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# Formulation of new iodophore preparation (chitosan iodine cream) and evaluation of its antimicrobial activity

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## ABSTRACT

A new iodophore preparation (chitosan iodine cream) was prepared. Its antimicrobial activity against different microbial standard strains was evaluated and compared against the antimicrobial activity produced by the commercially available povidone iodine (Betadine) ointment. The results revealed that chitosan iodine cream produced almost equal antimicrobial activity against the tested microbial standard strains to that produced by povidone iodine (Betadine) ointment. These results suggest the use of the new iodophore preparation (chitosan iodine cream) as an efficient antiseptic and as an alternative to the commercially available povidone iodine (Betadine) ointment.

## INTRODUCTION

Antiseptics have long and commonly been used on wounds to prevent or treat infection. They have multiple targets and a broader spectrum of activity, which include bacteria, fungi, viruses, protozoa, and even prions (McDonnell and Russell, 1999). Some antiseptics contain detergents, which render them too harsh for use on nonintact skin. The usefulness of antiseptics on intact skin is well established and broadly accepted. However, the use of antiseptics as prophylactic anti-infective agents for open wounds, such as lacerations, abrasions, burns, and chronic ulcers, has been an area of intense controversy for several years (Niedner, 1997). Iodine is recognized as an effective and useful germicide. It is active against a wide variety of microorganisms, such as viruses, bacteria, protozoa, yeast, and fungi. Despite the successes achieved with iodine, it was ascertained early that it also possesses properties unsuitable for practical applications. It has unpleasant odour; in addition, it stains the skin with an intense yellow-brownish colour and combines with iron and other metals. Furthermore, its suspensions are not stable, it irritates animal tissue and it is a poison. The adverse side effects of iodine, its painfulness on open wounds and the possibility of allergic reactions, led to the production of many iodine preparations with the aim of avoiding these incompatibilities without a significant loss of germicidal efficiency (Goebel, 1906).

It was reported that many of the undesirable qualities of iodine could be eliminated by combining it with polyvinylpyrrolidone (povidone). When it is combined with iodine, a complex is formed in which iodine's toxic properties are abolished without affecting its bactericidal activity. The complex, povidone iodine (PVP-I) has been used effectively as a surface disinfectant against various bacterial and mycotic infections (Shelanski and Shelanski, 1956). PVP-I differs from iodine in that it is less irritating to the skin and does not require iodides or alcohol to be dissolved. Additionally, its stains are water washable. PVP iodine is a stable complex which is water soluble and has a broad spectrum biocidal activity as well as it has a film forming capacity. It is safer and easier to use than classic iodine preparations and has low systemic toxicity. It has a prolonged non selective antimicrobial action. So, it is considered to be effective in treating mixed infection. Its effectiveness has been clinically proven for all types of topical applications in both human and veterinary medicine (Kumar *et al.*, 2009).

Chitosan is a linear polysaccharide composed of glucosamine and N-acetyl glucosamine units linked by beta (1-4)

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glycosidic bonds. Chitosan can be prepared commercially by deacetylation of the naturally occurring chitin (from crustaceans) under alkaline conditions. Chitosan is described of having properties such as non-toxic, biocompatible and biodegradable. Its degradation products are non-toxic, non-immunogenic and non-carcinogenic. Therefore, these properties made chitosan to have interesting biological properties and it was being researched for several applications including pharmacy, agriculture, food industry and biotechnology. However, chitosan is not soluble in water but soluble in diluted acids due to the large molecular weight of the polymer. Chitosan derivatives have been synthesized in order to improve the solubility of chitosan in physiological media, improve the biological properties and to widen the applications of chitosan (Aranaz *et al.*, 2010).

The exact mechanisms of the antibacterial activities of chitosan and its derivatives are still unknown. It is known that chitosan's antimicrobial activity is influenced by a number of factors that act in and orderly and independent fashion. The cationic amino groups of chitosan and its derivatives probably bind to the anionic groups of the microorganisms' surface, resulting in growth inhibition (Hirano, 1995).

Additionally, Studies of using chitosan in healing of wounds revealed that chitosan, having structural characteristics similar to glycosaminoglycans of the skin, could be considered for developing such substratum for skin replacement (Le *et al.*, 1996).

## MATERIALS and METHODS

### Media and chemicals

Glycerin was supplied by the Sigma Chemical Company, St. Louis, Mo, USA. Glacial acetic acid was supplied by B.D.H. Ltd., Poole, UK. Low molecular weight chitosan (70000), moderate molecular weight chitosan (750000) and high molecular weight chitosan (2000000) were the products of Fluka Chemie Laboratories, Buchs, Biochemica, Switzerland. Iodine, potassium iodide, stearic acid and polyvinyl pyrrolidone (povidone) were supplied by El-Nasr Pharmaceutical Chemicals Company. Potassium hydroxide was supplied by Prolabo laboratories, Paris, France. Povidone iodine ointment (Betadine ointment) was the product of Nile Company for Pharmaceutical and Chemical industry Cairo, Egypt. Müller-Hinton agar was obtained in dehydrated form from Oxoid, Hampshire, England.

### Bacterial strains

A total of twelve standard microbial strains were used. Six strains were obtained from the Egyptian Pharmaceutical Industries Company (EIPICO), Egypt which were *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosae* ATCC 9027, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumonia* ATCC 27736 and *Candida albicans* ATCC 10231. Six standard microbial strains were supplied by Prof. Dr Ashraf Ahmed Kadry, Professor of Microbiology and Immunology, Faculty of Pharmacy, Zagazig University, Egypt which were *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus agalactiae* ATCC 12386, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosae* ATCC 27853 and *Candida albicans* ATCC 90028. Isolates were maintained on Brain Heart Infusion agar or blood agar (for *Streptococcus agalactiae* ATCC 12386) slants at 4 °C. For long term maintenance of isolates, heavy inoculum of each isolate was prepared in brain heart infusion broth with adding glycerol (20%) and kept at –70°C.

### Preparation of water soluble chitosan derivative (chitosan HAC)

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One gram of chitosan was dissolved in 100 ml of 1% acetic acid (HAC) and left at 40°C with stirring until the whole chitosan is dissolved.

**Preparation of iodine/ potassium iodide (KI) solution**

Iodine (4 gm) and Potassium iodide (4 gm) were dissolved in absolute ethanol solution (50 ml) with addition of 1 ml of glacial acetic acid and the volume was completed to 100 ml by addition of distilled water.

**Formulation of chitosan iodine cream preparation and its control preparations**

**Chitosan HAC iodine cream**

Stearic acid (3 gm) was heated in water bath till become completely dissolved. Then, 5ml of chitosan HAC solution was taken and added to the molten stearic acid in water bath with continuous trituration for mixing. Then, 5ml of iodine/KI/ethanol solution was added to them in water bath with continuous trituration for mixing. Potassium hydroxide (0.4 ml) was added to glycerin (1 ml) and water (7 ml) in a test tube and heated to 70°C. After heating, this solution was added to the mixture present in the water bath 1ml by 1ml with continuous trituration in the same direction for mixing. Then, the mixture was removed from the water bath and triturated in the same direction till obtaining the consistency of the cream.

**Povidone iodine cream (control)**

The same procedure as mentioned above but with addition of 5 ml of povidone solution instead of chitosan HAC solution.

**Chitosan HAC cream (control)**

The same procedure as mentioned above but without addition of Iodine/KI/ethanol solution.

**Povidone cream (control)**

The same procedure as mentioned above but without addition of Iodine/KI/ethanol solution.

**50% ethanol cream (control)**

The same procedure as mentioned above but the disinfectant solution is replaced by 5 ml of 50% ethanol solution.

**1% HAC cream (control)**

The same procedure as mentioned above but the disinfectant solution is replaced by 5 ml of 1% acetic acid solution.

**Cream base (control) (without chitosan and iodine)**

The cream base was prepared as mentioned above but without chitosan or iodine.

**Antibacterial Activity of chitosan iodine cream against the different standard strains and comparison to that of povidone iodine (Betadine) ointment by agar diffusion method**

Antibacterial activity of the chitosan HAC iodine cream preparation against the different standard microbial strains was carried out according to the agar diffusion method (Heggers *et al.*, 1990). An overnight culture of the different standard microbial strains was adjusted to reach a cell density of approximately $10^7$ to $10^8$ cfu/ml and was surface inculcated onto Muller-Hinton agar plates (with addition of 5% sheep RBCs for *Streptococcus agalactiae*). Then, by sterile technique, 10 mm cups (wells) were made in the plate. Each topical cream or ointment preparation was introduced into each cup and labeled. After 24 hours of incubation at 37 °C, the susceptibility was determined by measuring the diameter of the zones of inhibition (in mm) around each cup. The test was made in triplicate.

## RESULTS

**Antibacterial Activity of chitosan iodine cream and povidone iodine (Betadine) ointment by agar diffusion method**

The results in (Table 1) revealed that chitosan HAC iodine cream gave antimicrobial activities approximately equal to that produced by povidone iodine ointment (Betadine ointment) and that produced by the prepared povidone iodine

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cream. Chitosan HAC cream did not produce antimicrobial activity against the standard microbial strains. Agents used in the preparation of chitosan HAC cream such as 50% ethanol and 1% HAC had a very little or almost no antimicrobial activity. The cream base had no antimicrobial activity. So, the antimicrobial activity of chitosan HAC iodine cream was due to the combination between chitosan HAC and iodine/potassium iodide.

**Comparison between different molecular weight grades of chitosan used in the preparations of chitosan HAC iodine cream and testing their antimicrobial activity by agar diffusion method**

The results in (Table 2) revealed that the different molecular weight grades of chitosan (low, moderate and high) used in the preparation of chitosan HAC iodine cream produced approximately equal or very close results of antimicrobial activity against the standard microbial strains and all of them produced almost equal antimicrobial activity to that produced by the povidone iodine (Betadine) ointment and the prepared povidone iodine ointment. Thus, using the high molecular weight chitosan in the preparation of chitosan HAC iodine cream might be beneficial as it had the lowest degree of chitosan degradation in its preparation. So, the beneficial properties of chitosan will be more utilized than in low or moderate molecular weight chitosan.

Table (1). Inhibition zones of chitosan HAC/$I_2$ cream compared to inhibition zones of povidone/$I_2$ (Betadine) ointment against the standard microbial strains

| Samples | Diameter (mm.) of inhibition zone against the standard strains | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *S. aureus ATCC 25923* | *S. aureus ATCC 6538* | *S. epidermidis ATCC 12228* | *Strept. agalactiae ATCC 12386* | *E. coli ATCC 25922* | *E. coli ATCC 10536* | *Salmonella typhimurium ATCC 14028* | *P. aeruginosa ATCC 27853* | *P. aeruginosa ATCC 9027* | *Klebsiella pneumoniae ATCC 27736* | *Candida albicans ATCC 90028* | *Candida albicans ATCC 10231* |
| Betadine ointment | 22 | 25 | 17 | 17 | 18 | 18 | 19 | 18 | 19 | 17 | 18 | 18 |
| Chitosan HAC/$I_2$ cream (high M.Wt. chitosan) | 21 | 24 | 19 | 19 | 18 | 18 | 18 | 18 | 18 | 17 | 18 | 18 |
| Chitosan HAC cream (high M.Wt. chitosan) (control) | | | | | | | | | | | | |
| Prepared PVP/$I_2$ cream | 22 | 25 | 18 | 18 | 19 | 18 | 19 | 19 | 20 | 18 | 19 | 20 |
| Prepared PVP cream (control) | | | | | | | | | | | | |
| 1% HAC cream (control) | 11 | | | | | | | | | 11 | | |
| 50% ethanol cream (control) | | | | | | | | | | | | |
| Cream base(no chitosan, no iodine) (control) | | | | | | | | | | | | |

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Table (2). Inhibition zones of chitosan HAC/$I_2$ creams with different molecular weights of chitosan (low, moderate and high) compared to inhibition zones of povidone/ $I_2$ (Betadine) ointment against the standard microbial strains

| Samples | Diameter (mm.) of inhibition zone against the standard strains | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *S. aureus ATCC 25923* | *S. aureus ATCC 6538* | *S. epidermidis ATCC 12228* | *Strept. agactiae ATCC 12386* | *E. coli ATCC 25922* | *E. coli ATCC 10536* | *Salmonella typhimurium ATCC 14028* | *P. aeruginosa ATCC 27853* | *P. aeruginosa ATCC 9027* | *Klebsiella pneumoniae ATCC 27736* | *Candida albicans ATCC 90028* | *Candida albicans ATCC 10231* |
| Betadine ointment | 22 | 25 | 17 | 17 | 18 | 18 | 19 | 18 | 19 | 17 | 18 | 18 |
| Prepared PVP/ $I_2$ cream (control) | 22 | 25 | 18 | 18 | 19 | 18 | 19 | 19 | 20 | 18 | 19 | 20 |
| Chitosan HAC/ $I_2$ (low M.Wt. chitosan) cream | 21 | 24 | 19 | 19 | 18 | 17 | 18 | 18 | 17 | 17 | 17 | 17 |
| Chitosan HAC/ $I_2$ (moderate M.Wt. chitosan) cream | 21 | 24 | 19 | 19 | 18 | 18 | 18 | 18 | 17 | 17 | 18 | 18 |
| Chitosan HAC/ $I_2$ (high M.Wt. chitosan) cream | 21 | 24 | 19 | 19 | 18 | 18 | 18 | 18 | 18 | 17 | 18 | 18 |

## DISCUSSION

Antiseptics and disinfectants are used extensively in hospitals and other health care settings for a variety of topical and hard-surface applications. In particular, they are an essential part of infection control practices and aid in the prevention of nosocomial infections (Larson, 1996).

This investigation aimed to formulate a new iodophore preparation (chitosan iodine cream), study its antimicrobial activity against different standard microbial strains and compare it with the antimicrobial activity produced by the commercially available povidone iodine (Betadine) ointment. Chitosan iodine cream produced antimicrobial activities against the majority of the tested standard microbial strains approximately equal to that produced by povidone iodine (Betadine) ointment and produced higher antimicrobial activity against some tested standard microbial strains than that produced by povidone iodine (Betadine) ointment. This was in agreement with that reported by (Kumar *et al.*, 2009) where povidone in PVP iodine serves only as a sustained release reservoir and carrier for iodine. The carrier augments dispersibility and penetration of povidone iodine as a topical microbial antiseptic which essentially retains the broad spectrum activity of iodine, yet is virtually free from the undesirable features associated with tincture of iodine and lugol's solution. Iodine is bactericidal, sporicidal, fungicidal, protozoacidal, cysticidal and virucidal, gram-positive and gram-negative bacteria are about equally affected. On the other hand, in the present study, the antimicrobial activity of water soluble chitosan was not

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detected but, chitosan had a significant antimicrobial activity and this was in agreement with that reported by Yang *et al.* (2005) and Xie *et al.* (2007) which stated that as environmental pH is below the pKa of chitosan and its derivatives, electrostatic interaction between the polycationic structure and the predominantly anionic components of the microorganisms' surface (such as Gram-negative lipopolysaccharide and cell surface proteins) plays a primary role in antibacterial activity. When the positive charge density of chitosan strengthens, the antibacterial property will increase consequently. On the contrary, if the polycationic property of chitosan is deprived or reversed, the corresponding antibacterial capacity will be weakened or lost. Thus, these theories might have been the cause of the increased antimicrobial activity produced by chitosan iodine cream than povidone iodine ointment against some of the tested standard microbial strains. On the other hand, chitosan iodine ointment had another advantage over povidone iodine ointment. In wound treatment, povidone iodine does not effectively promote good wound healing. In fact, it either impaired wound healing or reduced wound strength (Sheila, 1999). In addition, povidone iodine showed negative effects on wound healing similar to those of steroids (Ajaya, 1995). However, Studies of using chitosan in healing of wounds revealed that chitosan, having structural characteristics similar to glycosaminoglycans of the skin, could be considered for developing such substratum for skin replacement (Le *et al.*, 1996). Thus, chitosan iodine promotes wound healing more than povidone iodine. Another advantage of chitosan HAC cream over povidone iodine (Betadine) ointment is that by application of both of them on the skin, chitosan iodine cream had very little staining of the skin.

## CONCLUSSION

The new iodophore preparation (chitosan iodine cream) could be used as an alternative to the commercially available povidone iodine (Betadine) ointment. It also promotes wound healing and has less staining effect on the skin. Different molecular weight grades of chitosan (low, moderate and high) were used in this investigation. Preferably, using the high molecular weight chitosan in the preparation of chitosan HAC iodine cream might be beneficial as it had the lowest degree of chitosan degradation in its preparation. Thus, chitosan HAC iodine cream can be used as an effecient antiseptic cream.

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# صياغة مستحضر ايودوفوري جديد (كريم كيتوزان ايودين) وتقييم نشاطه المضاد للميكروبات

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من المعروف علميا أهمية استخدام مركب اليود كمطهر عام ضد جميع الميكروبات (البكتيرية والفيروسية والفطرية) من على سطح جلد الإنسان, ولكن ثبت من استخدام محلول مركب اليود فى عمليات التطهير لجلد الإنسان أن له رائحة غير مستحبة ، وأن استخدامه يؤدى إلى صباغة الجلد بلون بنى مصفر، ويعمل على تهيج أنسجة الجسم، ومؤلم عند استخدامه على الجروح المفتوحة، وكذلك توجد احتمالات لحدوث تفاعلات الحساسية نتيجة لاستخدامه.

هذا ما دعا المهتمين بالمطهرات من على سطح جلد الإنسان بالتفكير فى تحضير الأيودين فى صورة معقدة تقلل من العيوب الناتجة من استخدام اليود وحده.

وبالفعل تم عمل مستحضر يستخدم عالميا تحت اسم بيتادين كأقوى مطهر جلدى وآمن بدرجة عالية للاستخدام الخارجى لتطهير الأماكن الظاهرة وذلك بتحضير الأيودين فى صورة مركبة بواسطة مركب آخر يعرف بالبوفيدون ومن ثم تم تقليل عيوب استخدام مركب الأيودين وحده هذا بالإضافة إلى أنه أعطى تأثير طويل المدى لليود وهذا أتاح فرصة أكبر ومدة أطول لتطهير الجروح والحروق.

الهدف من هذا البحث هو تحضير مستحضر أيودوفورى جديد وهو كيتوزان أيودين كريم وتقييم المفعول التطهيرى له على العترات الميكروبية القياسية المختلفة.

أظهرت نتائج هذا البحث أن مستحضر الكيتوزان أيودين كريم له نشاط تثبيطى على العترات الميكروبية القياسية مساويا تقريبا للنشاط التثبيطى الناتج من استخدام مرهم البوفيدون أيودين (البيتادين مرهم). وتميز المستحضر الجديد عن مرهم البيتادين المتوفر فى السوق العالمى فى أن له صفة صبغية أقل بكثير عن الصفة الصبغية لمرهم البيتادين. كما أن له ميزة أخرى تنتج من استخدام الكيتوزان بدلا من البوفيدون فى مركب الأيودوفور وهى أن الكيتوزان من الثابت علميا أنه يساعد علي التئام الجروح لأنه يساعد فى تكوين الطبقة الأولية فى تجديد خلايا الجلد بينما البوفيدون أيودين لا يساعد فى التئام الجروح بل على العكس فإنه يعوق التئام الجروح كما توجد دراسات تشير إلى أن البوفيدون أيودين له تأثير سلبى على التئام الجروح يشبه تأثير مركبات الاستيرويدات.

نستنتج من هذا البحث إلى أنه يمكن استخدام الكيتوزان أيودين كريم كبديل جيد وفعال لمرهم البوفيدون أيودين.