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# IN VIVO ANTI-CANCER ASSESSMENT OF PLATINUM LOADED NARINGENIN NANOPARTICLES USING EAC CELL LINES

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**Abstract**

**Objective:** Cancer is one of the fatal life-threatening diseases in the modern world due to life style changes. Nanotechnology have gained wider application in the treatment of cancer as a novel drug delivery. The plant-based nanoparticles derived from green synthesis acquired much importance than conventional treatment procedures for cancer because of its several advantages like biological compatibility, toxicity reduction, eexcellent stability, increased permeability and precise targeting. Platinum can serve as an excellent carrier for loading biomolecules in novel cancer treatment.**Methods:** In this present study, the platinum loaded nanoparticles using the compound naringenin isolated from the seeds of the plant Mucuna pruriens was evaluated for hepatic carcinoma using EAC cell lines. **Results:** The results obtained were compared with standard anti -cancer drug Cisplatin. **Conclusion:** From the studies, it was concluded that the platinum loaded naringenin nanoparticles can be effectively used for liver cancer.

**Key words**: Nanoparticles, Platinum, Naringenin, Anti-cancer activity, Blood parameters

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## 1. Introduction

Globally cancer is becoming the foremost fatal disease due to its complexed pathogenesis. Nearly 277 different types of cancers have been identified.[1,2] Use of synthetic chemicals, various radiations, immunological products, hormones, stem cells are the various therapeutic treatment procedures following for cancer traditionally.[3]However, they are not specific, cytotoxic in its action and resistance associated with use of different drugs are the major factors to be considerable as difficulties in cancer therapy.**[4]**The emergence of nanotechnology have been turned the track of diagnostic and therapeutic struggles associated with cancer.The particles with 1–100 nm called as nanoparticles are in use for treating cancer possess several advantages compared to traditional treatments such as biological compatibility, toxicity reduction, eexcellent stability, increased permeability and precise targeting.**[5]** This is an Novel delivery system which is distinct in nature and uses only the area of cancer and its surroundings aspects in cancer treatment and thereby solving the disadvantages of traditional cancer ttherapy by over coming resistance associated in use of different drugs.**[6]**

Nanoparticles were technically engineered structures having 1 dimension below 100 nm having distinct characteristics. The Nanoparticles engineered using metals are now trending on“biological imaging” and specific drugdelivery due to their remarkable characteristics optically, magnetically and photothermally. Metals such as gold,silver,iron,platinum,copper etc are used widely to engineer as nanoparticles. All the metallic nanoparticles have been used in the cancer treatment but platinum nanoparticles are most promising one as the mechanism of action of platinum compounds is on acting with DNA.[7,8]If the destruction to the DNA is in increasing manner than the capacity of the repair mechanism of cells,it leads to programmed cell destruction called as apoptosis.[9]Several studies had found that platinum nanoparticles having the size of 50 nm possess anti-cancer activity,whereas the one with size of 5 nm and 20 nm does not have anticancer activity.Platinum nanoparticles are widely used in the treatment of various types of cancers.[10]

So the present research deals with Isolation of the compound Naringenin from the Plant *Mucuna pruriens* belonging to the family of fabaceae. The naringenin was made to engineered as nanoparticle using platinum metal and it was evaluated hepatocarcinoma by in-vivo method using Ehrlich ascites carcinoma **(**EAC) cell lines.

## 2. Materials and methods

EAC cell lines were obtained from the National Cancer Institute, Pune.It was properly maintained and in aseptic environment these cell lines were made to propagateby intraperitoneal transplantation serially.

### *Extraction, Isolation and Preparation of Platinum loaded nanoparticles using naringenin [11,12,13]*

The seeds of the plant *Mucuna pruriens* were collected and extracted using soxhlet apparatus by hydroalcoholic extraction. The compound naringenin was isolated by using column chromatography and confirmed by mass spectroscopy. About 1 mg/mL of naringenin was combined with 100 ml of ethanol and aqueous H2PtCl6. The mixture was refluxed at 95°C for 3 hours in a fully closed flask to prevent evaporation.The Pt nanoparticles was separated from the biomolecules by reduction in the Pt solution,then for ten minutes made to sonicate.The syringe filter was used to filter the solution. Centrifugation at 12000 rpm was used several times to separate the decreased Pt for 30 minutes. The pellets were washed by using distilled water to get rid of contaminants. The Prepared platinum loaded naringenin nanoparticles were subjected to in-vivo cytotoxic activities.

### *Acute toxicity studies[14,15]*

Female Swiss albino mices of weight 25 – 30 g were chosen for the studies and were maintained under specific controlled conditions. The oral acute toxicity study of drugs was assessed as of OECD guideline 423 on mice,by using the limit test dose of 500 mg/kg.The animals were made to divide to 5groups, each group consisting of 4 animals. The 1$^{st}$ group were the normal control. The 2$^{nd}$, 3$^{rd}$, 4$^{th}$ and 5$^{th}$ were the test groups receiving pure compounds at doses of 25mg/kg, 50 mg/kg, 100 mg/kg, 200 mg/kg by oral route. The weight of the animals was checked before

the administration for calculating the dose required. After the treatment with drug, the animal was under observation for next four hours and for 3 days for the evidence of toxic effects. There were no change in body weight on 25mg/kg, 50 mg/kg. Toxicological signs and mortality observed, whereas one animal died at 100mg/kg and 2animals were died at 200 mg/kg mg/kg. Thus 25mg/kg and 50mg/kg were selected as therapeutic doses.

***Anticancer Activity[14,15]***

2.5 - 3 months old female Swiss albino mice of weight 20-25 g were selected for this study. By intraperitoneal route,Viable EAC cells (2.5 $x10^6$) were injected to every animal by aseptically and was considered as day 0 as tumor is inoculated. The parent lines of the EAC cells parent line were procured from the National Cancer Institute, Pune and were made to maintain in Female Swiss albino mice as tumor cells and they were collected from 1 week old donor. The viable condition of EAC was confirmed and each mouse was implanted by subcutaneous route, about 0.1 ml of ascites fluid diluted in 20:80 ratio with normal saline) into the intraperitoneal cavity.

***2.4 Experimental design:***

40 adult female Swiss albino mice of weight 20-25 g were selected and divided into 5 groups consisting of 8 mice /group).

**Group (1)**: Mice under group I were injected intraperitoneally using sterile saline (0.1 ml / mouse) for fourteen days each day acting as normal group.

**Group (2):** Mice under group II were inoculated intraperitoneally using EAC cell line, (2.5× 10 6 cells/ 0.1 ml /mouse) in the first day only acting as negative control.

**Group (3):** Mice under group III were inoculated intraperitoneally using EAC cell line, (2.5× 10 6 cells/ 0.1 ml /mouse) +platinum nanoparticles – 25mg/kg) for 14 days

**Group (4):** Mice under group IV were inoculated intraperitoneally using EAC cell line, (2.5× 10 6 cells/ 0.1 ml /mouse) +(platinum nanoparticles – 50mg/kg) for 14 days

**Group (5):** Mice under group V were inoculated intraperitoneally with EAC cell line, (2.5× 10 6 cells/ 0.1 ml /mouse) +(standard drug Cisplatin 3mg/kg) for 14 days

After 14 days, 5 mice in every group were given anesthesia and made to sacrifice for assessing the anti-tumor Activity. The remaining mice were observed under alive condition to evaluate the mean survival time and percent increase in life span.

The tumour volume was assessed by the volume of the used measuring tube in milliliters (ml). The Mean survival time and Percentage increase in life span are calculated using the formula as follows

Mean survival time = (No. of days to firstdeath + No. of days to final death)/2

%Increase in life spa n = [(Mean survival time of drug group / Mean survival time of control group)-1] × 100

***Sample collection***

The blood was withdrawn from tail vein after each experiment and separeted from serum in a sterile test tube by centrifugation at 3000 rpm for 20 minutes.After centrifugation,the resulting supernatant was made to collect in a suitable container, it was stored at -20$^oC$.

***Histopathological assessment***

The remnants of the liver tissue were made to dissect andusing normal saline it was made to fix and submerged in paraffin. Then the sections were made and using Hematoxylin and Eosin undergoes staining process as follows.At first in surrounding temperature, the sections were dewaxed using xylene three times each for 5 mins, then hydrated using different concentrations of ethylalcohol each for 5 min.The hydrated sections were now stained with the stain hematoxylin for (20 sec),and rinsed with 1% HCl, then with ethanol for 35 sec,followed by staining with second stain eosin for 2 min.Now it was made to rinse with water, dehydrated again in different grades of ethanol,5 min for each.Using xylene the paraffin was removed and then mounted and covered using cover slips then were captured by an Olympus Binocular microscope.

Hematological measurements like Hb, RBC, WBC and evaluation of biochemical parameters like the levels of enzymes SGPT,SGOT,Alkaline phosphatase,Triglycerides

(TGL),SOD,Catalase,Glutathione peroxidase,Glutathione S-transferase, Total protein assay, Urea,Uric acid,creatinine were evaluated.

## 3. Results and Discussions

The histopathological examinations of liver shows that normal liver cells (Figure 1) have hepatic architecture with hepatic cords, sinusoids, Kupffer cells and polygonal shaped nuclei with central veins were observed. Cytoplasm was clearly visible with endoplasmic reticulum, Golgi apparatus, mitochondria and ribosome. The control group hepatocytes shown in Figure 2 were irregular size and shape with irregular border. There were also sign of necrosis, hemorrhagic zone surrounding the central vein and liver sinusoids. Liver revealed lysis in blood cells or infiltration of neutrophils, lymphocytes or macrophages. The Platinum loaded Naringenin NP added in the concentration of 25mg/kg have liver sections with hepatocytes shown in Figure 3 that was relatively reserved. The count of cells affected with necrosis and dysplastic liver cells were decreased,revealing pattern of recovery in this group.In addition the active Kupffer cells,eosinophilic granular cytoplasm, prominent nucleoli with round nucleusalso observed. The Platinum loaded Naringenin NP added in the concentration of 50 mg/kg shown in Figure 4have a normal portal vein with fewer hepatocytes and renal tubular degeneration. The standard drug cisplatinhas liver section shown in Figure 5 possess anoticeable thickening and inflammatory cell infiltration associated with degeneration, besides the potent congestion in the portal vein. Mild focal necrosis in hepatocytes and parenchymatous degeneration was observed.

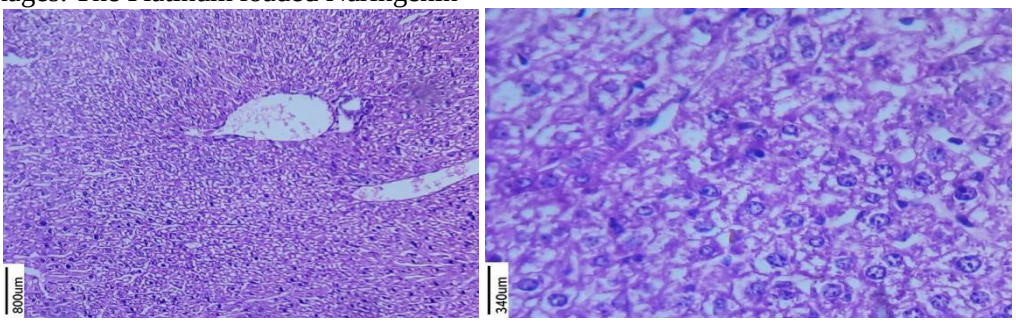


**Fig 1: Normal Liver Sections**

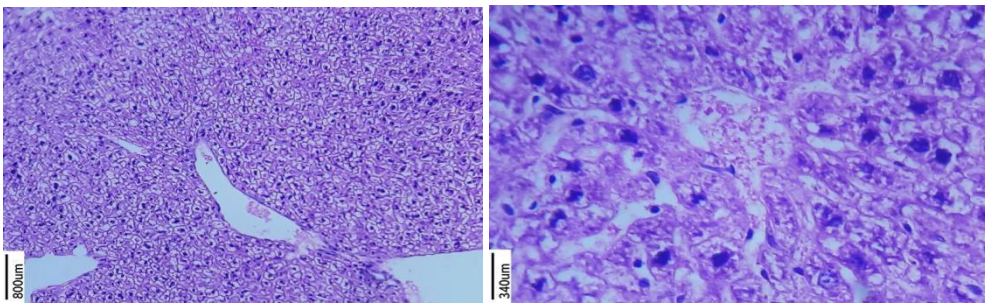


**Fig 2: Control Liver Sections**

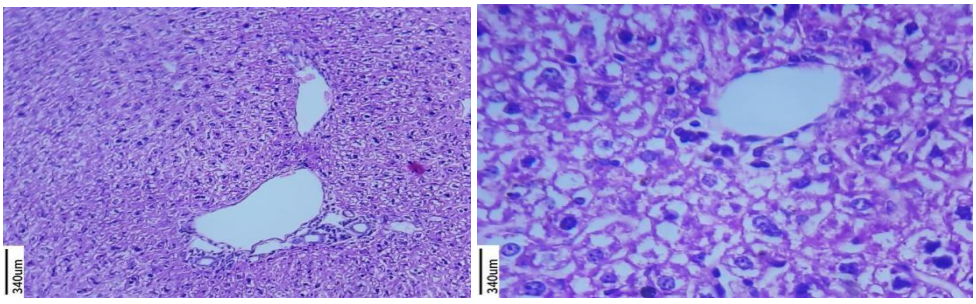


**Fig 3: Liver Sections of Platinum loaded naringenin nanoparticles (25mg/kg)**

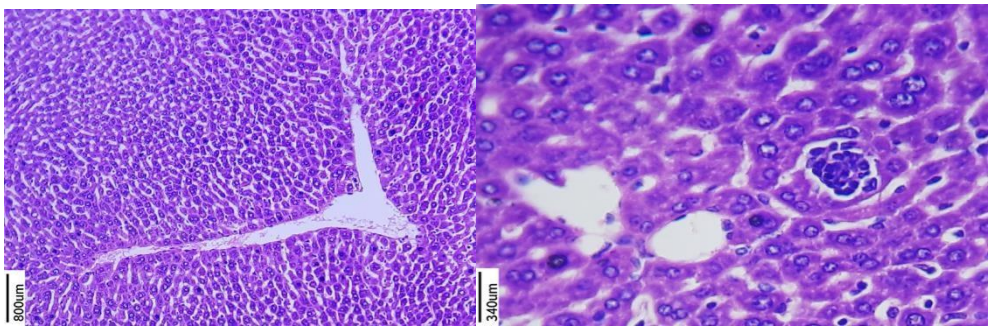


**Fig4: Liver sections of Platinum loaded naringenin nanoparticles (50mg/kg)**

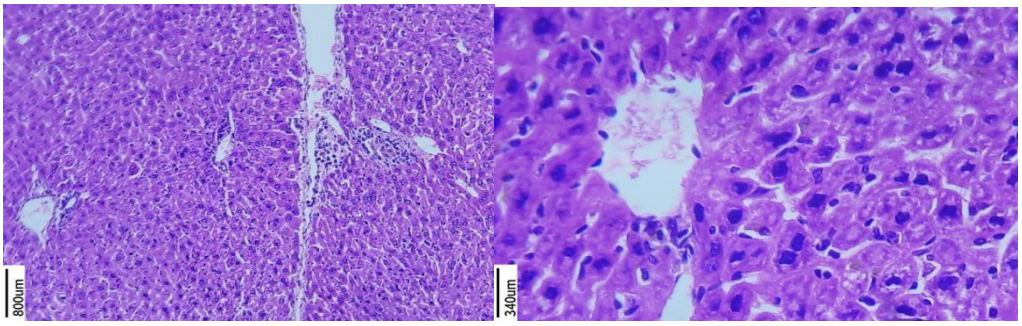


**Fig 5: Liver sections of Standard drug cisplatin**

***Haematological parameters***

Blood was withdrawn from the respective groups of animals and the hematological parameters like RBC count, WBC count, Hb level, ESR and CRP were determined. The results are tabulated in table 1. The Hb level in the liver homogenates were found to be 10.63 gm/100ml and11.53 gm/100mlfor animals treated with platinum loaded nanoparticles in the concentration of 25mg/kg and 50 mg/kg that was nearly higher to the Hb level of standard drug treated group 9.93 gm/100ml. Similarly, the RBC count was 7.36 cells /100 ml of blood and 7.76 cells/100 ml of blood for platinum loaded naringenin nanoparticles. Thestandard drug treated group was found to be 7.133cells /100 ml. The WBC count was found to be 11.93 cells / 100ml and 10.76 cells /100 ml for naringenin nanoparticles and 12.93 cells/100 ml for the standard drug cisplatin. The ESR rate for treated animals with platinum loaded naringenin nanoparticles 25mg/kg and standard drug cisplatin was nearly equal having 12.3 and 12.96.

**Table 1: Haematological Parameters**

| Group Name | Hb Level* Gm/100ml | RBC Count* | WBC Count* | ESR* | CRP* |
|---|---|---|---|---|---|
| **Normal** | 14.4±0.458 | 8.83±0.251 | 7.6±0.435 | 9.16±0.32 | 1.83±0.321 |
| **Control** | 7.9±0.435 | 4.33±0.152 | 18.16±0.873 | 19.46±0.737 | 11.3±0.80 |
| **PTNP-L (25mg/kg)** | 10.63±0.450 | 7.36±0.208 | 11.93±0.568 | 12.3±0.173 | 3.26±0.351 |
| **PTNP-L (50mg/kg)** | 11.53±0.602 | 7.76±0.896 | 10.76±0.057 | 10.53±0.585 | 2.33±0.115 |
| **Cisplatin** | 9.93±0.378 | 7.13±0.251 | 12.93±0.66 | 12.96±0.611 | 3.73±0.503 |

**Table 2: Tumor volume**

| Group Name | Tumor volume |
|---|---|
| **Normal** | 6.65±0.702 |
| **PTNP-L (25mg/kg)** | 3.20±0.30 |
| **PTNP-L (50mg/kg)** | 2.60±0.30 |
| **Cisplatin** | 1.46±0.152 |

The results of the tumor volume and lifespan were shown in the table 2 and 3. The concentration of 25mg/kg and 50 mg/kg of Platinum loaded naringenin nanoparticles shows a tumor volume of 3.20 ±0.30 and 2.60±0.30 whereas the standard drug have 1.46±0.152. Similarly, the life span parameter of the 25mg/kg and 50 mg/kg of Platinum loaded naringenin nanoparticles were 52.33±5.68 and 61.33±2.51 whereas for the standard drug cisplatin 17.33±2.08.

**Table 3: Life span**

| Group Name | Life span |
|---|---|
| **Normal** | 96.66±5.03 |
| **Control** | 25.66±2.51 |
| **PTNP-L (25mg/kg)** | 52.33±5.68 |
| **PTNP-H (50mg/kg)** | 61.33±2.51 |
| **Cisplatin** | 17.33±2.08 |

***Biochemical parameters***

In study like urea level, uric acid level, total protein, bilirubin level, albumin level, creatinine level, enzyme levels of SGPT, SGOT, ALP was to indicate the effect of tumour induction and also to evaluate the naringenin loaded nanoparticle on reducing the tumour. The results were shown in table 4. The blood urea and uric acid level was higher in the platinum loaded naringenin nanoparticles in the concentration range of 25mg/kg (2.63 ± 0.152 and 3.43 ± 0.251) when compared with standard drug cisplatin treated groups showing 3.13 ± 0.251 and 3.5 ± 0.360 respectively than the 50mg/kg PTNP. The concentration of 25mg/kg and 50 mg/kg of Platinum loaded naringenin nanoparticles shows a total protein level of 7.76±0.152 and 8.23±0.55, where the same for the standard drug cisplatin was 7.46±0.404. The creatinine level of concentration of 25mg/kg and 50 mg/kg of Platinum loaded naringenin nanoparticles were 3.43±0.251 and 2.96±0.404 and for the standard drug cisplatin was 3.5±0.360.25mg/kg of PTNP shows nearly equal creatinine level to that of cisplatin indicating the regaining action of kidney. The bilirubin level was nearly or more or less at 3.5 mg /dl for PTNP in both concentrations which were same as that for the standard drug treated groups. The SGPT and SGOT levels of the PTNP were 33.2 ± 0.964 and 43.56 ± 0.750 for 25mg/kg NP, 34.66±1.436 and 44.56±2.07 for 50mg/kg which was compared with the same for standard drug cisplatin having 37.46±1.530 and 44.56±2.07 respectively.

The ALP levels of standard drug treated groups was 33.46±0.929 which is nearly equal for the Platinum loaded naringenin nanoparticles especially high in the 25 mg/kg concentration (32.25±0.494). The albumin level was 4.43±0.251 for both the concentration of nanoparticles which is more when compared to the standard drug treated group with 4.25±0.40.

**Table 4: Biochemical parameters in vivo anticancer studies**

| Group Name | Total protein (g/dL) * | Creatinine (mg/dL) * | Bilirubin (mg/dL) * | SGPT (U/L) * | SGOT | ALP (U/L) * | Albumin (g/dL) * |
|---|---|---|---|---|---|---|---|
| **Normal** | 8.43±0.208 | 1.4±0.264 | 2.36±1.152 | 24.63±1.305 | 42.6±0.916 | 26.43±0.709 | 5.56±0.351 |
| **Control** | 3.46±0.305 | 4.26±0.288 | 6.3±0.20 | 60.36±1.850 | 92.76±2.136 | 47.53±0.709 | 2.36±0.305 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **PTNP-L (25mg/kg)** | 7.76±0.152 | 3.43±0.251 | 3.46±0.251 | 33.2±0.964 | 43.56±0.750 | 32.25±0.494 | 4.43±0.251 |
| **PTNP-H (50mg/kg)** | 8.23±0.55 | 2.96±0.404 | 3.36±0.208 | 34.66±1.436 | 44.56±2.07 | 30.4±0.854 | 4.43±0.208 |
| **Cisplatin** | 7.46±0.404 | 3.5±0.360 | 3.53±0.208 | 37.46±1.530 | 47.5±1.17 | 33.46±0.929 | 4.25±0.40 |

The enzymatic levels of various antioxidant enzymes were tabulated in table 5.SOD levels of Platinum loaded naringenin NP in the concentration of 25mg/kg and 50 mg/ kg were 103.46 ±1.700 and 112.06 ±2.715 and for the standard drug cisplatin 98.16±1.167. The catalase level was33.46 ±1.106 and 35.96±0.208 for the concentrationof 25mg/kg and 50 mg/kg PTNP and for the cisplatin it was 28.46±0.602. The GSH, GPH and GST levels were nearly more in values to that of the cisplatin. The Lipid peroxidation level for the 25mg/kg loaded NP and the standard drug cisplatin was 0.253 ± 0.035 and for the PTNP 50 mg/kg was 0.253 ± 0.030.

**Table 5: Biochemical Parameters for liver homogenates (In blood serum-II)**

| **Group Name** | **Urea (g/dL)*** | **Uric acid (mg/dL)*** |
|---|---|---|
| **Normal** | 1.6±0.2 | 16.5±1.65 |
| **Control** | 4.56±0.35 | 4.26±0.288 |
| **PTNP-L (25mg/kg)** | 2.63±0.152 | 3.43±0.251 |
| **PTNP-H (50mg/kg)** | 2.36±0.152 | 2.96±0.404 |
| **Cisplatin** | 3.13±0.251 | 3.5±0.360 |

| **Group Name** | **SOD*** | **Catalase*** | **GSH*** | **GPX*** | **GST*** | **Lipid Peroxidation *** |
|---|---|---|---|---|---|---|
| **Normal** | 128.4±1.333 | 39.23±0.850 | 63.6±1.113 | 19.5±0.721 | 1.53±0.086 | 0.16±0.026 |
| **Control** | 67.16±1.250 | 21.83±1.789 | 36.23±0.960 | 8.96±0.208 | 0.32±0.041 | 1.92±0.015 |
| **PTNP-L (25mg/kg)** | 103.46±1.700 | 33.46±1.106 | 56.43±0.907 | 14.56±1.123 | 0.84±0.062 | 0.253±0.035 |
| **PTNP-H (50mg/kg)** | 112.06±2.715 | 35.96±0.208 | 61.7±1.374 | 17.46±0.665 | 1.02±0.04 | 0.153±0.030 |
| **Cisplatin** | 98.16±1.167 | 28.46±0.602 | 53.60±0.964 | 12.53±0.602 | 0.74±0.051 | 0.253±0.030 |

## 4. Conclusion

The in vivo anticancer assessment of the isolated naringenin which was loaded as nanoparticle using platinum metal as a nanoparticle carrier can be effective in the treatment of hepatic carcinoma as a novel drug delivery system. Furthermore, it can be assessed to use as anti -cancer agent by allocating the research activities in future related to therapeutic distribution and bioavailability of the nanoparticle.

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