Control of *Salmonella Spp* isolated from Garmat Ali sewage by superoxide radicals produced in vitro.

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Abstract

A modified method was used in the present study to generate superoxide radicals ($O_2^{\cdot -}$) in vitro, the method involves exposing of riboflavin to lighting and generate $O_2^{\cdot -}$. *Salmonella Spp* was isolated from sewage of Garmat Ali region in Basrah city, Iraq. This study aimed to reduced *Salmonella Spp* by submitted it to superoxide radicals. A significant reduction in the number of bacteria was revealed after exposed to superoxide radicals at (10, 20, 30, 40 and 50) µg/mL of riboflavin for 10 min of lighting with fluorescent lamp (40 watt), as well as the reduction in bacteria number was observed when the bacteria was exposed to superoxide radicals at 30µg/mL of riboflavin for intervals (5, 10, 15, 20, 25 and 30) min of lighting with fluorescent lamp. This study suggesting that reduction in bacteria number was increased as increased riboflavin concentration or increased lighting time.

Introduction

Sewage contamination in drinking and recreational waters is of great concern, because the presence of enteric pathogens threatens public health, Among these organisms are *Salmonella*, which are pathogenic enteric bacteria that can cause salmonellosis in animals and humans, if concentrations able to give rise to infections are present. *Salmonella* is the bacterial pathogen most commonly studied in sewage . In addition, they can survive for long periods of time in sewage sludge and soil, perhaps years under cool moist conditions. Infected humans and animals shed salmonellae into the environment via faeces and ingestion of salmonellae-contaminated food and water (Clyde *et al*., 1997). Salmonellosis due to infected food handlers has been reported by many authors in different countries (Luby & Jones, 1993; Al-Turki *et al*., 1998; Senthilkumar & Prabakaran, 2005).

Free radicals such as $O_2^{\cdot -}$ and its derivatives are believed to be the cause of oxygen toxicity. $O_2^{\cdot -}$ is a strong oxidant factor and toxic to cells as the cause of killing bacteria (Kanafani & Martin, 1985). Damage to *Azotobacter chroococcum* and *Escherichia coli* by $O_2^{\cdot -}$ has been demonstrated (Buchanan, 1977; Van Hemmen & Meuling, 1977).

Riboflavin also known as vitamin B_2, is an easily absorbed micronutrient with a key role in maintaining health in humans and animals. Riboflavin has been used as part of the phototherapy treatment of neonatal jaundice, a muscle pain reliever, improve the safety of transfused blood by reducing pathogens found in collected blood and other uses (Powers, 2003; Ball, 2006).

Aim of the study

The aim of the present study was not to survey on presence of *Salmonella Spp* in sewage but the main aim was control of (reducing) this bacteria by submitted it to superoxide radicals produced in vitro according to the modified assay as shown in Fig (1).

Materials and Methods

Isolation and diagnosis:

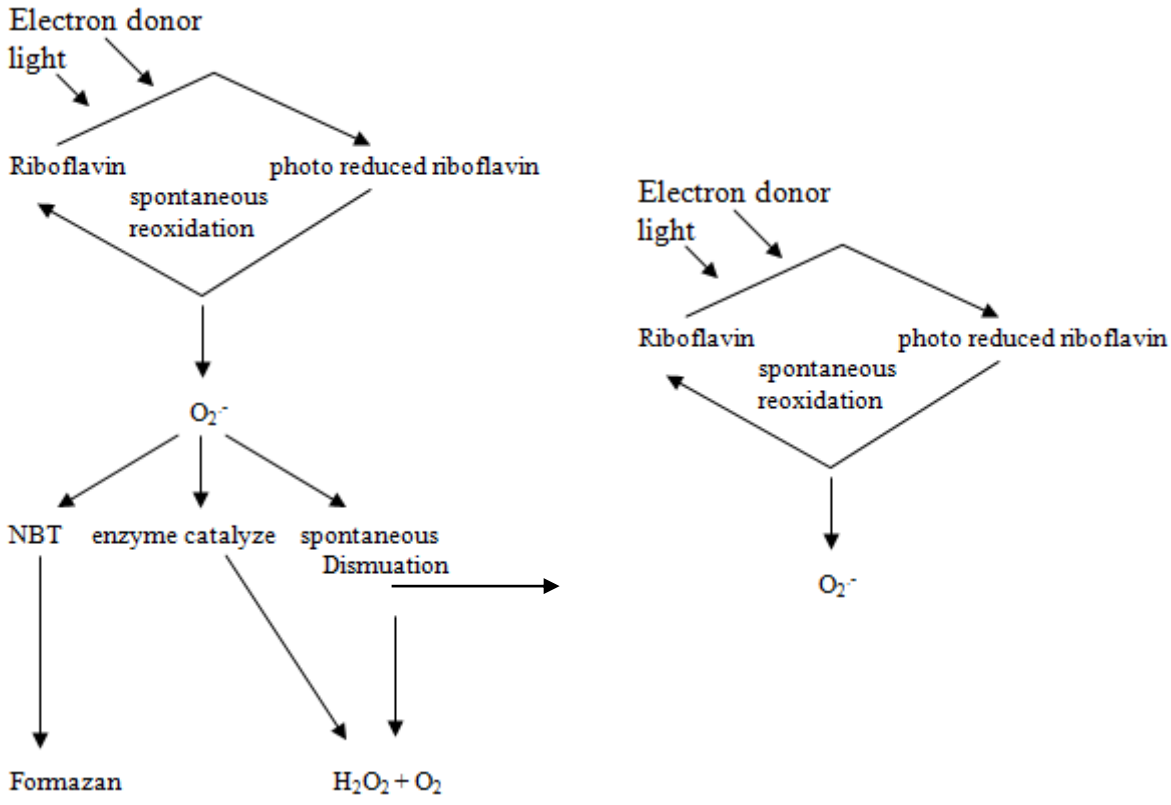
Ten sewage samples were collected from Garmat Ali region in Basrah city, and immediately cultured on *Salmonella Shigella* agar (*SS* agar), a differentially selective medium for the isolation of pathogenic enteric bacilli, especially those belonging to the genus *Salmonella*, then dishes were incubated at 37 Co for 24 - 48h to obtain on *Salmonella Spp* colonies. Preliminary diagnosis of *Salmonella Spp* depended on cultural and morphological characteristics, it appear as clear, translucent colonies, some with black centers indicating H_2S production.

biochemical tests:

After obtained of colonies from *SS* agar, in order to detect *Salmonella Spp*, biochemical tests such as gram stain, catalase activity, indole production, carbohydrate fermentation and other tests were used for this aim, table (1) was showed that.

Superoxide production:

For produce superoxide radicals in vitro, the assay of superoxide dismutase activity by Beyer & Fridovich, (1987) was modified as figure below. The procedure was required to the box padded from the inside with aluminum foil and contained fluorescent lamp (40 watt) in addition to riboflavin (B_2 vitamin) which reduced by light and then photo reduced riboflavin interacts with oxygen to generate superoxide radicals (fig 1).



Before modify After modify

(Donnelly *et al*., 1989)

Fig(1): Production of superoxide radicals in vitro.

Experiment:

Tubes of dilution $1x10^4$ of *Salmonella Spp* were prepared (according to World Health Organization, (2004)) with normal saline at concentrations (0, 10, 20, 30, 40 and 50 µg/mL riboflavine), the experiment tubes were exposed to lighting (inside the box) for 10 min and cultured on *SS* agar, then dishes were incubated at 37 C^o for 24h.

Tubes of dilution $1x10^4$ of *Salmonella Spp* were prepared with normal saline at concentration 30 µg/mL riboflavine. the experiment tubes were exposed to lighting (inside the box) for intervals 5, 10, 15, 20, 25 and 30 min and cultured on *SS* agar, then dishes were incubated at 37 C^o for 24h.

Results and Discussion

All sewage samples (10) that have been collected from Garmat Ali region were given a heavy growth of *Salmonella Spp* on *SS* agar, and the table (1) was shown the biochemical tests for diagnosis of *Salmonella Spp*. A significant reducing in Colonies Forming Unit (CFU) of bacteria was revealed after exposed to superoxide radicals at 30µg/mL riboflavin for 10 min of lighting (40 watt fluorescent lamp) as compared with non treatment bacteria (0 µg/mL riboflavin or control), and the reducing in CFU was increased as increased concentrations of riboflavin as it clear in Fig (2). The present study was also revealed a significant diminution in CFU of *Salmonella Spp*

after submitted it to the superoxide radicals at 30µg/mL of riboflavin for different times of lighting and the diminution in CFU was increased as increased lighting time (Fig 3), and the Fig (4) was showed a significant differences between treated and untreated *Salmonella Spp* with $O_2^{\cdot -}$ radicals. from these results we can suggesting that production of superoxide radicals were increased by increased riboflavin concentrations, and this was explained the reduction in CFU of *Salmonella Spp* was increased as increased of riboflavin concentrations (Fig, 2). As well as increasing of lighting time that's mean increasing of Exposing the bacteria for long period with superoxide radicals and reasoned CFU reduction (Fig, 3).

The present study was resembled to previous studies, such as the study of (Jaquette *et al*., 1996) which revealed a significant reduced in population of *Salmonella stanley* inoculated onto alfalfa seeds after the last was treated with different concentrations of chlorine, as well as the study of (Gandhi & Matthews, 2003) was revealed that chlorine or calcium hypochlorite were reduced of *Salmonella* population in alfalfa seeds artificially contaminated with *Salmonella*, also the study of (Zhuang & Beuchat, 1996) was revealed a significantly reduced in population of *Salmonella montevideo* in core tissue of tomatoes dipped in 4–15% TSP (trisodium phosphate), and the study of (Sapers & Jones, 2006) was revealed that hydrogen peroxide (H_2O_2) had efficacy in reduced population of *E. coli* on dip-inoculated tomatoes.

Table (1): biochemical tests for diagnosis of *Salmonella Spp*

The test	Result
Motility	+
Gram's stain	-
Cellular morphology	Rods
Catalase activity	+

Indole production	-
Voges proskauer	-
Triple sugar iron	K/A with H_2S
Sugar fermentation	
Glucose	+
Lactose	-
Maltose	-
Xylose	-

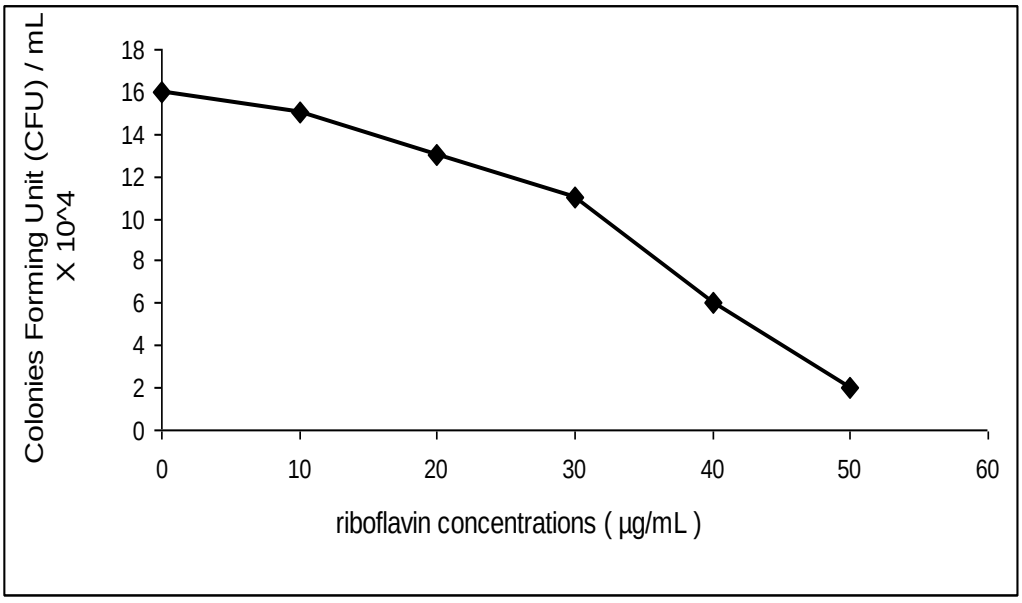
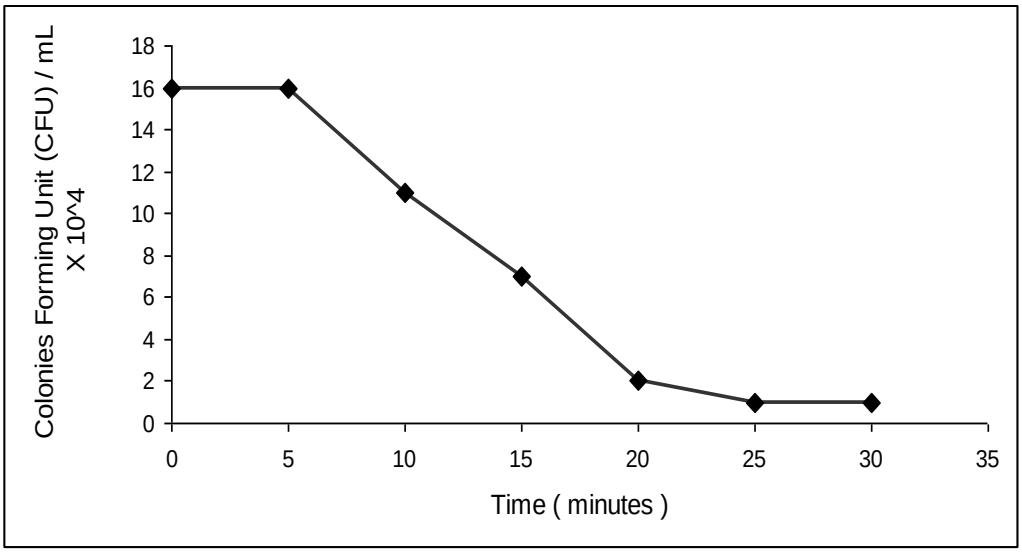
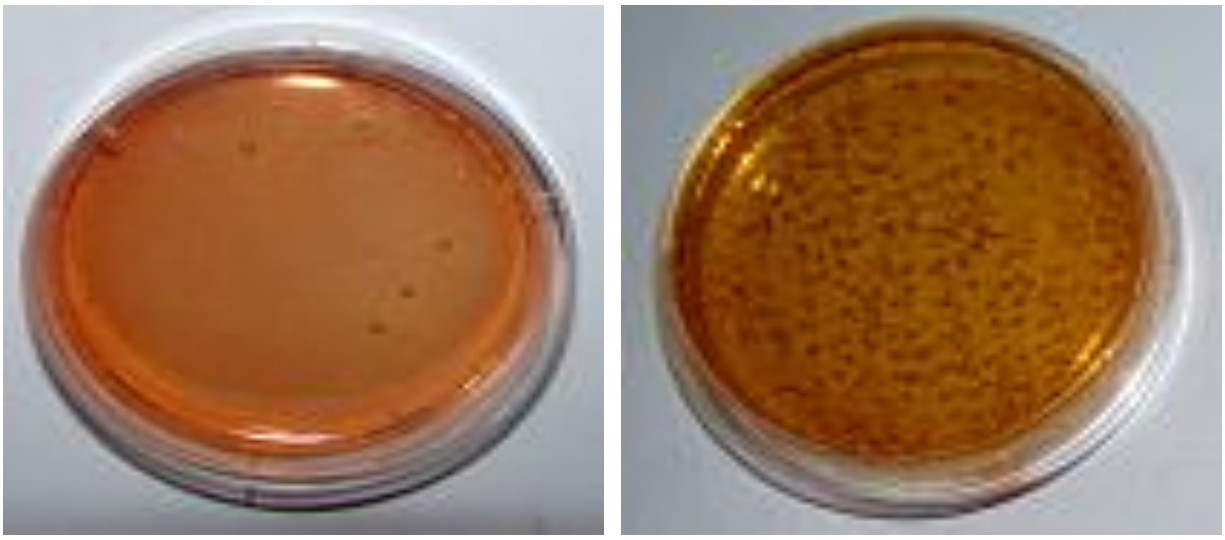


Fig (2): exposure of bacteria to superoxide radicals at different concentration of riboflavin for 10 min.



Fig(3): exposure of bacteria to superoxide radicals at 30µg/mL of riboflavin for different times.



(A) (B)

Fig(4): comparison between *Salmonella Spp* exposed and non exposed to superoxide radicals.

Where:-

(A) exposed to superoxide radicals at 30µg/mL riboflavin for 15min.

(B) non exposed to superoxide radicals.

Conclusion

From the present study, one can conclude that superoxide radicals have efficacy in elimination on *Salmonella Spp* in sewage samples from Garmat Ali region in Basrah city, and the modified method which was used in current study was very efficient in the production of superoxide radicals, as well as the production of superoxide radicals was increased as increased of riboflavin concentrations according to the modified method of this study.

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الخلاصة

السيطرة على بكتريا *Salmonella Spp* المعزولة من مياه الصرف الصحي من منطقة كرمة علي بوساطة جذور السوبرأوكساید المنتجة خارج الجسم الحي.

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تم أستخدام طريقة محورة في الدراسة الحالية لإنتاج جذور السوبرأوكساید ($0_2^{\cdot-}$) مختبريا، إذ تضمنت الطريقة معاملة الرايبوفلافين (B_2) إلى الإضاءة وتحرير جذور السوبرأوكساید. وهدفت الدراسة إلى استخدام هذه الجذور في السيطرة (قتل) على بكتريا *Salmonella Spp* التي تم عزلها من مياه الصرف الصحي من منطقة كرمة علي في مدينة البصرة. أظهرت الدراسة انخفاض واضح في أعداد البكتريا بعد تعريضها إلى جذور السوبرأوكساید عند تراكيز (10، 20، 30، 40 و50) مايكرو غرام/ مليليتر من الرايبوفلافين لفترة 10 دقائق من الإضاءة بمصباح فلوريسنت 40 واط . كما لوحظ أيضا انخفاض كبير في إعداد البكتريا بعد تعريضها إلى جذور السوبرأوكساید بتركيز 30 مايكرو غرام/ مليليتر من الرايبوفلافين لفترات إضاءة مختلفة (5، 10، 15، 20، 25 و 30) دقيقة بمصباح الفلوريسنت، وهذا يشير إلى إن الانخفاض في أعداد البكتريا يزداد بزيادة تركيز الرايبوفلافين أو بزيادة فترة التعرض للإضاءة.