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Antibacterial Activity of Titanium Dioxide (Tio_2) Doped with H_2O_2 against *staphylococcus aureus* Human pathogen in aqueos solution.

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Abstract

The effects of parameters such as amount of TiO_2, presence of H_2O_2, irradiation time, dark and light condition were studied and investigated their acted against *S.aureus* human pathogen in aqueous solution. The results show that the number of *S.aureus* was greatly reduced after treatment with a visible light and Tio_2 doping H_2o_2 better than after treatment with visible light only and visible light with Tio_2 only. In the treatment with Tio_2 and light the number of bacteria was decreased with survival ratio at 40% . The best result has been obtained at 0.33mg/ml Tio_2 concentration which is equal to survival ratio 30%. Two dark/H_2O_2 treatments have been done in presence and absence of Tio_2, in adding 10ppm H_2o_2 the bacterial number decreases (survival ratio 45%) and in adding 0.33mg/ml of Tio_2 with 10 ppm H_2O_2 in dark the survival ratio 40%. Two light/H_2O_2 treatments have been done in presence and absence of Tio_2. Survival ratio in presence of light /H_2O_2 was 23%, the survival ratio of *S. aureus* in presence of light/H_2O_2/Tio_2 was 11%. This study suggests that H_2o_2 doped Tio_2 can be used as disinfectant in water phase.

Keywords: photocatalytic killing of *S. aureus*, TiO2 photocatalyst, H_2O_2, sterilization, water phase, light, dark.

الخلاصة:

تم دراسة تاثير عدد من العوامل مثل كمية Tio_2، وجود H_2O_2. وقت التشعيع ،وجود او عدم وجود الضوء على بكتريا *S.aureus* الممرضة للانسان في الطور المائي .بينت النتائج ان اعداد البكتريا اختزلت بشكل كبير بعد المعاملة مع Tio_2/H_2O_2 بوجود الضوء افضل من المعاملة بالضوء فقط او المعاملة بالضوء و Tio_2 فقط . عند المعاملة Tio_2 والضوء انخفضت اعـــداد البكتريا بنسبة بقاء 40% . كانت افضل النتائج المستحصلة عند تركيز 0.33 mg/ml من Tio_2 اذ كانت نسبة البقاء 30% . تم اجراء تجربتين بظروف الظلام وH_2O_2 بوجود او عدم وجود Tio_2 ، عند اضافة 10 ppm من H_2O_2 والظلام انخفضت اعداد البكتريا (نسبة البقاء 45%) وعند اضافة Tio_2 كانت نسبة البقاء للبكتريا 40%. تم اجراء تجربتين بظروف الضـــوء و H_2O_2 بوجود او عدم وجود Tio_2 ، عند عدم وجود Tio_2 كانت نسبة بقاء البكتريا 23% في حين عند وجود Tio_2 بهذه الظروف كانت نسبة بقاء البكتريا 11%. توصي هذه الدراسة باستخدام H_2o_2 وTio_2 كمعقم في الطور المائي .

الكلمات المفتاحية : القتل الضوئي ، بكتريا *aureus*, TiO H_2O_2, التعقيم ، الضوء المائي ، الضلام

Introduction:

Staphylococcus aureus are capable of prolonged survival on a variety of environmental surfaces, it can be alive in distilled water and in all parts of hospital and it is resistant to chemical disinfectants and many of conventional antibiotics (Carson *etal*, 1988).

It is the most virulent of the many staphylococcus species and it is responsible for infections ranging from superficial skin to soft tissue infections (Kasper *etal*, 2005), and its exotoxin producing pathogen, can cause food-borne disease in human (Salyers and Whitt, 1994) it has the highest pathogenic effect in human and it is the first pathogen agent in hospitals (Gacesa and Russell, 1990).

The Bacteria can be destroyed by a number of different techniques including heat, radiation and chemical oxidizing agents such us H_2O_2,Which are used to treat microbial pollution of waters ,but they are not completely efficient on some of resisting microorganism such us *S.aureus* (Mills and Lettunte ,1997).

The wide spread use of antibiotics led to the emergence of more resistant and virulent strains of bacteria and this caused an urgent need to develop alternative sterilization technologies to disinfect water and waste water from hospital such us using photocatalytic effect of Tio_2,this method is feasible and inexpensive to act as disinfectants (Russell, 2004 and Manes *etal* 1999 and Aiello and Larson ,2003 and Block, *etal* 1997) and powerful biocide process due to production of redox reaction species from Tio_2, when irradiation Tio_2 particles are indirect contact with or close to microbes to initial oxidative attack (Daneshvar *etal*, 2007 and Lee *etal*, 2005) and photokilling action was associated with the reduction in the level of intracellular coA through photo oxidation (Mills & Lettunte 1997).

The oxidation processes such us Tio_2/Uv and H_2o_2/Uv are useful in water purification and surgical suites (Coates *etal*, 2007 , Julian *etal*, 2007 and Cho *etal*,2002).

Since 1981 many papers have been published about semiconductor, the most studies in this field have been done on bacteria especially *E.coli* and *S. aureus* and other bacteria. Mastsunaga and coworkers published first report about photocatalytic disinfection in 1985 (Mills & Lettunte 1997; Aiello & Larson 2003; Julain *etal* 2007; Hemraj *etal*,2014).

The action of this technology in aqueous phase is in presence of Tio_2 alone or with H_2o_2 upon Uv light excitations ,the photo energy excites valenee band electron and generate pairs of electrons and holes that diffuse and trapped on or near the Tio_2 surface (Wong *etal* 2006; Cheng *etal*, 2009; John *etal*, 2014) and these pairs have strong reducing and oxidizing oxygen to yield reactive species such us ^{-}OH &$^{-}O_2$ (Fujishima and Honda 1972) which are extremely reactive upon contact with organic compounds and bacterial cell (Saleh, 2011) and complete oxidation to carbon dioxide (Jacoby *etal*, 1998). These radicals operate in consent to attack poly unsaturated phospholipids in bacteria (Wong *etal*, 2006) Another related study was carried out by Hirakawa and coworkers (2004) which has shown that photo irradiation Tio_2 catalyzed site-specific DNA damage via H_2o_2 .These findings suggested that Tio_2 might exert antimicrobial effects similar to these of peroxygen disinfectant H_2o_2 (McDonnell and Russell, 1999).

The aim of this study is to investigate the effect of Uv visible light on the antibacterial activity in presence of H_2o_2/Tio_2 and using photo catalytic reaction for disinfecting water and waste water instead of chemical material.

Materials and Methods

Bacterial Strains and Culture

Basic bacterial cultured methods have been done (Johnson and Case, 1995). Clinical isolated *S. aureus* was collected from Babylon Hospital of pediatric and maternity.

In this study, bacterial concentrations were determined by standard plating count method (SPC).Afresh bacterial culture was diluted by factors 10^{-1} to 10^{-6} and bacterial concentrations of these dilutions were determined using SPC. After this step the right dilution has been selected to be used in these experiments and before every experimental the number of bacteria reading and this number is used as astandard number.

Chemicals

A:-Nutient agar was supplied from HIMDIA.

B:-$H_2O_2$was supplied from DDH at 30%.

C:-Titanium dioxide (Tio_2) was supplied from Degussa P25(Cheng *etal*, 2009; Julain *etal*, 2007).

2-3 Instruments: photo catalysis cell.

A:-Source of irradiation: use low pressure mercury lamp type OSRAM (160W) (306-750nm)

B: Reaction vessel: content photo cell (35 cm^3) with quartz window (2cm^2).

C: Regulator circulating thermostat (Desagafrigosta): using to control the temperature.

D: Oxygen gas container was connected with flowmeter (Rato) to control the rate of gas passing on the surface of aqueous solution.

E: Amagnetic stirrer (Abovolt) was used to homogenous suspension.

F: Centrifuge (Hettich) was used to remove Tio_2 practical and the supernal liquid, the instruments used in this work were previously described in detail (Gassim *etal*, 2004).

2-4 Photo Catalysis Experiments:-

In all photo catalytic experiments 30 ml of aqueous solution of *S. aureus* cell suspensions were added to a known weight of Tio_2 particles in photo cell quartiz window and suspended by using magnetic stirrer the oxygen was passed on the surface of aqueous suspension at the rate 10 ml/min. The temperature was controlled at $25c^o$ by using circulating thermostat. The suspension was irradiated for 40 min.

Other experiments have been done by adding 10 ppm of H_2o_2 *S. aureus* aqueous solution in absence and in presence of Tio_2 catalyst, dark and light condition. After each 10(min) samples of irradiated mixture were drawn by using syringe with along pliable needle and then centrifuged at 1000 rpm /5min in all experiments, 0.5 ml of the suspension was immediately added to 20ml nutrient agar media in petri dish with triplicates per each treatment. The culture was kept in the dark at $37c^0$ for 24h .Colony forming units (cfu) of *S. aureus* were counted by SPC (Saleh,2011).

The incident light intensity was measured by using parcker and Haut chard method (Maness *etal*, 1999) this method consists of irradiated potassium ferrioxalate actinometry k3fe (c_2o_4) 2.3 H_2o_2 for 3min after passing nitrogen gas for 15 min at $25c^0$. The average light intensity is 6.2×10^{-8} Einstein $L^{-1}S^{-1}$(Gassin *etal*, 2009).

Results & Discussion:-

In this study the effects of parameters such as amount of TiO_2, presence of H_2O_2, irradiation time, dark and light condition were studied and investigated their acted against *S.aureus* human pathogen. All experiments occurred in presence of oxygen.

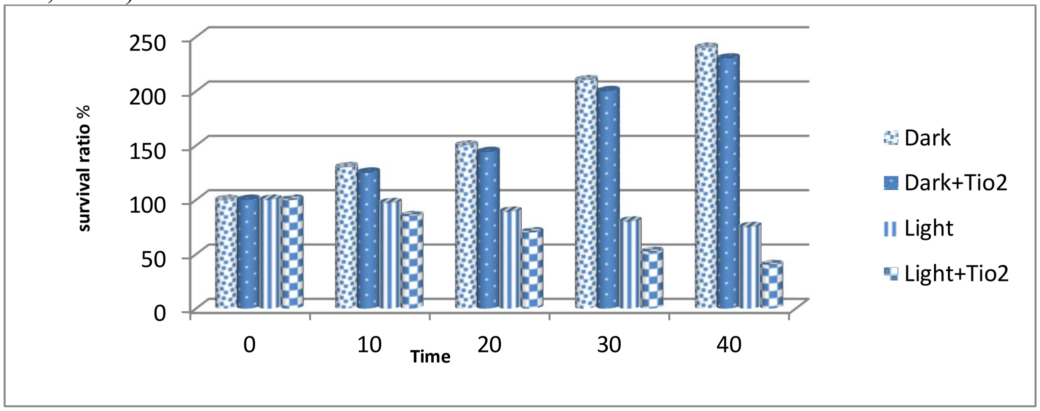
1-Determination of optimum conditions for photocatalytic reactions.

To determine the optimum condition, which led to high killing efficiency, four primary experiments have been done .Figure(1) shows that ,in the first treatment with dark only the number of bacterial cell was increased with time because the bacteria have acclimatized to their new environment and synthesis the enzymes needed to initialize the available materials (Johnson &case ,1995) and division by binary fission this leads to increase the number of cells with time in aqueous solution(Kwaadsteniet *etal*,2011).

In the treatment with Tio_2 in dark (Figure-2), there's no effect on bacterial number because Tio_2 are biologically and chemically invert and its antibacterial activity can be switched on and off or modulated by controlling the light intensity, and this agrees well with other studies (wong *etal*,2006; cheng, *etal,*2009; Kwaadsteniet *etal*,2011; Hemraj *etal*,2014).

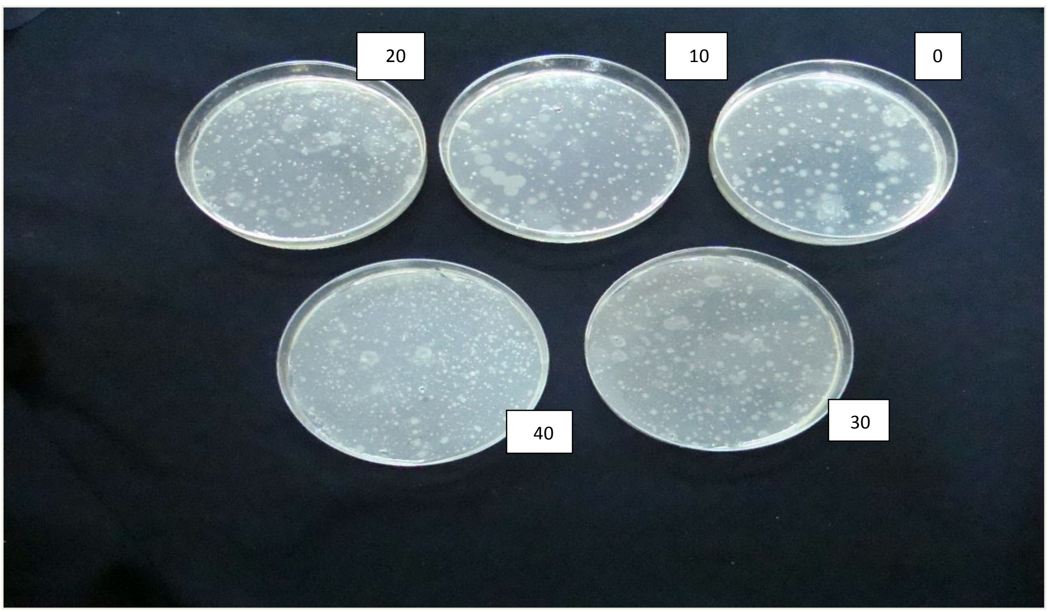
In the treatment with light only, the number of bacteria is decreased, because the light used in this work has wave length ranges from 306 to 750 nm which can cause damage to DNA resulting in the cell death by forming oxygen radicals within cell (Johnson &case,1995, Ireland *etal* 1993, Kruft, and Green 2011).

In the treatment with Tio_2 and light the number of bacteria was decreased with survival ratio at 40%. This means that the presence of light and Tio_2 catalyst was very essential for photocatalytic reaction due to the bacterial activity of Tio_2 act in presence of light. The study of pal &coworkers (2006) and cheng and coworkers (2009) found that light can activate the antibacterial activity of Tio_2 by exciting it (Saleh, 2011).



Fig(1) Primary experiments for determination of optimum conditions for photocatalytic reactions

Fig(2): Treatment with dark and Tio_2 (time 0,10,20,40 min.).



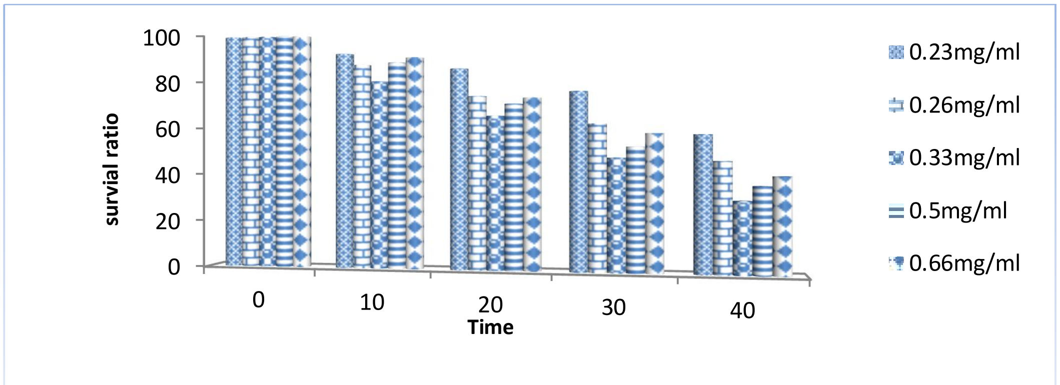
2- **Effect of Tio_2 amount on removal of *S.aureus*.**

Tio_2 is the most widely used as semiconduction photocatalyst due to its high photostability non-toxic high oxidizing potential and insolubility in water under different conditions (Saleh,2011).

Different amounts of Tio_2 (0.23, 0.26, 0.33, 0.5 and 0.66 mg/ml) were added to the samples with known initial number of bacterial cells and the results in figure (3) show that the increasing in Tio_2 concentration led to increase the removal of *S. aureus* and this result agrees with Kweedsteniet and coworkers (2011) and Saleh (2011) and Daneshvar and coworkers (2007) who found that the presence of Tio_2 in water has higher photocatalytic effects on *S.aureus* in the aqueous solution.

At high Tio_2 concentration (more than 0.33mg/ml) the removal efficiency of *S. aureus* was decreased. The turbidity of the solution prevents the effect of light, theTio_2 particles from inner filter which absorbs high portion of the incident light. The light scattering due to the turbidity of the solution which led to reduce the rate of photocatalytic reactions. (Gassim, 2009).

The best result has been obtained at 0.33mg/ml Tio_2 concentration which is equal to survival ratio 30% so that this concentration will be chosen to study the effect of H_2O_2/Tio_2 semiconductors on the killing efficiency of *S. aureus* in aqueous solution.



Fig(3) Effect of Tio_2 amount on removal of *S.aureus*

3- *S. aureus* photodegradation with H_2O_2 in different condition.

A number of experiments has been carried out including the adding 10 ppm of H_2O_2 to study its bactericidal effect on *S.aureus*. Two dark/H_2O_2 treatments have been done in presence and absence of Tio_2 as shown in figure (4).

In the adding 10ppm H_2o_2 the bacterial number decreases (survival ratio 45%) and in adding 0.33mg/ml of Tio_2 with 10 ppm H_2O_2 in dark the survival ratio 40%. The hydrogen peroxide decreases the respiratory activities that led to cell death (Johnson &case1995).There was no actual effect on survival ratio when Tio_2 was added to the treatment because it was inert in dark.

Two light/H_2O_2 treatments have been done in presence and absence of Tio_2 (figure 5) the number of bacterial cells decreases (survival ratio in presence light /H_2O_2 was 23% but the killing efficiency of *S. aureus* in presence of light/H_2O_2/Tio_2 was highest than when compared with light /H_2o_2 condition alone (survival ratio 11%).

This effect was explained by many studies (Matsunaga, *etal* 1985; Saleh, 2011; Djurisic, *etal* 2012 and Jaisai *etal* 2012) which found that both H_2O_2 and hydroxyl Radicals from Tio_2 are necessary for bactericidal effect, and H_2o_2 alone could not induce the same anti-bacterial effect when doping with Tio_2.

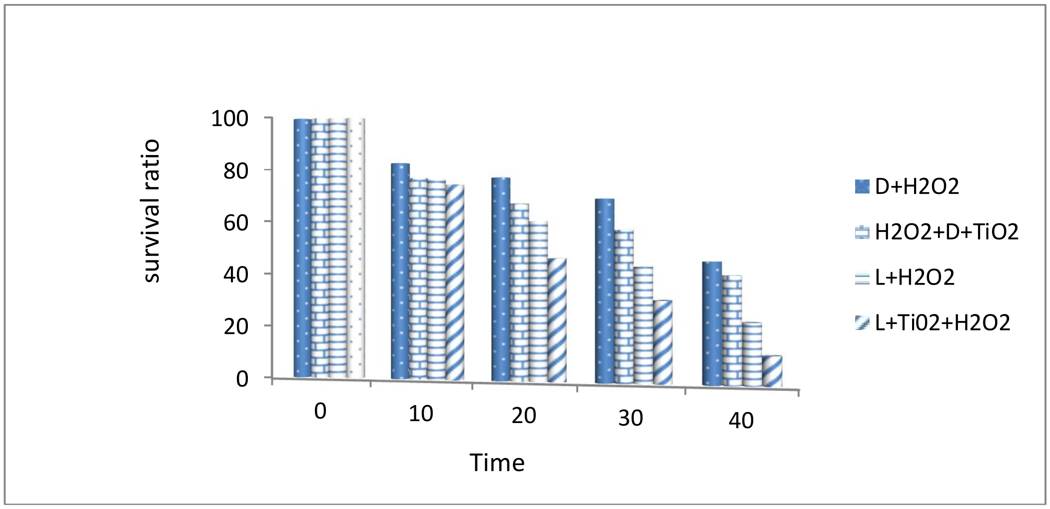
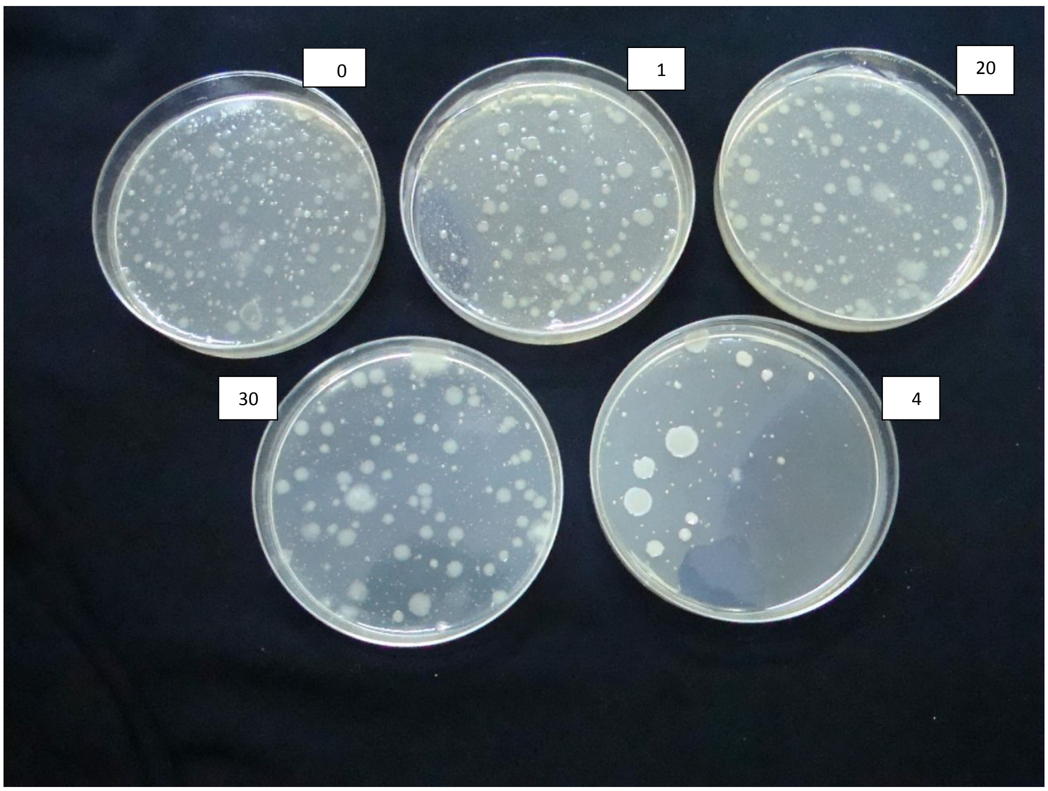


Fig (4): photodegradation effect of H_2O_2 on *S. aureus* in different condition



Fig(5):Treatment with H_2O_2 and Tio_2 and light.(time 0,10,20,30,40 min.)

4- The effect of presence and absence of H_2O_2 on *S.aureus* survival ratio

Figure (6) shows the comparison of survival ratio of *S.aureus* in aqueous solution treated with light/Tio_2 0.33mg/ml between the presence and absence of H_2O_2.

The killing efficiency of *S.aureus* was increased in both treatments but the higher killing rate was obtained when adding 10 ppm H_2o_2 (survival ratio 11%) the presence of H_2o_2 /Tio_2/light operates in concerted attack poly unsaturated phospholipids in bacteria cell surface and photocatalytic this surface first makes contact with whole cells, and cells suffering from cell-wall damage and increased cell permeability and then OH , O_2 and Tio_2 particles have easier access and photo oxidation of intracellular elements which cause cell death (Saleh,2011; Fujishima and Honda, 1972 and Wong *etal* 2006).

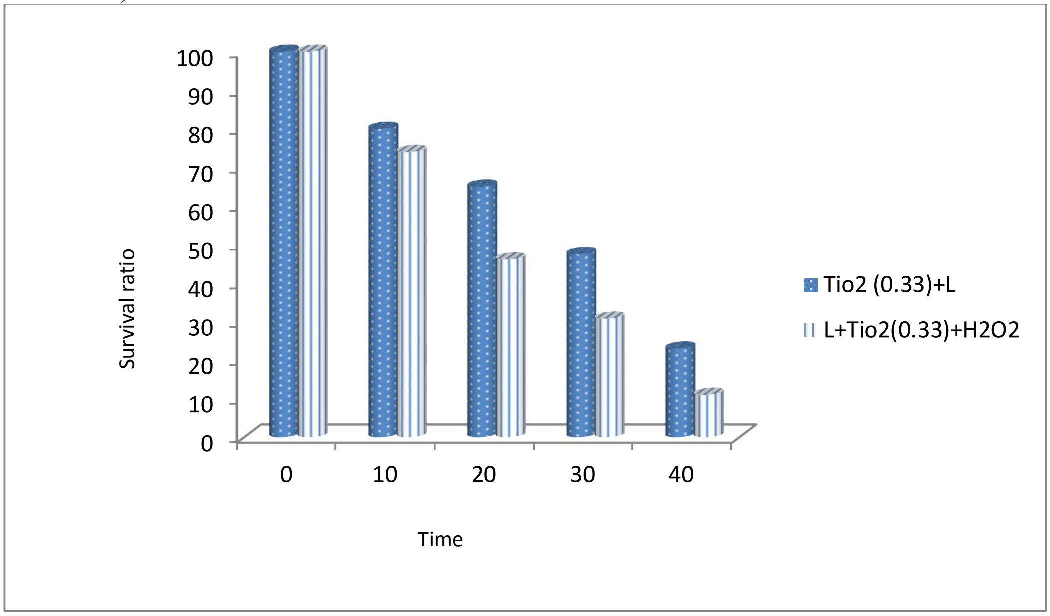


Fig (6): comparison of survival ratio of *S.aureus* in the presence and absence of H_2O_2.

These results show that the number of *S.aureus* was greatly reduced after treatment with a visible light and Tio_2 doping H_2o_2 better than treatment with visible light only and visible light with Tio_2 only.

This study suggests that we can use H_2o_2 doped Tio_2 as disinfectants in water phase.

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