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The use of *Bacillus cereus* Phospholipase C in Prophylaxis and Treatment of Thromboplastin Induced Thrombosis in Mice

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Abstract

Volumes of crude cell free filtrate and steps of partial purified phospholipase C were prepared from non-toxigenic *Bacillus cereus* isolate. *Also* concentrations of highly purified preparation (ICN) was used. Prophylaxis against greater than LD_{50} thromboplastin (0.0612mg/mouse) revealed recovery of mice with 97%, 100% and 97% for cell free filtrate, haemolysin cured & sephadex eluted phospholipase C respectively with no significant differences among injection time intervals while the pure preparation recovered 97% mice with a significant differences among time intervals. Treatment by post thrombosis induction revealed that cell free filtrate, partial purification steps and the highly purified phospholipase C recovered 98% mice with no significant differences among injection time intervals .The haematological parameters PCV, RBC count and platelet count remained within normal range after 12 hours of injection. These results suggested the possibility of using partially purified & highly purified phospholipase C as prophylactic agent against infarction in man.

Keywords: *B. cereus*, thrombosis, thromboplastin, prophylaxis.

Introduction

Blood coagulation is the process by which fibrin strands create a meshwork that cements blood components together [1]. Several therapeutic agents are being used to prevent thrombus formation. Aspirin was used to prevent arterial thrombi [2]. Warfarin, an oral anticoagulant, acts by decreasing prothrombin time [3]. Heparin is a thrombin inhibitor [4]. Tissue plasminogen activator, a thrombolytic drug which converts plasminogen to plasmin [5]. Urokinase, prepared from human kidney cells, it is now an effective thrombolytic drug [6]. Sterptokinase induced both localized and systemic proteolytic effects since fibrinolysis, fibrinogenolysis, degradation of plasmin-sensitive coagulation factors and proteolysis of other proteins can be observed [7]. *Staphylococcus aureus* staphylokinase was suggested to be therapeutic [8]. The fibrinolytic enzyme fibrolase from copperhead venom was a new fibrinolytic drug used [9]; it has been shown to degrade fibrin clots [10]. Tissue thromboplastin (tissue factor- TF- or factor III) is the most potent physiological trigger of blood coagulation known; it is a membrane glycoprotein surrounded by phospholipids that are essential for its procoagulant activity [11]. Nemerson [12] found that removal of 95% of the tissue factor phospholipid resulted in a loss of 98% of its biological activity. After major surgery and trauma, thromboplastin may be detected in the general circulation [13], and may be responsible for the thromboembolic episodes following such event [14].It has been shown that intravenous infusion of

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In rats, a marked activation of factor VII and a fall in the activities of factors V, VIII and thrombocytes count were observed; the main changes during the first 15 min. after injection being observed in the lungs were fibrin – containing thrombi and platelet aggregates [15,16]. In recent years, the ability of tissue factor to promote the activation of factor VII by factor VIIa has been investigated using both purified and cell- associated TF [17,18]. *Bacillus cereus* produces several types of phospholipase C specific toward phospholipids, these are phosphatidylcholine (PC), phosphatidylethanolamin (PE) and phosphatidyserine (PS) all good substrate for it [19,20]. Phosphatidylcholin specific phospholipase C (EC. 3.1 .4.3) is characterized by the site of cleavage of phosphoilpid to diglyceride and phosphorylcholin [21]. On solid media the production of phosphatidylcholin hydrolyzing enzyme has often been detected as a zone of opalescence surrounding colonies grown on an egg-yolk emulsion supplemented agar [22]. *B. cereus* phosphatidycholin specific phospholipase C retained the phospholipase C, but showed marked reduced sphingomyelinase, haemolytic, and lethal activities [23]. Phospholipase C led to an increase in the secretion of the granule enzyme lysozyme from human neutrophils. Stimulation of neutrophile degranulation is a potential contributing factor for tissue damage in infection caused by *B. cereus* phospholipase C [24]. Gollube [25] first reported that *B. cereus* phospholipase C protected rabbit against intravenous infusion of human thromboplastin. Otnaess *et al.* [26] showed that exposure of thromboplastin to phospholipase C of *Bacillus cereus* resulted in rapid loss of the thromboplastin procoagulant activity caused by hydrolysis of the essential phospholipids. Infusion of purified tissue thromboplastin in rats causes the accumulation of fibrin and platelets in the lungs and produce marked changes in the platelet count and in the coagulation factors V, VII and VIII. Intravenous administration of purified phospholipase C effectively prevents all these changes and protected rats against otherwise lethal doses of tissue thromboplastine [15,16]. Phospholipase C was found to protect mice against abortion and intrauterine fetal death induced by endotoxin (Lipopolysaccharide) caused fibrinogen accumulation, thrombosis and heamorrhage in the placental tissue then fetal death [27]. Rat, mouse, bovine and acetone powdered rabbit brain preparations of thromboplastin are more sensitive to attack by phospholipase C than human, sheep and standard rabbit preparations [28]. Highly purified phospholipase C from *B. cereus* reduces factor VII activity in pregnant women to the level similar to those in plasma from non-pregnant women [29,14]. The infusion of phospholipase C dose between 40-50mg/kg/hr inhibited most of the post-traumatic pulmonary microembolism in pig [30]. This study is a part of an investigation the possibility of using *B. cereus* phosphoplipase C as a prophylactic or therapeutic agent in thrombosis and intravascular coagulation induced by human tissue thromboplastin in mice.

Materials & Methods

Strains: Local soil non toxic isolate of *B. cereus* (isolate no.17) obtained by a previous study [31], this strain is negative to enterotoxicity, lethal factor, skin redness, skin necrosis, odema tests but with superproduction of phospholipase

C. phospholipase C: obtained as crude, partially purified and highly purified forms. Crude preparation was obtained as cell free filtrate in Brain-Heart infusion broth [32]. Partially purified was obtained as two steps of purification, the first was heamolysin- cured by

cholesterol cell free filtrate [33] and the next step obtained by filtration in sephadex G100 and dialysis against tris-HCl [33]. Pure phospholipase C was obtained by (ICN Sweeden) with activity of 50 Units/mg. Determination of total protein: achieved depending on biuret method [34]. Thromboplastin: (Biomerex).

Mice: albino mice (*Mus musculas*) were used as model for thrombosis induction (range weight 30-33gm). Phospholipase C activity: Cell free filtrate, different purification steps of partially purified and highly purified Phospholipase C was tested for lecithinase activity. Using plate method on Egg-Yolk salt agar, the mean radii of opalescence in millimeters around pores impregnated by test solutions was measured [35, 36]. Also it was tested turbidometrically at absorption spectrum 600nm [37]. Determination of thromboplastin lethal dose (LD_{50}): Human thromboplastin powder was prepared as mentioned by manufactured company. Different thrmboplastin preparations of 0.2, 0.4, 0.5, 0.6 and 0.7ml (each preparation for a single mouse) were applied with 10 duplicates were injected intravenously; the preparation doses were 0.0175, 0.035, 0.0437, 0.0525 and 0.0612mg thromboplastin /mouse respectively. LD_{50} was calculated as the dose, which kills 50% of injected mice. Prophylaxis protocol: This protocol included the prophylaxis by injection before the induction of thromboplastin-mediated thrombosis. A: Different volumes of chosen cell-free filtrate, 0.1, 0.25, 0.5, 0.75 and 1.0 ml were injected intravenously (each preparation for a single mouse with 10 duplicates). After three intervals (0, 5, 10 min.) an 0.7ml (a dose represents the lethal dose of thromboplatin) preparation was injected intravenously to each mouse of 10 duplications .The results were interpreted as the mean dead mice % (the residual mice recovered represented the % recovery). B: By the same method, preparations of 0.05, 0.1, 0.25, 0.5 and 0.75ml partially purified phospholipas C (Haemolysin-cured cell-free filtrate). C: By the same method, and the same preparations of partially purified phospholipase C (sephadex elution). D: By the same method, preparations of 0.005,0.01, 0.02, 0.03 and 0.04ml highly purified phospholipaseC. Treatment protocol: This protocol involved the prophylaxis by cell-free filtrate, partially purified and highly purified phospholipase C after induction of thrombosis. Doses of thromboplastin, phospholipas C preparations and the time intervals were the same as in prophylaxis protocol. Some blood parameters included packed cell volume PCV [38], red blood cell count [39], platelet count [40] were achieved to assay the healthiness of survivors after prophylaxis and treatment.

Results

The partial purification was started with 1250ml cell free filtrate. Phospholipase C activity showed 24, 23, 24 and 21 mm opacity zones for crude, haemolysin-cured, dialysis and sephadex eluted samples respectively. Turbidometrically, they showed activity with 0.88, 0.86, 0.87, 0.69 absorption for purification steps respectively while the pure phospholipase C showed 28mm-opacity zone and absorbency 1.12 at 600nm.Only crude preparation showed haemolysin activity (table1). The sephadex eluted samples showed higher peak at fraction 14, each fraction of 3ml (figure,1) The lethal dose (LD_{50}) of thromboplastin was 0.4 ml containing 0.035 mg human origin tissue thromboplastin (table 2), which found to make mice died. It was found that mice died within a period of 1-15min

Table (1): Partial purification steps of phospholipase C for *Bacillus cereus* (isolate No.17) cell-free filtrate. *

N0	Steps	Volume (ml)	Opacity zone PLC mm.	PLC Activity A_{600}	Total protein mg/dl	Haemolys in activity A_{540}
1	Cell-free filtrate	1250	24	0.88	347	0.12
2	Haemolysin cured cell free filtrate	1000	23	0.86	289	0.0
3	Dialysis	150	24	0.87	43.1	0.0
4	Column chromatography	15	21	0.69	5.19	0.0
5	Highly purified	-	28mm	1.12	-	-

*** The highly purified ready supplied preparation was included for comparison.**

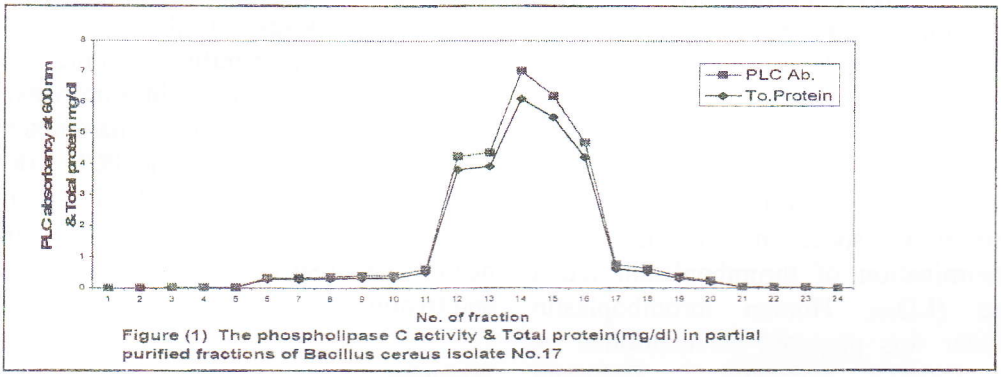

Figure (1) The phospholipase C activity & Total protein(mg/dl) in partial purified fractions of Bacillus cereus isolate No.17

Table (2): The LD_{50} test for human tissue Thromboplastin in mice.

Thromboplastin injected (ml/mouse)	Thromboplastin Dose (mg/mouse)	No. of mice tested	Dead animal %	Survivors %
0.1	o.oo87	10	0	100
0.2	0.017	10	0	100
0.3	0.026	10	20	80
0.4	0.035	10	50	50
0.5	0.043	10	70	30
0.6	0.052	10	90	10
O7	0.061	10	100	0

Injection of 1ml cell-free filtrate was the most convenient prophylactic dose, which saved mice from more than lethal dose (0.7ml) of tissue thromboplstin. There was a significant difference observed among doses of cell-free filtrates (F=640.2614), and among the injection time intervals also, (F=4.043479). The lower dose of haemolysin-cured cell free filtrate found to be prophylactic at 0.75ml per mouse in different time intervals, a significant difference among injections (F=844.707) and no significant difference among time intervals (F=0.176471) were seen. Injection of 0.5ml of partially purified (sephadex elution) phospholipase C per mouse previous to thrombosis induction recovered 95% of tested mice from lethal effect of tissue thromboplastin. Significant differences were observed among doses (F=517.68367), whereas no differences appeared among time intervals (F=0.5263). Purified phospholipase C showed higher efficiency in prophylactic activity against lethal effect of thrombosis than crude,

haemolysin-cured and partially purified phospholipase C that a dose of 0.04ml (150 units/kg) pure preparation per mouse (5.0 units) cured 98% mice from thrombosis. Pure phospholipase C was found to have higher activity than crude, haemolysin-cured and partially purified preparation when egg-yolk emulsion was used as substrate (table1). Significant differences observed among doses and time intervals (F=1408.611)(Table3).

Table (3): The prophylaxis experiments by different preparations of phospholipase C before different time intervals of induction thrombosis by tissue thromboplastin.

Injected different phospholipase C preparations (ml/mouse)	Thrombo -plastin (mg/mouse)	Time intervals (min.)	No. of mice injected	Mean dead mice (non recoverd)%***			
				cell free filtrate	Heamolysin cured cell free filtrate	Partial purefied(Sephdex eluted)	Pure phospholipase C
0.01	0.0612	0 5 10		----------	------	-----	92 97 97
0.02	0.0612	0 5 10	10	----------	-----	100 100 100	67 72 72
0.03	0.0612	0 5 10	10	----------	------	100 100 100	32 37 40
0.04	0.0612	0 5 10	10	----------	------	98 98 95	0 0 2
0.05	0.0612	0 5 10	10	----------	10	85 87 87	------
0.1	0.0612	0 5 10	10	97 100 100	97 97 82	57 45 42	-----
0.25	0.0612	0 5 10	10	95 85 83	45 47 47	12 15 17	-------
0.5	0.0612	0 5 10	10	52 45 45	3 8 5	0 0 2	-------
0.75	0.0612	0 5 10	10	7 7 7	0 0 0	-------	-------
1	0.0612	0 5 10	10	0 2 0	--------	-------	-------
Cell free filtrate* Heamolysin cured* Partial purified* Pure phospholipase C*	0 0 0 0	---------- ---------- ---------- -----	10 10 10 10	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
Brain Heart infusion broth**	0.0612	----------	10	100	100	100	100

* (+)ve control (mice injected with phospholipase C preparations without thromboplastin).

** (-) ve control (mice injected with brain heart infusion broth and lethal dose of thromboplastin).
*** the mean recovery % is equal to 100 – mean death %.

Table (4) showed all results of treatment protocols at three time intervals of phospholipase C preparation injection after induction of thrombosis. The 1ml/mouse was the best dose for cell-free filtrate with significant differences among doses (F=608.552) and no significance with respect to time intervals (F=0.052). The haemolysin-cured cell-free filtrate was found to have the same results; 0.75ml injection after induction of thrombosis was the best, with significant differences among doses used (F= 740.126), but with no significant differences among time intervals even after 10 min. of induction thrombosis (F=3.404). It was appeared that 0.75ml of partially purified phospholipase C per mouse have the best prophylactic activity, with a significant differences among doses used (F=414.907) but no significance among time intervals (F=0.111). The dose 0.04ml; 150units/kg of purified phospholipase C showed a best effectiveness with significant differences among doses used (F=699.5001) but no significant differences among time intervals (F=1.117647).

Table (4) the treatment experiments by different preparations of phospholipase C after different time intervals of induction thrombosis by tissue thromboplastin.

Thrombo-plastin mg/mouse	Injected different phospholipase C preparation (ml/mouse)	Time intervals (min.)	No. of mice injected	Mean dead mice (non recoverd)%***			
				cell free filtrate	Heamolysin cured cell free filtrate	Partial purefied(Sephdex eluted)	Pure phospholipase C
0.0612	0.01	0 5 10	10	---------- ---	------	-----	92 97 97
0.0612	0.02	0 5 10	10	---------- --	-----	--------	73 77 75
0.0612	0.03	0 5 10	10	---------- -----	------	--------	45 48 53
0.0612	0.04	0 5 10	10	---------- --	------	----------	3 0 0
0.0612	0.05	0 5 10	10	---------- --	97 100 100	97 95 95	------
0.0612	0.1	0 5 10	10	97 97 97	82 87 90	42 42 47	-----
0.0612	0.25	0 5 10	10	87 80 80	45 37 45	13 17 20	-------
0.0612	0.5	0 5 10	10	52 47 47	10 17 17	5 5 2	-------
0.0612	0.75	0 5 10	10	13 17 17	7 7 7	2 0 0	-------
0.0612	1	0	10	3	--------	-------	-------

		5 10		3 5			
0 0 0 0	Cell free filtrate(1)* Heamolysin cured(o.75)* partial purified(o.5)* pure phospholipase C(0.04)*	--------	10	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
0.0612	** Brain Heart infusion broth	--------	10	100	100	100	100

* (+)ve control (mice injected with phospholipase C preparations without thromboplastin).

** (-) ve control (mice injected with brain heart infusion broth and lethal dose of thromboplastin).

*** the mean recovery % is equal to 100 – mean death %.

There was no effect on PCV, RBC and platelet count after 12 hr of prophylaxis by partially purified phospholipase C, purified phospholipase C, negative control (mice neither treated by thromboplastin nor phospholipase C) and positive control (mice injected by phospholipase C only). Cell free filtrate was found to apparently decrease these parameters (Table 5).

Table (5): Effect of crude, haemolysin-cured, partial purified and pure preparations of *B. cereus* (isolate No.17) phospholipase C on some haematological parameters in mice after 12 hr of prophylaxis.

PLC Treatment (ml/mouse)	Thromboplastin (mg/kg)	PCV (%)	RBC count (10^6/mm3)	Platelet count ($x10^3$/mm3)
Crud (1ml)	0.612	37	6.85	6.88
Haemolysin Cured (0.75ml)	0.612	44	8.695	795
Partial purified PLC (0.5ml)	0.612	45	8.85	812
Pure PLC(0.04) (0.2ml)	0.612	45,44	8.80, 8.55	824, 822
(1000) spore	0.612	34,34	6.05, 6.11	608, 6.01
Normal mice	0	46	8.95	839

Discussion

Cholesterol has an effect on cereolysin O which is the main haemolysin (haemolysin I) produced by *B. cereus*, but it cannot affect the second haemolysin (haemolysin II or cereolysin AB) [41]. Phospholipase C is a part of synergistic activity of two enzymes phospholipase C and sphingomyelinase worked as a single toxin (cereolysin AB) which has less haemolytic activity differ from cereolysin O [21]. Death of mice after thromboplastin injection may have been due to a limb thrombosis or cerebrovein thrombosis [28] .The estimated LD_{50} was 1.05mg/kg mice. Intravenous infusion of human or rabbit thromboplastin was reported to cause pulmonary thrombosis in rats [15,16]. Infusion of human thromboplastin to rabbit caused alteration of the factor V and VIII levels, the estimated LD_{50} for standard human thromboplastin preparation was 1.5-1.8ml/kg [14]. *B. cereus* phospholipase C has a potential value as a prophylactic agent against intravascular coagulation triggered by thromboplastin [15,16]. There was a

clear effect of phosophlipase C in prophylaxis when compared with injection by the medium used for phospholipase C production (brain heart infusion broth) which has not been prophylacted thromboplastin-induced mice. Also, the prophylactic dose (1ml) was not lethal for mice when injected without induction of thrombosis. It was appeared that the prophylactic dose of haemolysin-cured and sephdex eluted partially purified phospholipase C is lower than crude cell free filtrate. Pure phospholipase C was shown to be the best prophylactic agent in comparison with other preparations when injected before induction thrombosis. All three preparations were tested to know whether other extracellular factors had a synergic or antagonistic effect on phospholipase C or not. Results revealed that phospholipase C alone is required as prophylactic agent and there was no any positive effect by using other *B. cereus* extracellular factors such as haemolysin and other toxins. The period considered in application treatment against thrombosis-mediated death is 1-15 min. which is the period in which death happened .It was found that partially purified and completely purified phospholipase C have a prophylactic activity after induction thrombosis. Gollub & coworkers [25] first reported that *B. cereus* phospholipase C protected rabbits against intravenous infusion of human thromboplastin. A protective effect against otherwise lethal doses of purified human thromboplastin given intravenously to rats has been demonstrated [15] at phospholipase C concentration well below the estimated LD_{50} for the enzyme (1.70mg/kg of rats)[28]. Haemolysin has a potential lethal effect [42] but cell-free filtrate in this study has no lethality in spite of its hemolytic activity. Cell free filtrate of *B. cereus* (isolate No.17) has no enterotoxin, vascular permeability and lethal factor activities but it was reported to has a mild haemolysin and elevated phospholipase C activity [31]. It is known that phospholipase C & sphingomyelinase act synergistically creating a duplex haemolysin called cerolysin AB [21,43]. The haemolysin activity of the isolate No.17 may be due to the cereolysin (haemolysin I) activity which is known to have a cytolytic effect on mammalian cells *in vitro* and *in vivo* [41]. *B. cereus* phospholipases affect membrane phospholipids through forming pores on the mammalian cells [22,44].This study revealed that haemolysin-cured cell-free filtrate have a lower effect than crude cell-free filtrate with respect to effect on PCV, RBC and platelet counts when thrombosis-induced mice tested .So, there is a mild effect of haemolysin which lost by treatment with cholesterol [41]. Partial purified phospholipase C caused less cell lysis than crude and haemolysin cured cell-free filtrate (Table5), this fall in toxic activity appeared to be due to the further purification steps, dialysis and column chromatography, which cured the possible cytolytic activity of other proteins. Incubation of 100fold excess (5units/ml) of phospholipase C in previous study result in 100% hemoglobin release from erythrocytes [44]. So that, purified phospholipase C was found to have a less cytolytic effect on RBC and platelet count and a mild decrease of PCV. Hitland & coworkers [28] reported that haematocrit, platelet count and blood pressure remained constant in monkeys after phospholipase C injection. They demonstrated also that rabbit's erythrocytes had more accessible sensitivity to phospholipase C toxicity and heamolysis. The half-life of phospholipase C in rabbit is about 90 min., the delayed disappearance of phospholipase C in rabbit's plasma is probably lead to increase toxicity than mice which show half-life of about 5.2-

5.4 min. [45]. The current results were in agreement with the work of Little *et al.* [46] respected the effect of phospholipase C on platelet lysis, which demonstrated that minimal cell lysis, had occurred under conditions of 4-days incubation at 37C° *in vitro*. Only the phospholipid of the outer layer of the platelet membrane were accessible to the phospholipase C but the phospholipase C attack to the inner membrane was limited [45], whereas reported that erythrocytes were not accessible to phosphatidylcholin specific phospholipase C [19]. The marked cell lysis may be due to the fact that erythrocyte membrane composed of phospholipids, about 40% of it was degraded by phospholipase C [45]. It was found that phospholipase C is of a great value in prophylaxis activity against thrombosis in spite of that previous reports pointed out phospholipase C causes a significant increase of several plasma enzymes in rabbits, but fortunately with no respiratory or circulatory changes [28]. From this study it was concluded that there is a significant prophylactic and treatment activity of phospholipase C against thrombosis in mice. The cell free filtrate and partial purification steps have the same activity on prevention thrombosis, but the pure preparation is typical. There was a mild effect of phospholipase C preparations on PCV, RBC and platelet count in mice. So that, the phospholipase C preparations, especially the pure one, is suggested to applied on infarction-risked humans, as volunteers, to test their prophylactic activity against infarction.

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بەکارهێنانی ئەنزیمی فۆسفۆلیپێز سی ی بەکتیریای *Bacillus cereus* لە پاراستن و چارەسەرکردن لە دژی مەیینی خوێن کە بە ترۆمبۆپلاستین هاندراوە لە مشک.

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پوختە

چەند قەبارەیەکی پاڵاوتەی چاندنگەی خاو و چەند قۆناغێکی پاککردنەوەی ناتەواوی ئەنزیمی فۆسفۆلیپێز سی ئامادە کران لە جیاکراوەیەکی بەکتیریای *Bacillus cereus* نەژەهراوی و زۆر بەرهەمهێنەری ئەنزیمەکە. چرکانی ئەنزیمی پاکی تەواویش (کۆمپانیای ICN) بەکارهێنرا. پاراستن لە دژی چڕی ترۆمبۆپلاستین کە لە ژەمی لە نیو کوژ بەرزتر (٠,٠٦١٢ ملگم/مشک) مشکەکانی رزگارکرد بەرێژەی ٩٧٪ و ١٠٠٪ و ٩٧٪ بە پاڵاوتەی چاندنگە و ئامادەکراوەی بێ شیکەرەوەی خوێن و ئامادەکراوەی بە سیفادیکس پاڵیوراو یەک لەدوای یەک بەبێ بوونی جیاوازی راستەقینە لە نێوان ماوەکانی کاتی لێدان بەڵام ئەنزیمی پاک توانیویەتی ٩٧٪ ی مشکەکان رزگار بکات لەگەڵ بوونی جیاوازی راستەقینە لەنێوان ماوەکانی کات. لەمەڕ چارەسەرکردن، پاش هاندانی مەیین، پاڵیوراو و ئامادەکراوەی پاکی ناتەواو و پاک مشکیان رزگارکرد بەرێژەی ٩٨٪ بەبێ ئەوەی جیاوازی راستەقینە لەنێوان ماوەکانی کاتدا هەبێت. پێوەرەکانی خوێن، PCV و RBC و ژمارەی پلێتەکانی خوێن لە مەدای ئاسایی خۆیان مانەوە پاش ١٢ کاتژمێر لە لێدان. ئەم ئەنجامانە پێشنیاری ئەوە دەکەن کە توانا هەیە بۆ بە کار هێنانی ئامادەکراوەی پاکی ناتەواو و پاکی تەواوی ئەنزیمی فۆسفۆلیپێز سی ی بەکتیریای *Bacillus cereus* وەکو هۆکار بۆ پاراستن لەدژی مەیینی خوێن لە مرۆڤدا.

أستخدام انزيم فوسفوليبيز سي لبكتريا *Bacillus cereus* في التحصين و العلاج ضد تجلط الدم المستحث بالثرومبوبلاستين في الفئران.

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الخلاصه

أحجام من طافي المزرعة الخام و مراحل للتنقية الجزئية لأنزيم فوسفوليبيز سي جرى تحضيرها من عزلة لبكتريا *Bacillus cereus* غير سامة و ذات أنتاج عالي للأنزيم. أستخدمت أيضا تراكيز من الانزيم المنقى بشكل كامل (شركة ICN). لقد أدى التحصين ضد تركيز للثرومبوبلاستين أعلى من الجرعة نصف القاتلة (٠,٠٦١٢ ملغم/فارة) الى شفاء الفئران بنسبة ٩٧٪ و ١٠٠٪ و ٩٧٪ بطافي المزرعة و التحضير الخالي من حال الدم و التحضير المرشح بالسيفادكس على التوالي مع عدم وجود فروق معنوية بين الفترات الزمنية المختلفة للحقن بينما شفا الأنزيم النقي نسبة ٩٧٪ من الفئران مع وجود فروق معنوية بين الفترات الزمنية للحقن. أما بالنسبة للعلاج، بعد حث التجلط، فقد أدى طافي المزرعة و مراحل التنقية و التحضير النقي الى شفاء ٩٨٪ من الفئران من دون وجود فروق معنوية بين الفترات الزمنية للحقن. بقيت المعايير الدموية PCV و RBC و عدد الصفيحات الدموية ضمن المدى الطبيعي بعد ١٢ ساعة من الحقن. تقترح هذه النتائج أستخدام التحضير شبه النقي و النقي بشكل كامل لأنزيم فوسفوليبيز سي لبكتريا *Bacillus cereus* كعامل تحصين ضد الجلطة في الانسان.

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