



ORIGINAL ARTICLE

Biodistribution and toxicity evaluation of sesbania mosaic virus nanoparticles in mice

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Abstract Sesbania mosaic virus (SeMV), a 30-nm spherical plant sobemovirus, is suitable for developing functionalized nanoparticles for biomedical applications. However, the *in vivo* behavior of SeMV and the clinical impact following its delivery via the oral or intravenous route are not known. To address this question, we examined the biodistribution, toxicity and histopathological changes in SeMV treated mice. No toxic effects were observed in mice administered high doses (100 mg and 200 mg per kg body weight orally or 40 mg and 80 mg per kg body weight intravenously) of SeMV, and they were found to be normal. Analysis of fecal sample showed that SeMV was cleared in 16 h when 20 mg of the virus per kg body weight was administered orally. RT-PCR analysis of blood samples showed that SeMV was present up to 72 h in mice inoculated either intravenously (8 mg/kg body weight) or orally (20 mg/kg body weight). Further, SeMV was found to be localized up to 72 h in spleen and liver tissues of intravenously inoculated mice only. Biochemical and hematological parameters were found to be normal at 6 and 72 h after administration of SeMV. Furthermore, no noticeable changes were observed in histological sections

of brain, liver, spleen, lungs and kidney tissue samples collected at 6 and 72 h from SeMV administered mice when compared to control mice. Thus, SeMV appears to be a safe and non-toxic platform that can be tailored as a nanocarrier for *in vivo* biomedical applications.

Introduction

Nanoparticles (NPs) inherently have varying biological behaviors dependent on their size, shape, composition, surface chemistry, and associated physical properties. These attributes can greatly influence deposition, clearance from the body, and the toxicological nature of the nanoparticle [27]. Recently, many efforts have been made to develop targeted NPs using nanospheres, quantum dots, dextrans, liposomes, antibodies, dendrimers and viral nanoparticles for a variety of biomedical applications [35]. Although these NPs are useful for various biomedical applications due to their unique physicochemical properties, many of them are not biocompatible or show slow tissue clearance and toxicity [8, 30]. The establishment of *in vivo* nontoxicity of NPs has gained importance due to the growing concern raised about their biosafety [18, 41]. Further, a detailed understanding of the body's response to the NPs will allow modification and optimization of their biodistribution and clearance [6].

Viruses are excellent examples of naturally occurring nanoparticles that can serve as ideal platforms for the development of virus-based nanomaterials for biomedical applications [43, 44]. Among the several virus nanoparticles (VNPs) that are being studied for such applications, plant-virus-based nanomaterials have exhibited excellent performance as nanocarriers in targeted drug delivery,

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bioimaging and biosensing [24, 43, 47] due to their several attractive features, such as size range (nanometers), high degree of symmetry, polyvalency, monodispersity in shape and size, and efficient and inexpensive production in plants, as well as their noninfectious, biocompatible and biodegradable nature in animals and humans. These VNPs can be designed and engineered using both chemical and genetic tools. Examples of plant VNPs developed for potential applications in nanobiotechnology include, cowpea mosaic virus (CPMV) [19], cowpea chlorotic mottle virus (CCMV) [2], brome mosaic virus (BMV) [14], tobacco mosaic virus (TMV) [7] and potato virus X (PVX) [37]. The fate of some of these plant VNPs was studied *in vivo* [11, 28, 33, 35], and much attention is still focused on the design of new nanoplatforms to promote their pharmacokinetic properties.

Sesbania mosaic virus (SeMV), which infects *Sesbania grandiflora*, is a single stranded RNA virus made up of 180 copies of a 29-kDa coat protein (CP) that forms a 30 nm-diameter icosahedral particle [32]. Large quantities of SeMV can be easily generated and purified from infected *Sesbania* leaves [21]. The crystal structure of SeMV has been determined at 3 Å resolution [5]. Over expression of the SeMV CP in *E. coli* generates T = 3 virus-like particles (VLPs) resembling native virus particles [22]. Both wild-type SeMV and *E. coli* expressed VLPs are monodisperse in nature upon purification and are stable over a wide range of pH, temperature and buffer conditions [22, 31]. These extraordinary properties allow SeMV to be an attractive candidate for nanobiotechnology applications.

Plant VNP utilization in the field of nanobiotechnology is in its nascent stage in India, and technologies and protocols have not been developed for any of the indigenous VNPs though there is a strong need for it. It was recently demonstrated that chimeric SeMV VLPs expressing the B domain of *Staphylococcus aureus* protein A can deliver antibodies inside mammalian cells and that the delivered antibodies are functional [1]. To use such VNPs/VLPs for potential biomedical applications, the *in vivo* behavior of VNPs needs to be investigated. In the present paper, we have established the non-toxic and non-pathogenic behavior of SeMV by studying its biodistribution, toxicity and histology in mice. This is the first report on *in vivo* behavior of a plant VNP from India.

Materials and methods

Procurement and maintenance of experimental animals

Adult female Swiss albino mice, 8–10 weeks of age with an average body weight of 25 grams, were obtained from

certified rodent vendor (M/s Raghavendra Enterprises, Bengaluru, India). The animals were housed (four mice per cage) in sterile polypropylene cages and were maintained under standard laboratory conditions (temperature 24 ± 2 °C, light and darkness 12:12 h). Mice were allowed free access to a standard pellet chow (Purchased from Sai Durga Agencies, Bengaluru, India) and water *ad libitum*. All experiments were performed in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, 2003). The experimental protocols were reviewed and approved by Sri Venkateswara University's Institutional Animal Ethical Committee (IAEC), Tirupati, India. (Resolution No. 58/2012-13/(i)/a/CPCSEA/IAEC/SVU/GPV-MH dated 08.07.2012).

SeMV production and purification

SeMV was purified from SeMV infected *Sesbania* leaves harvested 20–30 days post-inoculation by following a modified protocol described by Lokesh *et al.* [21]. The purity of the SeMV sample was determined by size-exclusion FPLC using a Sepharose 6 column (AKTA Purifier, GE Healthcare). The virus concentration was measured using a UV spectrophotometer (NanoVue plus, GE Healthcare).

Toxicity studies

SeMV maximum tolerance study

Each study group consisted of four mice and was labelled accordingly. Phosphate buffered saline (PBS) was used as a control. SeMV in PBS was administered in a total volume of 100 µL and 200 µL total volume intravenously and orally, respectively. Different doses of purified SeMV were prepared corresponding to 100 mg and 200 mg per kg body weight for oral administration and 40 and 80 mg per kg body weight per mouse for intravenous administration.

Monitoring of clinical signs

Each mouse was examined for some important clinical signs, such as lack of movement, ruffled fur, hunched posture, ataxia, rapid movement around the cage, dehydration, hypothermia and seizure. At first, mice were monitored continuously for 30 min and then up to 10 h at one-hour intervals, followed by 24 h intervals for a period of one week. A 0 to 3 scale was used for grading and indication of clinical signs: 0—no clinical signs; 1—mild; 2—serious; 3—extreme. A separate experiment was carried out to monitor the body weights of mice receiving similar SeMV dosages. Body weights were measured every week for a period of six weeks.

Distribution and clearance of SeMV

Mice were sorted into three groups ($n = 4$); control (C), oral (O) and intravenous (IV). SeMV was administered orally (20 mg/kg body weight) and intravenously (8 mg/kg body weight) in all subsequent experiments. Fecal samples were collected from each group up to 24 h at 2 h time intervals and suspended in PBS. Western blot analysis (TE 70 Semi-Dry transfer unit, GE Healthcare) was performed [16] for detecting the virus using SeMV CP specific anti-serum, which was raised in our laboratory by injecting recombinant SeMV CP into rabbit. ELISA was performed with fecal and serum samples to determine the amount of SeMV being cleared at various time points.

Blood was collected from the tail vein of mice at 0, 1, 6, 12, 24, 48, 72, 96, 120, 144 h post administration (hpa). Mice were euthanized at 1, 6, 12, 24, 48, 72 and 96 hpa from separate groups. The cadavers were immediately transferred onto ice packs to collect vital organs, including brain, kidneys, liver, lungs and spleen. RNA was isolated from 50 mg of fresh tissue and blood samples using TRIzol Reagent (Life Technologies, USA) as per manufacturer's instructions. RT-PCR (Veriti Thermal Cycler, Applied Biosystems) was performed using MMuLV reverse transcriptase (Fermentas, USA) and gene specific primers (SeMV CPF-5' GTTCTGCTGTGCAGCAG 3' and SeMV CPR-5' AGGTGCAGTAAATGCGTC 3') designed in the internal region of SeMV-CP, resulting in product size of 654 bp.

Hematology and histopathology

Blood samples were collected by cardiotocesis at 6 and 72 hpa for examination of blood cells, and the carcasses were quickly placed on wet ice and transferred to the laboratory for post-mortem examinations. Brain, kidneys, liver, lungs and spleen were fixed in 10 % buffered formalin for histology examinations. The tissues were trimmed and embedded in paraffin, and sections were made using a Leica microtome (Leica Biosystems, USA) and mounted on glass slides. Hematoxylin and eosin staining was performed for the mounted sections, which were examined by light microscopy.

Serum was separated from blood collected at 6 and 72 hpa from virus-inoculated and control mice. Serum creatinine was quantitated by the kinetic method [40]. Serum glutamic oxaloacetic transaminase (SGOT) or aspartate transaminase (AAT) and serum glutamic pyruvic transaminase (SGPT) or alanine aminotransferase (AlAT) levels were estimated on a Star 21 Plus Semiautomatic Biochemistry Analyzer by picric acid assay [39]. Lipid peroxidase (LPx), glucose-6-phosphate dehydrogenase (G6PD) levels were estimated from the tissue homogenates

and cytosolic fractions of brain, kidneys, liver, lungs and spleen by the TBARS (thiobarbituric acid reactive substances) method [25] and the Bergmeyer and Bernt method [4], respectively. The data were statistically analyzed for variation.

Splenocyte proliferation assay

Mice were administered 100 μ g (4 mg/kg) of SeMV intravenously. Mice were euthanized at 7 and 14 days, the spleen was isolated aseptically, and splenocytes were isolated by flushing the spleen with IMDM medium using a 22-gauge needle and a glass syringe. The splenocyte suspension was treated with RBC lysis buffer to remove the RBCs, and the cell count was estimated using a Neubauer chamber. Cells (0.2×10^6 cells per well) were seeded in a 96-well culture dish. The cells were treated with PBS, concanavalin A at 0.1, 1, and 2.5 μ g (corresponding to 0.9, 9.6 and 24 picomoles) per well as a positive control, and SeMV at 1, 5, 50 and 125 μ g (corresponding to 0.185, 0.9, 9.6 and 24 picomoles), respectively. The treated cells were incubated in a CO₂ chamber for four days, and MTT assay was performed to check for the proliferation of splenocytes [23].

DAC-ELISA

Direct antigen-coating ELISA was performed by following a modified protocol described by Hobbs et al. [13]. The primary antibody was used in dilutions from 10 fold to 2000 fold. Serum from PBS administered mice was used as a control. In the antigen control (Ag control), no antigen was coated onto ELISA strips, and in the antibody control (Ab control) no primary antibody was added to the ELISA strips.

Results

Toxicity

Careful monitoring of mice treated orally and intravenously with 100 and 200 mg and 40 and 80 mg of SeMV per kg body weight, respectively, at different time points showed no clinical signs different from those of PBS injected mice at each of the doses investigated (supplementary data). Viruses may bind to surface of red blood cells, leading to agglutination of RBCs or haemagglutination [17]. Haemagglutination was not observed even after high doses (100 and 200 mg/kg body weight) of SeMV were administered to mice. Significant differences in body weight were not observed in any of the virus treated animals when compared to the control animals (supplementary data).

Clearance and distribution of SeMV

Fecal samples were collected from each SeMV administered (20 mg/kg body weight) and control group up to 24 h at 2 h time intervals and subjected to SDS-PAGE, followed by western blot analysis using anti-SeMV CP antibodies. SeMV CP was observed in fecal matter from 6 to 14 hpa in orally administered mice. CP was found to be cleared in 16 h and was not observed at subsequent time points (Fig. 1). No signal was observed in fecal samples from intravenously administered mice (data not shown). To verify these results, the amount of SeMV CP present in fecal samples was also estimated by ELISA. Maximum clearance occurred at 10 h (2.4 μ g), and by 16 h, a negligible amount of CP (2 ng) was detected. These results demonstrate that SeMV is cleared in 16 h in orally administered mice. RT-PCR analysis performed for monitoring the circulation time of SeMV in blood revealed the presence of SeMV RNA in both orally and intravenously administered mice from 1 hpa to 72 hpa (Fig. 2A). Liver and spleen were found to be the sites of SeMV localization only in intravenously administered mice, where SeMV was found from 6 hpa to 72 hpa (Fig. 2B).

Hematology, histopathology and immune response

Hematology reports showed that the mice in the intravenous group had mild leukopenia with a slight reduction in white blood cell count at 72 hpa (control: 5.045 ± 0.187 ; oral: 4.953 ± 0.145 ; IV: $4.817 \pm 0.114 \times 10^3$ cells/mm 3). Histological examination of vital organ tissues including brain, kidneys, liver, lungs and spleen revealed

no pathological changes and no signs of toxicity (tissue degeneration or necrosis) in the tested vital organ tissue sections of SeMV-inoculated and control animals (Fig. 3). Serum creatinine, SGOT and SGPT levels were normal in major organs of SeMV-inoculated animals when compared to control animals, and no significant difference was observed in any of the tested doses, either orally or intravenously (Table 1). Lipid peroxidase (LPx) and G6PD levels were assayed in the cytosolic fraction of mice tissues, and no significant difference was observed in their levels in SeMV administered mice when compared to the control group (Tables 2 and 3). These results demonstrate that the administration of SeMV is non-toxic and does not cause any changes in the vital organs.

In the splenocyte proliferation assay, 0.2×10^6 cells/well were treated with PBS (buffer control) or concanavalin A at 0.1, 1, and 2.5 μ g (corresponding to 0.9, 9.6 and 24 picomoles) per well as a positive control and SeMV at 1, 5, 50 and 125 μ g (corresponding to 0.185, 0.9, 9.6 and 24 picomoles), respectively, for 96 hours and treated with MTT for 4 h. The absorbance was measured at 570 nm. As shown in Fig. 4A, in cells treated with buffer alone, there was no proliferation of the splenocytes from control, 7-day- and 14-day-treated mice as expected. Further, there was no significant increase in the proliferation of splenocytes treated with SeMV compared to the cells treated with concanavalin A at equimolar concentrations (Fig. 4A), suggesting that the administration of SeMV does not result in a significant immune response. However, DAC-ELISA results showed that SeMV antibodies can be detected at a low dilution of the serum (1:10), suggesting that there is a humoral response upon administration of single dose of SeMV (Fig. 4B).

Fig. 1 Western blot analysis of fecal samples collected from orally administered mice showing the presence of SeMV CP from 6 to 16 hpa. Degradation of SeMV CP was observed at 14 and 16 hpa. Experiments were carried out twice with duplicates for each sample. Numbers on lanes represent the time points of sample collection

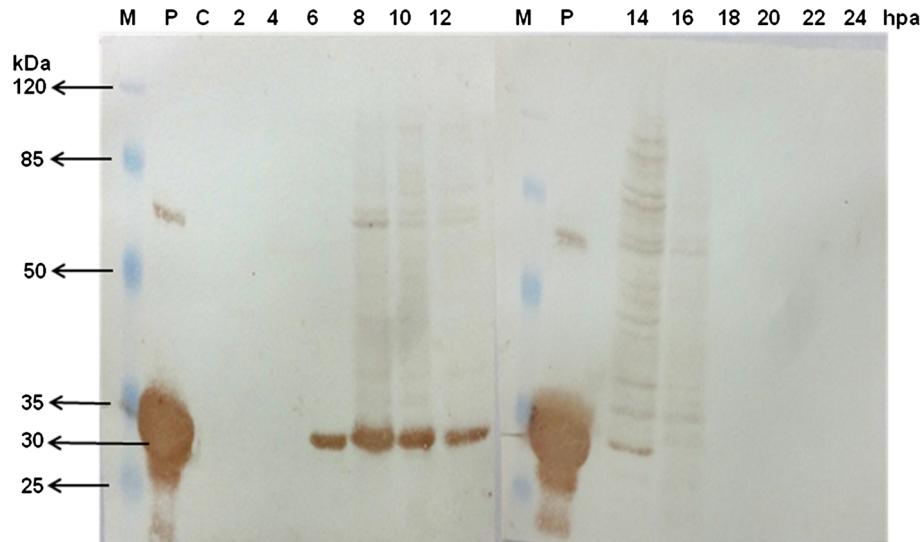


Fig. 2 (A) RT-PCR analysis of the total RNA collected from whole blood from orally and intravenously administered mice at different time points showing the presence of virus from 6 to 72 hpa. (B) RT-PCR analysis of the total RNA isolated from liver and spleen collected from orally and intravenously administered mice at different time points, showing the presence of virus from 6 to 72 hpa. Experiments were carried out twice with duplicates for each sample. Numbers on lanes represent the time points of sample collection

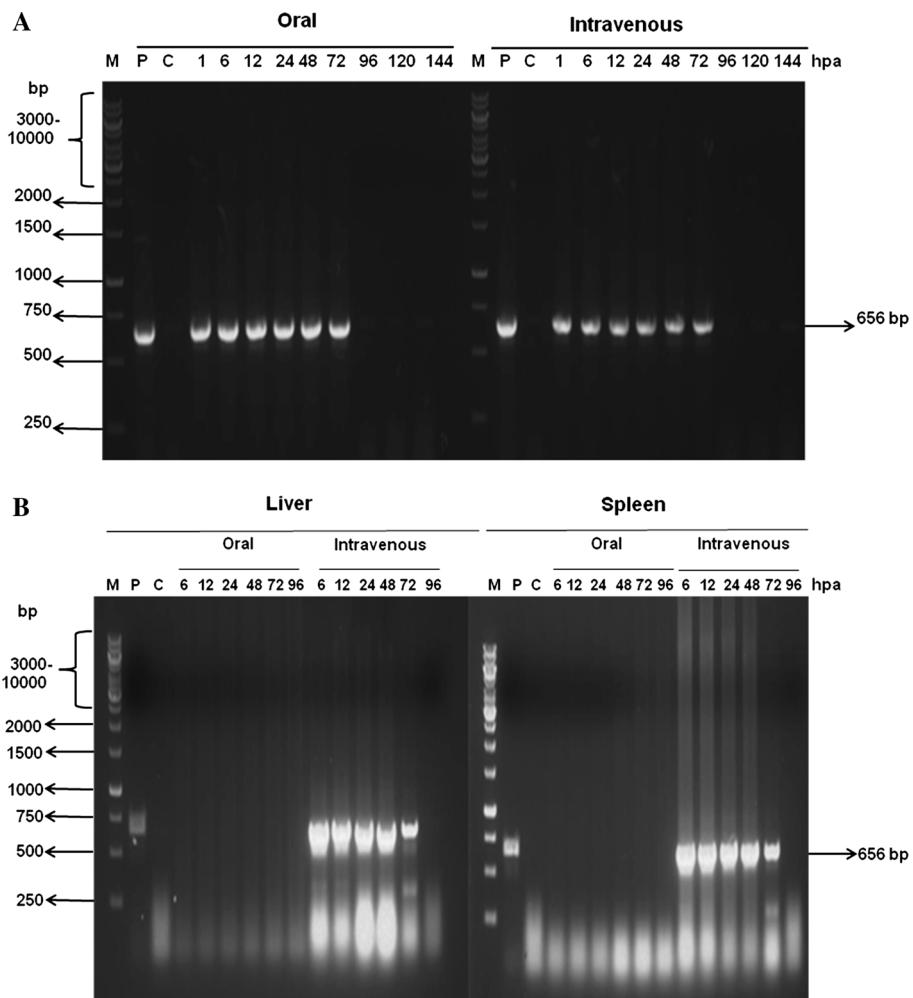


Fig. 3 Histology of vital organs performed at 72 hpa. The sections were stained with hematoxylin and eosin. There were no signs of tissue necrosis or degradation at 72 hpa (400x magnification)

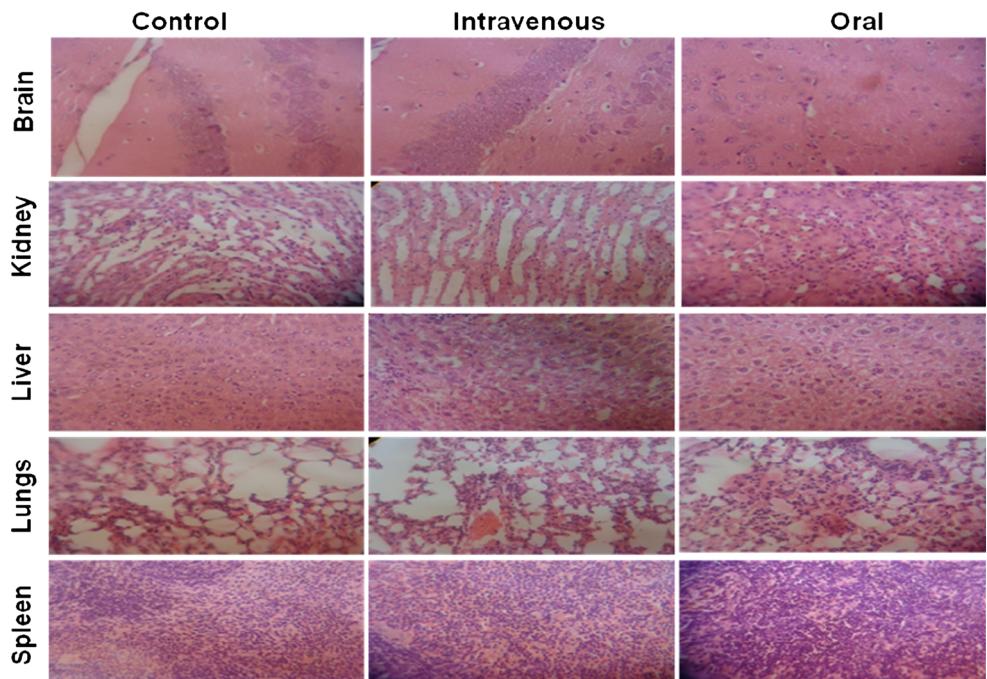


Table 1 Changes in the levels of serum creatinine (mg/dL), SGOT and SGPT (IU/L) in the serum of mice administered with SeMV both orally and intravenously at 6 and 72 hpa

Parameter	Control	Oral 6 h	Oral 72 h	IV 6 h	IV 72 h
Serum creatinine	0.54 ^a ± 0.03	0.58 ^a ± 0.03 (7.4)	0.59 ^a ± 0.02 (9.25)	0.55 ^a ± 0.03 (1.85)	0.55 ^a ± 0.02 (1.85)
SGOT	100.39 ^a ± 5.53	93.95 ^a ± 4.96 (-6.41)	92.49 ^a ± 6.06 (-7.86)	94.85 ^a ± 5.18 (-5.51)	93.51 ^a ± 5.42 (-6.85)
SGPT	120.76 ^a ± 6.92	116.23 ^a ± 7.69 (-3.75)	115.42 ^a ± 10.75 (-4.42)	114.39 ^a ± 8.22 (-5.27)	115.06 ^a ± 7.45 (-4.72)

Values are the mean ± SD for four animals

Values in parentheses are % change from the control

Values with the same superscript within a row do not significantly differ from each other ($P < 0.05$)

Table 2 Changes in the levels of lipid peroxidation (micromoles of malondialdehyde per gram of tissue) in different tissues of SeMV-inoculated and control mice

Organ	Control	Oral 6 h	Oral 72 h	IV 6 h	IV 72 h
Brain	35.4 ^a ± 2.39	34.85 ^a ± 2.30 (-1.55)	34.2 ^a ± 2.34 (-3.38)	34.79 ^a ± 1.37 (-1.72)	34.76 ^a ± 1.42 (-1.8)
Kidney	16.97 ^a ± 1.92	15.32 ^a ± 1.08 (-9.72)	13.84 ^a ± 1.24 (-18.44)	14.82 ^a ± 1.28 (-12.69)	16.36 ^a ± 1.51 (-3.59)
Liver	19.48 ^a ± 1.69	17.00 ^a ± 0.99 (-12.73)	17.32 ^a ± 1.25 (-11.08)	20.47 ^a ± 2.04 (5.08)	19.67 ^a ± 1.76 (0.97)
Lungs	19.02 ^a ± 1.43	17.80 ^a ± 1.3 (-6.41)	16.89 ^a ± 1.3 (-11.19)	17.66 ^a ± 1.36 (-7.15)	17.39 ^a ± 1.56 (-8.56)
Spleen	26.02 ^a ± 1.43	24.59 ^a ± 2.10 (-5.49)	23.96 ^a ± 2.08 (-7.91)	23.84 ^a ± 1.68 (-8.37)	24.02 ^a ± 1.82 (-7.68)

Values are the mean ± SD for four animals

Values in parentheses are % change from the control

Values with the same superscript within a row do not significantly differ from each other ($P < 0.05$)

Table 3 Changes in the levels of G6PD (micromoles of formazan formed per gram of tissue) in different tissues of SeMV-inoculated and control mice

Organ	Control	Oral 6 h	Oral 72 h	IV 6 h	IV 72 h
Brain	2.14 ^a ± 0.17	2.09 ^a ± 0.16 (-2.33)	2.04 ^a ± 0.24 (-4.67)	2.02 ^a ± 0.19 (-5.6)	2.06 ^a ± 0.17 (-3.73)
Kidney	2.90 ^a ± 0.22	2.66 ^a ± 0.17 (-8.27)	2.61 ^a ± 0.26 (-10.00)	2.70 ^a ± 0.25 (-6.89)	2.68 ^a ± 0.21 (-7.58)
Liver	3.07 ^a ± 0.19	3.04 ^a ± 0.12 (-0.97)	3.01 ^a ± 0.13 (-1.95)	3.00 ^a ± 0.14 (-2.28)	2.98 ^a ± 0.15 (-2.93)
Lungs	2.02 ^a ± 0.21	1.85 ^a ± 0.14 (-8.41)	1.94 ^a ± 0.19 (-3.96)	1.98 ^a ± 0.19 (-1.98)	1.89 ^a ± 0.22 (-6.43)
Spleen	2.2 ^a ± 0.21	2.09 ^a ± 0.17 (-5.0)	2.06 ^a ± 0.17 (-6.36)	2.19 ^a ± 0.17 (-0.45)	2.17 ^a ± 0.20 (-1.36)

Values are the mean ± SD for four animals

Values in parentheses are % change from the control

Values with the same superscript within a row do not significantly differ from each other ($P < 0.05$)

Discussion

Demonstration of *in vivo* non-toxicity of nanoparticles is becoming increasingly important for their application in clinical settings. Their pharmacologic profiles, routes of administration, biodistribution patterns, and dosage are all important considerations that are only beginning to be addressed in detail [3, 9, 20]. In this study, no relevant clinical signs and no significant differences in body weight were observed in mice administered with high doses of SeMV (Supplementary data). Overall, the animals appeared essentially normal. The potential for toxic side

effects that may be associated with VNPs related to human pathogens has caused concern about the use of such virus-based platforms. For instance, adenovirus particles were found to cause liver injury when injected at 2×10^{11} particles/kg body weight in humans and non-human primates [12]. Preclinical studies in mice have shown that CPMV can be administered at doses of up to 100 mg (10^{16} VNPs) per kg body weight without signs of toxicity [35]. In the case of SeMV, administration of similar doses did not result in noticeable toxicity.

Western blot analysis of fecal samples revealed that SeMV was present in the fecal samples up to 14 hpa in

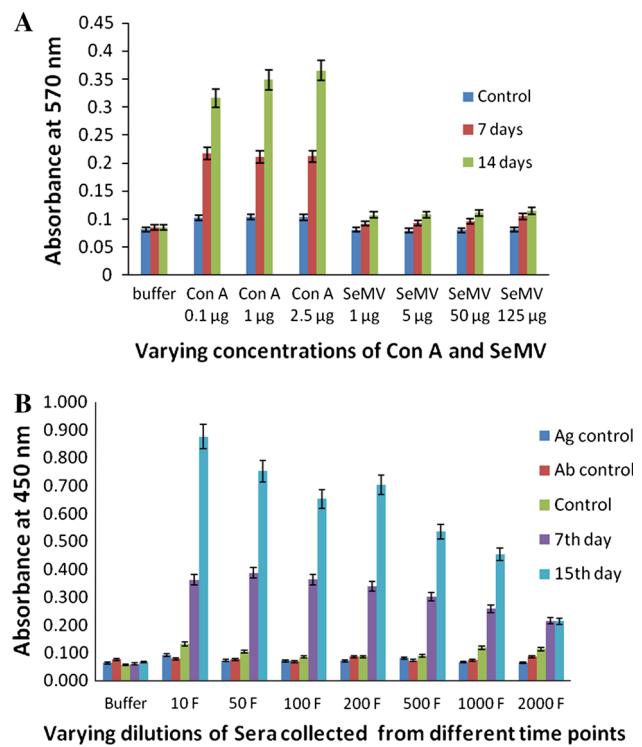


Fig. 4 (A) MTT assay was performed with splenocytes isolated from mice on the 7th and 14th day after administration of SeMV, showing significantly less proliferation than in mice treated with concanavalin A. Error bars represent the standard deviation of the mean. The assay was repeated twice with triplicates for each sample. (B) DAC-ELISA for serum collected from mice administered with SeMV at 7 and 15 days after administration. SeMV antibodies were detected at the lowest dilution (10 F) and significantly reduced upon further dilution. Ag Control, antigen control; Ab Control, antibody control

orally administered mice, although SeMV CP appeared to be cleared by 16 h (Fig. 1). Fecal samples collected from mice receiving SeMV intravenously showed no signal for virus presence, and the reason for this could be that the virus enters the blood circulation directly and therefore, a major portion may get cleared from the body through the reticulo-endothelial system (RES). Further, the virus was detected up to 72 hpa in the blood samples collected from both orally and intravenously administered mice, and it was cleared from the blood circulation beyond this point (Fig. 2A). Generally, particulate materials including viruses are cleared swiftly from the bloodstream by RES of the liver and spleen. For example, CPMV particles were shown to be cleared from blood circulation within 30 minutes with an average half-life of 4-7 minutes in plasma [35]. Adenovirus (Ad) particles administered intravenously in mice were found in the plasma for 10-15 minutes, with a half-life of more than 5 minutes [12]. Ad particles appear to be sequestered from circulation in part by RES in mice, presumably by interaction with scavenger receptors [10, 26] or platelets [38]. Intravenously administered

phage particles declined to negligible amounts within one hour [36], whereas lambda phage particles survived longer in circulation [42], suggesting that surface interactions between phage, the immune system and vascular endothelial cells have a significant effect on plasma clearance. In the present study, SeMV shows a longer half-life, which could be an advantage if these particles are to be used for various biomedical applications. In fact, some of above mentioned VNPs have been modified by PEGylation to increase their half-life. ELISA data of fecal and serum samples showed that the majority of the SeMV was cleared through fecal matter (2.2 µg). The biodistribution of SeMV within various tissues following oral and intravenous dosing was determined by RT-PCR analysis, and the results showed that the SeMV VNPs were localized in liver and spleen until 72 hpa in only intravenously administered mice (Fig. 2B), and no detectable SeMV was found in other tissue samples (data not shown). In the orally administered animals, most of the virus would have been digested or excreted from the body, and the amount of virus that was assimilated into the blood stream would not be sufficient to reach the organs. Quantitative biodistribution studies have indicated that labeled CPMV particles mostly accumulate in the liver, with some accumulation in the spleen, with no associated toxicity [35]. Similarly, a broad distribution and movement throughout most tissues and organs, rapid excretion, absence of long-term persistence and no toxicity were observed after a single injection of ¹²⁵I-labeled CCMV [15]. A study of tobacco mosaic virus (TMV), a typical rod-like VNP in both normal and tumor-bearing mice, showed that virus particles were cleared from the liver and spleen within days, with no apparent changes in histology, that TMV spheres were cleared more rapidly from tissues than rods, and that no sign of toxicity was observed in any of the examined tissues. A similar biodistribution pattern was observed in tumor bearing mice [6]. PVX also accumulated not only in the liver but also was also present in significant amounts in the spleen. This is in good agreement with our results (Fig. 2B). Uptake and accumulation of VNPs in organs with filtration function such as liver and spleen are expected. These organs are part of the RES, which is a component of the immune system. Its function is to remove antigens such as proteinaceous nanoparticle structures from circulation [34].

Histological sections of the major organ tissues (brain, kidneys, liver, lungs and spleen) of SeMV treated mice showed no apparent tissue degradation or necrosis (Fig. 3). Hematology reports of the intravenously administered mice showed they were somewhat leukopenic, with a relative decrease in white blood cells after 72 hpa. In the case of CPMV administered mice, hematology was essentially normal except that the mice were leukopenic at the highest

dose examined, and this condition was not considered a significant problem. No pathological signs were noted on histological examination of CPMV-inoculated animal tissues, in accordance with the results presented here [35]. The rapid clearance and liver-selective trafficking of CPMV particles suggested the requirement of CPMV modification with PEG or other immune masking agents for specific targeting to other tissues or cells. Liver is a vital detoxifying organ that processes endogenous and exogenous substances; hence, hepatotoxicity is considered as a serious health problem [46]. Numerous investigations have linked elevated transaminase levels to tissue damage [29]. Serum creatinine is an important indicator of renal health and kidney function, as creatinine is an easily measured byproduct of muscle metabolism and is excreted by kidneys. Serum creatinine, SGOT and SGPT levels were normal in major organs of SeMV-inoculated animals when compared to control animals, and no significant difference was observed in any of the tested doses, either orally or intravenously, indicating that there was no effect on renal function (Table 1). Lipid peroxidation is another indicator in the pathogenesis of various liver injuries and subsequent liver fibrinogenesis in experimental animals and humans [46]. No significant difference in the lipid peroxidase levels was observed in major organs of SeMV administered mice, indicating that there is no disturbance of the balance between the oxidant and anti-oxidant system (Table 2).

Although nanoparticles can be used for targeted drug delivery, their effect on non-target tissues needs to be studied. G6PD is the first and rate-limiting enzyme of the pentose phosphate pathway, and chief source of NADPH, a major cellular reductant that is essential for cell survival. Several studies have shown that partial or complete inhibition of G6PD activity leaves tissues extremely susceptible to oxidant stress [45]. Major vital organ tissues were collected from SeMV-inoculated mice, and G6PD levels were found to be within the range when compared to control animals (Table 3). All of the above results indicate that SeMV administration does not disturb any of the vital processes in mice and is non-toxic.

Splenocytes consists of a variety of cell populations, including T and B lymphocytes, dendritic cells, and macrophages, which have specific immune functions. The presence of the mitogen like Con A stimulates the proliferation of the splenocyte population. However, the presence of SeMV did not elicit significant levels of proliferation compared to Con A, even at the highest amount of SeMV (125 µg) added to the splenocytes, which shows that SeMV is less immunogenic (Fig. 4A).

While studying the toxicity of SeMV, an attempt was made to measure antibody production against SeMV by DAC-ELISA at 7 and 15 days post-administration, and the results indicate that there is a humoral immune response

compared to 7 and 15 days (Fig. 4B). The results of the present study indicate that the SeMV can serve as a safe bio-nanoparticle for biomedical applications and further studies are in progress to engineer exterior and interior portions of the SeMV capsid with a variety of effector molecules by using chemical and genetic tools.

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Compliance with ethical standards

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Conflict of interest The authors declare no conflicts of interest.

Ethical approval All of the institutional guidelines for the care and use of animals were followed.

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