Overproduction Of Fibrinolysis Enzyme Produced By Mutant Pseudomonas Aeruginosa Compared To Wild Type Pseudomonas Aeruginosa

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KEYWORDS
Expression, thrombolytic enzyme and bacteria.

ABSTRACT
Objective: The aim of the study is to increase the production of fibrinolytic enzyme produced by pseudomonas aeruginosa after mutagenesis with physical mutagens compared to the wild type.
Study design: Cross-sectional in descriptive study design and case–control in analytical study design.
Backgrounds: P. aeruginosa is a ubiquitous gram-negative aerobe. Its opportunistic pathogen that infects patients with cystic fibrosis, burn, wounds, immunodeficiency, chronic obstructive pulmonary disorder (COPD), cancer. Fibrinolytic enzyme is a factor that lysis fibrin clots. This fibrinolytic factor has prospective use totreat cardiovascular diseases such as stroke and heart attack. Thrombotic disorders are the main cause of death world.
Methodology: Study patients, specimens, collection of bacterial isolates. Plasma agar plate assay, Skim milk agar assay, Radial caseinolytic assay, Preparing magnetizing Water. Exposure P. aeruginosa to radioactive sources, lasers and magnetic water. Determination of optimal bacterial isolate for the fibrinolytic enzyme production.
Results: Increased when P. aeruginosa were exposed to Na23 at an efficiency of 10 µci. Also exposed to 10 ml of magnetic water during 48 hr., then exposed to a cobalt radioactive source during 3 hr., another radioactive source is Cs137, Co60 and Sr90 in 1 hr. during 48 hours with exposure to laser and magnetized water.
Conclusion: Conclude that the overproduction of fibrinolytic enzyme (clot-dissolving enzyme) increased when P. aeruginosa were exposed to Na23, Cs137, Co60 and Sr90 with exposed to laser and magnetized water in certain period.

1. Introduction

P. aeruginosa is present gram-negative aerobe pertinence to the family Pseudomonadaceae, rod constituted and happen singly, in pairs or tiny series [1]. Its opportunistic pathogen which contract patients to cystic fibrosis, wounds, burn, chronic obstructive pulmonary disorder (COPD), immunodeficiency, cancer and severe contagious demand ventilation like COVID-19 [2]. Its occasion disease in vulnerable human, additionally its ecological resilience to the capability to conform to and flourish in a various of environmental status [3]. Excreted virulence agents inclusive dyes (pyocyanin, pyoverdin), enzymes (protease IV, elastase, phospholipase C) and toxins (Exoenzyme S, exotoxin A) [4].

Universal death-rate is mainly imputed to cardiovascular diseases (CVDs), position as the first agent beyond disaster [5]. The numeral of person dying of throe sickness has mortal of further unless two million states in 2000 to almost 17.9 million during 2019 [6].

The extreme cause widespread cardiovascular disorders for instance peripheral vascular disease (PVD), ischemic heart disease (acute coronary syndrome), stroke is thrombosis, venous thromboembolism (VTE) and pulmonary embolism (PE) generating in the obstructing of artery or vein via a blood clot (thrombi) [7].

Fibrinolytic enzyme is a agent that dissolve fibrin clots. Such fibrinolytic factor has expected utilize to therapy cardiovascular diseases like heart attack and stroke. Thrombotic disorders are the major occasion of doom universe. The overall charge of enzyme output is solitary the main defy about the charge efficient manufacturing apply of the thrombolytic Enzymes [8]. Streptokinase is a thrombolytic drug produce of *Streptococci spp*. fundamentally utilized to therapy pulmonary thromboembolism and acute myocardial infarction. Its technicality of effect evolves transforming plasminogen to plasmin, consequently humanitarian the body's naturalistic fibrinolytic operations. While, as it constructs of a hemolytic bacterial resource, it outrage an antigenic restraint and depend refined conduct outcomes in the formulation of equalizing antibodies and occasions allergic

responses [9].

2. Methodology

Medical study design

The clinical study design of this research is a case-control for the analytical and cross-sectional for the descriptive.

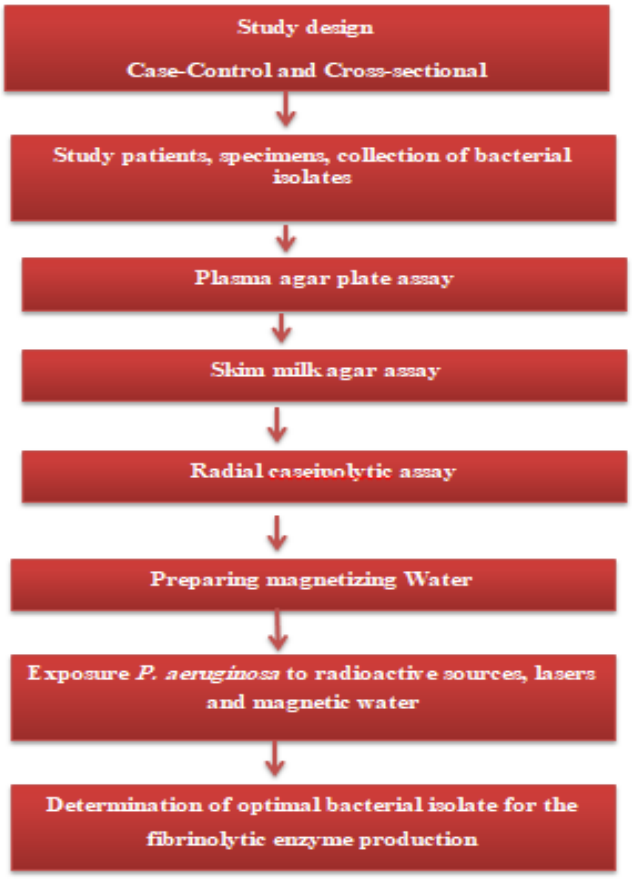

Figure (1): Medical study plan for this research.

Study patients, specimens, collection of bacterial isolates

One-hundred fifty of Pseudomonas isolates were collected from patients with burns, wounds, sepsis and cancer patients from Baghdad hospitals during the period 2023/2024 and were diagnosed by biochemical and molecular methods [10, 11].

Plasma agar plate screening assay

The culture medium was prepared based on [12] by adding 20% human blood plasma to the culture medium of Nutrient Agar at a temperature of 56°C for 20 minutes, then left to solidify. After that, holes were made in the culture medium and 5 ml of bacterial suspension were added to the holes and incubated in the incubator at a temperature of 37°C for 24 hours. The decomposition area around the holes was measured to examine the bacteria producing the decomposition enzyme.

Skim milk agar screening assay

The culture medium was prepared based on [13] by adding 20% skimmed milk to the culture medium of Nutrient Agar at a temperature of 56°C for 20 minutes, then left to solidify. After that, holes were made in the culture medium and 5 ml of bacterial suspension were added to the holes and incubated in the incubator at a temperature of 37°C for 24 hours. The decomposition area around the holes was measured to examine the bacteria producing the decomposition enzyme.

Radial caseinolytic screening assay

The culture medium was prepared based on [14] by adding 1% fresh human serum, casein to the culture medium of Nutrient Agar at a temperature of 56°C for 20 minutes, then left to solidify. After that, holes were made in the culture medium and 5 ml of bacterial suspension were added to the holes and incubated in the incubator at a temperature of 37°C for 24 hours. The decomposition area around the holes was measured to examine the bacteria producing the decomposition enzyme.

Preparing magnetizing Water

Magnetic water was prepared by exposing a glass or plastic container containing sterile water to a magnet for 72 hours [15].

Exposure *P. aeruginosa* to radioactive sources, lasers and magnetized water

One ml *P. aeruginosa* were exposed to different radioactive sources sodium, cobalt, cesium and strontium. The bacteria were also exposed to laser at different times and to magnetized water at different times [16].

3. Results and discussion

Radiance is power that derives of a resource and wandering during several matter or during area. Light and heat are kinds of ray. The type of ray known ionizing radiance in order that it has sufficient power to elminate an electron of an atom, action which atom an ion [17].

Table (1): Sources of mutant isolates, diameter of the lysis zone, production of clot-dissolving enzyme.

No.	Sources of mutant isolates	Diameter of caseinolysis or fibrinolysis zone (Production of staphylokinase)				Explain of results
		P1	P2	P3	P4	
1	*P. aeruginosa* exposed to Na^{23} 10 µci for 3 hr. without alminium produce staphylokinase through 24 hr.	11	10.5	11.5	9.5	Increase production in triplicate (P3)
	P. aeruginosa exposed to Na^{23} 10 µci for 3 hr. without alminium produce staphylokinase through 48 hr.	19	10	19	0	Increase production in replicate (P1) and triplicate (P3)
2	*P. aeruginosa* exposed to Na^{23} 10 µci for 2 hr. without alminium produce staphylokinase through 24 hr.	11	9.5	8	8.5	No increase production
	P. aeruginosa exposed to Na^{23} 10 µci for 2 hr. without alminium produce staphylokinase through 48 hr.	12	0	0	0	No increase production
3	*P. aeruginosa* exposed to Na^{23} 10 µci for 1 hr. without alminium produce staphylokinase through 24 hr.	0	0	0	0	No increase production
	P. aeruginosa exposed to Na^{23} 10 µci for 1 hr. without alminium produce staphylokinase through 48 hr.	0	0	0	0	No increase production
4	*P. aeruginosa* exposed to magnetic water 8 ml produce staphylokinase through 24 hr.	9	9.5	10	8.5	No increase production
	P. aeruginosa exposed to magnetic water 8 ml produce staphylokinase through 48 hr.	10.5	10.5	11	0	No increase production
5	*P. aeruginosa* exposed to magnetic water 10 ml produce staphylokinase through 24 hr.	10.5	10	12	0	No increase production
	P. aeruginosa exposed to magnetic water 10 ml produce staphylokinase through 48 hr.	15	20	17	0	Increase production in replicate (P1), duplicate (P2) and triplicate (P3)
6	*P. aeruginosa* exposed to Co^{60} 1 µci for 3 hr. without alminium produce staphylokinase through 24 hr.	11.5	12	9.5	8	Increase production in duplicate (P2)
	P. aeruginosa exposed to Co^{60} 1 µci for 3 hr. without alminium produce	17	18	18	0	Increase production in replicate (P1), duplicate (P2) and triplicate

	staphylokinase through 48 hr.					(P3)
7	*P. aeruginosa* exposed to Co^{60} 10 µci for 3 hr. with alminium produce staphylokinase through 24 hr.	10	8.5	9	7	No increase production
	P. aeruginosa exposed to Co^{60} 10 µci for 3 hr. with alminium produce staphylokinase through 48 hr.	11	14	0	0	Increase production in duplicate (P2)
8	*P. aeruginosa* exposed to Cs^{137} 10 µci for 1 hr. without alminium produce staphylokinase through 24 hr.	9	10	8	8.5	No increase production
	P. aeruginosa exposed to Cs^{137} 10 µci for 1 hr. without alminium produce staphylokinase through 48 hr.	19	19	18	18	Increase production in replicate (P1), duplicate (P2), triplicate (P3) and tetraplicate (P4)
9	*P. aeruginosa* exposed to Cs^{137} 10 µci for 1 hr. with alminium produce staphylokinase through 24 hr.	11	10	9.5	9	No increase production
	P. aeruginosa exposed to Cs^{137} 10 µci for 1 hr. with alminium produce staphylokinase through 48 hr.	22	22	18	0	Increase production in replicate (P1), duplicate (P2) and triplicate (P3)
10	*P. aeruginosa* exposed to Cs^{137} 10 µci for 3 hr. with alminium produce staphylokinase through 24 hr.	9.5	10	9.5	9	No increase production
	P. aeruginosa exposed to Cs^{137} 10 µci for 3 hr. with alminium produce staphylokinase through 48 hr.	11	16	18	18	Increase production in duplicate (P2), triplicate (P3) and tetraplicate (P4)
11	*P. aeruginosa* exposed to Cs^{137} 10 µci for 3 hr. without alminium produce staphylokinase through 24 hr.	14	10.5	9.5	8	Increase production in replicate (P1)
	P. aeruginosa exposed to Cs^{137} 10 µci for 3 hr. without alminium produce staphylokinase through 48 hr.	14	11	9.5	9.5	Increase production in replicate (P1)
12	*P. aeruginosa* exposed to laser in 10 min produce staphylokinase through 24 hr.	0	0	0	0	No increase production
	P. aeruginosa exposed to laser in 10 min produce staphylokinase through 48 hr.	0	0	0	0	No increase production
13	*P. aeruginosa* exposed to laser in 20 min produce staphylokinase through 24 hr.	14	13	0	0	Increase production in replicate (P1) and duplicate (P2)
	P. aeruginosa exposed to laser in 20 min produce staphylokinase through 48 hr.	25	26	23	23	Increase production in replicate (P1), duplicate (P2), triplicate (P3) and tetraplicate (P4)
14	*P. aeruginosa* exposed to Cs^{137} 1 µci for 2 hr. with alminium produce staphylokinase through 24 hr.	10.5	12	12	11.5	Increase production in duplicate (P2), triplicate (P3) and tetraplicate (P4)
	P. aeruginosa exposed to Cs^{137} 1 µci for 2 hr. with alminium produce staphylokinase through 48 hr.	21	21	18	0	Increase production in replicate (P1), duplicate (P2) and triplicate (P3)
15	*P. aeruginosa* exposed to Cs^{137} 10 µci for 2 hr. without alminium produce staphylokinase through 24 hr.	13	12	11	9	Increase production in replicate (P1), duplicate (P2) and triplicate (P3)
	P. aeruginosa exposed to Cs^{137} 10 µci for 2 hr. without alminium produce staphylokinase through 48 hr.	16	16	13	10.5	Increase production in replicate (P1), duplicate (P2) and tetraplicate (P4)
16	*P. aeruginosa* exposed to Co^{60} 10 µci for 2 hr. with alminium produce staphylokinase through 24 hr.	9	8	8	0	No increase production
	P. aeruginosa exposed to Co^{60} 10 µci for 2 hr. with alminium produce staphylokinase through 48 hr.	18	0	0	0	Increase production in replicate (P1)
17	*P. aeruginosa* exposed to Co^{60} 1 µci for 1 hr. without alminium produce staphylokinase through 24 hr.	9	9.5	0	0	No increase production
	P. aeruginosa exposed to Co^{60} 1 µci for 1 hr. without alminium produce staphylokinase through 48 hr.	20	20	21	20	Increase production in replicate (P1), duplicate (P2), triplicate (P3) and tetraplicate (P4)

	P. aeruginosa exposed to Sr^{90} 1 µci for 1 hr. produce staphylokinase through 24 hr.	10	8.5	0	0	No increase production
18	*P. aeruginosa* exposed to Sr^{90} 1 µci for 1 hr. produce staphylokinase through 48 hr.	10	13	0	0	Increase production in duplicate (P2)
	P. aeruginosa exposed to Sr^{90} 1 µci for 2 hr. produce staphylokinase through 24 hr.	9.5	8.5	8.5	8.5	No increase production
19	*P. aeruginosa* exposed to Sr^{90} 1 µci for 2 hr. produce staphylokinase through 48 hr.	9.5	8.5	10	0	No increase production
	P. aeruginosa wild type produce staphylokinase after 24 hr.	12	11.5	10.5	10	
Control	*P. aeruginosa* wild type produce staphylokinase after 48 hr.	12	11	13	0	

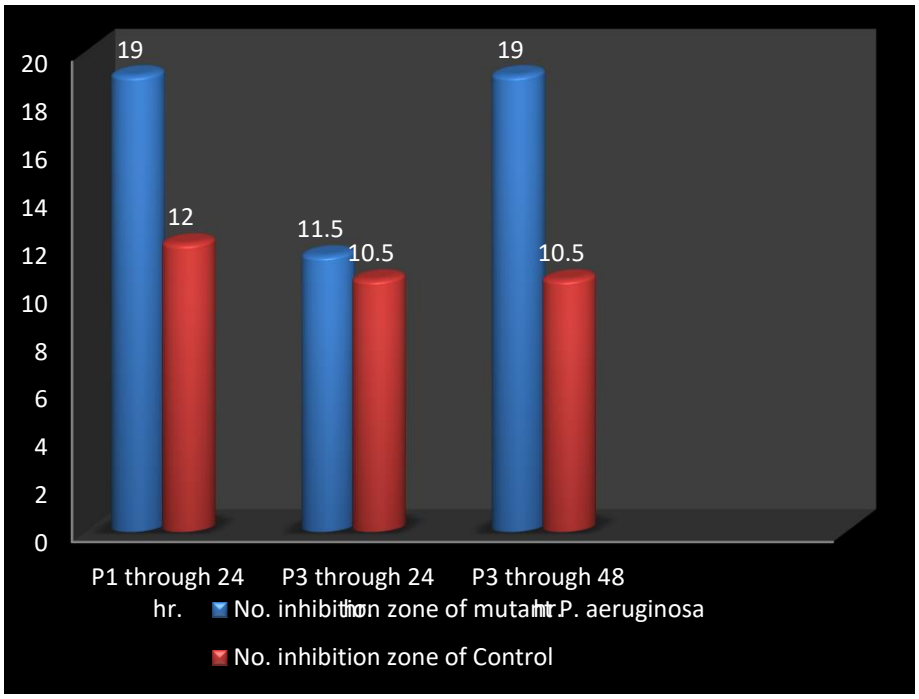

Figure (2): Diameter of inhibition zone of fibrinolytic enzyme produced from mutant *P. aeruginosa* exposed to Na^{23} with activity 10 µci without alminium for isolates No. 1.

Radiance indicates to exposing to ray take place while whole or portion of the organism is roofless to radiance of a resource. Rays does not produce a individual radioactive [18].

The results in table (1) with figure (2) show an increase in the diameter of the lysis zone of the clot-dissolving enzyme produced by *P. aeruginosa* after exposure to the Na^{23} radioactive source at an efficiency of 10 µci without aluminum for isolate (1) in which the increase in enzyme production reached (11.5, 19, 19) compared to the control in which the lysis zone was (10.5, 10.5, 12).

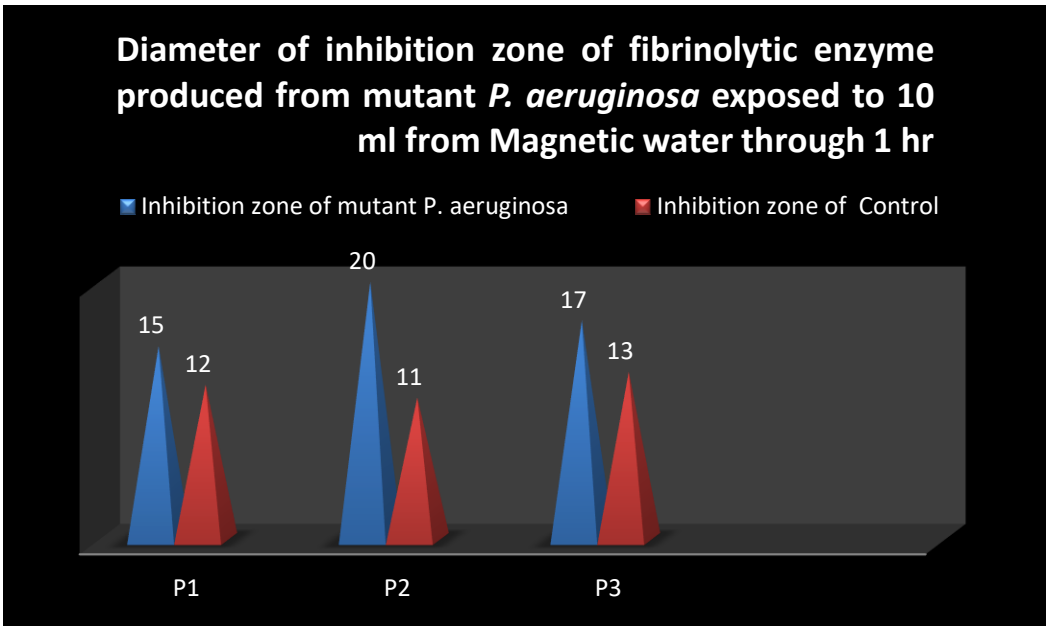

Figure (3): Increase production with Diameter of inhibition zone of fibrinolytic enzyme produced from mutant *P. aeruginosa* exposed to 10 ml from Magnetic water through 1 hr. for isolates No. 5.

Magnetized water is paramagnetic concept which carry a magnetic charge. Biological impact of constant magnetic field was scrutinized via utilizing ferrite magnets to arrive a magnetic field exposing trial on species of bacteria [19].

A previous study by [20] on the biological effect of the magnetic field on bacterial growth, the results showed a decrease in the growth rate of *S. aureus* and *S. mutants* when grown in anaerobic conditions and did not inhibit its growth in aerobic conditions.

The results in table (1) with figure (3) show an increase in the diameter of the lysis zone of the clot-dissolving enzyme produced by *P. aeruginosa* after exposure to the magnetized water at an efficiency for isolate (5) in which the increase in enzyme production reached (15, 17, 20) compared to the control in which the lysis zone was (12, 13, 11).

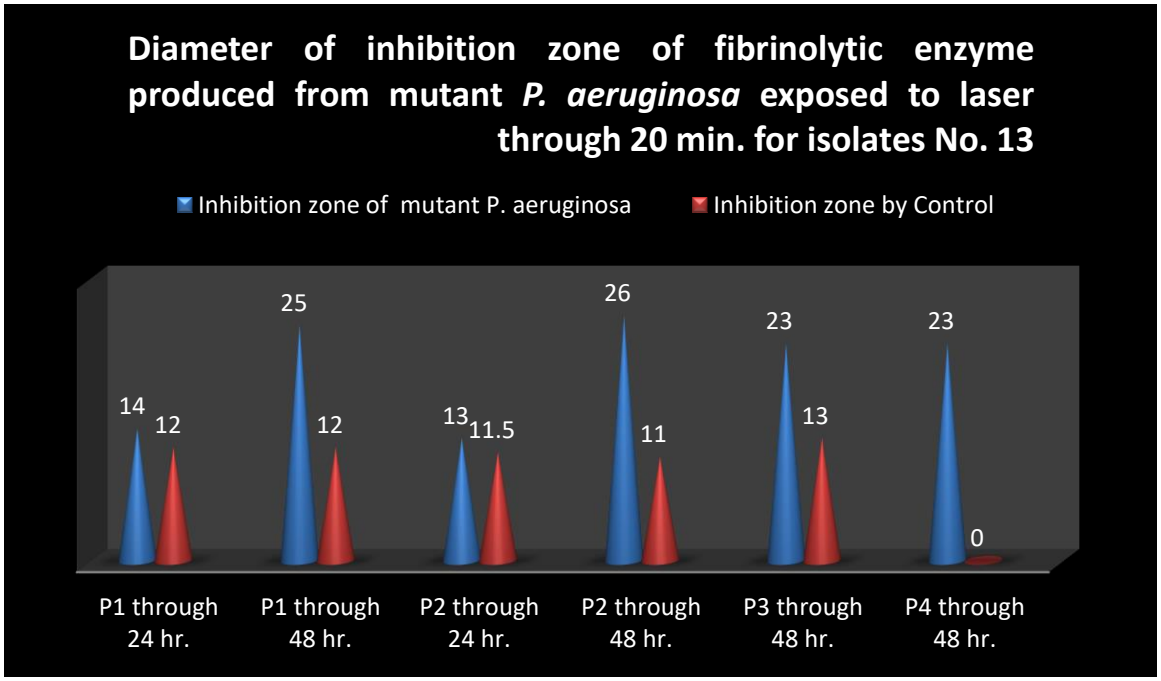

Figure (4): Increase production with Diameter of inhibition zone of fibrinolytic enzyme produced from mutant *P. aeruginosa* exposed to laser through 20 min. for isolates No. 13.

Medical application of laser include melanin, oxhemoglobin, hair obstruction, water (in tissues), retina surgery, retina tear, cataracts, therapy of glaucoma, correction of myopia, urolithiasis, condylomata, prostate Cancer, benign prostatic hyperplasia (BPH), suture removal, Vasovasostomy [21, 22].

The results in (figure 4) show an increase in the diameter of the lysis zone of the clot-dissolving enzyme produced by P. aeruginosa after exposure to laser at an efficiency for isolate (13), in which the increase in enzyme production reached (13, 14, 23, 23, 25, 26) compared to the control, in which the lysis zone was (11.5, 12, 13, 0, 12, 11,).

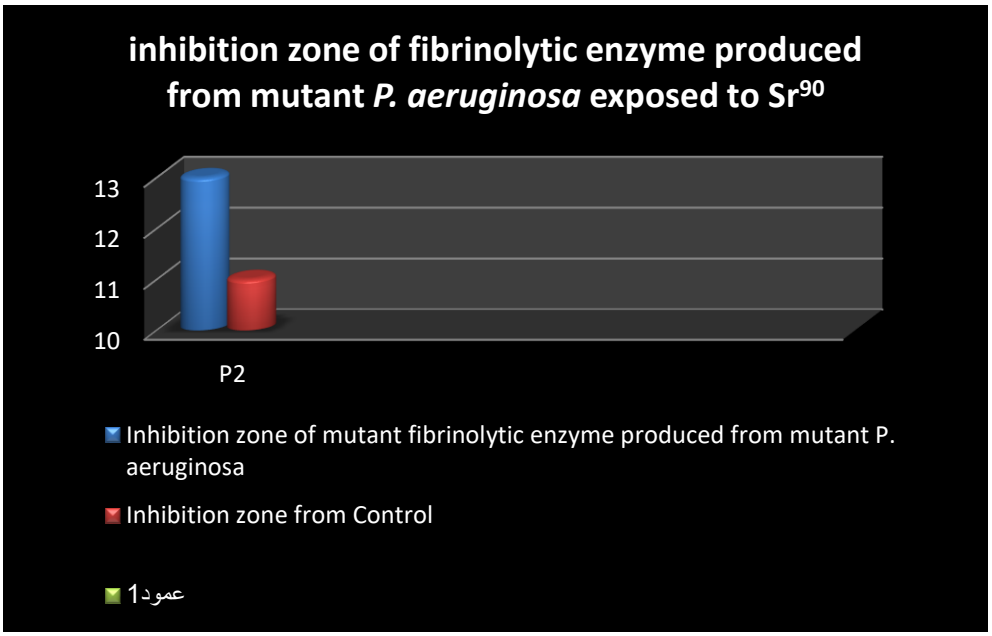

Figure (5): Diameter of inhibition zone of fibrinolytic enzyme produced from mutant *P. aeruginosa* exposed to Sr^{90} with activity 10 µci for isolates No. 18.

Beta radiance drive meters in air and is reasonably perspicacious. Gamma radiance and X-radiations are electromagnetic radiance various solitary in the quantities of power that fit to drive several meters in air and much centimeters in human tissue. It easily perspicacious utmost matter [23, 24].

The results in table (1) with figure (5) show an increase in the diameter of the lysis zone of the clot-dissolving enzyme produced by P. aeruginosa after exposure to Sr^{90} at an efficiency for isolate (18) in which the increase in enzyme production reached (13) compared to the control in which the lysis zone was (11).

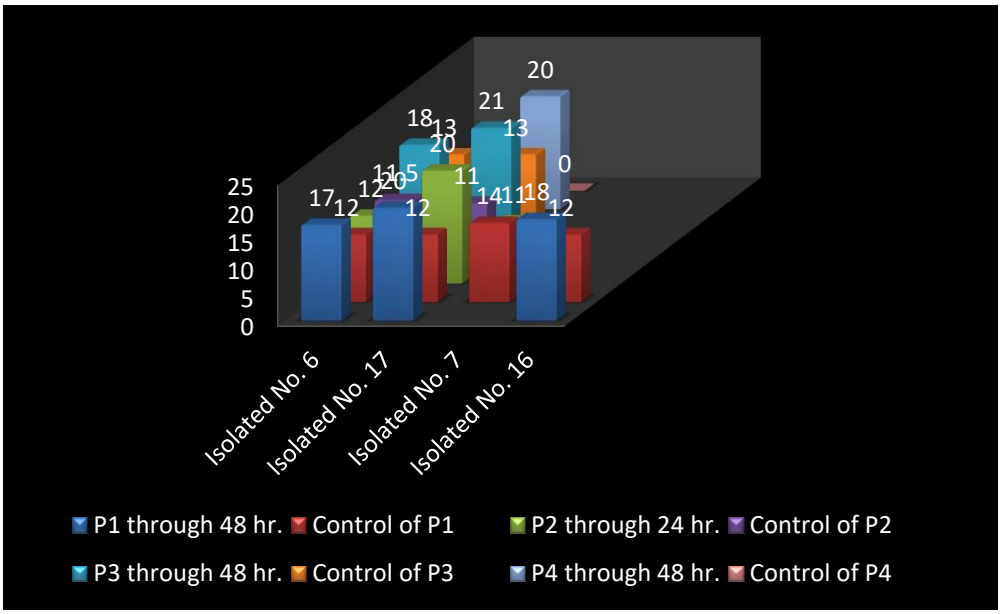

Figure (6): Diameter of inhibition zone of fibrinolytic enzyme produced from mutant *P. aeruginosa* exposed to Co^{60} with activity (1 and 10) µci with and without alminium for isolates No. 6, 17 by Co^{60} 1 µci and 7, 16 by Co^{60} 10 µci.

Ionizing radiance reason important physical and chemical alterations that ultimately cause to biological impacts in the bared tissue or organism. Ultimately, a wide extent of biological impact of the various depending on types of radiation [25].

The results in table (1) with (figure 6) show an increase in the diameter of the lysis zone of the clot-dissolving enzyme produced by *P. aeruginosa* after exposure to Co^{60} with activity (1, 10) µci at efficiency for isolate (6), (17) ،(7), (16) in which the increase in enzyme production reached (12, 17, 18, 18) (20, 20, 21) (14) (18) compared to the control in which the lysis zone was (10.5, 12, 11, 13) (12, 11.5, 10.5) (11.5) (12)

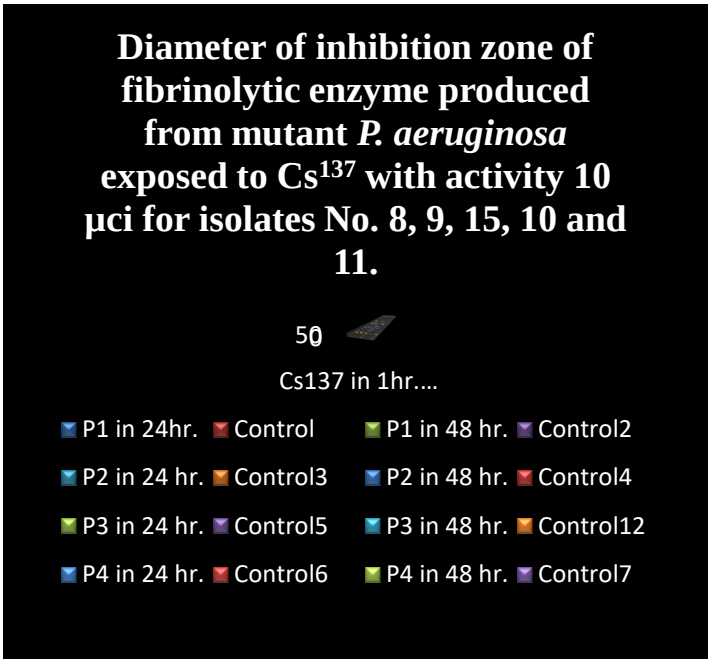

Figure (7): Diameter of inhibition zone of fibrinolytic enzyme produced from mutant *P. aeruginosa* exposed to Cs^{137} with activity 10 µci for isolates No. 8, 9, 15, 10 and 11.

Radiance is power, in the compose of mote or electromagnetic radiations emitted of radioactive

atoms, there are types of radiance include alpha, beta particles and gamma radiance [23].

Iradiation biology is the treatise of the impact of ionizing ray on biological tissues and dashing organisms. It combines radiation physics and biology [25]. It voyage in the compose of energy waves or elevation velocity particles. Radiance competence take place naturalistically or caused by human beings. Two types of radiance include non-ionizing and ionizing radiance [24].

The results in table (1) with figure (7) show an increase in the diameter of the lysis zone of the clot-dissolving enzyme produced by *P. aeruginosa* after exposure to Cs^{137} at an efficiency for isolate (8), (9) ،(10), (11), (15) in which the increase in enzyme production reached (19, 19,18,18), (22, 22, 18), (16, 18, 18), (14, 14), (13, 12,11 and 16, 16, 10.5) compared to the control in which the lysis zone was (12, 11.5, 10.5, 10 and 12, 11, 13, 0).

4. Conclusion and future scope

Conclude that the overproduction of fibrinolytic enzyme (clot-dissolving enzyme) increased when P. aeruginosa were exposed to Na23, Cs137, Co60 and Sr90 with exposed to laser and magnetized water in certain period.

Ethical approval

All examination protocols were confirmed by the College of Ibn Sina University of Medical and Pharmaceuticals Sciences. All screening was achieved following the confirmed guidelines.

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There was no financial disclosure.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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