

Salmonella Typhimurium Treated by Gamma Radiation as an Approach for Cancer Management

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Abstract

Background: Salmonellae are gram-negative, facultative anaerobes, it can survive and colonize in hypoxic environment of the tumor, leading to a substantial reduction in the tumor size. Bacteria can be attenuated by radiation which resulted in complete loss of replicative ability but remained metabolically active.

Aim of the Work: This study was conducted to evaluate the efficiency of ψ -radiation as a tool to decrease the virulence of Salmonella typhimurium wild type strain ATCC 14028 (S. typhimurium.WT.) capable of tumor regression.

Methods: Exposure of Salmonella typhimurium wild type strain (S.typhimurium.WT.) to different doses of ψ -radiation, testing S.typhimurium.WT. that exposed to different doses of ψ radiation for cytotoxic activity in vitro, and its pathogenicity in mice as well as the wild type strain was assessed. The antitumor activity of the ψ -irradiated Salmonella in vivo was tested as well. Sixty adult male weighty 20-25g were used for this purpose and the mice divided into 6 experimental group, three of these groups were mice bearing EACs solid tumor, after the tumor volume reached approximately 1cm^3 , the mice were treated with S.typhimurium.WT. parent strain and S.typhimurium.WT. exposed to 1 and 4kGy ψ -irradiation dose histopathological analysis, as well as, proliferation and immunological biomarker, was assessed. The study has been carried out for last three years in the National Center for Radiation Research and technology, Atomic Energy Authority, Egypt.

Results: Results showed a reduction in S.typhimurium.WT. viable count with the increase of ψ -radiation exposure dose, also a highly significant inhabitation in EAC cells count % was occurred when S.typhimurium.WT. exposed to ψ radiation at dose of 1 and 4kGy, it was found S.typhimurium ψ - 1kGy give a high inhabitation percentage in cell line and became less virulent to mice than S.typhimurium wild type strain, results revealed that, treatment with S. typhimurium ψ 1kGy caused retardation in tumor growth in mice bearing tumor groups more than the wild type strain do. This was leading to a reduction in tumor volume accompanied with a significant increase in caspase-3 activity. In addition, histopathological

analysis revealed that irradiated Salmonella achieve improvement of the muscular thigh tissue compared to EAC tumor bearing mice.

Conclusion: The exposure of S.typhimurium.WT. to ψ -radiation decreases its virulence in mice and increase its antitumor activity in vivo and in vitro.

Key Words: Salmonella typhimurium – Gamma radiation – Antitumor activity.

Introduction

SALMONELLA enterica serovar typhimurium is a facultative anaerobic bacterium, gram-negative, flagellated which generally develops and grows preferably in a wide variety of tumor cells. In recent studies, it was shown that attenuated strains of S. enterica typhimurium served as antitumor agents, preferably growing within tumors in a ratio of 1000:1 when compared to normal tissues [1]. The preference for tumor tissues can be explained for some facts such as; there is the adaptation of S. enterica typhimurium to grow in areas with low percentage of oxygen; increased availability of nutrients released by necrotic cells and decreased bactericidal activity of macrophages and granulocytic neutrophils in regions with low oxygen concentration. Positive pressure and irregularities in vascularization of the tumor mass also inhibit, but not entirely, the entry of antibodies and complement factors favoring the growth of S. enterica typhimurium [2,3].

Although in some experimental systems, activation of innate immunity can protect against tumor development, in most systems effective cancer immune surveillance responses require the additional expression of tumor antigens capable of propagating the expansion of effector CD^4+ and CD^8+ T-cells. Thus, coordinated and balanced

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activation of both innate and adaptive immunity is needed to protect the host against a developing tumor, in addition, the tumor regression due to enhancement of apoptosis pathway the activation of caspas-3 activity [4,5].

The attenuation of this species in order to create antitumor carrier molecules have been widely used, thereby increasing the LD 50 causing shrinkage of melanoma in mice that received such attenuated strain [6]. Furthermore, attenuated strains which produce cytokines with the potential to stimulate the host immune response against tumors [7,8].

Ultimately production of a promoter induced by hypoxia (HIP-1) enabling modulation of genes with their transcription increased in the areas of hypoxia in the tumor mass [9].

Ionizing and non-ionizing radiation has been used for years to produce bacterial mutants with same different traits, and characters such as attenuation to be less virulent in mice [10].

Aim of the work:

The present study aimed to search for the attenuated Variants of Salmonella typhimurium induced by gamma-irradiation as a simple low cost effective method, capable of tumor regression; suggesting a great potential for cancer therapy.

Material and Methods

- *Microorganisms:* Salmonella typhimurium 14028 ATCC (American Type Culture Collection) was obtained from Microbiological Resources Center, Cairo, Egypt, MIRCEN.

- *Media:* The following media were used for cultivation, numeration, recovery and determination of Salmonella typhimurium viable counts. The media were sterilized by autoclaving at 121° for 20 minutes; nutrient broth, nutrient agar, Luria Bertani broth and Luria Bertani agar.

- *Experimental animals:* One hundreds and seventy of male Swiss Albino mice weighted about 23-25g were obtained from Holding Company for Biological Products and Vaccines, Vacsera, Cairo, Egypt.

Animals were housed in animal house of National Center for Radiation Research and Technology (NCRRT) Cairo, Egypt. They were carried in accordance with the standard out line in guide for the care and use of laboratory animals (DHHS, publication 85-23) and regularly, received standard pellet feed and drinking water.

- *Cell lines:* Ehrlich Ascites Carcinoma cells (EAC). Cell line used in this study was kindly supplied by the National Cancer Institute (NCI), Cairo University, Egypt. Tumor cells were maintained by weekly intraperitoneal (i.p) transplantation of 2.5×10^6 cells per male mice [11].

- *Tumor transplantation:* Solid tumors were obtained by intramuscular (I.M) injection of 0.2ml of AEC cells (contains 2.5×10^6 viable EAC cells) in the right thigh of the lower limb of each Swiss Albino mouse (Gupta et al., 2004) [12]. Then, the tumor was left to grow until become in palpable appearance to reach volume of 1cm³ in control group and the volume was measured by caliper.

- *Chemicals, reagents and kits:* All chemicals obtained from sigma (USA), Mouse Caspase-3 Colorimetric Assay Kit manufactured by Abcam.

- *Gamma irradiation facility:* The Gamma (γ) irradiation unit holding up to several thousand curries of Cobalt⁶⁰ manufactured by Bhabha (INDIA) were used. The irradiation volume was approximately of 4000CC. The dose rate at the time of the experiments was found to be 1kGy/24 minutes.

1- Determination of sensitivity of Salmonella typhi.

WT parent strain towards Gamma radiation:

This procedure was carried out to investigate the sensitivity of Salmonella typhimurium.WT. strain towards γ -radiation according to [13]. S. typhimurium.WT. strain was grown in nutrient broth and incubated for 18hr at 37°C. The cell culture was divided equally into dry sterile screw capped plus test tube, immersed in ice water, and exposed to different γ -irradiation doses in CO₆₀ (Indian cell) as follow (0.5, 1, 2, 3, 4, 5, 6, 7,kGy). Immediately after irradiation, the irradiated samples and their control (non-irradiated one) were removed and serially diluted in physiological saline (0.9%). Samples were shaken very well and 0. 1ml of each dilution was plated onto nutrient agar plated as triplicate, to estimate survival fraction at each dose. All plates were incubated at 37°C for 24hr. and the D₁₀-value was calculated for S.typhimurium.WT. strain from the dose survival curve. The samples were irradiated in CO₆₀ facility at gamma radiation unit (4000A) at National Center for Radiation Research and Technology (NCRRT), whereas the average dose rate was 1kGy/24min. at the time of the experiments. The elapsed time from irradiation exposure to plating did not exceed 15min., and un-irradiated control was held for equivalent time periods [14].

2- *Antitumor activity in vitro; cytotoxic activity against Erihlich Ascites Carcinoma (EAC) after one hr. incubation using Trypan Blue Method:*

According [15], tumor cells (EAC cells) were diluted several times with normal saline to obtain final concentration of EAC cell suspension contains 2.5×10^6 viable cell/ml. Salmonella typhimurium wild type which exposed to ψ -radiation at doses of 1, 2, 3, 4, 5 and 6kGy were cultured in LB broth medium for 18hrs/37°C to a late logarithmic phase ($OD_{600} = 0.9$). A preliminary calibration of the cell density shows that a cell suspension with OD_{600} of 1.0 is equivalent to a cell concentration of 7.5×10^8 CFU/ml.

Bacteria were harvested by centrifugation at 4000rpm for 20min., then washed twice by BPS and diluted to a concentration of 5.0×10^6 CFU/ml in BPS [16]. The all groups were assayed in triplicates. Cells were then incubated for 1hr./37°C under a constant overlay of 5% CO₂. EAC viable cells were counted by trypan blue exclusion using hemocytometer. Cells were examined under microscope [17].

3- *Evaluation of irradiated Salmonella typhi. WT toxicity in mice:*

The irradiated Salmonella typhimurium which show a higher anti-tumor activity in vitro were tested for their toxicity in mice; S. typhimurium ψ -1kGy: S.typhimurium.WT. exposed to ψ -radiation at dose of 1kGy and S.typhimurium ψ -4kGy: S. typhimurium.WT. exposed to ψ -radiation at dose of 4kGy. As well as, Salmonella typhi.WT. The previous mentioned bacteria were cultured in L.B. broth at 37°C for 18 hours to the log phase. The formula of $1 \text{ } OD_{600}$ equivalents to 7.5×10^8 CFU/ml was used to estimate the bacterial concentration. Bacteria were centrifuged at 4000rpm for 15 minutes, washed twice with PBS and diluted in PBS to yield the concentration of 10^3 , 10^4 , 10^5 , 10^6 , 10^7 , 10^8 CFU/ml. Thirty six male mice were tested for each variant toxicity. All these dilutions of the cell suspensions were injected into the mice intraperitoneal ($200 \mu\text{l}$), and the mice were scored for viability for a period of 3 weeks.

4- *Preparation of Sal. typhi. WT and its tested irradiated ones for in vivo studies:*

S. typhimurium. WT. exposed to ψ -radiation at dose of 1kGy (S. typhimurium ψ -1kGy) showed least toxicity in mice was tested in vivo for antitumor activity, as well as, S. typhimurium. WT., S. typhimurium ψ -1kGy. The bacterial cells were inoculated into L.B broth for 18hours (late log

phase) at 37°C, centrifuged at 4000rpm for 20min., washed twice by BPS and suspended in it (BPS). The cell suspensions were adjusted to a concentration of 2×10^4 CFU/ml for S. typhimurium. WT. and 2×10^6 CFU/ml for S. typhimurium. ψ -1kGy. The injection of bacterial cell suspensions were started at the first day of the experiment which last for 21 days long in all groups, except for the control and tumor groups, the injection were Intraprotenial (IP) twice and Intratumoral (IT) 3 times 4 days apart [18].

5- *Experimental design:*

Group 1: Animals were allowed 10 days for adaptation, then randomly equally distributed into 10 groups. Control Group 1; animals left without any treatment.

Group 2 (tumor group): Animals bearing solid Ehrlich tumor left without any treatment.

Group 3 (S. typhimurium. WT.): Animals not bearing solid Ehrlich tumor and injected with $100 \mu\text{l}$ of S. typhimurium. WT. at dose of 1×10^5 CFU/ml (IP) 4 days apart.

Group 4 (S. typhimurium. WT. + tumor); animals bearing solid Ehrlich tumor and injected with $100 \mu\text{l}$ of S. typhimurium. WT. at dose of 2×10^5 CFU/ml twice (IP) and $200 \mu\text{l}$ at same dose 3 times (IT) 4 days apart.

Group 5 (S. typhimurium ψ 1kGy): Animals not bearing solid Ehrlich tumor and injected with $100 \mu\text{l}$ of S. typhimurium. ψ -1kGy at dose of 2×10^6 CFU/ml twice (IP) 4 days apart.

Group 6 (S. typhimurium. ψ 1kGy + tumor): Animals bearing solid Ehrlich tumor and injected with $100 \mu\text{l}$ of S. typhimurium. ψ -1kGy at dose of 2×10^6 CFU/ml twice (IP) and $200 \mu\text{l}$ at the same dose (IT) 3 times 4 days apart.

6- *Measurement of tumor size (mm^3):*

The solid Tumor Volume (TV) mm^3 was measured every four days by using Vernier Caliper according to the following equation: $T.V (\text{mm}^3) = 0.52 (\text{length} \times \text{width}^2)$ [19], where; length, is the greatest longitudinal diameter, and width, is the greatest transvers diameter.

7- *Sample collection and preparation:*

At the end of the experiment (after 3 weeks) all mice were scarified and the blood samples were prepared for biochemical analysis, and tissue samples were collected and stored in 10% formalin for histopathological findings.

8- Caspase-3 activity assay:

A549 cells were seeded in 24-well tissue culture plates and were allowed to grow until confluence and after that; the cells were infected in the conditions described on the adhesion assay. The caspase-3 activity assay followed the design described by R & D Systems (Minneapolis, MN). After five hours of incubation, the cells were scraped and equivalent protein aliquots of each bacterial strain were determined for caspase-3 activation. All the assays were performed in triplicate. As a negative control, non-infected A549 cells were used [20].

9- Statistical analysis:

Statistical analysis of results including the mean, Standard Error (SE), Standard Deviation (SD) and *p* value were performed using Statistical Package for Social Science (SPSS) Version 15.0 for windows, Chicago, SPSS Inc, released 2006. All data were given as means \pm SD. Differences were considered significant at $p < 0.05$.

Results

1- Sensitivity of *Sal. typhi*. Wild Type parent strain towards γ -radiation: The dose survival curve of *Sal. typhi*. WT strain was illustrated in Fig. (1). It is clear that the survival fraction of *S. typhimurium*. WT. was decreased by the increase of the exposure dose to γ radiation.

A significant reduction ($p \leq 0.001$) of *S. typhimurium*. WT. count was recorded compared to non-irradiated control, and the wild type strain exhibited an exponential rate of death. The D_{10} -value was 0.7 for the *S. typhimurium*. WT. parent strain.

2- Cytotoxic effect of *Sal. typhi*. WT that exposed to γ -irradiation against EAC cells after 1 hr. incubation using trypan blue method: The cytotoxicity data of *S. typhimurium*. WT. which exposed to different doses of γ -radiation against EAC cells after one hr. incubation was illustrated in Fig. (2). *S. typhimurium*. WT. exposed to 1kGy recorded the highest mortality percent (66%) followed by that was exposed to 4kGy which recorded a mortality percentage of 56. On the other hand, *S. typhimurium*. WT. and those which exposed to 0.5kGy recorded the least mortality percentage of 21 and 32, respectively.

3- Toxicity and lethality of *S. typhimurium*. WT. ATCC (14028) exposed to 1 and 4kGy γ -radiation in mice: After 21 days of injection, the mortality incidence percentage in all groups was recorded and illustrated in Fig. (3). Results showed that no

mortality occurred in all mice after the injection by concentrations 10^2 , 10^3 and 10^4 CFU/ml of *S. typhimurium*. WT., *S. typhimurium*. γ -1kGy and *S. typhimurium*. γ 4kGy. The data indicated that *S. typhimurium*. WT. was more virulent to mice than those which subjected to 1 and 4kGy. This result was obtained since the wild type strain had recorded 100% mortality in mice when injected by a concentration of 10^7 , 10^8 CFU/ml, meanwhile, the wild type one which exposed to 1kGy was less virulent to mice than the wild type and that which exposed to 4kGy.

4- Tumor volume: After the tumor volume reached, approximately 1 cm^3 , the measurements were obtained for consecutive treatments of the animals with *S. typhimurium*. WT and *S. typhimurium*. γ -1kGy for 21 days and the results were illustrated in Fig. (4). A marked suppression in tumor growth was noticed on days of 10, 14, 17 and 21 due to the bacterial treatments.

Measurement of tumor volume at day 21 in mice bearing tumor that treated with *S. typhimurium*. WT. and *S. typhimurium*. γ -1kGy revealed a significant ($p \leq 0.001$) regression in the growth comparing with tumor control group (1672 ± 123 vs $3726 \pm 102 \text{ mm}^3$ and 1482 ± 98 vs $3726 \pm 102 \text{ mm}^3$, respectively).

5- Serum Caspase-3 activity in different mice groups (unit/ml): The results in (Table 1) revealed that the treatment of mice bearing tumor with *S. typhimurium*. γ -1kGy showed highly significant increase ($p \leq 0.001$) in caspase-3 activity levels compared to its level in mice bearing tumor group treated with *S. typhimurium*. WT.

6- Histopathological findings:

A- Group of mice kept as control: There was no histopathological alteration and the normal histological structures of the bundles were recorded in Photo (1).

B- Group of mice bearing solid tumor: Most of the Ehrlich tumor cells in the thigh muscles were histological intact Photos (2-4). Inflammatory cells infiltration was observed in between the muscle bundles Photo (5).

C- Group of mice bearing solid tumor treated with *S. typhimurium*. WT.: The result of histological analysis illustrated that the Hyalinization was noticed in the muscle bundles associated with inflammatory cells infiltration as well as between the tumor cells Photo (6). Nuclear pyknosis was detected in some of the Ehrlich tumor cells Photos (7,8). There were inflammatory cells infiltrations,

as well as, focal hemorrhages between the muscle bundles Photo (9).

D- Group of mice bearing solid tumor treated with *S.typhimurium*. γ -1kGy: Defined demarcation line of inflammatory cells and collagen fibers was noticed and separating the multiple fragmented and nuclear pyknotic nuclei of the Ehrlich tumor cells from the musculature Photos (10-12). There was inflammatory cells infiltration in between the muscle bundles.

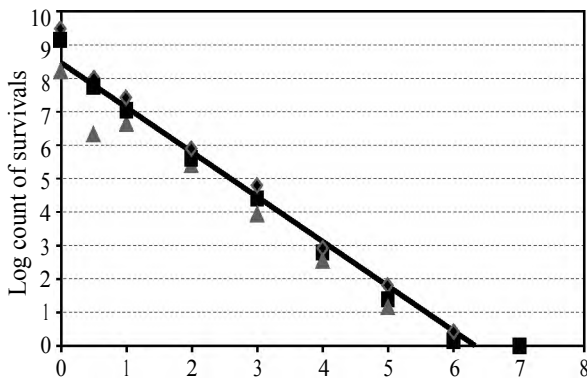


Fig. (1): Dose response curve of Salmonella typhimurium WT towards Gamma irradiation.

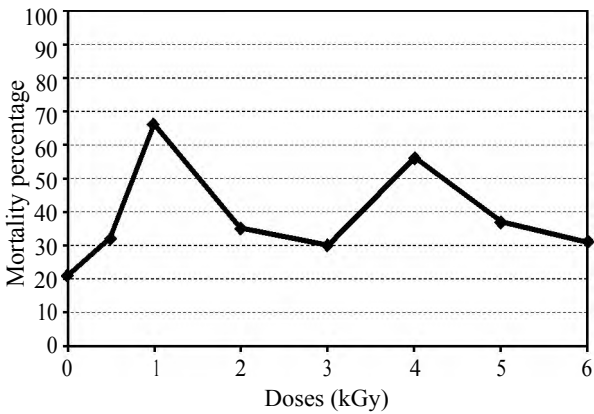


Fig. (2): Cytotoxicity of Salmonella typhimurium.WT. strain exposed to different doses of Gamma-irradiation against EAC cells after 1hr. incubation period.

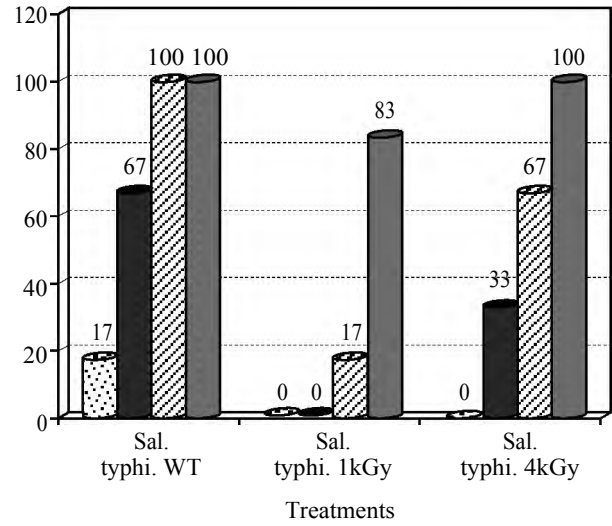


Fig. (3): Percentage of mortality of Salmonella typhimurium. WT. strain and its variants in mice.

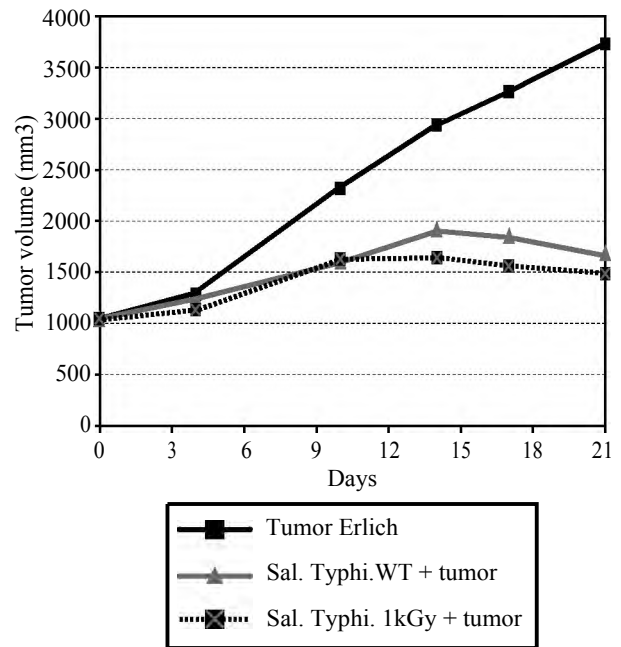


Fig. (4): Values of tumor volumes (mm³) in mice bearing tumor groups.

Table (1): Caspase-3 activity in the different mice groups treated (unit/ml).

Group	Mean \pm SD	Probability value (<i>p</i> -value)						% change from control
		<i>p</i> vs 1	<i>p</i> vs 2	<i>p</i> vs 3	<i>p</i> vs 4	<i>p</i> vs 5	<i>p</i> vs 6	
1. -ve control	1.1 \pm 0.04							
2. Tumor	3.7 \pm 0.23	0.001						+260
3. <i>S. typhimurium</i> . WT.	1.3 \pm 0.12	N.S	0.001					+18
4. <i>S. typhimurium</i> . WT. + Tumor	4.3 \pm 0.29	0.001	0.001	0.001				+290
5. <i>S. typhimurium</i> . γ -1kGy	1.45 \pm 0.1	0.001	N.S	0.001	0.001			+32
6. <i>S. typhimurium</i> γ -1kGy + Tumor	5.3 \pm 0.3	0.001	0.001	0.001	0.018	0.002		+382

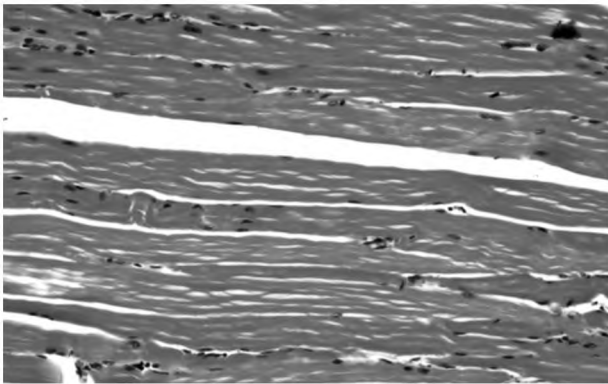


Photo (1): The muscle of mice in group showing normal histopathological structure of the muscle bundles.

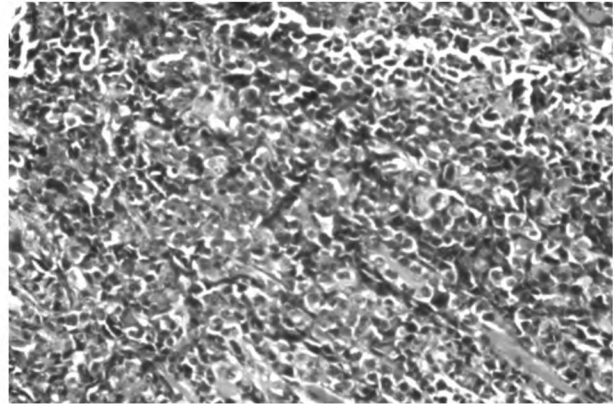


Photo (2): Ehrlich tumor cells thigh muscle of mice groups showing interact histopathological structure of most of the tumor cells (ec).

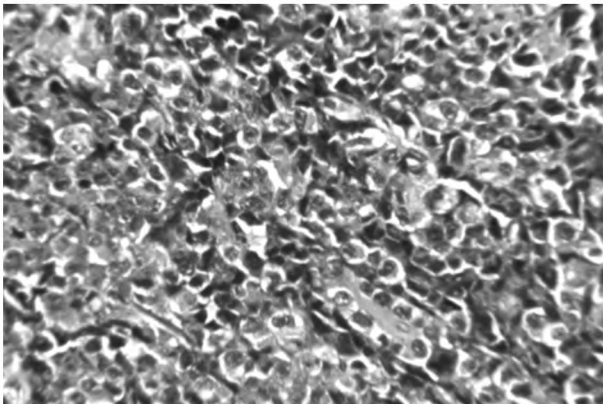


Photo (3): Ehrlich tumor cells in thigh muscle of mice group (2) showing the magnificent of Photo (2) to identify the histological structure (cc).

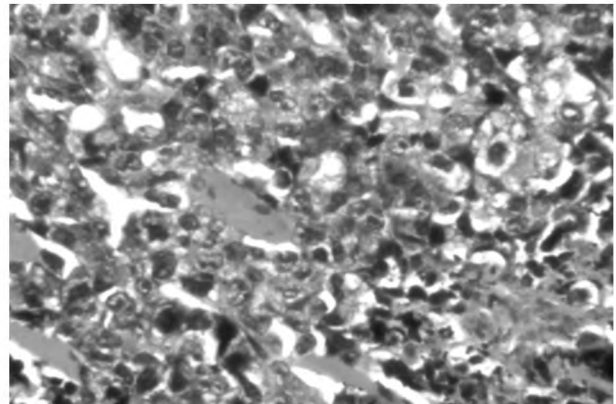


Photo (4): Ehrlich tumor cells in thigh muscle of mice groups (2) showing the magnificent of Photo (3).

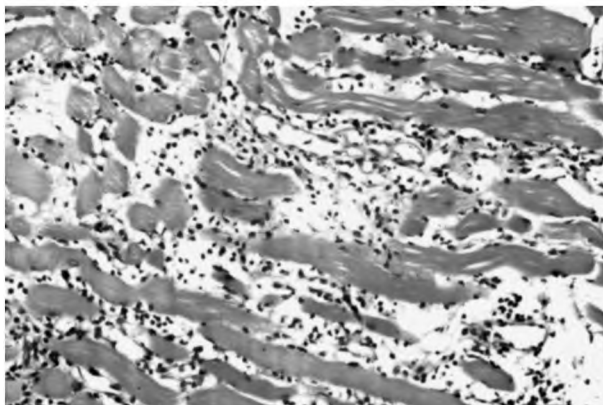


Photo (5): Thigh muscle of mice group (2) showing inflammatory cells infiltration (m) in between the bundles (ml).

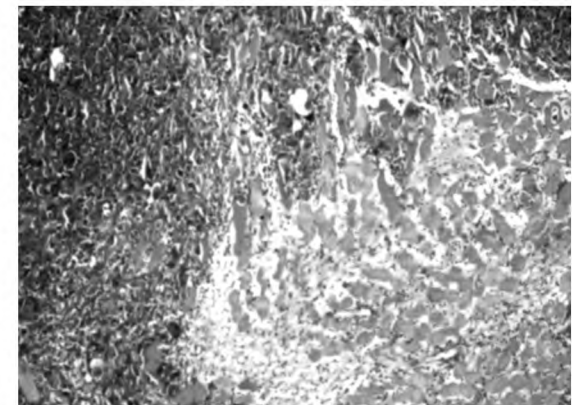


Photo (6): Ehrlich tumor cells m thigh muscle of mice group (4) showing hyalinization in skeletal muscle bundle (ml) with inflammatory cells infiltration (m) in between the tumor cells and muscle bundles.

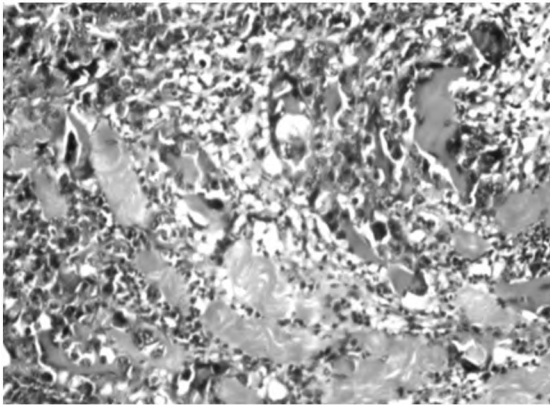


Photo (7): Ehrlich tumor cells in thigh muscle of mice group (4) showing pyknosis in the muscle of some tumor cells (cc).

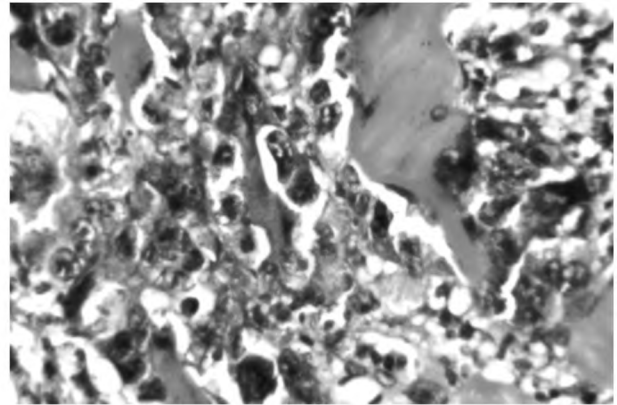


Photo (8): Ehrlich tumor cells in thigh muscle of mice groups 1 showing the magnification of Photo (7) to identify the pyknosis in nuclei of tumor cells (cc).

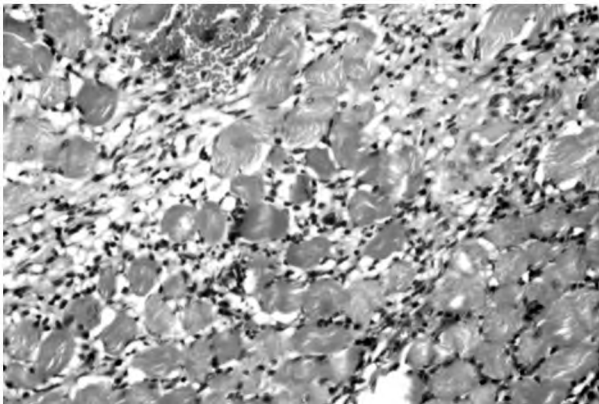


Photo (9): Skeletal muscle in mice group (4) showing inflammatory cells infiltration (m) and focal hemorrhage (b) in between the hyaline zed muscle bundles.

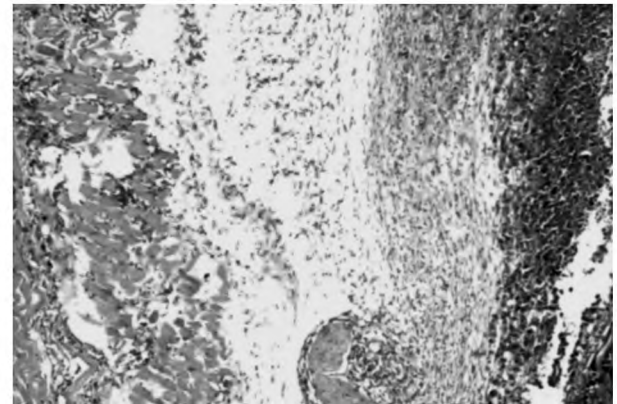


Photo (10): Ehrlich tumor cells in thigh muscle of mice group (6) showing inflammatory cells infiltration (m) with collagenous proliferation defined the skeletal muscle (m) of the necrosis in the tumor cells (NCC).

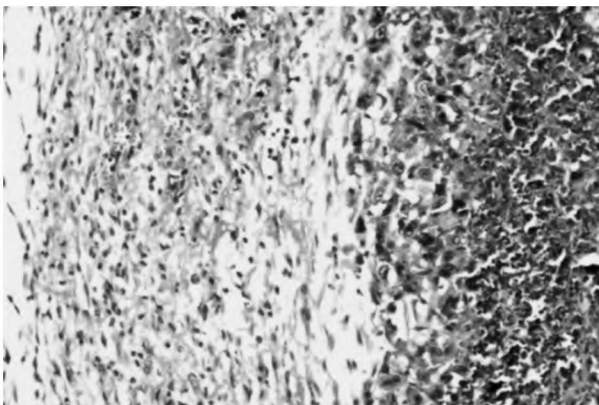


Photo (11): Ehrlich tumor cells in thigh muscle of mice group (6), showing the magnification of Photo (10) to identify the inflammatory reaction of collagen (m) with the necrosis in tumor cells (NCC).

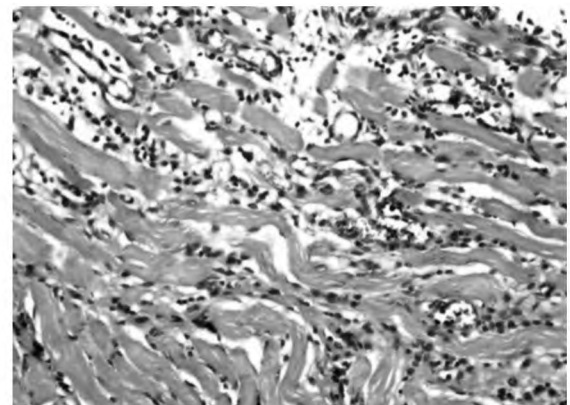


Photo (12): Thigh muscle of mice m group (6) showing inflammatory cells infiltration (m) in between the bundles.

Discussion

The present investigation was aimed to study the potential of enhancing the *S. typhimurium* . WT. by radiation on preventing solid tumor incidence.

It was found that *S. typhimurium*. Exerted a direct intrinsic toxic effect on EAC cells by different level according to its ability to invade and affect the EAC cell line but the maximum inhibition was for *S. typhimurium*. ψ -1kGy this results supported by [5].

Once Salmonella has infected the eukaryotic cell line, bacterial proliferation and expression of certain bacterial proteins during the first hours of infection induce cells death [21]. This prove and support the results obtained in the present study.

The present experimental data indicated that the exposure of *S. typhimurium*. WT. strain ATCC 14028 to 1KGy Gamma radiation dose become partially inactivated which leads to decrease its virulence in mice, which was in agreement with [22] who explained this results as follow; the *S. typhimurium*. WT. attenuation was due to cells surface damage as result of interaction of extra cellular hydroxyl radicals and oxygen, and intra cellular DNA damage.

Since 50% to 70% of bacterial cell mass is water, it absorbs much of the radiation. As a result hydroxyl radiates and hydrated electrons, which are important in irradiation-included cell inactivation, are produced [23].

[24] proved that ionizing irradiation can produce a variety of damaging effect in base and sugar moieties of the plasmid in DNA which lead to mutation and inactivation.

Irradiation damage of DNA is considerable a major cause of cell inactivation, which those of protein, lipid and RNA contribute less, since reaction of DNA with hydroxyl radicals may result in single-and/or double strand breaks, protein-DNA cross-linkage, and base alternation leading to cellular inactivation [25].

This investigation cleared that visual inspection of the treated tumor bearing mice, as well as, analysis of histopathologic section of tumor showed a control necrosis with growth of *S. typhimurium*. ψ -1 kGy variant within the tumor, as well as significant reduction ($p < 0.001$) of tumor growth was seen in mice receiving *S. typhimurium*. WT. and *S. typhimurium* ψ -1kGy compared with tumor control. These was in agreement with [26-28] who

reported that possible mechanism to explain the slowing of tumor growth by Salmonella include the consumption of nutrients by bacteria needed of tumor growth, the production of enzymes by bacteria such as asparaginase that can deplete essential amino acids needed by the tumor, the secretion of local toxins into the extra cellular environment, or the stimulation of tumor necrosis factor-alpha a production, which can import tumor vasculature. The non-specific inflammatory Responses seen at the site of bacterial growth could also potentially activate antitumor T-cells.

[29] reported that the activation of the caspase-3 is enough to come to the conclusion that Salmonella typhimurium is inducing apoptosis on the adenocarcinomic cell. Furthermore, it causes programmed cellular death on the tumor makes this bacteria an important and promising candidate for the development of a new therapy based on living organisms.

The treatment with *S. typhimurium*.WT. exposed to 1kGy improved the outcome in solid tumor regression, and induces apoptotic process through caspase-3 activation [30].

This is consistent with our previous results which reveals that *S. typhimurium* may activate specific apoptotic enzymes including caspase-3 through *S. typhimurium* effector SipA. The activation of caspase-3 in turn increases the infectivity of this pathogen by enhancing the secretion of effectors Furthermore, the activation of cleaved caspase-3 is linked to tumor cell apoptosis [5].

In the present work, the results showed a significant increase in caspase-3 activity, in mice that treated with *S. typhimurium* ψ -1 kGy more than those that treated with *S. typhimurium*.WT. These findings was in agreement with [31] and [11] who reported that *S. typhimurium* is being used to target and destroy tumors through sensitizing the immune system to the presence of tumors, and also directly activate caspase-3, a key enzyme of the apoptotic pathway.

The Hematoxylin and Eosin (H & E) staining of tumor tissues showed that the Salmonella typhimurium colonization in mice solid tumor resulted in a sharper increase in the number of necrotic solid tumor cells [30,32].

In this study, histopathological finding revealed that treatment of mice bearing tumor groups with *S. typhimurium* that exposed to ψ radiation showed, a histopathological improvement compared to tumor group.

In the present study histopathological finding of muscular thigh tissue of the mice bearing tumor treated with *S. typhimurium* 1-kGy showed approximately normal Histological structure compared to the control healthy group which agreement with muscular and biochemical parameters.

Conclusion:

It was concluded that the exposure of *Salmonella typhimurium* wild type to gamma irradiation produced an attenuated *Salmonella typhimurium* capable of tumor targeting, colonizing and eliciting growth suppression of solid tumor in mice.

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السالمونيلا تايفيموريوم المعالجة بالإشعاع الجامي كوسيلة للتحكم في السرطان

يعتبر مرض السرطان من الأمراض التي يصعب السيطرة عليها نتيجة للطبيعة الفريدة للبيئة المكونة للخلايا السرطانية، خاصة في بعض حالات الأورام الصلبة. ويعزى هذا للنقص الشديد في الأكسجين بها فضلا عن عدم وجود أوعية دموية مما يصعب الوصول إليها وعلاجها.

وجد أن السالمونيلا تايفيموريوم لها القدرة على الإختراق والنمو والتكاثر في الخلايا السرطانية، كوسط غذائي لها حيث أنها تتميز بدرجة عالية من التخصص في الإنتشار والتكاثر في النسيج الورمي، وتهدف هذه الدراسة إلى بحث إمكانية استخدام الأشعة المؤينة (أشعة جاما) لضعاف السالمونيلا تايفيموريوم الأصلية تكون لها القدرة على إستهداف وتقليص حجم الورم، ولتحقيق هذه الهدف ولمعرفة مدى إمكانية استخدام السالمونيلا المعرضة للإشعاع في إنحسار وتقليل حجم الأورام الصلبة، تم إختبارها وحقنها في مجموعات في فئران الألبينو السويسرية الذكور المحملة بورم الأريلخ الصلب بعد تعريضها لجرعات مختلفة من أشعة جاما.

قد أوضحت النتائج أن تعرض السالمونيلا تايفيموريوم للإشعاع قد أدى إلى إضعافها، وبالتالي أصبح تأثيرها أقل سمية لفئران التجارب. وقد وجد أن إختبار السمية الخلوية للسلاسل المعرضة للإشعاع على خلايا ورم الأريلخ خارجيا أدى إلى تثبيط معدل نمو وإنقسام هذه الخلايا وسجلت أعلى نسبة تثبيط عند تعريض السالمونيلا تايفيموريوم الأصلية للإشعاع الجامي لجرعة واحد وأربعة كيلو جراي. كما تم حقن السلالة المعرضة للإشعاع الجامي لجرعة واحد كيلو جراي والتي قد أظهرت أقل سمية في مجاميع الفئران الذكور المحملة بورم الأريلخ الصلب لدراسة مدى تأثيرها على حجم الورم. كذلك تم تقدير مستوى نشاط كاس باس 3 في نسيج الورم بالإضافة إلى دراسة قطاعات الأنسجة العضلية المحملة بالورم. أظهرت النتائج زيادة معنوية في نشاط الكاس باس-3، كما أوضح تحليل الهستوباثولوجي تحسن في تركيب النسيج العضلي وزيادة في عدد الخلايا في طور الموت المبرمج للخلايا السرطانية.