The use of propolis and black cumin in treatment of some wounds and burn infections.

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الخلاصة

بالنظر الى الاستخدام العشوائي للمضادات الحياتية في السنوات الاخيرة ضد الممرضات وصعوبة العلاج والشفاء لاصابات الجروح وخصوصا في المرضى الراقدين ،كذلك بدأ الاهتمام لايجاد الطب البديل مثل استخدام مستحضرات النباتات الطبية او مواد طبيعية للتقليل من الاثار الجانبية التي قد تنتج من العلاج بالمضادات الحياتية .

الهدف هو دراسة تأثر (داخل وخارج الجسم الحي) لمادة طبيعية (البروبوليس _شمع النحل) ونبات طبي (الحبة السوداء) بصورة منفردة او مزدوجة تجاه بعض العزلات المرضية الملوثة للجروح والحروق.

جمعت مسحات حروق وجروح من المرضى الراقدين في مستشفى الديوانية التعليمي لغرض عزل وتشخيص المسببات المرضية لاصابات الجروح والحروق باستخدام الطرق الروتينية الزرعية. اختبر تاثير مستخلص بذور الحبة السوداء الزيتي والمستخلص الكحولي للبروبوليس باستخدام طريقتي الانتشار بالاقراص والتخفيف تجاه عزلات مختلفة من الحروق والجروح. حسبت نصف الجرعة المميتة للمستخلصات المحضرة وكفاءة هذه المستخلصات داخل الجسم الحي كمستحضرات علاجية (9%) تجاه فئران مختبرية اصيبت تجريبيا بالعزلات المرضية.

بينت النتائج ان مستخلص الحبة السوداء يحتوي على مركبات مثل الفلافونيدات والفينولات والتانينات والكومارين والكلايكوسيدات بينما مادة البروبوس احتوت على الفينولات والتانينات والكومارين والكلايكوسيدات بينما مادة البروبوليس احتوت على الفينولات والتانينات والفلافونويدات . كانت بكتريا المكورات الذهبية اكثر تاثيرا تليها المكورات المسبحية القيحية ، القولونية ثم الزوائف الزنجارية تجاه المستحضرات المختبرية وبمعنوية ($P<0.05$) مقارنة بالجنتامايسين كسيطرة . واعطى المستحضر (9%) كفاءة عالية في الشفاء بالحيوانات التجريبية مقارنة بالتجربة الضابطة .

Abstract

Background : Due to the randomly uses of antibiotics at the last decades against pathogenic microbes and the difficulties of treatment and recovery of burns and wounds infections especially in the hospitalized patients , an attention have been awarded to find an alternative medicine such the uses of as a medical plants preparations or natural materials to minimize the side effects that may resulted from the antibiotics therapy .

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Aim : **If study the effects in vitro& in vivo of the natural material (propolis = bees wax) and the medicinal plant (black cumin) as alone or in their combination against some pathogenic isolates of burns and wounds contamination.**

Materials & methods : Burns and wounds swabs were collected from hosted in patients in Al-Dwiania teaching hospital in order to isolate and identified the causative agents of burns and wounds infections using the routine culture methods .The effect of oily extract of black cumin seeds and alcoholic extract of propolis using disk diffusion and dilution methods of antibiotics susceptibly test were done against different isolate of bacterial causes of burns and wounds infections .The lethal dose (LD50) for prepared extracts was calculated .The efficacy of these extracts (propolis & black cumin) were also tested as in vivo an ointment preparation (9%) against experimentally infected skin of mice with bacterial isolates .

Results : The main medical active ingredients in black cumin's extract were flavonoids , phenols , resins , coumarins and glycosides, while in propolis were phenols , resins and flavonoids .*S. aureus* was highly (P<0.05) susceptible to the action of each extract alone or in both , followed by *Strep. pyogenes* , *E. coli* and *P. aeruginosa* than the ointment of gentamcin as a control .The prapared ointment (9%) showed high qualification to treat such experimentally wounds in mice (8-9 days) in comparison with control group (15-19 days) .

Conclusions & recommendations: The prepared mixture ointment from propolis and black cumin revealed a high efficacy (P<0.05) in treatment of wounds of laboratory infected mice with tested isolates and with 100 toxicity than drug control (Gentamicine) , So , the use of these medicinal and natural material in treatment can be recommended after a widely and deeply studies on voluntaries to document and establish the usage of this ointment .

Introduction

Wounds and burns infections still yet represent a huge challenge in medicine especially for hospitalized patients though the application all of chemoprophylaxis in hospitals or patients which eventually lead to septicemia and sometime to death resulted from infection by bacteria such as *Staphylococcus aureus* , *Pseudomonas aeruginosa* , *E. coli* , *Klebsiella spp.* and *Proteus spp.* (Baron& Finegold , 1990).

Propolis has a various biological and medical activities due to its contain a high ratio of flavonoids and phenols (Chang et al ., 2002) .

Different authors have been recorded the activity of propolis extraction as anti bacterial (Grange & Davey , 1990) antifungals (Kujumgiev *et al* .,1999) antiviral (Serkedjieva *et al* .,1992),anti protozoa ,(Decastro & Higshi, 1995) , anti oxidant (Pascual *et al* ., 1994) , anticancer (Mastsuno *et al* ., 1997) and anesthesia action (Sosnowski .,1974). The precious studies have been established the contain of black cumin on phenolic compounds such as thymol have antibacterial antifungal action (El-Fatatry *et al* ., 1975) .

The randomly uses of antibiotics in chemotherapy is one of causes of the profileration of resistant stains of microbes toward antibiotics (Pagnie *et al* ., 2002; Lakkis & Fleiszing , 2001) for this reason , an at tension has been paid to find the alternative medicine to minimize the side effects of antibiotics and work to extract of natural products of bees (e.g. propslis = beetglue) (El-Faham & Sawisan ,1994) or medical herbes (e.g. black cumin) (Invanov *et al* ., 1998) .

Due to the medicinal role of propolis and black cumin and to use the synergistic effects by its combination , the design of this article was aimed to examine their effect in vitro & in vivo of propolis and black cumin's preparation on growth of common microbes that contaminated the , causes wounds and burns infection especially in hospitalized patients.

Materials and methods

Patients and samples collection : A total of 150swabs were collected from wounds and burns inpatients who hosted in AL-Dwiania teaching hospital during the period Jan uary to May 2009 .Swabs were grown on suitable and differential media, incubated under optimal growth condition to isolate and identify the causative agents (Baron &Finegold, 1999) the cultural characteristics and biochemical features were recorded (Sneath *et al* ., 1988).

Collection &preparation of propolis and black cumin : Bee glue or propolis was collected from pores of bee cells during march ,2009 .These samples were cleaned from dust and debris than preserved in freeze (-20C^{o}) for 12 hrs. to solidify the propolis .After that a powder of propolis was prepared using electric blender , then preserved in clean &sterile screw-capped vials at room temperature until use .

Seeds of black cumin (Nigella sativa linn.)was collected from local market during may,2009after cleaned and blended by electric blender, samples were preserved in clean & sterile screw – capped until use .

Alcoholic extraction of propolis was prepared by mixing of 50 gl of prepared propolis with 450 ml of ethanol (70%) using hot plate with magnetic stirrer at room temperature .The mixture were filtered using Whatmann filter paper no.2 (0.45mm).alcohol evaporation was done under 45c^{o} in oven, then the resultant was weighed in grams and preserved in refrigerator (4 C^{o})until use (Krell, 1996).

The oily extraction of black cumin was prepared using fixed oil expressed with hydraulic press (400 bar) then the collection was filtered using Whatmann no.2 filter paper (0.45 Mm) and preserved in sterile container until use.

Different standard dilutions of alcoholic propolis by using ethylene glycol (70%) as diluents and no antimicrobial agent against microbial growth (Krell, 1996) .Ten graduated concentration of propolis extraction (10-100)mg were diluted with ethylene glycol ,then the volume was completed to one milliliter's gain a ratio 1-10% (w/v)(Charles *et al* .,1969)

Chemical detection of medicinal components in propolis & black cumin .Differed methods were followed to detect of resins flavonoids; phenols; coumarians; glycosides; Alkaloids and Terpenes (Harborne, 1973) .

Test of black cumin & propolis against microbes in vitro the microbial suspension of tested microbes (*Staphylococcus. aureus ;Pseudomonas aeruginosa* and *Escherichia coli* was added to prepared plates contained nutrient agar , then 5 pores (6 mm. in diam)for each plate were done on culture media using pasture pipette .0.1ml of prepared extraction was added to each pore ,incubated under 37C^{o} for 18-24C^{o} then the growth inhibition zone (mm) were recorded for triplicate reads .Also , the minimal inhibitor concentration (MIC) and minimal bactericidal concentration (MBC)were determined using a serial dilution (2-20mg/ml) of alcoholic propolis extraction in tubes contained bacterial suspension (1.6×10^{6} cell \ml)cell/ml.in addition to tube control tubes were then incubated at 37c^{o} for 48 hrs .after incubation , the MICs value were determined as the low concentration of extraction that prevent the appearance of a clear turbidity that seen by naked eye , while the MBCs were determined by taking 0.1ml from all tubes that had no turbidity and cultured on nutrient agar plates , incubated at 37C^{o} for 24hrs , then the calculation of MBC was determined as a low concentration that diminishing the no of colonies in 99.9% of stock culture (Baron *et al* .,1994)

Test the Combination effect of propolis and black cumin in vitro

A standard dilution of extracts (propolis &black cumin)in concentration (2.5, 5,7.5 ,10)% were prepared , then a standard dilution of mixture were done using equal volume of prepared concentration of both extracts in ratio 1:1 by mixing in a magnetic stirrer for several minutes to homogenate (2.5-10)% , then preserved in sterile tubes until use (Miorin *et al* ., 2002) .

Test of Effect of propolis & black cumin in vivo

The albino mice (Mus musculus)type Balb-c were used to study the activity of propolis & black cumin extracts in vivo .Sixteen mice (6-8 weeks) in age and 20-25g in weight of males were experimentally scratched in back region with tested bacterial suspension after shaving and sterilizing with 70% .ethyl alcohol the development of inflammation and infection were monitored , and a skin smears were taken after 24 and 48hrs of infection and cultured on blood and Meconkeys agar to detect the causative agent in the lesion .The experiment mice groups (20mice)were divided into five groups : ist group was as a control (inoculated but leave it without treatment); 2nd group was inoculated and treated with black cumin alone , 3rd .group was inoculated and treated with propolis alone; 4th group was inoculated and treated with propolis & black cumin , the 5th .group treated with gentamcin ointment (0.3%).

The treatment was applied topical in twice every day until perfect recovery of all groups.

Determination of lethal dose (LD50)via oral route

A total of 24 mice (albino , balb-c) , their age (six weeks) and weight (20-25)g , were distributed into 6 groups (each group included 4 mice) the 1st to 5th groups were grally administrated with graduated doses of prepared propolis and black cumin mixture extract dissolved in diluted ethylene glycol(3000,4500,6000,7500,9000 mg/kg of body weight , while the 6th group was administrated with diluted ethylene glycol alone as a control.

Statistical analysis

The analysis of variance (ANOVA) table was used to ensure if there was a significant differences ($P \leq 0.05$) between the various treatments (Daniels , 2000).

Results

• Bacterial isolation & identification

The routine laboratory techniques that used in diagnosis of causative agents of contaminated wounds and burns samples revealed that the *Staphylococcus aureous* was the most common of contamination followed by *Pseudomonas aeruginosa* then *Escherichia coli* and *Streptococcus pyogenes* .

• Extraction & detection of medicinal compounds:

The result showed that the oily extraction using the mechanical press gave an extraction ratio (12%)for black cumin as where was 33% for propolis extraction using ethyl alcohol (70%) from dry weight of both materials flavonoids ,glycosides, alkaloids and resins (table 1).The chemical analysis of prepared black cumin and propolis showed the presence of numerous compounds such as phenols ,f

• Effect of black cumin extraction on bacterial growth in vi vitro

The results showed that the oily extract of black cumin was inhibited the growth of contaminated bacteria of wounds and burns of human in a significant differences (P≤ 0.05) when compared with the zone of inhibition of growth (mm) at different concentration of extraction (table 2) and in comparison with control (gentamcin) .

• Effect of alcoholic extraction of propolis on bacteria growth in vitro

The results revealed a significant differences (P≤ 0.05) of activity of propolis against tested bacterial growth according to the concentration of extract and tested bacterial species in comparison with gentamcin as control(table3) .

• Effect of mixture extracts (black cumin & propolis) on bacterial growth in vitro

The activity of mixture extract against tested bacterial growth in vitro showed a significant differences (P≤ 0.05) based on concentration of mixture and bacterial species (table 4) when compared with the use of black cumin or propolis alone in addition to gentamcin as a control.

• The MICs and MBCs of tested extractions in vitro :

Table (5). shows the results of MICs &MBCs of the used extracts alone(black cumin& propolis) in addition to the MICs& MBCs of mixture extract against the tested bacteria .

• Activity of prepared exraction in vivo

After 48-72hrs of experimentally infection of mice with *Staph. aureus* and *Pseudomonas aeruginosa*. the skin lesions showed erythema with allegic reaction at lesion site .Then and after applying the treatment of experimental mice wounds with prepared ointment (mixture of black cumin and propolis) in a concentration of 9% , the infected wounds with *S.aureus* began to heal and recovery during 5days in comparison with control group (without treatment) that need to 15days while the group treated with gentamcin ointment need to 8days to recovery of infected mice .in respect with *Pseudomonas aeruginosa* the period of recovery was 8 days when treated with prepared ointment in compared with 18 days for control and 10 days for gentamcin ointment (Table 6).

• Lethal dose (LD50) determination

Due to analysis of result statistically according to probity method, the value of LD50 was 6000 mg/kg of body weight after 14hrs of oral administration.

Table (1) Chemical detection of medicine active compounds of oily extract of black cumin and alcoholic extract of propolis.

Extract / Compound	Black cumin	Propolis
Flavonoids	+	+
Glycosides	+	-
Alkaloids	+	-
Phenols	+	+
Resins	+	+
Terepins	+	+

(+) presence to (-) absence

Table (2)Effect of oily extract of black cumin on tested bacterial growth in vitro.

Control (mg/ml)	Mean (mm)±SE of zone growth inhibition	
	S. aureus	*Pseudomonas aeruginosa*
25	*9±0.2	*7.2±0.1
50	15±0.3	10.2±0.2
75	18.5±0.3	12.5±0.2
100	25±0.2	16.3±0.3
Gentamcin	20±0.2	17±0.12
Ethylene glycol	0.0	0.0

***F test (P < 0.05) significant**

Table (3)Effect of alcoholic extract of propolis in tested bacterial growth in vitro.

Control (mg/ml)	Mean (mm)±SE of zone growth inhibition of growth	
	S. aureus	*Pseudomonas aeruginosa*
25	*9.58±0.27	*7.66±0.13
50	15.5±0.14	11.58±0.14
75	18.08±0.14	13.16±0.15
100	21.25±0.17	17.33±0.13
Gentamcin 30mg/ml	20±0.26	17±0.12
Ethylene glycol (70%)	0.0	0.0

***F test (P < 0.05) significant**

Table (4) Effect of mixture extract in tested bacterial growth in vitro.

Control (mg/ml)	Mean (mm)±SE of zone growth inhibition of growth	
	S. aureus	*Pseudomonas aeruginosa*
25	*9.58±0.14	*7.66±0.13
50	15.16±0.15	11.58±0.14
75	19.16±0.15	13.16±0.15
100	26.08±0.07	17.33±0.13
Gentamcin 30mg/ml	20±0.26	17±0.12
Ethylene glycol (70%)	0.0	0.0

***F test (P < 0.05) significant**

Table (5) Mics and MBCS of prepared extract tested against bacteria in vitro .

Bacterial species	Black cumin (mg\ml)		Propolis mg\ml		Mixture (black cumin &propolis)	
	MICs	MBCs	MICs	MBCs	MICs	MICs
Staph. aureus	1.25	10	2.5	7.5	2.5	10
Psendomonas aeroginosa	2.5	15	15	20	7.5	15

Table (6) Mean of recovery period (days) for experimentally mice infected which treated with prepared mixture of black cumin and propolis.

Bacterial species Treatment material	Period of recovery (days)	
	Staph .aureus	*Pseudomonas aeruginosa*
Black cumin & Propolis mixture (9%)	*5	*8
Gentamcin(0.3%) ointment	8	6
Control (without treatment)	15	18

F test (P<0.05) significant

Discussion

The study showed that the oily extraction using the mechanical damage of seed of black cumin gave 12% as a ratio extraction .This may due to that technique is consider a natural method to gain that high ratio of ingredients without changing or denaturing the chemical structure (configuration) in addition to the maintaining the physical nature of the prepared compounds that may denaturing by using temperature or organic solvent n extraction process .The second advantage of this technique is that the yield of extraction had a high viscosity which aid to use this extract as a drug for skin infection without using the fixed and phores of drugs (wound &burn infection)as a topical ointment (EL-Fatatry *et al*.,1975).

On the other hands , the use of ethyl alcohol (70%) in propolis extraction gave a high ratio (33%) with viscous yield .This may be due to the presence of flavonoids and this may aid to use it easier as topical ointment for treatment of wounds & burns infections (Stepanovi *et al* .,2003; Taylor ,2002) .

The chemical detection of medical active compound in black cumin seeds revealed the presence of main phenol compounds (thymol and thymoquinone)which have the antibacterial effect on G+ve &G-ve bacteria and fungi in addition , the glycosides and alkaloids that contain the nigellimine (Rhmana &Malik, 1985)

In addition to that various authors noticed that the black cumin contain flavonoids which have an inhibitor effect for bacteria and fungi when synergistic with phenols and resins (Hasan *et al*., 1989; Hanafy, 1991; Eliopoulos, 1988). The presence of flavonoids in propolis extract have many biological effects such as antioxidants (Scheller *et al* .,1990), antibacterial (Kujumgiev *et al* .,1999) and antifungal (Cafarchia *et al* .,1999) .this may due to that propolis contain 40 compounds of flavonoids (Maciejewiez, 2001)while the presence of resins in propolis gave it the viscous nature (Taylor , 2002)and the phenols gave which have the propolis the character of antibacterial effect which have the synergistic nature with flavonoids (Bankova *et al* .,1996).

The results also revealed that the combination between the extract of black cumin and propolis gave a synergistic effect than use of each one alone when tested against the bacterial contaminated the wounds & Burns infections .This is also established by authors (Prescott *et al* .,2000) and this effect was highly significant on G+ve than G-ve bacteria .This may due to that G-ve bacetria have a barrier contain lip polysaccharide integrated with complex proteins which together may prevent the entrance the antibacterial compounds into bacteria than G+ve bacteria (Hugo&Russel,1983).

The study of efficacy of prepared mixture of black cumin & propolis (9%preparation) as a topical ointment in wounds treatment of experimentally infected mice with *Staphylococcus aureus* & *Pseudomonas aeruginosa* than the gentamcin ointment as a control revealed a significant effect ($P \leq 0.05$) in healing and recovery of wounds which aid to inhibit or kill the contaminated bacteria of wounds , but also the ingredients of this mixture through the synergistic effect may induce the synthesis of damage tissues through the activation of cell division (Gaberys *et al* .,1986) .On the other hands , the use of this mixture (the viscosity nature)may play a role in the formation of physical barrier between atmospheric air and site of infection which may impairment the arrival of oxygen and block the metabolism to the contaminated strict aerobes (Scheller *et al* .,1977).

Conclusions

1.The prepared mixture of black cumin & propolis had a various medicinal active compounds especially the flavonoids , phenols and resins that have antimicrobial effects .

2.The prepared mixture gave a significant effect on the growth of tested bacteria in vitro & in vivo than control (Gentamcin).

Recommendations

1. Purification of the medicinal active compound separately and tested on other microorganisms.

2. The use of combination effect between the extracted material and the traditional antibiotics in vitro in vivo against the pathogens

3. Test the prepared mixture after a deeply studies on voluntaries patients with wounds or burns infection.

4. Encourage the specialist to use the medicinal plants as a alternative drug of toxic or serious side effect of antibiotics.

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