The Efficacy of Dextran 70 on Reticuloendothelial System in Systemically Infected Mice with Klebsiella Pneumoniae

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ABSTRACT:

BACKGROUND:

The reticuloendothelial system (RES) play an important role in immunity against bacterial infection and *Klebsiella pneumonia*e one of the most common causes of hospital-acquired infections. Dextran70 (D70), a polysaccharide, may alter functions of this system through changing many biological activities in the tissues.

OBJECTIVE:

This study focuses on the prophylactic effects of D70 on RES in systemically challenged mice with *K. pneumonia*e which has been isolated from urine sample of patient with urinary tract infection.

METHODS:

Four groups of adult white mice were intravenously injected with *K.pneumoniae*. Three groups of them was preinjected intraperitonealy for three successive days with 0.5 , 1, and 2 mg D70/100 g body weight, and the fourth group was challenged only as a control group. The ratio of liver and spleen weights to body weight was calculated and histological sections for heart, lung, and kidney were examined after 24 and 48 hours from challenge in all groups.

RESULT:

The results revealed that an increasing in the ratio of liver weights to body weight in control group, while there is slight increase in the three groups treated with D70. The ratio of spleen weights to body weight was more increasing after 24 hours in both 0.5 and 2 mg D70/100 g body weight of treated groups. The histological study demonstrated suppurative lesions and abscesses in the heart, lung, and kidney due to *K. pneumoniae* infection in the control group, while pretreated mice with D70 have clearly demonstrated less pathological changes and more integrity of the tissues comparative to control group.

CONCLUSION:

This study confirmed that D70 has a protective role and may be prophylactic effects against systemic bacterial infections.

KEY WARDS: Dextran – prophylactic effects - *Klebsiella pneumoniae* – Infection- Immunomodulator.

INTRODUCTION:

*Klebsiella pneumonia*e, one of the most causative agent cause hospital-acquired infections. It causes urinary tract infection, bacterial pneumonia, and bacteremia with focal lesions in debilitated patients [1]. It produces many enzymes causing sever damage to tissues [2].

Dextran70, D70 (Mo. Wt. 70 000), is a polysaccharide given intravenously during or just after surgery to reduce the incidence of postoperative venous thrombosis. It alters platelet function and prolong the bleeding time [3]. It is a potential therapeutic agent for cerebral ischemia because it increases local cerebral blood flow [4]. It causes inhibition of many bacterial infection nonspecifically due to blocked attachment of bacteria to epithelial cells in respiratory tract, e.g. *S. aureus*, Group A streptococci, *H. influenzae*, and *P. aeruginosa* [5,6]. Administration of the polymyxinB-dextran70 conjugate causes significant protection against endotoxemia, in reduced toxicity of polymyxinB but retention of its endotoxin-nutralizing ability [7]. PolymyxinB-dextran70 conjugate resulted a significant amelioration of sepsis [8]. Dextran an inexpensive and nontoxic agent and may be useful in patients with cystic fibrosis to prevent colonization and infection with *P. aeruginosa* [5].

The present study focuses on the effectiveness of dextran70 on reticuloendothelial system to protect tissues against the systemic infection. Pretreated mice with several doses of dextran70 were challenged intravenously with *K. pneumoniae* that has been isolated from urine sample of patient with urinary tract infection.

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MATERIALS AND METHODS:

●**Dextran 70 (D70):** a stock solution of dextran 70(BDH) was prepared in sterile distilled water. Three different doses of D70 (0.5, 1, and 2 mg/100g body weight of mice) were injected intraperitonealy at three successive days prior to bacterial challenge to each dose.

●**Bacterial challenge:** *K. pneumoniae* isolated from urine sample of patient with urinary tract infection was employed in this study. Bacterial suspension diluted in sterile saline to provide 10^4 viable cell/0.1 ml saline [9]. This suspension was injected intravenously with 0.1 ml/mouse after two days from the last dextran injection.

●**Mice:** Twenty adult white mice (18-20 g body weight) were breeding in a relatively controlled environment at room temperature. The animals were divided into equal five groups. Four groups of them pretreated with (0.5, 1, and 2) mg D70 per 100 g body weight for three successive days, after two days from the last injection, the mice challenged intravenously with *K. pneumoniae*. The fifth group of mice kept without infection as a control group. Half of all groups were dissected after 24 h and the remaining half was dissected after 48 h from bacterial challenge. The ratio of liver and spleen weights (mg) to the body weight (g) for all mice groups were calculated.

●**Histological Examination:** Samples of heart, lung, and kidney were taken from all mice groups and sections from paraffin block of these organs were prepared and stained with hematoxylin-eosin stain [10], and examined under high power light microscopy.

●**Statistical Analysis:** was carried out using F-test [11].

RESULTS:

The ratio of liver weights to body of infected mice (control group) show significant increase, especially after 48 h and slight increase in pretreated mice with D70 (Fig .1).

The ratio of spleen weight to body weight shows different responses from the liver especially in mice groups pretreated with D70, there are reversible effect of D70 in both pretreated mice groups 0.5 and 2 mg D70/100 g body weight (Fig .2).

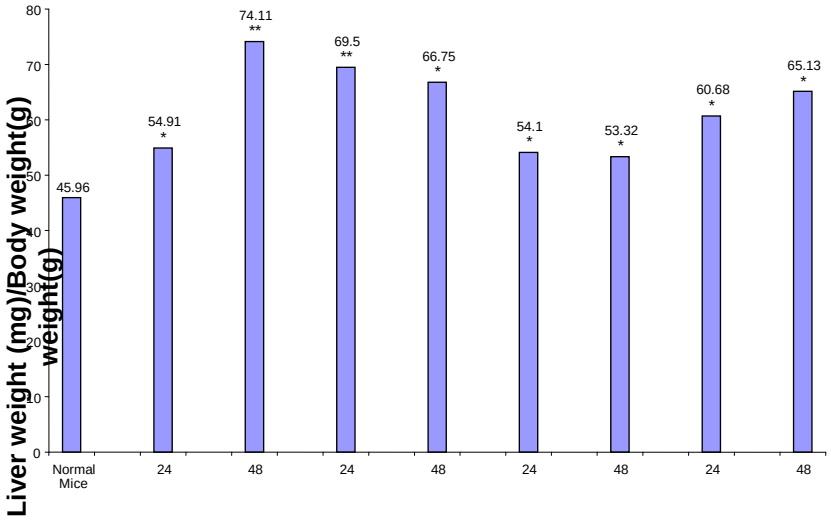


Fig .1: The ratio of liver weight (mg) to body weight (g) of infected mice i.v. with *K. pneumoniae* and pretreated i.p. with (0.5, 1, and 2) mg D70/100 g body weight. *: **Significant; p< 0.05.**

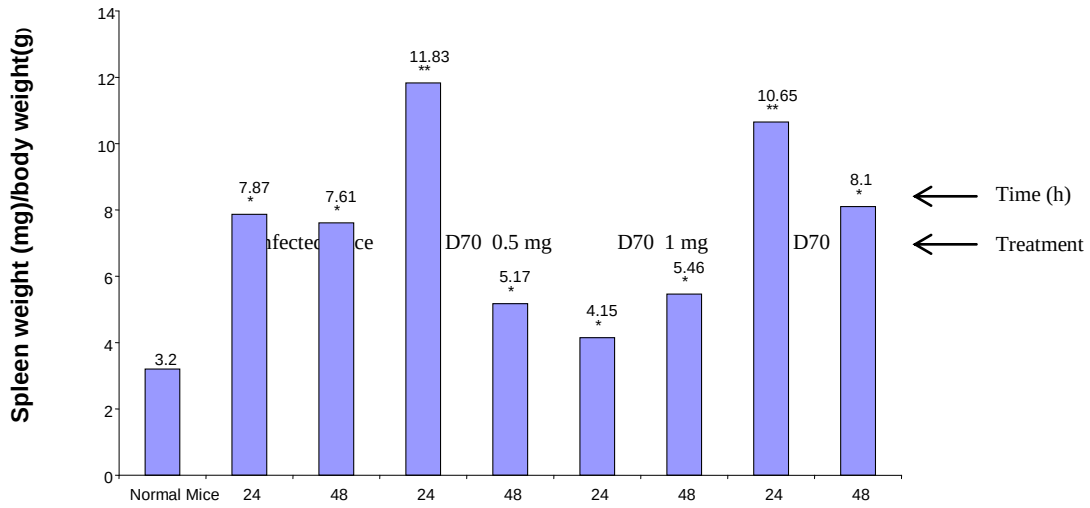
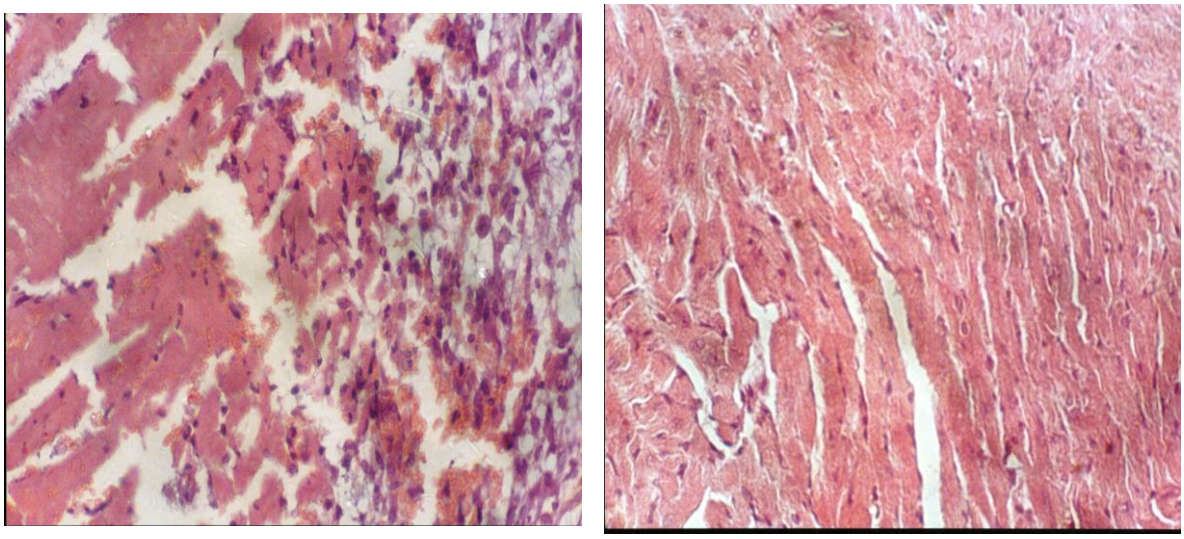


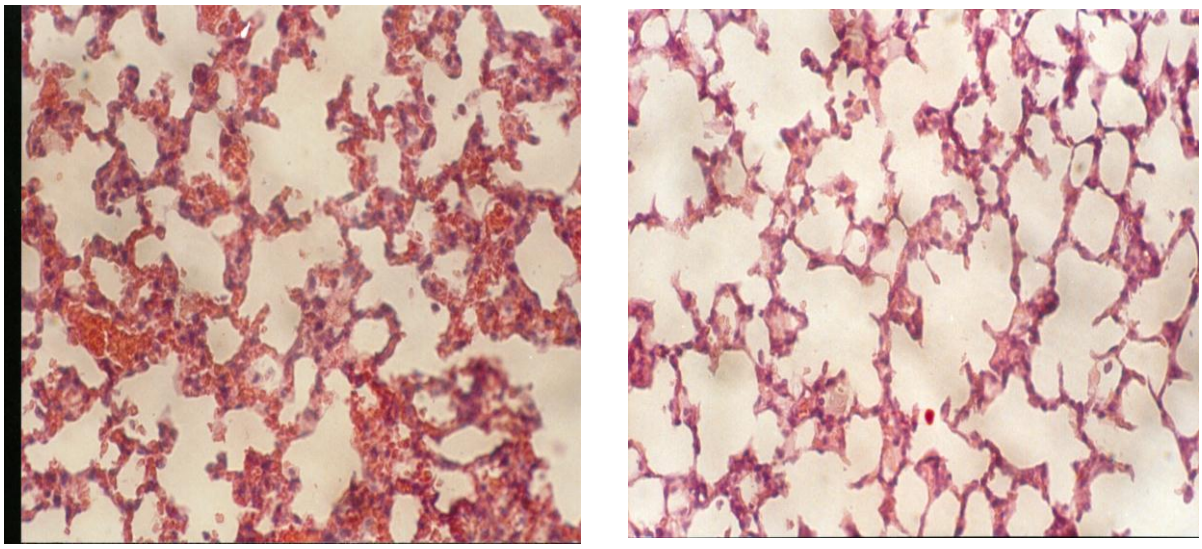
Fig .2: The ratio of spleen weight (mg) to body weight (g) of infected mice i.v. with *K. pneumoniae* and pretreated i.p. with (0.5, 1, and 2) mg D70/100 g body weight. *: Significant; p< 0.05.

The histological examination of heart, lung, and kidney showed that systemically infected mice with *K. pneumoniae* after 24 and 48 h suffered marked pathological changes. The heart sections showed suppurative myocarditis and microabscesses (Fig 3-A). A diffuse acute inflammation with congestion of small blood vessels and hemorrhage were seen in the lung sections (Fig .4-A). The kidney showed acute inflammatory changes with enlargement of glomeruli and presence of pus cells in the renal tubules (Fig .5-A). The heart, lung, and kidney sections from pretreated mice with D70 revealed less or no histopathological changes with more integrity of the interstitial tissues comparative to control group (Fig .3-B, Fig .4-B, and Fig .5-B).



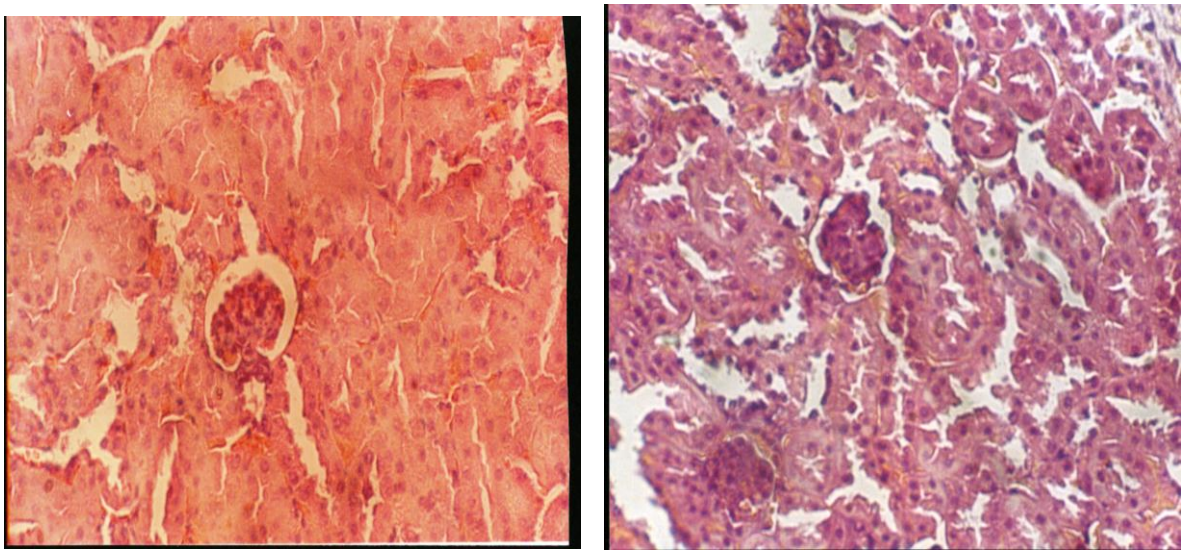
(A) **(B)**

Fig .3: Heart sections of: (A), infected mice after 48 h i.v. challenge with *K. pneumoniae* ;(B), pretreated mice i.p. with 2 mg D70/100 g body weight. Hematoxylin-eosin stain, 200X.



(A) **(B)**

Fig .4: Lung sections of: (A), infected mice after 48 h i.v. challenge with *K. pneumoniae*; (B), pretreated mice i.p. with 2 mg D70/100 g body weight. Hematoxylin-eosin stain, 200X.



(A) **(B)**

Fig .5: Kidney sections of: (A), infected mice after 48 h i.v. challenge with *K. pneumoniae*; (B), pretreated mice i.p. with 2 mg D70/100 g body weight. Hematoxylin-eosin stain, 200X.

Histopathological changes in mice tissues of the control group that has been challenged i.v. with *K. pneumoniae* after 48 h more sever than at 24 h, while prior i.p. injection mice with D70 has significantly ameliorated the histopathological changed observed (Table .1). It has been noted that the three doses of D70 cause less histopathological changes, but the treatment with 1 mg D70/100 g body weight is more effective in reducing the severity of these changes than the other treatment.

Table .1: Histopathological changes score of some reticuloendothelial organs in infected mice i.v. with *K. pneumoniae* and pretreated i.p. with (0.5, 1, and 2) mg D70/100 g body weight.

Treatment mice groups	Heart		Lung		Kidney	
	24 h	48 h	24 h	48 h	24 h	48 h
-Control (*K.pneumoniae*)	++	+++	++	+++	++	+++
-D70 0.5 mg/100 g body weight	++	+	++	+	++	+
-D70 1 mg/100 g body weight	+	-	+	-	+	-
-D70 2 mg/100 g body weight	++	+	++	+	++	+

-: No pathological changes; +: Mild changes; ++: Moderate changes; +++: Sever changes.

DISCUSSION :

The reticuloendothelial system plays an important role in removing bacteria, toxins, and immuncomplexes from the circulation [12].liver, spleen, and kidney act alike, as bacterial filters [13], through phagocytosis by fixed-tissue macrophages [14].This may explain the obvious heptosplenomegaly in control group mice compared to other groups and which may be due to inflammatory responses to *K.pneumoniae* infection. These pathological effects were less in D70 pretreated mice groups which may be the results of increase in neutrophil oxidative burst activity caused by D70 [15], and caused transient changes in the function of reticuloendothelial system with increased number of granulocytes [16], these changes may help in clearness of bacteria, especially the D_{70} itself prevent bacterial colonization and infection[5] .

Previous studies have shown that dextrans strongly inhibited *Staphylococcus epidermidis* and can efficiently block bacterial attachment [17], also can eradicated *Helicobacter pylori* load in mice [18]. So in this study D70 was used as a prophylactic agent for systemic bacterial infection.

D70 is compatible with many solutions and drugs reportedly has no drug interactions that are clinically significant, also can be degraded by dextranase in the spleen then metabolized to carbon dioxide and water [19]. Morever D70 has been used in hypertonic saline as an effective treatment for septic shock secondary to pyometra in dogs [20], and in prevention of hemorrhagic shock-induced leukocyte-endothelium adherence [21]. Hypertonic saline with D70 provided the fastest method in reversing the effects of hyperkalemia [22] and may be of value in treating critically ill septic patients [23], especially it has advantages on homodynamic and safety [24]. So it used for treating hypovolumic shock [25], and in resuscitation following traumatic injury [26]. Also it improves survival in early phase of porcine endotoxin shock [27].

D70 can be used with iron for treatment iron-deficiency anemia it is efficacious and safe [28,29], without any adverse reactions [30]. Many studies have shown the role of dextran sulfate to prevent viral infections [31,32] even HIV infection [33], bacterial infections [5,6,17,34], and parasitic infection [35].

Histological examination of heart sections revealed the efficacy of D70 in protecting the heart tissues against systemic bacterial infections in mice. This effect may be in part due to muscle regeneration which enhanced *in vivo* by treatment with dextran [36],normalization of the hemocrit and restoration of the cardiac index [37]. This study also shows that D70 prevent lung damage may be, as one of reasons, due to decreases inflammatory cytokine responses to subsequent pneumonia-related sepsis [38], stimulation of immune functions of lung epithelial tissue [6], and metabolism, thereby reducing reperfusion injury of the lung [39].

Histological examination of kidney sections revealed the role of D70 in protecting kidney from damaging through the bacterial infection may be, at least, because dextran improve healing by promoting cell adhesion and encouraging proliferation in wound [40], and increasing wound strength by stimulation of cell to produce growth factors [41], also prevent bacterial overgrowth and translocation by maintaining vascular endothelial barrier integrity [42]. For all reasons mentioned previously, D70 have clearly demonstrated less pathological effects when used against bacterial infection and causes more integrity of the reticuloendothelial system tissues than control mice group .

CONCLUSION:

Our observations confirmed the prophylactic effects of D70 on reticuloendothelial system against systemic *K. pneumoniae* infection in animal model. This prophylactic agent may act as a immunomodulator in bacterial infections and may depends on dose and duration of application. So the possibility of D70 as a prophylactic and therapeutic agent for human against serious diseases may need more and extensive studies, especially D70 is safe and used in many medical applications.

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