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ORIGNAL ARTICLE

Detoxification of doxorubicin by nanosponge in the animal model (rabbit)

Sadaf Ishtiaq, Faqir Muhammad*, Bushra Akhtar, Abdul Aleem and Majid Anwar

Institute of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan

Abstract

Toxicity induced by drugs is the main complication with medicines that are being used in the treatment of cancer. Doxorubicin is an anthracycline antibiotic. However, use of doxorubicin (Dox) as a therapeutic agent is restricted due to cardiotoxicity, narrow therapeutic range and multi-drug resistance. So, the extra amount of doxorubicin should be removed from the blood to avoid adverse effects. The current research was planned to investigate the effect of nanosponge in the detoxification of doxorubicin in animal model (rabbits). PLGA-COOH (polylactic-co-glycolic acid) nanoparticle was coated with red blood cell membrane to form nanosponge. Nanosponge was characterized for size and charge distribution. The *in vitro* absorption study was done with doxorubicin + water and doxorubicin + blood. For *in vivo* study, rabbits were separated into three groups (n=3). 1st Group was administered IV doxorubicin (7 mg/kg) and 2nd group was administered doxorubicin and PLGA NPs while 3rd group was administered doxorubicin and nanosponge. Blood was taken on 1st, 3rd, 5th and 7th day from all groups fand serum was separated. Biochemical parameters of heart, liver and kidney were measured. At 7th day, survived animals were sacrificed. Heart tissues were collected for histopathological observation. Cardiac function parameters showed significant increase in LDH and CK-MB levels due to doxorubicin and nanosponge showed significantly decrease of these parameters while no effect on liver and kidney enzymes was found. Cardio toxic effects of doxorubicin were further confirmed by histopathological examination. All results showed that nanosponge might be used for treatment of doxorubicin induced dose-dependent cardiotoxicity.

Keywords

Cardiotoxicity
Doxorubicin
Nanosponge
Red blood cells
LDH

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Introduction

Cancer is the most important reason of death throughout the world and approximately 6 million people die every year with cancer. Chemotherapy is the leading treatment approach for different kinds of cancers (Abd El-Dayem *et al*., 2010). Chemotherapy stops the development of primary and secondary malignancies due to their cell damaging effects against rapidly dividing cells (Pavlova *et al*., 2014). Chemotherapy is very effective to treat cancerous conditions but have certain limitations like narrow therapeutic range and toxic effects. So, chemotherapeutic agents should be used vigilantly (Gandhi *et al*., 2005). Toxicity induced by drug is the main complication occurring as a result of chemotherapy. Chemotherapeutic agents can cause different side effects e.g. cardiotoxicity, altered lower limb function, necrosis and ulceration (Vieira and Gamarra, 2016). About 40% drug induced toxicity is due to overdose in humans (Nguyen *et al*., 2016).

Doxorubicin, semi synthetic anthracycline, included in essential drugs used to treat cancer according to World Health Organization (WHO). It is economical to use. Therefore, many hematopoietic malignancies and solid

***Corresponding author:** Email: faqirmuhammad33@gmail.com

tumors including Hodgkin disease, Non-Hodgkin lymphoma, stomach cancer, lung cancer, and leukemia, neoplasm of bladder, bone and soft tissue sarcoma, ovary and breast cancer are being treated with it (Rahman *et al*., 2007). Their use at clinical level is restricted due to various side effects which include toxicities of acute and chronic levels like myelosuppression, cardiomyopathy, congestive heart failure, multi-drug resistance and narrow therapeutic range. To minimize side effects of doxorubicin, extra amount of doxorubicin should be removed from the blood (Joseph *et al*., 2014).

Nanosponge can be used to minimize the above-mentioned side effects of doxorubicin. Nanosponge is net like entity having cavities of nanometer composed of microscopic particles in which vast different types of materials can be integrated. These particles are employed to carry hydrophilic and lipophilic substances and to improve water solubility of less soluble substances (Jilsha and Viswanad, 2013).

Preparation of Nanosponge includes the coating of membrane of red blood cell on PLGA nanoparticles. PLGA (polylactic-co-glycolic acid) polymer is biodegradable and utilized for the formation of nanosponge. This nanosponge is capable of absorbing different cationic drugs such as doxorubicin resulting into the decreased toxic effects on normal body cells and enhanced ability to treat different kind of cancers (Danhier *et al*., 2012).

Current research was planned to examine the detoxifying effect of nanosponge on doxorubicin-induced toxicity.

Materials and Methods

Chemicals: Doxorubicin HCL (10 mg/5 ml) injection and water for injection were bought from Moon Medical Store near Allied Hospital, Faisalabad. Dr. Santosh Aryl from Kansas State University, USA, gifted prepared PLGA nanoparticles for this research. Nanosponge was formed in Nanomedicine Laboratory of Institute of Pharmacy, Physiology and Pharmacology, University of Faisalabad.

Extraction of RBCs membrane: Heparinized blood about 0.5 ml was taken in two eppendorf tubes and diluted each with 0.5 ml of distilled water. Eppendorf were sonicated for 2-3 minutes. Then these tubes were centrifuged at 13000 rpm for 3-4 minutes, supernatant was separated and pellets were resuspended in 1 ml of distilled water and centrifuged again at 13000 rpm for 3 minutes. The above step was repeated 5-6 times until clear pellets of membrane of RBCs remained. Then pellets of the membrane of RBCs were transferred into one eppendorf tube and 1 ml of distilled water was used to resuspend the pellets. Suspension of the extracted membrane of RBCs was freeze-dried and stored until utilization (Hu *et al*., 2011).

PLGA nanoparticles formation: Dr. Santosh Aryl from Kansas State University, USA, gifted PLGA nanoparticles. PLGA nanoparticles were formed by nano precipitation method. In short, the stock solution of PLGA in acetonitrile (10 mg/ml) was formed and 100 µl of PLGA stock solution was incorporated to 1 ml Milli-Q water dropwise with continuous stirring. The resulting mixture was continuously stirred overnight which allowed the preparation of PLGA nanoparticles and evaporation of acetonitrile. Amicon Ultra-4 centrifugal filter with a molecular weight of 10 kDa was employed to purify PLGA nanoparticles.

Nanosponge formation: nanosponge formation was done by the fusion of Purified PLGA NPs with 100 µl of RBCs membrane suspension (1 mg/ml). The resulting mixture was stirred for 5 minutes at 1200 rpm. Then this mixture extruded through 100 nm polycarbonate porous membrane using Avanti mini extruder. The suspension was loaded into one extruder glass syringe. Extruder glass syringe containing suspension and second extruder glass syringe was fitted at opposite sides. Plunger of suspension containing syringe was pressed for movement of suspension through extruder membrane and then plunger of second syringe was pressed for backflow into the first syringe through the extruder membrane. This step was revised 15-21 times to accomplish the coating of RBC membrane onto PLGA NPs. Storage of prepared nanosponge was done at 4°C until further use.

Characterization of PLGA NPs and Nanosponge: PLGA-NPs and nanosponge were characterized for size and zeta potential from National Textile University, Faisalabad.

***In vitro* chemotherapeutic absorption study**

Preparation of standard: Doxorubicin standard solutions with varying concentrations i.e. 1, 2, 3, 4 and 5 µg/ml were prepared in distilled water. Fluorescence was checked at 580 nm in Multiskan GO™ (Thermo Scientific). Standard curve was drawn which was used to calculate the concentration of doxorubicin.

***In vitro* drug absorption ability of PLGA-NPs and nanosponge:** For checking absorption ability of nanosponge, four different concentrations of nanosponge and PLGA-NPs (62.5, 125, 250 and 500 µg/ml) were incubated with a known amount of doxorubicin in water and rabbit plasma respectively. The amount of absorbed drug into these respective particles was quantified by using Multiskan GO™ (Thermo Scientific).

PLGA NPs and nanosponge incubation with doxorubicin

In distilled water: In distilled water, known amount of doxorubicin (1.5 µg/ml) was incubated with varying concentrations of PLGA NPs and nanosponge (62.5, 125, 250 and 500 µg/ml) for 30 min. Samples were centrifuged for 10 min at 10,000 rpm after incubation.

Absorbed and unabsorbed quantities of drug were determined using separated pellets and supernatant by measuring the doxorubicin fluorescence (λ_{ex} = 490 nm, λ_{em} = 580 nm) (Nguyen *et al*., 2016).

In rabbit plasma: In rabbit plasma, known amount of doxorubicin (1.5 µg/ml) was incubated with varying concentrations of PLGA NPs and nanosponge (62.5, 125, 250 and 500 µg/ml) for 30 min. Samples were centrifuged for 10 min at 10,000 rpm after incubation. Absorbed and unabsorbed quantities of drug were determined using separated pellets and supernatant by measuring the doxorubicin fluorescence (λ_{ex} = 490 nm, λ_{em} = 580 nm) (Nguyen *et al*., 2016).

***In vivo* chemotherapeutic absorption study**

Animals: Twelve (12) healthy albino rabbits having body weight between 1000-2000 g were housed at the animal house of Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad, under standard conditions of relative humidity and temperature with 12-hour light and dark cycle. Animals were fed routine diet and water.

Experimental design: Rabbits were divided into three groups (n=3), each group was consisted of 4 rabbits (Table 1). They were accommodated for one week. Animals of group 1 were administered doxorubicin alone intravenously at the dose rate of 7 mg/kg and that of group 2 were administered doxorubicin (7 mg/Kg) and PLGA NPs (2 ml/kg) intravenously. Animals of group 3 were administered doxorubicin (7 mg/Kg) and nanosponge (2 ml/kg) intravenously. visual signs were observed for toxicity in rabbits. Blood was collected from rabbits on 1st, 3rd, 5th and 7th day for cardiac, liver and kidney biochemical parameters. At 7th day, remaining rabbits were decapitated. Hearts were collected and preserved for histopathological examinations.

Treatment protocol

Blood sampling: 3 ml of blood from each rabbit via jugular vein was withdrawn on 1st (before treatment), 3rd, 5th and 7th day in gel and clot activator tubes for the determination of cardiac, liver and kidney biochemical parameters. Serum was obtained by centrifuging the blood samples for 10 minutes at 3000 rpm. Serum was stored at -20^{o}C until biochemical analysis.

Biochemical parameters: Cardiac, liver and kidney enzymes such as creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), alanine transaminase (ALT), aspartate transaminase (AST), urea and creatinine were determined by serum. Biochemical kits and an automated biochemistry analyzer were used to quantify the biochemical parameters.

Cardiac function analysis

Creatinine kinase-MB (CK-MB; IU/L): Eppendorf tubes were labeled and 400 µl of R_1 (antibody) reagents and 100 µl of R_2 (substrate) were added using a micropipette. 50 µl of serum was added in eppendorf tube. Vortex mixer was used to homogenize the reagent mixture. This mixture was incubated for 1 min at 30^{0}C in water bath. The incubated reagents mixture was used as blank. For calibration, absorbance of reaction solution of R_2 and R_1 was checked at 340 nm wavelength, 1 cm optical and 30^{o}C temperature. Then absorbance of serum sample mixture and reaction solution was checked at a wavelength of 340 nm and 30^{o}C temperature.

Lactate dehydrogenase (LDH; IU/l): An automated analyzer was used to measure LDH levels.

Liver Function Analysis

Alanine transaminase (ALT; U/l): Quantification of alanine aminotransferase (ALT) levels in the serum was done by commercial kit of Merck Pvt. Ltd., Pakistan.

Aspartate transaminase (AST; U/l): Quantification of aspartate aminotransferase (AST) levels in serum was done by commercial kit of Merck Pvt. Ltd., Pakistan.

Kidney Function Analysis

Creatinine (mg/dl): Quantification of creatinine in serum was done spectrophotometrically by using commercial kit (Creatinine Jaffe Ecoline® diagnostic kit, Merck).

Histopathological Examination: At 7th day, animals were sacrificed to get heart for histopathological examination. Organs were washed with normal saline to remove blood from organs and stored in 37% formalin.

Statistical analysis: All results were expressed as mean ± SEM. One way ANOVA was employed. Duncan's Multiple Range (DMR) test at 5% level of significance ($p \leq 0.05$) was used (Steel *et al*., 1997). Statistical Package for the Social Sciences (SPSS) was utilized for the data analysis.

Results

Size and zeta potential of PLGA NPs: Size of PLGA NPs was found to be 207.8 nm with zeta sizer while zeta potential was found to be -39.0 as shown in Figure 1 and 2.

Size of and zeta potential of nanosponge: Size of nanosponge was found to be 222.6 nm with zeta sizer while zeta potential was found to be -12.5 as shown in Figure 3 and 4.

Standard curve: After taking absorbance at 580nm, standard curve was drawn between absorbance and concentration of Doxorubicin as shown in Figure 7.

***In vitro* absorption of doxorubicin by PLGA-NPs and nanosponge**

In distilled water: Percentage absorption of Doxorubicin (known amount) by different concentrations of PLGA-NPs and nanosponge in distilled water showed dose-dependent increase in Doxorubicin absorption as shown in Table 2.

Table: 1. Treatment protocol for control and treated albino rabbits.

Groups	Treatment
Group 1	Doxorubicin alone at (7 mg/kg) single IV injection.
Group 2	Doxorubicin (7 mg/kg) and PLGA-NPs (2 ml/kg) single IV injection.
Group 3	Doxorubicin (7 mg/kg) and nanosponge (2 ml/kg) single IV injection.

Table 2: Showing *in vitro* absorption of drug (known amount) by different concentrations of PLGA-NPs and nanosponge.

Concentrations (µg/ml)	*In vitro* percentage absorbance			
	Distilled water		Rabbit plasma	
	PLGA-NPs	Nanosponge	PLGA-NPs	Nanosponge
62.55	22.29±0.48	23.77±0.29	6.86±0.36	8.19±0.49
125	34.63±0.26	36.96±0.45	14.63±0.36	24.55±0.54
250	46.52±0.21	56.01±0.44	31.00±0.30	35.40±0.37
500	57.47±0.52	65.49±0.45	45.98±0.30	58.07±0.64

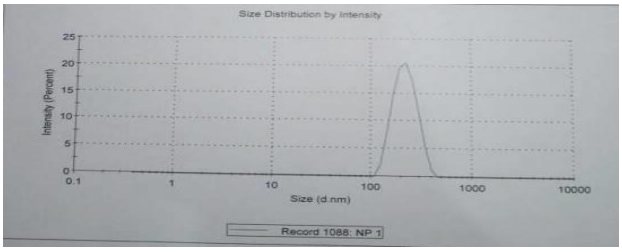

Figure 1: showing size distribution (nm) of PLGA NP before coating.

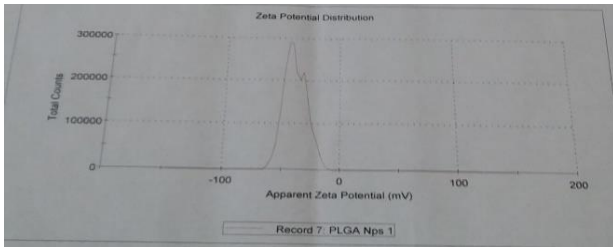

Figure 2: showing zeta potential (mV) of PLGA NP before coating.

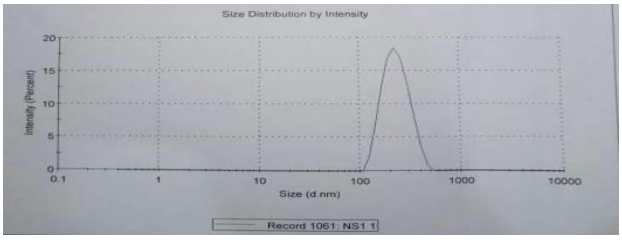

Figure 3: showing size distribution (nm) of nanosponge.

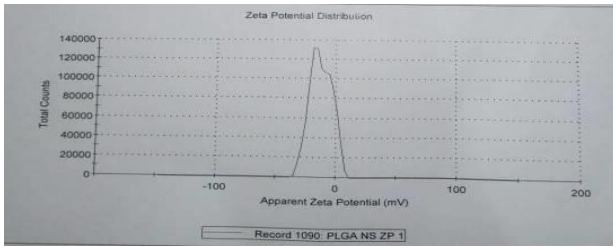

Figure 4: showing zeta potential of nanosponge.

In rabbit plasma: Percentage absorption of Doxorubicin (known amount) by different concentrations of PLGA-NPs and nanosponge in rabbit plasma showed dose dependent increase in Doxorubicin absorption as shown in Table 2.

***In vivo* absorption of doxorubicin by PLGA-NPs and nanosponge**

Cardiac enzymes

Creatinine kinase-MB (CK-MB; IU/l): Average± SEM levels of creatinine kinase-MB (IU/l) in different groups of rabbits are given in Table 3 and Figure 8 on different days (post doxorubicin administration). Results indicated that average CK-MB levels in doxorubicin alone group were 427.75±12.07, 1397.75±42.92, 1145.25±13.44 and 890.75±19.26 on day 1st, 3rd, 5th and 7th repectively. The CK-MB levels in Dox+PLGA NPs group were 464.75±18.96, 1149±0.71, 818±38.84 and 668±82.65 on day 1st, 3rd, 5th and 7th respectively. While average CK-MB levels in Dox+Nanosponge group were 459.75±4.49, 985±4.49, 798±2.74 and 662±31.83 on day 1st, 3rd, 5th and 7th respectively.

Lactate dehydrogenase (LDH; U/l): Average± SEM levels of Lactate dehydrogenase (LDH; U/l) in different groups of rabbits are given in Table 3 and Figure 9 on different days (post doxorubicin administration). Results indicated that average LDH levels in doxorubicin alone group were 532.25±8.08, 1054.75±7.69, 983.5±5.75 and 771.25±13.23 on day 1st, 3rd, 5th and 7th repectively. The LDH levels in Dox+PLGA NPs group were 537.25±2.17, 980.05±1.71, 885.25±4.27 and 719.25±8.94 on day 1st, 3rd, 5th and 7th respectively. While average LDH levels in Dox+Nanosponge group were 547.75±2.95, 786.25±5.04, 648.5±13.97 and 536±18.87 on day 1st, 3rd, 5th and 7th respectively.

Liver Function Tests

Alanine aminotransferase (ALT; U/l): Average± SEM levels of alanine aminotransferase (ALT; U/l) in different groups of rabbits are given in Table 4 and Figure 10 on different days (post doxorubicin administration). Results indicated that average ALT levels in doxorubicin alone group were 53.75±1.25, 46.25±4.44, 52.25±4.27 and 42±3.49 on day 1st, 3rd, 5th

Table 3: Showing average±SEM creatinine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) levels in 3 groups on different days.

Sampling time (Days)	Creatinine kinase-MB			Lactate Dehydrogenase		
	Treatments			Treatments		
	Doxorubicin	Doxrubicin + PLGA NPs	Doxrubicin + Nanosponge	Doxrubicin	Doxrubicin + PLGA NPs	Doxrubicin + Nanosponge
1st	427.75 ± 12.07	464.75 ± 18.96	459.75 ± 4.49	532.25 ± 8.08	537.25 ± 2.17	547.75 ± 2.95
3rd	1397.75 ± 42.92	1149 ± 0.71	985 ± 4.49	1054.75 ± 7.69	980.5 ± 1.71	786.25 ± 5.04
5th	1145.25 ± 13.44	818 ± 38.84	798 ± 2.74	983.5 ± 5.75	885.25 ± 4.27	648.5 ± 13.97
7th	890.75 ± 19.26	668 ± 82.65	662 ± 31.83	771.25±13.23	719.25 ± 8.94	536 ± 18.87

Table 4: Showing average±SEM alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in 3 groups on different days.

Sampling time (Days)	Alanine aminotransferase			Aspartate aminotransferase		
	Treatments			Treatments		
	Doxorubicin	Doxorubicin+ PLGA NPs	Doxorubicin+ Nanosponge	Doxorubicin	Doxorubicin+ PLGA NPs	Doxorubicin+ Nanosponge
1st	53.75±1.25	52.75±1.55	53.25±0.85	22.75±0.85	23.5±0.87	23.75±0.96
3rd	46.25±4.44	67.25±2.17	69.5±1.04	40.5±1.71	42±0.41	71.75±0.48
5th	52.25±4.27	47.5±8.15	36.75±1.55	46±3.03	67.25±9.00	36.5±0.87
7th	42±3.49	55.75±8.82	50.5±2.90	68.25±10.50	60.5±10.37	55.5±8.54

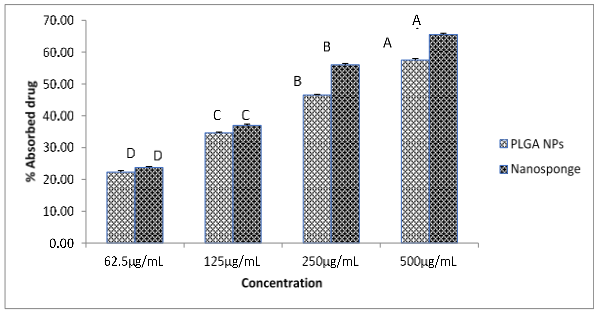

Figure 5: Percent absorption of doxorubicin by PLGA NPs and nanosponge in distill water after 30 min of incubation.

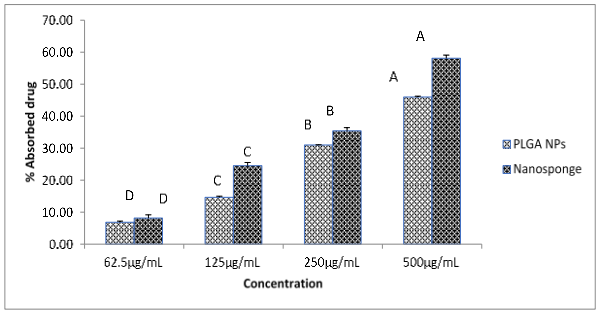

Figure 6: Percent absorption of doxorubicin by PLGA NPs and nanosponge in rabbit plasma after 30 min of incubation.

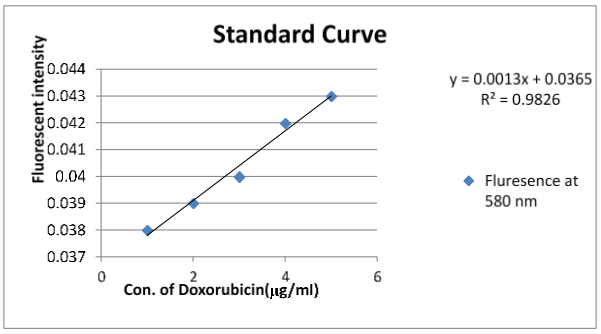

Figure 7: Showing standard curve for doxorubicin.

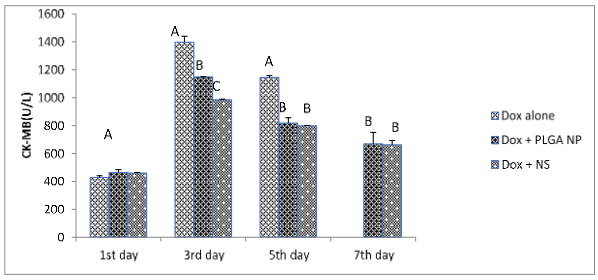

Figure 8: Showing creatine kinase-MB (IU/l) levels (Mean ± SE) at different days post doxorubicin, PLGA NPs and nanosponge administration in rabbit.

and 7th repectively.The ALT levels in Dox+PLGA NPs group were 52.75±1.55, 67.25±2.17, 47.5±8.15 and 55.75±8.82 on day 1st, 3rd, 5th and 7th respectively. While average ALT levels in Dox+Nanosponge group were 53.25±0.85, 69.5±1.04, 36.75±1.55 and 50.5±2.90 on day 1st, 3rd, 5th and 7th respectively.

Aspartate aminotransferase (AST; U/l): Average± SEM levels of aspartate aminotransferase (AST; U/l) in different groups of rabbits are given in Table 4 and Figure 11 on different days (post doxorubicin administration). Results indicated that Average AST levels in doxorubicin alone group were 22.75±0.85, 40.5±1.71, 46±3.03 and 68.25±10.50 on day 1st, 3rd, 5th and 7th respectively. The AST levels in Dox+PLGA NPs group were 23.5±0.87, 42±0.41, 67.25±9.00, 60.5±10.37 on day 1st, 3rd, 5th and 7th respectively. While

Table 5: Showing average±SEM creatinine and urea levels in 3 groups on different days.

Sampling time (Days)	Urea			Creatinine		
	Treatments			Treatments		
	Doxorubicin	Doxorubicin+ PLGA-NPs	Doxorubicin+ Nanosponge	Doxorubicin	Doxorubicin+ PLGA-NPs	Doxorubicin+ Nanosponge
1st	37.25±1.25	47.75±0.85	33.75±0.85	0.275±0.09	0.25±.06	0.5±0.07
3rd	38.5±1.32	44.5±1.71	54±1.08	0.675±0.06	0.725±0.03	0.775±0.07
5th	41.75±0.48	38.25±0.25	54.75±1.89	0.5±0.04	0.475±0.05	0.925±0.03
7th	46.25±1.03	29±2.35	47.75±1.89	1.375±0.10	0.275±0.05	0.65±0.14

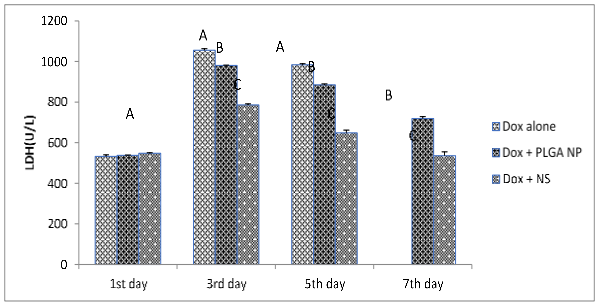

Figure 9: Showing lactate dehydrogenase (LDH; U/l) levels (Mean ± SE) at different days post doxorubicin, PLGA NPs and nanosponge administration in rabbits.

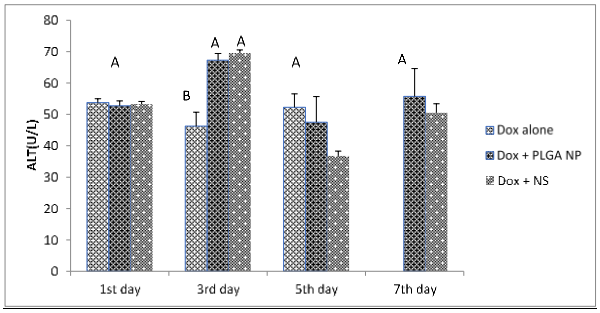

Figure 10: Showing alanine aminotransferase (ALT; U/l) levels (Mean ± SE) at different days post doxorubicin, PLGA NPs and nanosponge administration in rabbits.

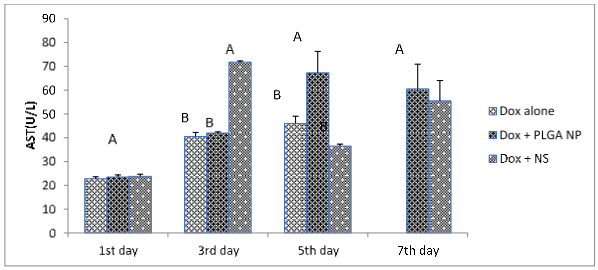

Figure 11: Aspartate aminotransferase (AST; U/l) levels (Mean ± SE) at different days post doxorubicin, PLGA NPs and nanosponge administration in rabbits.

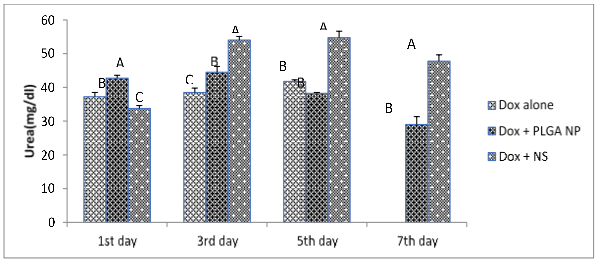

Figure 12: Showing urea (mg/dl) levels (Mean ± SE) at different days post doxorubicin, PLGA NPs and nanosponge administration in rabbits.

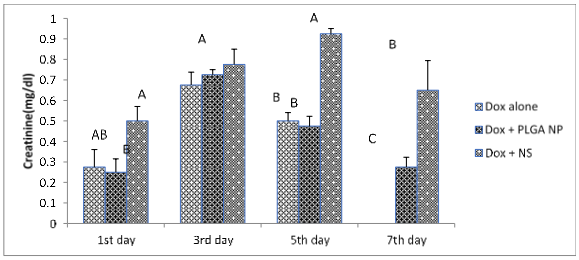

Figure 13: showing creatinine (mg/dl) levels (Mean ± SE) at different days post doxorubicin, PLGA NPs and nanosponge administration in rabbits.

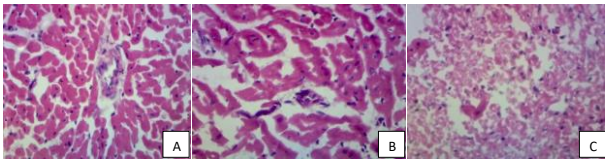

Figure 14: Hematoxylin and eosin stained heart tissues. A) Doxorucicin treated group. B) Doxorubicin and PLGA-NPs group. C) Doxorubicin and nanosponge treated group.

average AST levels in Dox+Nanosponge group were 23.75±0.96, 71.75±0.48, 36.5±0.87 and 55.5±8.54 on day 1st, 3rd, 5th and 7th respectively.

Kidney Function Tests

Urea (mg/dl): Average± SEM levels of Urea (mg/dl) in different groups of rabbit are given in Table 5 and Figure 12 on different days (post doxorubicin administration).

Results indicated that average urea levels in doxorubicin alone group were 37.25±1.25, 38.5±1.32, 41.75±0.48 and 46.25±1.03 on day 1st, 3rd, 5th and 7th respectively. The urea levels in Dox+PLGA NPs group were 47.75±0.85, 44.5±1.71, 38.25±0.25 and 29±2.35 on day 1st, 3rd, 5th and 7th respectively. While average urea levels in Dox + Nanosponge group were 33.75±0.85, 54±1.08, 54.75 ±1.89, 47.75±1.89 on day 1st, 3rd, 5th and 7th respectively.

Creatinine (mg/dl): Average± SEM levels of creatinine (mg/dl) in different groups of rabbit are given in Table 5

and Figure 13 on different days (post doxorubicin administration). Results indicated that Average creatinine level in doxorubicin alone group was 0.275±0.09, 0.675±0.06, 0.5±0.04, 1.375±0.10 on day 1st, 3rd, 5th and 7th respectively. The creatinine levels in Dox+PLGA NPs group were 0.25±.06, 0.725±0.03, 0.475±0.05 and 0.275±0.05 on day 1st, 3rd, 5th and 7th respectively. While average creatinine levels in Dox+Nanosponge group was 0.5±0.07, 0.775±0.07, 0.925±0.03 and 0.65±0.14 on day 1st, 3rd, 5th and 7th respectively.

Histopathological examination: Hematoxylin and Eosin staining of heart tissues of doxorubicin treated group showed more disorganization of heart muscle fibers and interstitial edema as compared to other groups either doxorubicin and PLGA-NPs treated or doxorubicin and nanosponge treated as shown in Figure 14.

Discussion

Doxorubicin is used in solid tumors, lymphoma and acute leukemia. Unfortunately, clinical use of doxorubicin is limited due to acute and chronic toxicities like myelosuppression, cardiomyopathy, congestive heart failure, development of multidrug resistance and narrow therapeutic window (Joseph *et al*., 2014). In this study, doxorubicin was chosen to reduce its dose-dependent toxicity by nanosponge. Nanosponge can absorb the excess of doxorubicin from blood. Nanosponge was formed with coating of RBCs membrane on PLGA nanoparticles described by (Nguyen *et al.*, 2016). Size distribution of PLGA NPs before the coating was 207.8 while size distribution of nanosponge was increased to 222.6 due to bilayer of RBC membrane on PLGA NPs that is an indication that coating of RBC membrane has been done on PLGA NPs as described by (Nguyen *et al*., 2016). *In vitro* study showed a dose-dependent increase in percent absorption of Dox was observed (Table 2, Figure 5 and 6). The results indicated that about 57.47% of Dox was absorbed by PLGA NPs and 65.49% of Dox was absorbed by nanosponge from a hydrophilic environment. Nanosponge has a relatively higher absorption capacity for Dox as compared to PLGA alone as reported by (Nguyen *et al*., 2016). As Prabathina *et al*. (1974) documented vitamin-E, morin, rutin and quercetin have antioxidant properties and can decrease the chances of doxorubicin-induced cardiomyopathy in rabbits while in our research nanosponge decreased doxorubicin dose-dependent toxicity. Nanosponge can absorb an extra amount of doxorubicin.

Komolafe *et al*. (2013) and Chopra *et al*. (1995) investigated that leaf extract of *Parkia biglobosa* (PBE) and Propolis reduced cardiotoxicity induced by doxorubicin in rats through their antioxidant properties. PBE caused a reduction in levels of serum creatinine kinase-MB, lactate dehydrogenase, glutathione peroxidase and superoxide dismutase. Propolis (bee glue) is rich of flavonoids that have antioxidant properties. In the present research, PLGA NPs caused a significant decrease in elevated CK-MB on the 3rd, 5th and 7th day as compared to doxorubicin alone group. While nanosponge caused further decrease in elevated CK-MB levels in comparison with PLGA NPs group and rabbits in which doxorubicin alone induced cardiac damage (Table 3, Figure 8).

Elberry *et al*. (2010) and Mahesh *et al*. (2013) found that cranberry extract (CRAN) and ethanolic extract of *Boswellia ovalifoliolata* leaf and bark prevented doxorubicin-induced cardiotoxicity by significantly decreasing levels of creatinine phosphokinase (CK), serum lactate dehydrogenase (LDH) and creatinine kinase-MB (CK-MB). In the present research, PLGA NPs caused a significant decrease in elevated LDH levels on 3rd, 5th and 7th day as compared to doxorubicin alone group. While nanosponge caused a further decrease in elevated LDH levels in comparison with PLGA NPs group (Table 3, Figure 9).

Al-zubaidy and Khattab, (2014) documented that Pentoxifylline has the potential to reduce doxorubicin-induced hepatotoxicity in rabbits by significantly decreasing transaminase enzymes (alanine aminotransferase and aspartate aminotransferase). In the present research, PLGA NPs and nanosponge caused no effects in ALT level on 3rd, 5th and 7th day (Table 4 Figure 10). Saad *et al*. (2001) found that deferoxamine has a preventive effect against cardiac, renal and liver toxicity caused by doxorubicin in rats. Biochemical parameters like CK-MB, LDA, ALT, AST levels of rats were changed. In the present research, PLGA NPs and nanosponge did not affect AST level on 3rd, 5th and 7th day (Table 4 and Figure 11).

Ajith *et al*. (2008) documented that aqueous ethanol extract of *Zingiber officinale* has the potential to decrease doxorubicin-induced nephrotoxicity by significantly decreasing creatinine and serum urea level. In the present research, urea (mg/dl) level was increased but PLGA NPs and nanosponge caused no effect (Table 5 and Figure 12).

Elsherbiny and El-Sherbiny, (2014) found that thymoquinone (TQ) had a protective effect against nephrotoxicity induced by doxorubicin. Increased values of creatinine, albumin, serum urea and histopathological examination indicated severe injury to renal tissues. Treatment of animals with TQ significantly decreased renal injury. In the present research, creatinine (mg/dl) level was not significantly increased in doxorubicin-induced cardiotoxicity rabbit group. PLGA NPs and nanosponge caused no effect on creatinine level on 3rd, 5th and 7th day (Table 5 and Figure 13).

Ziêba *et al*. (2003) studied the detoxification potential of Carnosine (CAR) in the cardiotoxicity of Doxorubicin in rabbits. Histopathological evaluation showed smaller injury to the heart muscle in rabbits that administered with doxorubicin and Carnosine as compared to those administered doxorubicin. In recent research, histopathological examination showed nanosponge has a protective effect against cardiotoxicity induced by doxorubicin as shown in Figure 14.

Conclusion: Based on results obtained in the present study it is concluded that nanosponge has the potential to absorb doxorubicin both in *in vitro* and *in vivo* condition. Nanosponge can prevent doxorubicin-induced cardiotoxicity by decreasing CK-MB, LDH and CPK levels. Nanosponge showed smaller damage to the heart muscle and can decrease doxorubicin-induced cardiotoxicity.

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