The activity of alcoholic extract of Garlic on the growth of Staphylococcus aureus with estimation of median lethal dose in lab. Mice

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Abstract

The study was intended to investigate the in vitro activity of alcoholic extract of Garlic on the inhibition of growth of Staph. aureus which was isolated from skin infections, and determine the median lethal dose (LD 50) of the extract in lab. mice. The Garlic was extracted by ethyl alcohol 95%, the ratio of ethanolic extraction amounted 44% of the weight of dry substance. Graduated concentration were prepared from alcoholic extract of Garlic from 10-100 mg/ml. Their activities were checked up against Staph. aureus by agar diffusion method using ethylene glycol as control. The results showed that the sensitivity of the test bacteria was gradually increased with increasing the extract concentration, the concentration 10-30 mg/ml were rather low active in preventing the growing of Staph. aureus in culturing media, the concentrations 40-70 mg/ml were moderately active, meanwhile the concentrations 80-100 mg/ml were highly active against the growing of Staph.aureus. The results also showed that the LD 50 of the ethanolic extract of Garlic when it is orally administered to the lab mice by gradual concentrations was about 8000 mg/kg body weight. The toxic signs during 24 hrs after initial feeding with the extract were rapid breathing followed by dullness, then death.

Introduction

Many plant-derived substances touted to improve health are sold around the world, Garlic (Allium sativum) is believed by many people to be useful for diseases prevention. The Garlic belongs to the Family "Liliaceae", and the important components are: volatile oils that contain diallyl disulfate, alliin and allicin with allinase and many vitamins (Hussein 1986). In ancient Egypt, Garlic was given to laborers and soldiers to mitigate fatigue or to prevent recovery from physical exhaustion (Essman et al 1984). Louis Pasteur was the first to describe the anti-bacterial effect of Garlic juice and found that it exhibit a broad anti-bacterial spectrum against both Gram-positive and Gram-negative bacteria (Sivam et al 2001). Garlic also long been known to have anti-fungal, anti-protozoal, antiviral, and anti-bacterial properties (Bakri et al 2005). Researches have been recently focused on the prevention and curative effects of Garlic on cancer (Black et al 1994), cardiovascular disease (Jacob et al 1993), and skin disease (المحلة إيناس 2003). The purposes of this study are to detect the in vitro activity of Garlic extract in the growth inhibition of Staphylococcus aureus isolated from skin diseases, so this study carried out to:

1- Prepare alcoholic extraction of Garlic in ethyl alcohol 95%.
2- Detection of inhibitory effects of different concentrations of extract on the growth of Staph. aureus by using agar well diffusion method.
3- Determination of median lethal dose (LD 50) of extract in lab. mice.
Materials and Methods

Materials:-

1- Culture media:
   Are prepared according to the producing companies instructions and sterilized in autoclave at 121 °C under pressure of 15 pounds/ inche after incubation at 37 °C for 24 hrs , used for culture and diagnosis of bacteria used in this study.

2- Chemicals and reagents:
   Specific chemicals and reagents are used for biochemical tests to confirmed the diagnosis of bacteria (Forbes et al).

3- Equipments:
   Different equipments were used such as:- analytical balance, autoclave, incubator, microscope, sensitive balance, spectrophotometer, and blender.

Methods:-

1- Preparation of plant:
   Garlic were collected from the local market and authenticated as Allium sativum (University of Baghdad Herb Centre).

2- Extraction methods:
   Garlic was skinned and sliced, 50 gm sliced Garlic were crushed in awarding blender for 1 minute, then soaked in 450 ml ethanol 95%. It was naturally extracted for 3 months at room temperature, the mixture was separated in test tubes by centrifugation 3000 rpm, the filtrate was dried in oven 37 °C for 24 hrs. The final product was stored in freezer at -20 °C (Krell et al 1996).

3- Culture preparation:
   The bacteria were activated by re-culturing on nutrient agar and kept in the incubator for 24 hrs at 37 °C, then transferred in to sterilized tubes containing heart infusion broth, then placed in the incubator for 24-72 hrs at 37 °C. Total bacterial count was estimated by using spectrophotometer, the percentages of light transmittance was 26% at a wave length of 580 nanometer, while the light transmittance was 100% for nutrient broth used to prepare the bacteria (Jassim 2005).

4- Preparaton of standard dilutions of Garlic extract:
   The dilution were prepared by using ethylene glycol which is inert solvent against microorganism (Charlas et al ), and by using serial concentrations from 10-100 mg from the extract, then dilute it with ethylene glycol and the volume was completed to 2 ml to get the final concentrations from 1-10 %.

5- Garlic extract activity test “well diffusion method”:
   Screening of the anti-bacterial activity was performed by well diffusion technique (Saeed et al). The Mueller-Hinton agar plates were seeded with 0.1 ml of the standardized inoculums of the bacteria. The inoculums was spread evenly over plate with sterile glass spreader. The seeded plates were allowed to dry in the incubator at 37 °C for 20 minutes. A standard crack border of 8 mm diameter was used to cut uniform wells on the surface of the plates, and 0.1 ml of each concentration was introduced in the well with ethylene glycol as a control. The inoculated plates were incubated at 37 °C for 24 hrs and zone of inhibition diameter was measured to the nearest millimeter (mm).

6- Determination of LD50 of Garlic extract:
   Thirty-six albino mice 6 weeks age weighing 20-25 g were classified into 6 groups, the first five groups were orally administered by gradual doses of Garlic extract (1000,3000,5000,7000, and 9000 mg/kg body weight) respectively. The sixth group was orally administered by ethylene glycol as a control group (the dose volume was 0.4 ml /20 g body weight). The toxic signs and mortality rate were observed within 24 hrs after extract administration (Dixon et al 1980).
Results

1- Identification of bacteria:
   a- The bacteria grew well on mannitol salt agar.
   b- Microscopic examination: Gram-positive, spherical in shape.
   c- Biochemical tests confirmed the identification of Staph. aureus, catalase and gelatinase +ve, oxidase -ve, blood agar (B-haemolysis, and production of local golden pigment).

2- The inhibitory effect of Garlic extract:
   The sensitivity of the previously mentioned bacteria gradually increased with the increment of concentration of extract. The zone of the inhibition was 8.2 + 0.2 mm was recorded for the concentration of 10 mg/ml, and 19.6 + 0.5 mm was for the concentration 100 mg/ml. The concentrations 10-30 mg/ml were rather low active in preventing the growth of Staph. aureus, the concentrations 40-70 mg/ml were moderate active, while the concentrations 80-100 mg/ml were highly active (Table 1, and Fig.1). There was a proportional relation between the concentrations of extract and the diameters of inhibition zones of the growth of Staph. aureus. The ethylene glycol has no effect on the growth of Staph. aureus (Fig. 2). The statistical analysis show significant differences in the diameters of inhibition zones at the concentrations of 30, 60, and 100 mg/ml respectively (P<0.05) compared to ethylene glycol as a control.

3- Determination of LD$_{50}$ of Garlic extract:
   The results showed that the LD$_{50}$ of the ethanolic extract of Garlic in lab mice was about 8000 mg/kg body weight. The toxic signs during 24 hrs after the orally feeding of the extract were rapid breathing, dullness, then death.

Table 1 The in vitro inhibitory effect of different concentrations of garlic extract on the growth of Staph. aureus measured by the diameter of zone of inhibition (mm).

| Concentrations (mg/ml) | Diameters of inhibition zone (mm) | Mean + SE |
|------------------------|----------------------------------|-----------|
| 10                     | 8                                | 8.2 + 0.3 |
| 20                     | 8.8                              | 9.4 + 0.2 |
| 30                     | 9.8                              | 10.2 + 0.3|
| 40                     | 12.2                             | 12.5 + 0.1|
| 50                     | 12.8                             | 13.5 + 0.3|
| 60                     | 14.8                             | 15.2 + 0.2|
| 70                     | 16.4                             | 16.8 + 0.3|
| 80                     | 18.6                             | 18.7 + 0.1|
| 90                     | 18.8                             | 19.6 + 0.3|
| 100                    | 19.2                             | 19.9 + 0.2|
Figure (1): The inhibitory effect of different concentrations of Garlic extract on the growth of *Staph. aureus*.

Figure (2): The relationship between the concentrations of garlic extract and the diameter of inhibition zones in the growth of *Staph. aureus*.
Discussion

In this study, Garlic possessed anti-bacterial effect against Staph. aureus, and the sensitivity of the bacteria was gradually increased with the increasing of extract concentrations (Table 1). Bacterial drug resistance is a world problem, a high number of bacterial species have become resistant to anti-bacterial drugs (Garau et al 1994). Thus, there is a need to evaluate the efficacy of plant chemicals concerning with the growth of bacteria by extracts of plants to be used, with dichloromethane extraction (Laenger et al 1996), maceration and soxhlet fluid extraction with hexane (Vilegs et al 1997). These preparations are unavailable to person for self medication, with these consideration the activity of Garlic extract on the growth of Staph. aureus were studied. Garlic has been known to have anti-bacterial, anti-fungal, and anti-viral activity (Bakri et al 2005). The present results are in fair correlation with the study above and the study carried out by Reuter et al 1996 in which Garlic has been reported to inhibit the growth of Staphylococcus and many other species. In another study crude juice of Garlic has been found to be high active against E. coli and Salmonella typhi (Abdon et al 1972). Sasaki et al 1999 found the Garlic activity against methicillin-resistant Staph. aureus and candida albicans. Garlic extract possesses anti-bacterial activity against H.pylori at moderate concentration, thus it has protective effect against stomach ulcer (Satiawane et al 2005). This observation needs many studies and investigations. Allicin is biologically active compound responsible for the anti-microbial properties of Garlic. The inhibitory effect of Garlic on the growth of Staph. aureus in this study is due to the important allicin compound in the Garlic extract. Pure allicin was effective against many clinical isolates of Aspergillus in vitro study (Shadkchan et al 2004). Further studies are clearly required to investigate the Garlic extract effects on fungi, yeasts, and mycoplasma species. The study also showed that the LD50 of Garlic extract was about 8000 mg/kg body weight, and this is considered that the extract is very safe when taken orally. There were no previous toxicological studies that focused on the LD50 of Garlic extract for comparison. The determination of LD50 may differs in its value among other studies which were achieved, this is due to the differences in Garlic sources and consequently differences in the chemical composition, the differences in lab. animals used, their species and numbers, the method of LD50 calculation and other circumstances that related to the researchers.

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"فعالية المستخلص الكحولي للثوم في نمو جرثومة المكورات العنقودية الذهبية" 

مع تقدير الجرعة المميتة النصفية للمستخلص بالفئران المختبرية

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الخلاصة

اجرعت هذه الدراسة لمعرفة فعالية المستخلص الكحولي للثوم في تثبيط نمو جرثومة المكورات العنقودية (خارج الجسم) والمعزلة من اصابات جلدية. ومعرفة الجرعة المميتة النصفية للكوراجي من الفئران المختبرية. استخلص الثوم باستخدام الكحول الأثلي 95% وقد بلغت نسبة الاستخلاص 44% من وزن مسحوق المادة الجافة. وحضرت تراكيز متدرجة من المستخلص الكحولي (0.01-10 ملغم/مل) واختبرت فعاليتها بطريقة الانتشار بالحفر. أظهرت النتائج ان اطفل تثبيط نمو الجرثومة يزيد بزيادة تراكيز المستخلص الكحولي وكانت التراكيز 0.10-0.75 ملغم/مل منخفضة الفعالية والتراكيز 0.80-1.00 ملغم/مل فكانت ذات فعالية مؤثرة ضد نمو المكورات العنقودية الذهبية. عند تعيين الجرعة المميتة النصفية 50LD للمستخلص الكحولي بعد اعطاءه بجرع متردية للفئران المختبرية عن طريق البيرل، أظهرت النتائج ان الجرعة تبلغ 800 ملغم/كم من وزن الجسم وقد تلخصت الأعراض السمية خلال 24 ساعة بعد التجربة بزيادة التنفس والحمول ثم حلا للحيوان.