of entrapment
1	G_1	13.39	66.50
2	G_2	11.15	72.12
3	G_3	7.67	80.82
4	G_1s	10.02	74.95
5	G_2s	5.25	86.87
6	G_3s	2.44	93.90

***In vitro* Drug Release Studies:** The *in vitro* release profile of all prepared nanoparticles showed typhical bimodal behaviour. In the present study nanoparticles prepared with lower polymer concentration were released faster than nanoparticles prepared with higher concentration of polymer. Drug - polymer ratio of 1:1 showed maximum cumulative drug release in 24 hours. Higuchi and korsemeyer Kinetic study equation confirms usnic acid nanoparticles release was zero order.

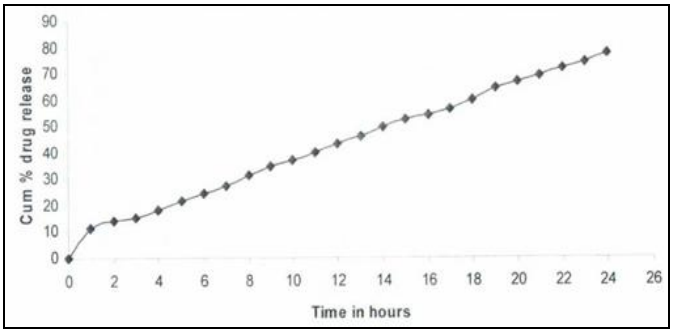

FIG. 6: *IN VITRO* RELEASE OF OPTIMIZED FORMULATION G_2S BY U.V. METHOD

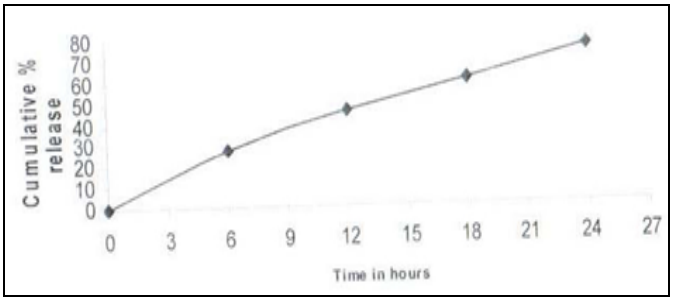

FIG. 7: *IN VITRO* RELEASE OF OPTIMIZED FORMULATION G_2S BY HPLC METHOD

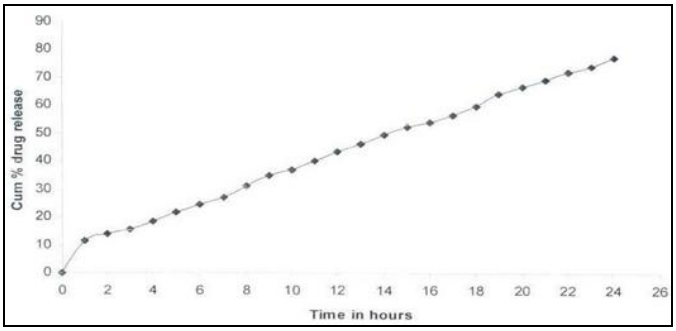

FIG. 8: ZERO ORDER PLOT OF OPTIMIZED FORMULATION G_2S

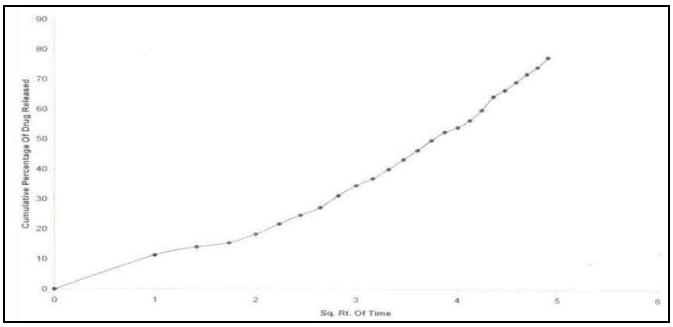

FIG. 9: HIGUCHI'S PLOT FOR OPTIMIZED FORMULATION

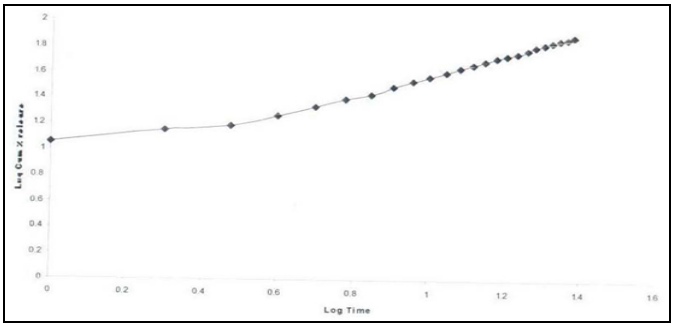

FIG. 10: KORSMEYER PLOT FOR OPTIMIZED FORMULATION

Effect of Usnic Acid and Usnic Acid Loaded Nanoparticles: The effect of usnic acid and usnic acid loaded nanoparticles on the survival of DAL tumour bearing mice is shown in **Table 4**. The percentage of ILS of the cancer control group was 44%, where as it was 90%, 67%, 78% for the group with 5FU 20mg/kg, usnic acid and usnic acid loaded nanoparticles respectively. The effect of usnic acid and usnic acid loaded nanoparticles on the average increase in body weight is shown in **Table 4**.

The average weight gain of tumour bearing mice was 8.16 ± 1.4gm, whereas it was 1.90 ± 0.09gm, 2.68 ± 1.32gm and 2.16 ± 1.11gm for the groups treated with 5FU, usnic acid, usnic acid loaded nanoparticles respectively. Usnic acid and usnic acid loaded nanoparticles were significantly reduced the packed cell volume and viable tumour cell count when compared to tumor control group (**Fig. 11, 12** and **13**).

TABLE 3: TREATMENT PROTOCOL CYT OTOXIC EVALUATION

Groups	Description	Dose	Treatments		
			Cancer Cells	Drug	Food / Water
1	Normal Control		----------		Access
2	Tumor Control		----------		Access
3	Positive Control	20mg/kg	Induced	5-Fluoro Uracil	Access
4	Treatment Control	5mg/kg	Induced	Usnic acid	Access
5	Treatment Control	5mg/kg	Induced	Nanoparticle	Access

TABLE 4: EFFECT OF USNIC ACID AND USNIC ACID LOADED NANOPARTICLE ON THE LIFE SPAN BODY WEIGHT AND CANCER CELL COUNT

Group	Treatment	No of Animals	% ILS	Increase in Body Weight (gms)	Cancer Cell Count Ml X 10^6
1	Normal control	6	≥30days	1.40±0.06	-
2	Cancer control	6	44%**[(a)]	8.16±1.4**[(a)]	1.92±0.26
3	Positive control	6	90%	1.90±0.09	0.70±0.12
4	Usnic acid	6	67%**[(b)]	2.68±1.32**[(b)]	1.12±0.30**[(b)]
5	Nanoparticle	6	78%**[(b)]	2.16±1.11**[(b)]	0.86±0.22**[(b)]

All values were expressed as Mean ± SEM

Values were find out by using ONE WAY ANOVA. Followed by Newman keul's multiple range test.

TABLE 5: EFFECT OF USNIC ACID AND USNIC ACID LOADED NANOPARTICLE ON SOLID TUMOR VOLUME

Group	Treatment	15^{th} day	20^{th} day	25^{th} day	30^{th} day
1	Tumor control	6.6±0.48	8.4±0.30	10.4±0.38	12.01±0.42
2	Usnic acid	5.42±0.42**[(a)]	5.64±0.38**[(a)]	7.22±0.26**[(a)]	7.30±0.34**[(a)]
3	Nanoparticle	5.06±0.39**[(a)]	5.18±0.46**[(a)]	5.13±0.36**[(a)]	5.01±0.40**[(a)]

Values are expressed as Mean ± SEM

Values were found out by using ONE WAY ANOVA. Followed by Newman keul's multiple range test.

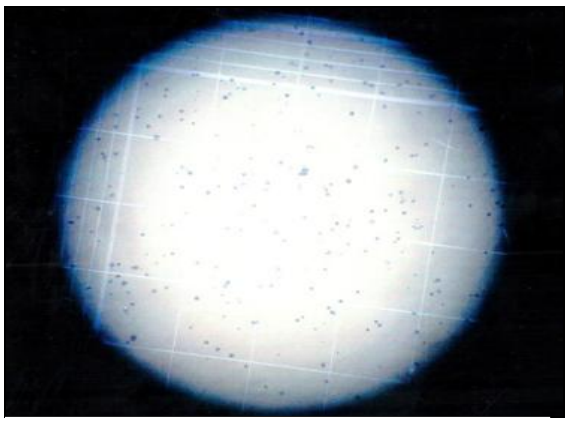

FIG. 11: VIABLE CANCER CELL IN DAL CELL LINE INOCULATED MICE

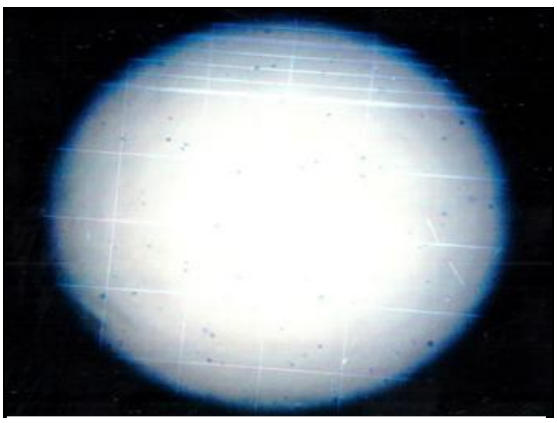

FIG. 12: VIABLE CANCER CELL IN USNIC ACID TREATED MICE

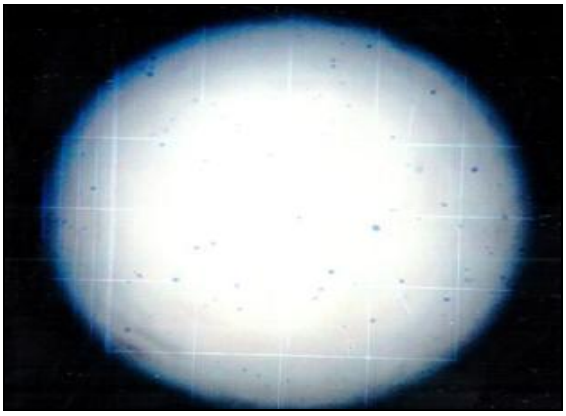

FIG. 13: VIABLE CANCER CELL IN NANO TREATED MICE

CONCLUSION: In this study usnic acid loaded nanoparticles were prepared by nanoprecipitation method with sonication technique with biodegradable polymer PLGA exhibited controlled drug release for 24 hours. Usnic acid loaded nanoparticles showed more antitumor activity and reduced the packed cell volume than unloaded free usnic acid. This study clearly showed that nanoparticulate system could be used to introduce usnic acid into chemotherapy and PLGA act as a better candidate to attain usnic aid in controlled manner at targeted site. Therefore developed usnic acid nanoparticles could be considered an effective anti-cancer drug delivery for cancer chemotherapy.

ACKNOWLEDGEMENT: We thank to K. M. College of Pharmacy to provide all the facilities for this research.

CONFLICT OF INTEREST: The authors have no conflict of interest to declare.

REFERENCES:

1. Cansaran D, Kahya D, Yurdakulola E, Identification and quantitation of usnic acid from the lichen Usnea species of Anatolia and antimicrobial activity, Z Naturforsch C 2006; 61(11-12): 773-6.
2. Behera BC, Verma N, Sonone A and Makhija U: Experimental studies on the growth and usnic acid production in ‘‘lichen’’ *Usnea ghattensis in vitro*, Microbiological Research 2006; 161: 232 -237.
3. Demet CD, Sümer A and Orhan A: Determination of Usnic Acid Content in Some Lichen Species Found in Anatolia, Journal of Applied Biological Sciences 2008; 2(3): 41-44.
4. Stark JB, Walter ED and Owens HS: Method of isolation of usnic acid from *Ramlina reticulates*. J Amer Chem Soc. 1950; 72: 1819.
5. Ribeiro-Costa RM, Alves AJ, Santos NP, Nascimento SC, Gonçalves EC, Silva NH, Honda NK and Santos-Magalhães NS: *In vitro* and *in vivo* Properties of Usnic Acid Encapsulated into Plga-Microspheres. J Micro-encapsul. 2004; 21(4): 371-84.
6. Kumar KCS and Muller K: Lichen metabolites. Antiproliferative and cytotoxic activity of Takai M, Uehara Y, Beister JA. Usnic acid derivatives as potential antineoplastic agents. J Med Chem. 1979; 22(11): 1380-1386.
7. Takai M, Uehara Y and Beister JA: Usnic acid derivatives as potential antineoplastic agents. J Med Chem. 1979; 22(11): 1380-1386.
8. Koparal AT, Tuylu BA and Turk H: *In vitro* cytotoxic activities of (+) usnic acid and (-) usnic acid on V 79, A 549 and human lymphocyte cells and the their non-genotoxicities on human lymphocytes. Natural Product Research 2006; 20(4): 1300-1307.
9. Kupchan SM and Kopperman HL: L- usnic acid: tumor inhibitor isolated from lichen. Experientia 1975; 31(6): 625.
10. Ingolfdosittir K: Review of usnic acid. Phytochemistry 2002; 61(7): 729-736.
11. Van Eerdenbrugh B, Vermant J, Martens JA, Froyen L, Van Humbeeck J, Van den Monter G and Augustijns P: Solubility increases associated with crystalline drug nanoparticles: Methodologies and significance. Mol. Pharmaceutics 2010; 7(5): 1858−1870.
12. Muller RH, Madar K and Gohla S: Solid lipid nanoparticles (SLN) for controlled drug delivery- a review of the state of art. Eur J Pharm Biopharm. 2000; 50(1): 161-177.
13. Sooyeon K, Singh RK and Wojciech C: Silica-based mesoporous nanoparticles for controlled drug delivery. J Tissue Eng. 2013; 4: 1–35.
14. Kedar, U, Phutane P, Shidhaye S and Kadam V: Advances in polymeric micelles for drug delivery and tumor targeting. Nanomed. Nanotechnol. Biol. Med. 2010, 6(6): 714−729.
15. Budhian A, Siegel SJ and Winey KI: Haloperidol - loaded PLGA nanoparticles: Systematic study of particle size and drug content. Int J Pharm. 2007; 336: 367-375.
16. Mainardes RM and Evangelista RC: PLGA nanoparticles containing praziquantel: effect of formulation variables on size distribution. Int J Pharm. 2005; 290: 137-144.
17. Perugini P, Simeoni S, Scalia S, Genta I, Modenta T, Copnti B *et al*.: Effect of nanopartticle encapsulation on the photo stability of the sun screen agent, 2-ethyl hexyl-p-methoxy cinnamate. Int. J. Pharm. 2002; 246: 37-45.
18. Hsu CH, Cui Z, Mumper RJ and Jay M: Preparation and characterization of novel coenzyme Q_{10} nanoparticles engineered from microemulsion precursors. AAPS Pharm Sci Tech. 2003; 4(3): 32.
19. Dillen K, Vandervoort J, Mooter GV, Verheyden L and Ludwig A: Factorial design, physicochemical characterization and activity of ciprofloxacin-PLGA nanoparticles. Int J Pharm. 2004; 275: 171-187.
20. Mangesh AM and Shanmukkappa: UV Spectro-photometric estimation of phenytoin sodium in pure and pharmaceutical formulation: Asian Journal of chemistry 2010; 22(3): 2447-2449.
21. Namdeo RJ, Ratan ST, Preeti VI and Sameer JN: Development and characterization of gelatin based Nanoparticles for targeted delivery of zidovudine, International Journal of Pharmaceutical Investigation 2013; 3(3): 26-30.
22. Reddy LH and Murthy RSR: Etoposide-loaded nanoparticles made from glyceride lipids: Formulation, characterization, *in vitro* drug release and stability evaluation. AAPS Pharm. Sci. Tech. 2005; 6(2): E158-E166.
23. Z KE, Zhu Z, Xu Z, Fang C and Hu S: Formulation design and *in vitro* evaluation of berberine- loaded self-nanoemulsifying drug delivery system, Tropical Journal of Pharmaceutical Research 2015; 14(5).
24. Wong HL, Rauth RM, Bendayan R and Yuwu X: *In vitro* evaluation of a new polymer-lipid hybrid nanoparticle (PLN) formulation of doxorubicin in a murine solid tumormodel. Eur J Pharm Biopharm. 2007; 65: 300-308.
25. Peppas LB and Blanchette JO: Nanoparticle and targeted systems for cancer therapy. Adv Drug Del Rev. 2004; 56: 1649-1659.
26. Kankanit P, Sineenart S, Wanichaya M and Wisanu P: Synthesis of CuO Nanoparticles by Precipitation Method Using Different Precursors, Energy Procedia 2013; 34: 740 -745.

27. Hamid RG, Ferdos PM, Hossein P and Behrad MR: Synthesis of ZnO nanoparticles by precipitation method, oriental journal of chemistry 2015; 31 (2): 1219-1221.
28. Sneha MM, Arul PF and Thiyagarajan D: *In situ* isolation and characterization of nano-usnic acid for medical applications. International Journal of Pharmacy and Pharmaceutical Sciences 2014; 6(6): 222-226.
29. Mu L and Feng SS: Vitamin E TPGS used as emulsifier in the solvent evaporation/extraction technique for fabrication of polymeric nanospheres for controlled release of paclitaxel. J. Control Release 2002; 80: 129-144.
30. Segundo EP, Quintanar AG, Perez VA and Guerrero DQ: Preparation and characterization of triclosan nanoparticles for periodontal treatment. Int. J. Pharm. 2005; 294:217-32.

How to cite this article:
Pandian MS, Karthikeyini SC and Nagarajan M: Fabrication, characterization and pharmacological activity of usnic acid loaded nanoparticles. Int J Pharm Sci Res 2017; 8(11): 4758-66.doi: 10.13040/IJPSR.0975-8232.8(11).4758-66.