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ROLE OF ZONTIVITY LOADED BY PLGA COATED BY TPGS IN AMELIORATING INDUCED CARDIOVASCULAR AND PULMONARY DISORDER IN ADULT MALE RATS

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ABSTRACT

This study was aimed to evaluate the role of PLGA nanoparticle that was prepared by two procedures involved nanoprecipitation methods. The study evaluated the effect of the organic phase with different concentration and different type of aqueous phase on particle size and Encapsulation Efficiency (EE), Polydispersity index (PDI) and Loading Efficiency (ID). Our data showed that using of acetone alone as well as when mixed with Dimethyl Sulfoxide (DMSO) and 0.03% TPGS that produced small diameter particle size as well as with suitable ID and EE reached ED to 110 nm, 9.89 %, and 95.9% , respectively.

Fifty adult male rats were divided randomly in to five equal groups (5 animal /each group), the first group (CNTR) was a negative control , the second group (T1) administrated high fat die 2 % with 0.5 % of H_2O_2 as a positive group, third group (T2) was received high fat die 2 % with 0.5 % of H_2O_2 treated with a daily dose of 1.1mg/kg empty nanoparticles, fourth group (T3) administrated high fat die 2 % with 0.5 % of H_2O_2 and treated with a daily dose of 0.38mg/kg of ordinary Vorapaxar, and five group administrated high fat die 2 % with 0.5 % of H_2O_2 and treated with a daily dose of Zontivity Loaded by PLGA at dose of 0.11mg/kg. The experiment was persistent for 10 weeks. *In vivo* study showed that Zontivity loaded PLGA appear has ability to promote antioxidant parameter as SOD and GPX, as well as reduction serum troponin. The data of serum chloride showed a significant reduction in positive control and empty PLGA groups than treated groups.

Moreover, histopathological changes of nanoparticles presented a cardiac hypertrophy and infiltration of inflammatory cells that didn't show any vaculation or necrosis in comparison with other groups, and that may support the histopathological architecture of pulmonary tissue which showed only minor thickening in alveolar septa.

INTRODUCTION

Poly lactic-co-glycolic acid (abbreviated as PLGA), is a copolymer that has been widely studied in the last ten years. PLGA is a biocompatible copolymer and has been recently approved by the Food and Drug Administration (FDA) for use in human medical purposes. It is biodegradable, and its degradation time can be controlled [1]. PLGA can be moulded into roughly any shape, and its surface can be handily adjusted [2].

The highly beneficial properties of PLGA have attracted much attention, especially in the scope of drug delivery and tissue engineering. Surprisingly, rarely have research articles investigated pure PLGA particles or Vitamin E TPGS-coated drugs at the level of atherosclerosis and cardiovascular dysfunction. In fact, D-α-tocopheryl polyethylene glycol succinate (vitamin E TPGS or TPGS) has been approved by FDA as a safe adjuvant and is being used more widely in drug delivery systems because of its high biocompatibility, increased drug solubility, and the fact that it serves to ameliorate drug penetration [3]. PLGA is a non-immunogenic biocompatible polymer and can be biodegraded to exist as non-toxic compounds in nature [2]. However, the hydrophobicity of PLGA and rapidly filtrated in liver and captured by the reticuloendothelial system. These properties can be masterfully evaded with the aid of TPG [4].

American Heart Association (AHA) reported that more than 17 million people died in 2013 due to cardiovascular disease, representing the first aetiology of death in the world [5]. In the United States, cardiovascular disease occupies approximately 31% of all deaths in2013 [5]. The value cost for the treatment of CVD can be as elevated as 200 billion Euro every year. It predictable that by 2030, nearly 23.6 million people will die from CVD worldwide (WHO statistics, 2012).

Zontivity functions as a thrombin receptor antagonist, working against the protease activated receptor PAR-1 to inhibit platelet aggregation without affecting haemostasis [7]. Ungar and coworkers [29] confirmed that treatment with Zontivity was associated with an increased rate of hemorrhagic stroke especially intracranial hemorrhage type. Moreover, Zontivity has many adverse effect such as bloody or black feces, constipation, severe stomach pain and signs of an allergic reaction [8]. The recent studies suggested that high dose of Zontivity lead to iron deficiency anemia via interfere with absorption of calcium from intestine. The aim of this study was designed to prepare an important new drug used in veterinary field to treat one important cardiovascular disease. In addition to Preparation of PLGA nanoparticles loading with Zontivity to overcome the side effects of ordinary Zontivity via reduce the therapeutic dose.

MATERIALS AND METHODS

MATERIALS

PLGA 50:50 (PURASORB PDLG 5010), Vitamin E TPGS NF Grade and Zontivity were obtained as from medical express chemistry (MEC, USA). Nanoparticles were prepared by the nanoprecipitation technique according to Gaonkar,2017 [10] and Jasim et al. (2019) [11] with some modification in the organic phase replace type of solvent. Briefly, an organic solution of PLGA (10 mg) and Zontivity (1 mg) in a acetone (3 ml and 1ml) respectively, then added to an aqueous part of 0.03% vitamin E TPGS solution (20 ml, different w/v) under highly magnification. The solvent was permitted to evaporate overnight. The suspension obtained was filtered (Whatman filter paper 1) to discard any precipitate and centrifuged at 14,000 rpm at 4°C.The free drug in

supernatant part was removed; the pellet obtained was washed 3-4 times with deionized water and lyophilized for two days to get a free-flowing powder. Empty PLGA were prepared according to the same above manner. Stability of PLGA was determine by long-term stability of Zontivity -NPs destined by nanoprecipitation method, and were stored at RT, 4 ± 2 °C for two months. Samples were analyzed, after these periods to determine zeta potential value that was record (−30 ± 2). and particle size as per the already described method. Experiments were performed in triplicates showed that particle size was 110 ± 6.5 nm of nanoparticles prepared by acetone as organic phase while after storage showed at 119.3 ± 7.3 nm fig.(D).

Induction of oxidative stress:

Seventy-two albino male rats were supplied by the animal house of AL –Qasim Green University. Their ages at the start of experiments were 15 weeks, and their weight was 250 -300 grams. In this experiment sixty-six rats were given hydrogen peroxide 0.5% in drinking water and high cholesterol fed diet (1.50% cholesterol) daily for three weeks. In addition, single a dose of triton 10mg/kg were added at end last three weeks of the experiment, the animals of control group were fed with standard laboratory diet the experiment was persistent for four weeks(9-12).The heart and lung tissue were smoothly collected and were put in 10% formalin solution for histopathological study.

Experimental design:

Fifty rats were randomly divided into five equal groups. First group (CONTR): animals in this group received normal saline as negative control. Second group (T1): animals in this group were offer to oxidative stress and treated with distilled water as a positive group. Third group (T2): animals in this group exposed to oxidative stress and treated with a daily dose of 1.1mg/kg empty nanoparticles given orally. Fourth group (T3) rats in this group was offer to oxidative stress and received of 0.38mg/kg of ordinary Zontiviy. Fifth group (T4) rats in this group offer to oxidative stress and received Zontivity loaded with PLGA daily at dose of 0.11mg/kg given orally by stomach tube. Finally, the experiment was carried out for 10 weeks.

RESULTS

Synthesis and characterization of Zontivityloaded PLGA NPs: Table (1) summarized the effective of average particle diameter, drug loading, and encapsulation efficiency of Zontivity-NPs. It can be seen from the figure and the table that nan precipitation technique states formation of Zontivity-NPs with almost narrow particle size distribution (110± 7 to 910 ±68) with poly dispersity index value around (0.26 to 0.005 and favorable zeta potential (−30 ± 2). The average diameter of the obtained NPs evaluated by SEM showed diameter range from 28 to 200 nm. On the other aspect TEM images had a distinct spherical form and mono scatter size distribution of NPs with crystallite size varied from 59 to 200 nm. The free-flowing powder of Zontivity-NPs was getting after lyophilization at 4 °C for for detection particle size assessment. The particle size was negligible elevated (Table 1) around 100 nm with a slightly change in PDI value, whereas this suggested the satisfactory stability of Zontivityloaded PLGA NPs during storage. Thus, utilization of low concentration of emulsifier at a concentration 0.03 %Vitamin E-TPGS in the emulsifying phase led to production of smaller particles range from(264 ±3.7 to 110± 6.5) as well as the higher concentration of emulsifier, In general, the experiments have been appeared that acetone produces smaller and more uniformly sized nanoparticles (264 ±3.7) after using DMSO.

Table 1

Effect of type of organic phase and emulsifier concentration on the polydispersity index (PDI), particle size distribution, encapsulation efficiency (EE) and drug loading (DL) of Zontivity loaded PLGA nanoparticles using solution of different concentrations of aqueous phase of Vitamin E TPGS

Ref	Aqueousphase (TPGS)	Organic phase	Drug: polymer	Particle size (nm)	PDI*	DL %**
a (5-0)	0.03%	DMSO + acetone	1:10	264 ±3.7	0.102	9.89
b (5-1)	0.03%	Acetone	1:10	110± 6.5	0.264	9.74

polydispersity index (PID), drug loading, dimethyl sulfoxide DMSO

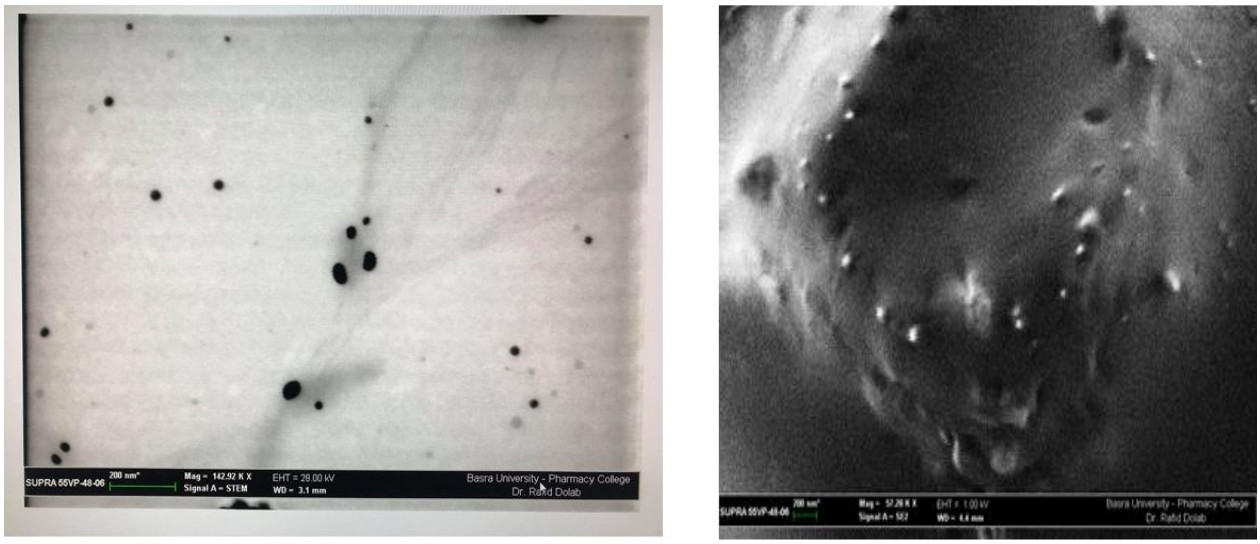

Figure (a), (b) refer to TEM of zontivity nanoparticles, appear ovoid shape PLGA loaded zontivity under SEM at size 200 nm

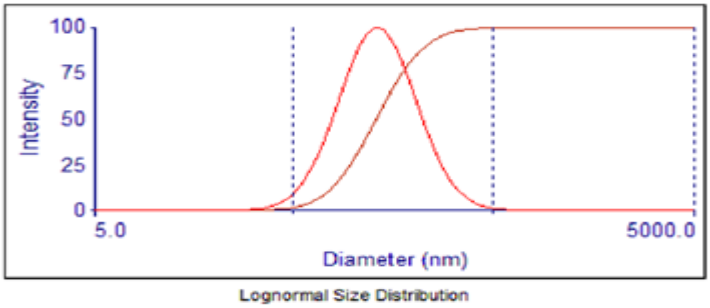

Figure (C) refer to long term stability of PLGA nanoparticles to recorded nano size at 127nm using zeta sizers

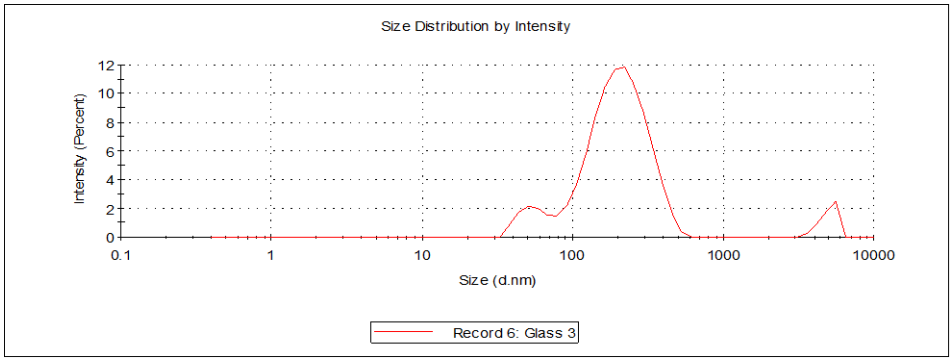

Figure (C) refer to particles sizers of nanoparticles to recorded nanosize at 131nm after loaded by PLGA

Table 2

Effect of Zontivity and PLGA loading Zontivity in electrolyte and antioxidant parameter in male rats high feed diet

Groups Parameters	T1	T2	T3	T4	T5	LSD
Chloride	108.2 ± 2. 1 A	88.4±3.1 C	90.1±3.1 C	99.4±1.7 B	101.1±1.3 B	5.7
Superoxide dismutase pg/ml	1.88±0.66A	0.67±0.14C	0.63±0.11C	1.30±0.19B	1.70±0.15A	0.161
Glutathione peroxidase IU/ml	72.73±5.66A	38.28±7.0C	41.34±4.9C	57.58±2.99aB	63.03±3.15aA	11.72
Highly sensitive troponin I (TNHS)ng/dl	2.75±0.60B	19.47±4.65A	17.91±4.63A	8.82±2.52B	6.045±1.07B	7.60

The value represents mean±SE
N=10 for each group
Different capital letters indicate significant (P<0.05) among groups

The antioxidant data of superoxide dismutase and glutathione peroxidase serum concentration of T1 and T2 were significantly (P<0.05) reduction as compared to the negative control group and other treated group. The long-term administrated with Zontivity at 0.38 mg/kg B.W and PLGA loading Zontivity at 0.11 mg/kg B .W and showed no a significant difference at (p<0.05) in SOD (1.30±0.19 and 1.70±0) and GPX (57.58±2.99 and 63.03±3.15) in mean, respectively as compared with negative control group.

The results of serum troponin I concentration in this study showed there is no significant difference (p<0.05) among the following treated groups (T3, T4 and control group T1), in mean values (8.82±2.5, 6.045±1.07 and 2.75±0.60) respectively. Whereas there is a significant increase (p<0.05) in serum troponin I concentration of untreated groups (T2, T3) when compared to all other groups in mean values (19.47±4.65 and17.91±4.63) respectively. As explained in table (2).

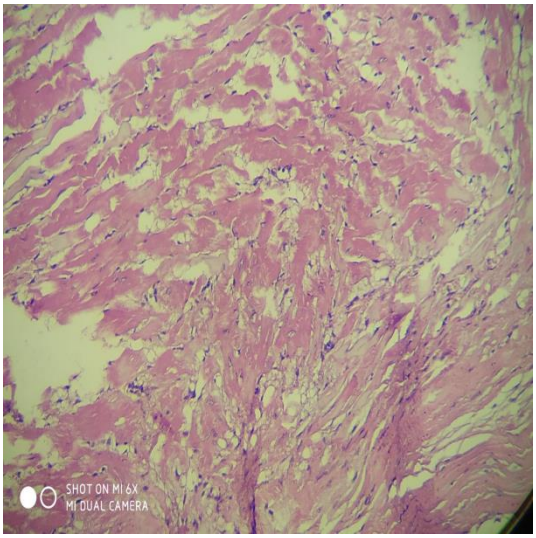

Figure 1. Histopathological change in the section of the heart of control positive groups show necrosis with infiltration inflammatory cells with vacuolation within cardiac muscles fibers (H & E stain, X20).

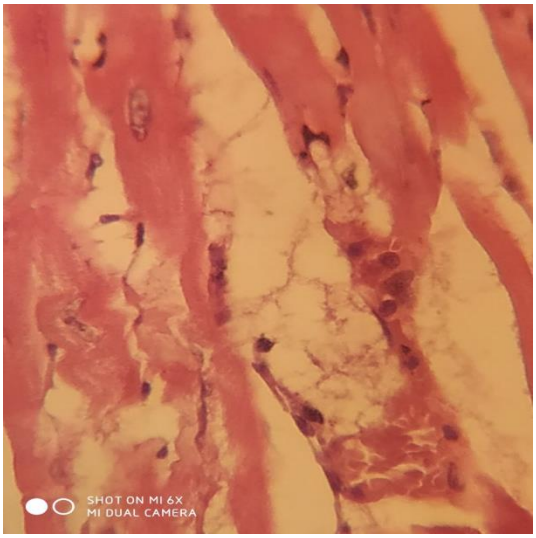

Figure 2: Histopathological change in the section of the heart group treated with empty nanoparticles show cardiac hypertrophy, vaculation and infiltration inflammatory cells loss myofibers (H & E stain, x40).

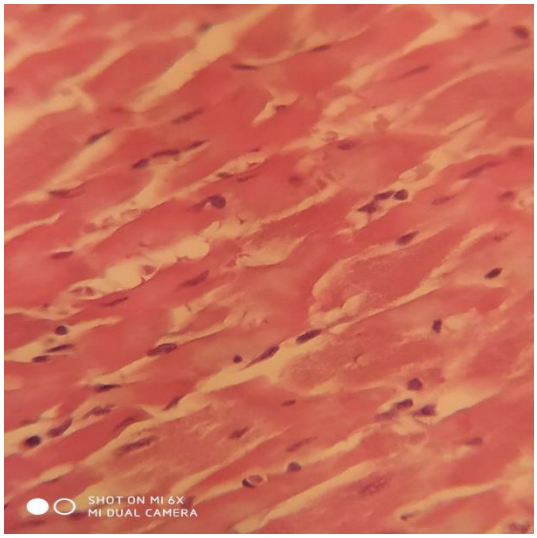

Fig 3. Histopathological change in the section of the hart group treated with Zontivity show cardiac hypertrophy and variations in myocyte size, infiltration inflammatory cells with vacuolation (H & E stain, stain, X40).

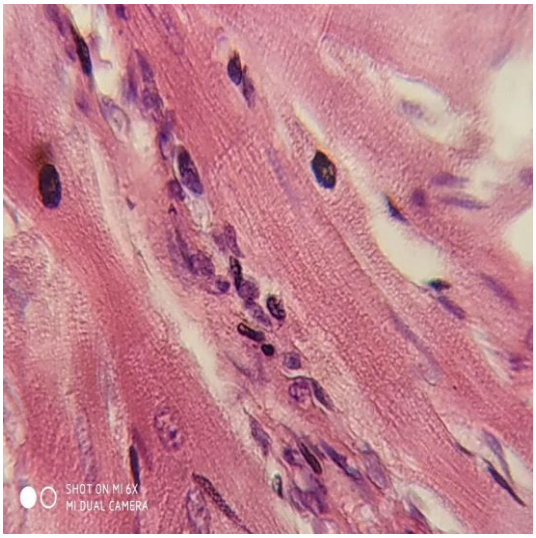

Fig 4. Histopathological change in the section of the heart group treated with empty nanoparticles show cardiac hypertrophy and infiltration inflammatory cells (H & E stain, x40).

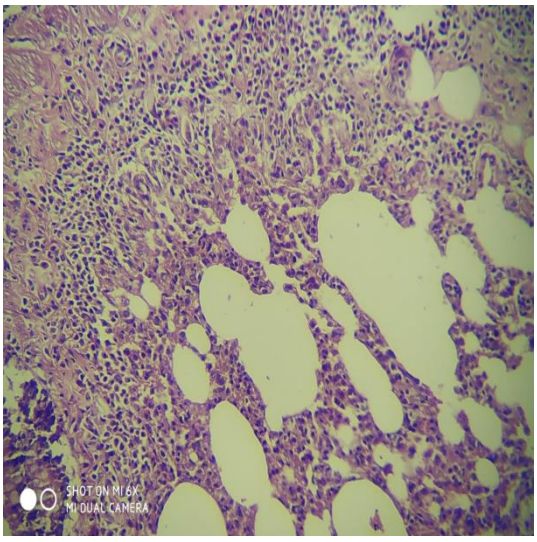

Figure 5: Histopathological change in the section of the lung section of the lung of control positive show infiltration inflammatory cells and deposition of protein material with congestion of blood vessels (hematoxylin-eosin stain, x20).

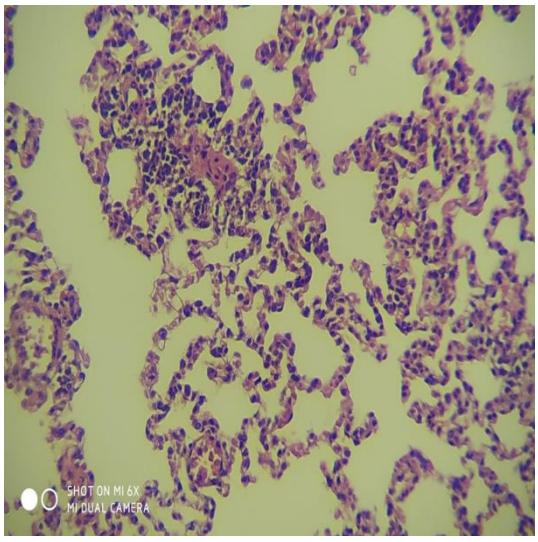

Figure 6: Histopathological change in the empty nanoparticles show lymphoid tissue hyperplasia of peribronchiolar diffuse cellular infiltrate within the interstitial (H&E stain, X20).

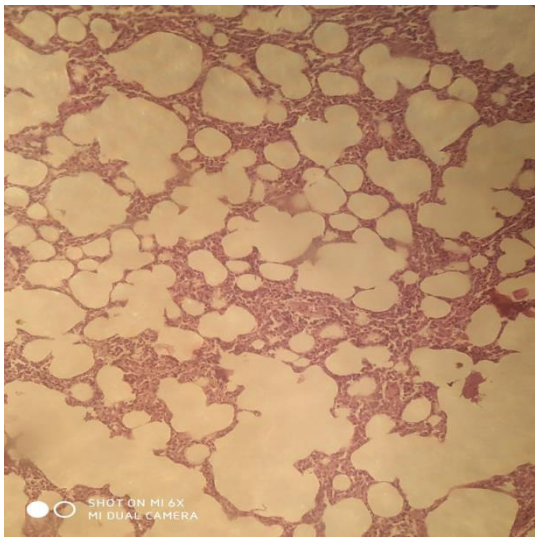

Figure 7: Histopathological change in the section of the lung group treated with zontivity show infiltration inflammatory cells and alveolar emphysema with thickness alveolar septa (H &E stain, X20).

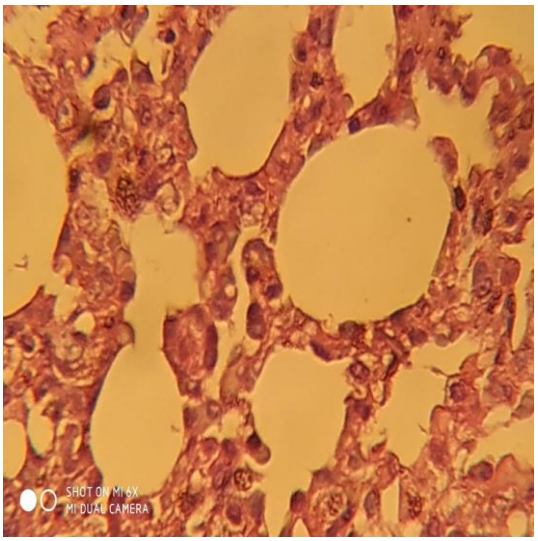

Figure 8: Histopathological change in the section of the lung group treated with nanoparticles show thickness alveolar septa with infiltration inflammatory cells (H&E stain, X40).

DISCUSSION

The nanoprecipitation technique produced nanoparticles (NPs) of approximately 110 nm in size, which is optimal for the ability of NPs to be used for oral administration and passive targeting to the aorta; this result was in an agreement with [12].The present study and others have used a variety of emulsifying and stabilizing agents, including vitamin E-TPGS9 and poloxamers[1].Our study used a 1:10 a drug-polymer ratio , this result is agreement with result reported by Gaonkar*et al.*[10] who demonstrated that a 1:10 ratio is optimal for a PLGA delivery system for Garcinol loading drug rather than 1:20, and the percentages of DL and EE were more significant for a drug-polymer ratio of 1:10. The size of NPs had a direct influence on mechanisms of particle internalization by cells, as well as their distribution in tissue, which can be resulted in dramatic differences in delivery effectiveness[13]. Delivery of small NPs might also be facilitated passive targeting of vessels via the enhancing permeation and retention effect as well as reducing drug administration frequency [14]. In present study the nanoprecipitation technique produced a drug encapsulation efficiency varies widely depending on the properties of the specific drug, the size of the particle, and the emulsifier.

The present study showed that concentration of SOD and GPX were more markedly high in T4 that long-term administrated with Vorapaxar at 0.38 mg/kg B.W. and T5 that received the PLGA loading zontivity at 0.11 mg/kg B.W. This result can be attributed to antioxidant activity of Vorapaxar and was in agreement with [11] who reported that Zontivity has reduce DPHH oxidative stress in vitro and recorded scavenger activity near adcorbic acid acted as standard antioxidant. Furthermore, Zontivity acted as a potent inhibition of platelet. Thus, that inhibition of platelet may become synergism of antioxidant that also confirmed in Zontivity in vitro study [11].

Moreover[2] confirmed GPx activity potentiates the inhibition of platelet activation by nitric oxide in healthy contributor. A case report study arterial thrombosis set reduction in levels of plasma GPx activity, an increased scale of H2O2, and mitigation of platelet inhibitory response to NO. As well as deficiency antioxidant GPx-3 related with a prothrombotic state and dysfunction of vascular special thickening of the aorta that encourage platelet-dependent arterial thrombosis [15]. Many of reports clarify the importance of this enzyme, antioxidant in plasma about platelet activity arrange, platelet-dependent thrombosis, vascular thrombotic propensity and endothelial function [4,16]. The present study was an agrees with [28] that reported that the efficacy of liver antioxidant enzymes GPX, glutathione reductase (GR) and GST recorded a significant reduction in atherosclerosis induced rats when compared to ordinary rats.

The results of serum troponin I concentration in this study showed there is no significant elevation in both treated (T3, T4) groups as compared with control group. Our result confirmed there is positive relationship between baseline levels of serum troponin and cardiovascular defect. These data was come with an agreement with [17] that reported that elevated serum levels of cardiac troponin I and inflammatory biomarkers celebrated different aspects of unstable coronary artery disease early in the course of non-ST elevation acute myocardial infarction. Some clinical studies showed there is a significant relation between the level of troponin and the existence of atherosclerotic injury to the coronary arteries[18,19]. Eisen*et al.*[20] reported that animals with stable atherosclerosis,taken Zontivity showed

absolute improvement in net clinical outcome. Although high-risk animals with prior MI fixed by elevation hs-TnI had before received zontivity as well as an essential, absolute ameliorate after treatment with Zontivity [20]. Our result revealed that Zontivity nanoparticle appeared clear, prominent at the level of serum troponine I, that considered gold parameter for evaluating efficacy of cardiovascular system. Novel antiplatelete Zontivity, that only ready PAR-1 antagonist, may be supplied clinicians with an effective treatment for patients with strong risk factors, such as diabetes and PAD [21].

The present study of histopathological section of heart appear change in control positive groups show necrosis with infiltration inflammatory cells with vacuolation within cardiac muscles fibers while nanoparticles showed clear improvement in the histological section with minor inflammatory response.Jasim*et al.*,[11] reported that animal received zontivity at dose 0.38 mg/kg showed mild fatty degeneration with presence of radially arrangement of hepatocyte around of central vein, some hepatocyte showed normal with hexagonal chain , Moreover hereported that may be due to zontivity had antioxidant activity that confirmed in vitro and in vivo through return the antioxidant enzyme to a value near from control such as anti-inflammatory cytokines (IL10), SOD and GPX. the same result recorded by [22] when they confirmed that PAR1 exerted a pro inflammatory turn in colitis in both humans and animal by elevating a Th17-type immune response, potentially by supporting the output of IL-23. The use of vitamin E-TPGS offers several possible advantages by improved drug stability, emulsification and encapsulation efficiency [23]. Other groups have reported utilized of Vitamin E-TPGS, including the inhibition of P-gp, a transmembrane efflux protein, which is known to has role in drug resistance by shuttling drugs out of target cells [23]. Our study was in agreement with recent study noted that PAR-1 expression is elevated in upon diabetes. A stimulation of PAR-1 in mesangial cells induced their proliferation and leads to the production of extracellular matrix [24]. Waasdorp*et al.* [24] confirmed that Vorapaxar treated diabetic mice diminished albuminuria and mesangial expansion, thus prevented deposition glomerular fibronectin, as well as prevented kidney damage with return the level of glucose to normal limits. Finally, recent the modern studies reported that Vorapaxar had the ability to potent inhibit PAR-1 and prevented the bleomycin induce pulmonary fibrosis [26]. The histopathological change in the section of the lung group treated with nanoparticles show thickness alveolar septa with infiltration inflammatory cells, the outcome was perfect rather than lymphoid tissue hyperplasia of peribronchiolar diffuse cellular infiltrate within the interstitial in the rats received only empty nanoparticles [27]. Finally, recent the modern studies reported that Vorapaxar had the ability to potent inhibit PAR-1 and prevented the bleomycin induce pulmonary fibrosis [25].

CONCLUSION

From this study concluded the concentration (0.03%) of Vitamin E -TPGS consider optimal as a stabilizer and coating PLGA. Using TPGS- PLGA loading Zontivity leads to reduce particle size especially with organic phase acetone rather than DMSO. In vivo study showed that Zontivity loaded PLGA appears improvement on antioxidant status to rats, as well as improve function and tissue repair of the cardiopulmonary system through reducing cardiomyopathy and alveolar damage induced by oxidative stress.

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