RESEARCH PAPER

Evaluation of Encapsulation Efficiency, in vitro Crude Extract Release and Pharmacokinetic Studies of Vitis vinifera Loaded Polymeric Nanoparticles (VV-PNPs)

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ABSTRACT

Background: Liver-related diseases are a global concern due to factors such as alcohol consumption, environmental pollutants, viruses, and drug overdoses. Hepatotoxicity, caused by various chemicals and drugs, poses a significant threat to liver health, with around 900 drugs identified as potential hepatotoxins. Chronic viral hepatitis, particularly Hepatitis C, affects a substantial portion of the world population, leading to liver failure, cirrhosis, and hepatic cellular carcinoma.

Objective: The limitations of modern therapeutic approaches have sparked an interest in herbal drugs, particularly those derived from plants like Vitis vinifera (V. vinifera). V. vinifera, a major fruit crop, has a rich history of traditional use and is known for its nutritional and medicinal values. The plant is abundant in flavonoids, anthocyanins, proanthocyanins, and other bioactive compounds that exhibit antioxidant properties.

Nanotechnology Approach: In the emerging field of nanotechnology, the synthesis of nanoparticles from plants, including V. vinifera, has gained attention. Polymeric nanoparticles (PNPs) derived from biodegradable and biocompatible polymers show promise in drug delivery, medicine, and environmental applications. These PNPs offer advantages such as improved drug stability, increased circulation time, high encapsulation efficiency, and targeted drug distribution.

Keywords: Hepatotoxicity, Nanoparticles, Vitis Vinifera, Acetaminophen, Poly (lactic acid-glycolic acid)

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INTRODUCTION

Diseases related to liver have become a global concern. The liver can often be abused by many factors such as daily consumption of alcohol, pollutants, environmental toxins, viruses, etc. Even an overdose of medicinal agents or intake within the therapeutic limits can lead to liver damage (Thomson *et al* 2017). Other chemical agents used in industries and laboratories can also cause liver damage. The chemicals that can lead to liver injuries are known as hepatotoxins. Different types of lethal chemicals such as carbon tetrachloride (CCl4), antibiotics, thioacetamide and acetaminophen cause hepatic cell damage. Around 900 drugs have been identified causing liver injury, which is the prime cause for removal of such drugs form the market (Chen *et al* 2015)

Viral hepatitis is one of the most significant health-related issue in India. Towards the management and prevention of HIV infection, lawmakers of our country have given great importance in last few years but there was no attention towards viral hepatitis in our country, although it is an equally important health-related problem in India.

About 8% of the entire world population (5% HBV and 3% HCB) has been affected by chronic viral hepatitis. Chronic infection of each type cause to failure of liver, cirrhosis, and liver cancer (Ringehan et al 2017)., due to HCV, around 399 thousand deaths were reported across the globe, together with more than 5 million people suffering from it in Europe alone according to World Health Organization (WHO) (2016). Serious long-term health problems occur due to chronic HCV and according to reports, 30% of patients will be affected by progressive liver damage with cirrhosis (scarring of the liver and damage), which places them at risk for liver cancer and liver malignancy (Ringehan et al 2017; Pinter *et al* 2016)

The liver injuries caused due to drug exposure or other non-infectious agent is known as hepatotoxicity. Basic cellular components can react with agents which are hepatotoxic and can cause almost every type of liver disease. Both the *in vivo* and *in vitro* conditions chemicals such as carbon tetrachloride, thioacetamide, acetaminophen, and galactosamine are the main model substances causing liver injury (Sharma *et al* 2015; Eidi

et al 2012). At present liver disorders have become startling diseases in the world for which most effective medicinal therapeutics or complementary and alternative medicines (CAM) are needed which are either natural products or their derivatives.

To treat liver diseases, herbal drugs are being increasingly used. These herbal drugs are safe and don't cause any undesirable reactions of serious nature as well are natural and readily available. The therapeutic properties of modern medicines are limited and their therapeutic successes are disappointing (Arige *et al* 2017). In the earlier studies, researchers have examined the traditional use of plants to treat liver diseases, indigenous healers as well as herbalists to brace the functioning and treatment of diseases related to liver. Bioactive compounds such as phenols, terpenes, flavonoids, polyphenolic, reductase, alkaloids, etc. are found in plants, they act as reducing agents.

In the field of Nanotechnology, Nanoparticle synthesis from plants is extremely promising area as the plants themselves behave as a capping agent together with a reducing agent. Various plant parts are used for nanoparticle synthesis and are mostly used in applications such as agriculture, biomedical, delivery of drug, anticancer, destruction of tumor, antibacterial, and antifungal activities (Haleemkhan *et al* 2015). It is reported that plants provide an eco-friendly method for the nanoparticle synthesis. Plants contain various phytochemicals that have various health-related benefits such as antipyretic, antioxidant, analgesic, anti-proliferative, wound healing, cytotoxic, neuroprotective, induce apoptosis, etc (Haleemkhan *et al* 2015).

In the field of research, polymeric nanoparticles (PNPs) have received huge attention over the past few years. Polymers-derived nanoparticles typically exhibit unique properties due to their interconnected nature. The biodegradable and biocompatible polymers, ranging in size from 10 to 1000 nm, are utilized in the preparation of PNPs, where the drugs are dissolved, encapsulated, entrapped, or attached to a nanoparticle matrix. PNPs have been rapidly expanding and playing a vital role in wide range of areas such as photonics, conducting materials, medicine, electronics, biotechnology, sensors, control of pollution and environmental technology.

Polymeric nanoparticles (PNPs) offer several benefits, including enhanced stability of acid-sensitive drugs, high drug encapsulation efficiency, consistent distribution at the intended site, prolonged circulation time in the body, and responsiveness to stimuli (Han et al., 2018; Moritz et al., 2015). these nanoparticles can efficiently penetrate different biological barriers and offer enhanced thermodynamic stability to the system. (Moritz et al., 2015; Derman et al., 2018).

Vitis vinifera L. (V. vinifera), a widely cultivated fruit crop, originally hails from southern Europe and western Asia. Today, it is grown across the globe in a variety of climates.

In 1300 AD it was introduced in India by invaders from Afghanistan and Iran. *V. vinifera* have health benefits and therapeutic properties, so it is in use for thousands of years. It is abundant in proanthocyanins, flavonoids, anthocyanins, sugars, salts, mineral, vitamins, organic acids and tannins. *V. vinifera* is an excellent source of phenols especially the skin and in the seeds of the fruit, which acts as an antioxidant (Alawi *et al* 2018). Flavonoids that are present in *V. vinifera* have many health-promoting properties such as- it increases the level of intracellular Vitamin C, reduces capillary fragility as well as permeability, and looks for free radicals and oxidants. According to studies, black grape juice, skin and seeds contain a large amount of resveratrol which is called the strongest natural antioxidant. *Vitis vinifera* exhibits various medicinal benefits, including anti-sclerotic properties. Its leaves possess vaso-protective, diuretic, astringent, and venotonic effects, while its fruits offer tonic, vitamin-rich benefits, support hair growth, and help prevent ischemic conditions. Additionally, the seeds are known for reducing vascular permeability and providing hypolipidemic effects. V. vinifera also demonstrates anticancer, antioxidant, and antidiabetic properties (Bonepally *et al.,* 2013). In present developments, nanotechnology motivated advancements are seen in each and every area of life, therefore nanoparticles synthesis by using the route of biosynthetic is always a safer and best alternative and conservative method. Therefore, now a day developing more effective and less toxic hepatoprotective agents to prevent the process of hepatotoxicity is required.

Developing nanoparticles from Vitis vinifera is essential due to their significant potential in enhancing health and medical treatments. Vitis vinifera, commonly known as grape, is rich in antioxidants such as resveratrol, which possess anti-inflammatory, anti-cancer, and cardioprotective properties. By converting these compounds into nanoparticles, their bioavailability and stability are significantly increased, leading to more effective delivery and absorption in the body. This innovation can improve therapeutic outcomes in treating diseases like cancer and cardiovascular conditions. Furthermore, the use of Vitis vinifera nanoparticles can advance wound healing and skin care products by

harnessing their natural anti-inflammatory and regenerative properties. Overall, the development of these nanoparticles offers a promising, natural, and efficient approach to enhancing health and medical treatments. This study hypothesizes that nanoparticles synthesized from *Vitis vinifera* will enhance hepatoprotective effects by improving the bioavailability and stability of its bioactive compounds, such as resveratrol and flavonoids. These nanoparticles are expected to mitigate liver toxicity caused by hepatotoxins, drugs, and viral infections, providing a natural and efficient therapeutic alternative. By leveraging nanotechnology, the study aims to develop a safer and more effective hepatoprotective agent to prevent liver damage and improve overall liver health.

MATERIALS AND METHODS

A random selection of Wistar albino rats (with an average body weight of 180 ± 10g) was made from the animal facility in our department and each group contain 6 rats (n=6). The rats were housed in polypropylene cages under controlled conditions for 2 months to obtain appropriate weight, with a 10-hour dark cycle, a 14-hour light cycle, relative humidity maintained between 60-70%, and a temperature of 25 ± 2°C. The animals were provided with a commercially available dry pellet diet (sourced from Ashirwad Industries Ltd., Chandigarh, India) and water ad libitum. The experimental procedures received approval from the Institutional Animal Ethical Committee (IAEC) (CPCSEA/574/02/ab) at Banasthali Vidyapith, India, and adhered to the guidelines established by the Committee for Control and Supervision of Experiments on Animals (CPCSEA), Chennai, India.

Random selection of Wistar albino rats was employed in this study to minimize selection bias and ensure that the sample is representative of the broader population. This approach enhances the validity and reliability of the findings by reducing potential confounding variables associated with individual physiological or genetic differences. Furthermore, randomization improves reproducibility and allows for unbiased distribution of subjects across experimental groups, thereby strengthening the study's generalizability.

Chemicals and reagents

Carbon tetrachloride (CCl_4), Acetaminophen, Poly (lactic acid-glycolic acid) (PLGA), and Silymarin were acquired from Sigma-Aldrich (USA). For the research, all analytical grade chemicals were acquired and used from E-Merck Company (India), Hi-media, and Sigma-Aldrich, etc.

Preparation of fruit extract of *Vitis vinifera* in different solvents and its phytochemical screening

Preparation of aqueous extract of *Vitis vinifera* (*V. vinifera)* by cold maceration method Fresh, healthy, and ripe V. vinifera fruits were carefully washed with tap water followed by a rinse with 70% ethanol. They were then dried in an oven at 50°C. After drying, the material was ground into a fine powder and sifted through a mesh to ensure uniformity. A 10-gram portion of the powdered material was then mixed with 100 ml of water (aqueous), methanol, and ethanol in a 1:10 ratio. Furthermore, all three samples were set on a Rocker shaker (MAC, Cat: MSW-309, Model No.: 0116-094) for 72 hr. Then, Whatman paper no. 1 was used to filter extracts. Through filtration with Whatman no. 1 filter paper, residue was detached as well as the aqueous extracts were lyophilized furthermore stored in airtight containers at 4ºC and aqueous sample is used for the further study.

Evaluation of encapsulation efficiency, *in vitro* crude extract release and pharmacokinetic studies of *Vitis vinifera* loaded polymeric nanoparticles (VV-PNPs) (Figure 1)

Evaluation of encapsulation efficiency

The amount of unentrapped crude extract was determined by calculating the encapsulation efficiency (EE). The same was established upon evaluating the drug absorbance in the supernatant, which was achieved by centrifugation of the suspension of VV-PNPs, after that the following formula was applied:

$EE\ (\%) = 100(W1 - W2)/ W1$

Where, EE= % encapsulation efficiency, W_1= (crude extract) total, W_2= (crude extract) supernatant [Mura *et al* 2013, son *et al* 2017].

The obtained value through the above equation was compared with the crude extract in entrapped condition by spectrophotometric analysis at 287nm absorbance.

Evaluation of *in vitro* crude extracts release study

The in vitro release study was conducted using a diffusion cell system with a dialysis membrane. An inverted cylindrical test tube served as the donor compartment. The recipient section contains the phosphate buffer (pH 1.2, 37 °C) in a beaker placed over a water bath and phosphate buffer was changed after 2 hr. A dialysis membrane was placed at the lower end of the cylindrical setup and it estranged the donor section from the recipient section. Extract that could hold nanoparticles was suspended into 5 ml of pH 7.4 buffer furthermore positioned in the donor section. Using a stirrer, the system was stirred. Sample were detached from the receiver section furthermore substituted with new

medium. At 287 nm the samples were investigated spectrophotometrically as mentioned in the previous literatures (Rafiei and Haddadi, 2017).

Pharmacokinetic studies

The study was conducted in two groups with each group having six rats

Group 1: VV *per se* (300 mg/kg orally) Group 2: VV-PNPs (100 mg/kg orally) as mentioned in previous papers.

Blood samples were taken at different time points over a 24-hour period. Plasma drug concentrations were measured using HPLC, and a calibration curve for the drug in plasma was created. The UV detection was carried out at a wavelength of 368 nm, and the mobile phase consisted of an ammonium acetate aqueous solution and acrylonitrile. Pharmacokinetic parameters were determined using the Winnonlin software, including: parameters were determined:

- The elimination rate constant (ke)
- The area under the curve (AUC)
- Elimination half-life ($t_{1/2}$)
- Clearance (CL)
- The volume of distribution (V_d)

RESULTS

Evaluation of encapsulation efficiency, *in vitro* drug release and pharmacokinetic studies of *Vitis vinifera* loaded polymeric nanoparticles (VV-PNPs)

Evaluation of Encapsulation Efficiency

The encapsulation effectiveness as well as loading of drug were the two major indices for the assessment of encapsulated NPs. Raising the dosage was one of the major techniques to progress the encapsulation effectiveness (Kalimouttou *et al* 2009). As shown in Table 1, it was found 20.55% in the VV-PNPs with 25 mg, which indicates that the very low amount of drug trapped in the polymer, 74.12% was found in 50 mg which shows the increase in the entrapment of the crude extract and further 85.48% was found in 75 mg and then the best entrapment of drug was found in 100 mg *i.e.* 94.72%, there was a noteworthy discrepancy observed in the last two higher concentration of VV-PNPs.

Evaluation of *in vitro* drug release study

In vitro release pattern of PNPs of all four formulations were studied over a period of 120 hr. The *in vitro* drug release experiment was done with phosphate buffer saline (PBS) at pH 6.8 and pH 1.2 using a diffusion cell setup across a dialysis membrane. Table 2 and Figure 2, clearly indicates that lower doses of VV-PNPs (25 mg and 50 mg) were faster in the release, which clearly shows that the burst and the fast drug release takes place in the 25 mg concentration of VV-PNPs, as the amount of crude extract increases (50 mg) with the constant amount of polymer, again it was observed that the fast release of the drug started within few hr. In the 75 mg dose of VV-PNPs showed sustained release of crude extract up to 24 hr and the highest sustained release of crude extract was observed in the 100 mg concentration of VV-PNPs, therefore there is a noteworthy difference observed in the sustained drug liberation of VV-PNPs at 75 mg and 100 mg. From the above outcomes, it was established that the PNPs had the result of extending the release of drug evaluated with lower doses of VV-PNPs..

Pharmacokinetic studies

In this investigation, by using pharmacokinetic data analysis software, different parameters were determined such as Elimination rate constant (ke), Clearance (CL), Elimination half-life ($t_{1/2}$), Volume of distribution (V_d) as well as Area Under the Curve (AUC). The plasma concentration-time profile of V. vinifera was generated after oral administration of crude V. vinifera suspension and VV-PNPs (100 mg/kg), at a dose of 5 mg/kg, to rats. The study revealed a significant difference in the pharmacokinetic profiles between the crude extract of V. vinifera suspension and the VV-PNPs nano formulation (Table 2). Following oral administration of the crude extract suspension, the drug was rapidly detected in the plasma during the initial hours, likely due to the enhanced permeability coefficient of VV-PNPs in the superior gastrointestinal tract (GIT). However, the plasma drug concentration quickly reduced to negligible levels after 8 hours. In contrast, with VV-PNPs, the highest concentration of V. vinifera was observed at 12 hours post-administration. The polymer in the nano formulation prolonged the residence time of V. vinifera, leading to a gradual decrease in drug concentration over the next 12 hours. This indicated an extended presence of drug in the colon, with slow release into the systemic circulation due to lower permeability and reduced surface area. The pharmacokinetic profile in rats following oral administration of crude *V. vinifera* and VV-PNPs are revealed in Table 3, VV-PNPs caused an intense alteration in the pharmacokinetics of the remedy. The half-life ($t_{1/2}$) and volume of distribution (V_d) of *V. vinifera* were increased when it was in the multifaceted form with nanoparticles in addition to ultimately the clearance of the molecule in complex form was also reduced. A reduced clearance (CL) is anticipated if the circulating drug is adequately limited to the blood compartment as a

consequence of being limited within circulating micelles. The area under the curve (AUC0–24) after oral administration of VV-PNPs was 22.12 µg.h/ml, while 5.24 µg.h/ml for AUC0–24 of crude *V. vinifera*.

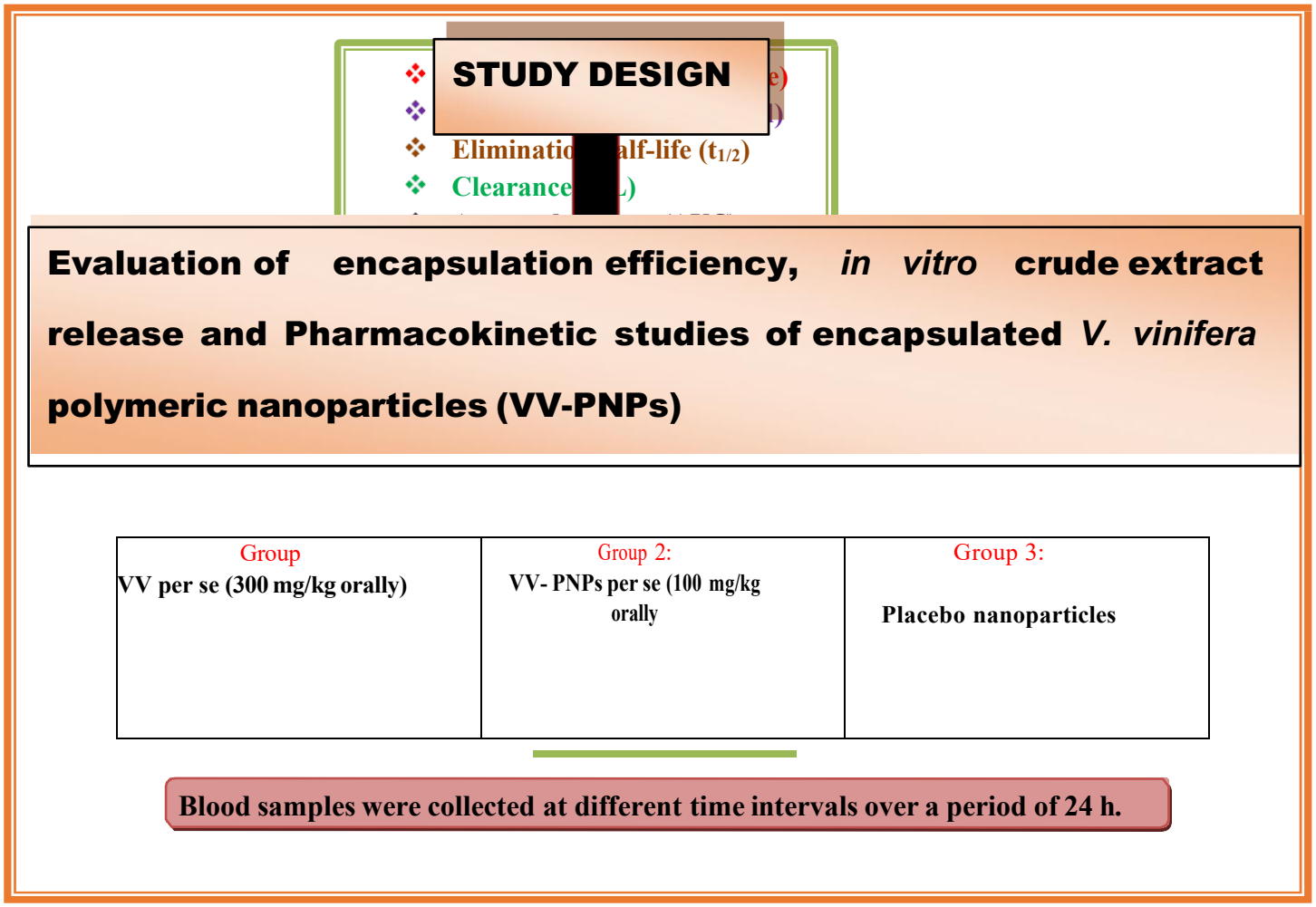

Figure 1: Study Design

Table 1: Entrapment efficiency of encapsulated *V. vinifera* in polymeric nanoparticles.

S.N.	Concentration of *V. vinifera* crude extract (mg)	PLGA concentration (mg)	Amount of entrapped drug (%)
1.	*V. vinifera* (control)	100	96%
2.	*V. vinifera* (25)	100	20.55 %
3.	*V. vinifera* (50)	100	74.12 %
4.	*V. vinifera* (75)	100	85.48 %
5.	*V. vinifera* (100)	100	94.72 %

Abbreviations: *V. vinifera* = *Vitis vinifera*; PLGA= Poly (lactide co-glycolides) acid; %= percentage.

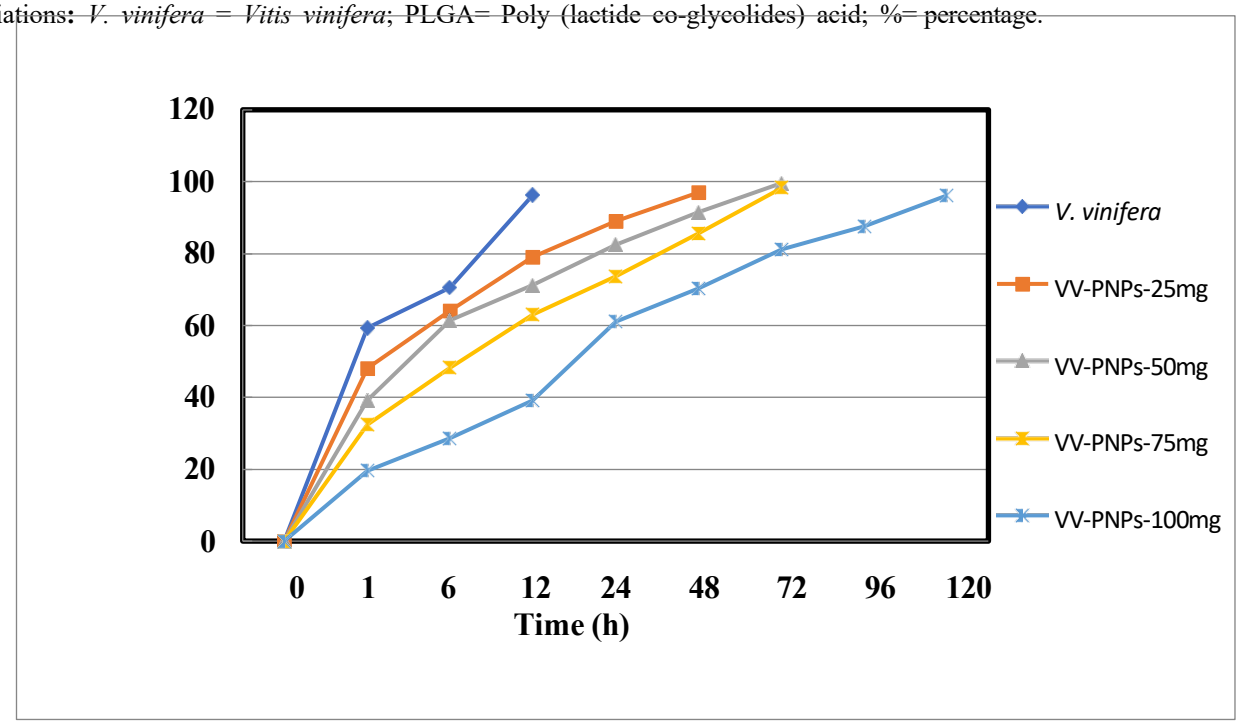

Figure 2: *In vitro* drug release of different formulations of VV-PNPs

Table: 2: *In vitro* drug release of different formulations of VV-PNPs

Time (h)	***V. vinifera* ± SD**	**VV-PNPs-1 (%) ± SD**	**VV-PNPs-2 (%) ± SD**	**VV -PNPs-3 (%) ± SD**	**VV-PNPs-4 (%) ± SD**
0	-	-	-	-	-
1	59.3 ± 7.72	48.2 ± 6.26	39.2 ± 5.09	32.4 ± 4.35	19.6 ± 2.67
6	70.5± 9.27	64.3 ± 8.49	61.4 ± 8.12	48.3 ± 6.18	28.7 ± 4.0
12	96.3 ± 12.11	79.4 ± 10.06	71.3 ± 9.45	63.1 ± 8.05	39.2 ± 5.20
24	-	89.7 ± 11.33	82.5 ± 10.56	73.7 ± 9.29	61.0 ± 7.45
48	-	97. ± 12.21	91.6 ± 11.244	85.6 ± 10.77	70.4 ± 8.92
72	-	-	99.6 ± 12.31	98.2 ± 12.26	81.1 ± 10.39
96	-	-	-	-	87.6 ± 11.07
120	-	-	-	-	96.2 ± 12.12

Abbreviations: VV-PNPs-1 = *V. vinifera* loaded in polymeric nanoparticles (25mg); VV-PNPs-2 = *V. vinifera* loaded in polymeric nanoparticles (50mg); VV-PNPs-3 *V. vinifera* loaded in polymeric nanoparticles (75mg); VV-PNPs-4 = *V. vinifera* loaded in polymeric nanoparticles (100mg); SD= standard deviation

Table 3: Pharmacokinetic studies *of V. vinifera* and VV-PNPs.

Parameter (0-24hr.)	***V. vinifera***	**VV-PNPs**
AUC(µg.h/ml)	5.24 ± 0.29	22.12 ± 1.22
t ½ (H)	2.1 ± 0.11	5.23 ± 0.29
Vd (ml/gm)	0.88 ± 0.04	2.98 ± 0.16

CL (ml/min)	0.34 ± 0.18	0.16 ± 0.008
$K_e(h^{-1})$	0.51 ± 0.28	0.38 ± 0.021

Abbreviations: **AUC=** area under curve, **t ½ =** Elimination half-life, **Vd=** volume distribution**, CL=** Clearance, **Ke=** elimination rate constant, **VV=** *V. vinifera*, **VV-PNPs =** polymeric nanoparticles.

DISCUSSION

The encapsulation efficiency and drug loading capacity were the primary parameters used to assess the performance of VV-PNPs. Increasing the drug concentration was an effective strategy to enhance encapsulation efficiency (Kalimouttou et al., 2009). As shown in Table 1, the encapsulation efficiency was 20.55% for 25 mg of crude extract, indicating minimal drug entrapment within the polymeric matrix. At 50 mg, encapsulation efficiency increased to 74.12%, suggesting improved drug entrapment. Further increases to 75 mg and 100 mg resulted in encapsulation efficiencies of 85.48% and 94.72%, respectively, demonstrating a statistically significant improvement in drug entrapment at higher concentrations. However, the difference between 75 mg and 100 mg was comparatively smaller, suggesting a potential saturation point in encapsulation efficiency.

The observed increase in encapsulation efficiency with higher crude extract concentrations is attributed to a decrease in drug loss during formulation, possibly due to an extended diffusional pathway in the aqueous phase (Pandya et al., 2016). This trend is consistent with findings from prior studies (Nagarajan et al., 2015; Son et al., 2017).

The in vitro drug release profile of all formulations was studied over 120 hours. As illustrated in Table 2 and Figure 1, the 25 mg and 50 mg formulations exhibited rapid release, characterized by an initial burst phase. In the 25 mg formulation, a substantial portion of the drug was released within the first few hours, whereas the 50 mg formulation also demonstrated rapid drug release. The burst phase is attributed to surface-adsorbed drug molecules.

In contrast, the 75 mg formulation exhibited a more controlled release, with sustained drug liberation over 24 hours. The most prolonged sustained release was observed with the 100 mg formulation, indicating a statistically significant difference between 75 mg and 100 mg formulations. The controlled release observed in higher concentrations is likely due to the formation of a bilayer structure within the nanoparticles, which retards drug diffusion. Additionally, the increased particle size at higher concentrations reduces the available surface area for immediate drug release, thereby contributing to prolonged drug liberation (Mura et al., 2015; Sharma et al., 2020). Similar findings have been reported in previous studies (Bohrey et al., 2016; Rafiei and Haddadi, 2017).

Bioavailability, a crucial pharmacokinetic parameter, was assessed by analyzing the concentration of crude extract in plasma following oral administration of VV-PNPs. Pharmacokinetic analysis revealed significant differences between the crude V. vinifera suspension and the VV-PNPs formulation. The crude extract suspension exhibited a rapid increase in plasma concentration, peaking within the first few hours, followed by a steep decline with negligible drug presence after 8 hours. In contrast, the VV-PNPs formulation showed a sustained release, with peak plasma concentration occurring at 12 hours, followed by a gradual decline over the next 12 hours. This suggests enhanced drug retention in systemic circulation, likely due to improved permeability and extended residence time in the gastrointestinal tract (Manikandan and Kannan, 2017).

Pharmacokinetic parameters further confirmed these findings. As presented in Table 3, the elimination half-life (t1/2) and volume of distribution (Vd) of V. vinifera were significantly

higher in the nanoparticle formulation compared to the crude extract suspension. Specifically, the AUC0–24 for VV-PNPs was 22.12 ± 1.22 µg.h/ml, markedly higher than 5.24 ± 0.29 µg.h/ml for the crude extract. This fourfold increase in bioavailability demonstrates the superior pharmacokinetic profile of the nanoparticle formulation. Additionally, the reduced clearance (CL) in VV-PNPs suggests improved drug retention in the

bloodstream, possibly due to nanoparticle-mediated drug stabilization within circulating micelles.

Overall, these findings indicate that VV-PNPs significantly enhance encapsulation efficiency, prolong drug release, and improve pharmacokinetic parameters compared to the crude extract suspension. This suggests the potential of VV-PNPs as an effective oral drug delivery system for V. vinifera.

CONFLICT OF INTEREST

The authors confirm that there are no conflicts of interest.

Conclusion

The elevated plasma concentration of *V. vinifera* for VV-PNPs may be an effect of nanoparticles size that remains the formulation in the circulation for an extensive period of time. These results might be because of the accumulation of NPs as well as the sustained release of the drug. Furthermore, properties of the particles (such as charge, size, shape along with hydrophilicity) can extend the withholding of them in the blood compartment. Thus, this study indicates that administration of VV-PNPs leads an extended plasma half-life as well as improved distribution to liver tissue in comparison to the crude extract of *V. vinifera* alone (Manikandan and Kannan 2017; Sun *et al* 2017) also documented similar findings.

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