Antibacterial effects of mixture (honey , Nigella sativa oil and propolis) on experimental animals infected with *Pseudomonas aeruginosa*

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Received **Accepted**
29/05/2007 **17/07/2007**

الخلاصة

بينت الدراسة الحالية امكانية استخدام مزيج العسل ، زيت حبة السوداء والعكبر في علاج الجرذان المصابة تجريبيا بجرثومة *Pseudomonas aerugiosa* والتي احدثت خراجات جلدية خلال 48 ساعة في منطقة الاصابة بعد حقن خلاياها بتركيز 10^8 وحدة تكوين المستعمرة /مل في منطقة الجلد المحترقة .

اظهرت الدراسة ان استخدام خليط من العسل ، زيت حبة السوداء والعكبر وبشكل متجانس اظهر فعالية جيدة في علاج هذه الاصابات اذ بينت الدراسة ان فعاليته في العلاج اكثر من المضادات الحيوية وخلال فترة48–72 ساعة بينما كانت 96 ساعة عند استخدام المضادات (Gentamicin, ampicillin).

Abstract

The present study showed the possibility of using a mixture of honey , Nigella sativa oil and propolis in the treatment of experimentaly infected rats occurred after induction of *Pseudomonas aerugiosa* in aburned area on the thigh at a concentration of 10^8 cfu / ml , the abscess appeared after 48hr .

The study showed that mixture of honey, Nigella sativa oil and propolis showed a good activity in the treatment of the abscess with in 48 – 72 hr ., compared with the antibiotics (Gentamicin , ampicillin) which gives the same results with in 96 hr

The aim of study

The present study aims to investigate the efficacy of amixture (honey , Nigella sativa oil and propolis) in treating infections caused by strains of *Pseudomonas aeruginosa*

Introduction:

Burns damage the body's mechanical barrier , neutrophil function and immune response, burn wounds are sterile immediately after the burn is inflicted but inevitably become colonized within hours with a mixed bacterial flora .

Burn injuries cause direct damage to the mechanical barriers of the body and abnormalities in neutrophil function and immune responses .

In addition there is a major physiologic derangement with loss of fluid and electrolytes (1)

Pseudomonas aeruginosa is an opportunist gram – negative aerobic rods that has along and infamous association with burn infections . It grows well in the moist environment of a burned wound , producing a foul , green – pigmented discharge and necrosis (2) (3) .

Honey anatural product that is generating renewed interest for its therapeautic application , it offers a suitable and better alternative in managing infected burn wounds and other forms of infected wounds as well as prophylaxis in trauma wounds. (4) (5) .

It has been suggested to enhance the healing of wounds and pressure sores and its antibacterial and antifungal properties have been well documented (6).

Nigella sativa has hypertensive , carminative and anthelminthic properties , and it is regarded as one of the greatest forms of healing medicine available , the prophet Muhammed once stated that the black seed can treat every disease except death(7) .

The use of Nigella sativa is considured a novel methods for modulating bacterial growth by administering, preferably topically, an effective amount of a composition comprising the Nigella sativa oil (8) (9).

Thus, the use of the oil as flavoring agent in food and as antiseptic agent in topical pharmaceutical preparations can be recommended (10) .

Propolis is a natural product derived from plant resins collected by honeybees, it is a resinous substance of a yellow green to brown or dark red colour (11).

It is known that propolis posses a wide spectrum of therapeutic effects as anti-inflammatory and hypotensive agent , immune system stimulant and bacteriostatic and bactericidal agent among many other uses , therefore , propolis has attracted much attention in recent years as a useful or potential substance used in medicine (12).

Material and methods :

1- Bacterial strains

(9) Strains of *Pseudomonas aeruginosa* had been taken from Al – Zahrawii hospital in Mosul which were isolated from swabs collected from burned patients, and diagnosed by using oxidase test and gelatinase test. Strains were subcultured from a starter culture incubated at 37C^{o} under aerobic condition for 24hr in brain heart infusion broth and stored in refrigerator of 4C^{o} until used (13).

2- Preparation of the mixture :

The mixture consists of :

1- Honey 25ml
2- Nigella sativa oil 25ml .

3- Propolis 50 gm

These materials were obtained from private market, a mixture was prepared by mixing the above mentioned concentration after propolis had been heated.

Our study consist of:

A- In vivo experimental study.

B- In vitro experimental study.

A- In vivo experimental study.

(9) rats were used in this study their ages ranged between 4-5 months.

The thigh of rat was shaved and clipped of for all the rats , these areas were burned using heated scalple , burned areas were infected with an inoculum of *Pseudomonas aeruginosa* at concentration of 10^8 cfu/ml subcutaneously and these rats were left for the following 2 days to ensure the occurrence of infection (image 1) .

Image (1) Site of wound and appeared the abscess

All the (9) infected rats were divided into (3) groups (3 rats/group).

- The first group (3 rats) were treated topically with the prepared mixture twice daily morning and evening for 10 days .
- The second group (3 rats) was treated topiccally with antibiotics ointment (Gentamicin , ampicillin) as the first group .
- The third group (3 rats) was treated topically with saline as above .

B- In vitro experimental study included :-

1- *Pseudomonas aeruginosa* was inoculated in nutrient broth , incubated for 24hr at 37 C^o .

2- Nutrient agar plates were seeded with 0.1 ml of liquid inoculum prepared for the strains of *Pseudomonas aeruginosa* disc impregnated with the tested materials were placed on the surface of the nutrient agar plates, the first disc was impergnated in a mixture (honey , Nigella sativa oil , propolis) by taking swab of it, the second disc was impergnated with antibiotics (Gentamicin, ampicillin) by concentration 500 mg, while the third was impergnated in saline and used as a control .

Results were recorded after incubation for 24hr . at 37C^O

Results and Discussion

The results of our study was estimated the healing rate , the first group treated with a mixture healed with in 48-72 hr .

Compared with the second group which was treated with gentamicin and ampicillin 96 hr , while the healing of the third group (Control group) take 120 hr .

The results reported in the figure (1) , indicate that the wounds treated with mixture showed complete healing after 2-3 days (Image 2)

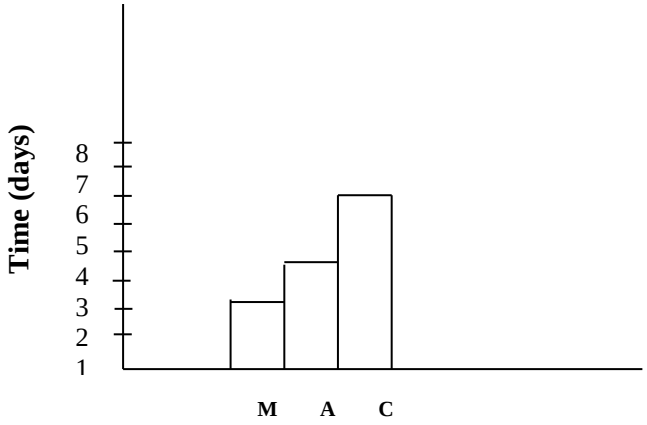
Image (2) Healing of wound by mixture (Honey , Nigella sativa oil , propolis)

Other wise wounds treated with Gentamicin and ampicillin healed after 3-4 days (Image 3) .

Image (3) Healing of wound by antibiotics (Gentamicin , ampicillin)

Also wounds treated with saline as a control group healed completely after 4-6 days (Image 4)

Image (4) Healing of wound by saline (Control group)



Type of treatment

M= Mixture A= Antibiotics C= Control

Figure (1) Relation between time of healing and type of treatment of wound

Significant differences in the rate of wound healing were observed between control wound treated with saline and wound treated with either mixture , antibiotics , mixture gave the fastest rate of healing compared with others, this was the results of invivo experimental study , the same results was obtained invitro experimental study , Zone of inhibition against the *Pseudomonas aeruginosa* for the mixture was higher than that inhibition for antibiotics against *Pseudomonas aeruginosa* . but control disc (saline) showed no zone of inhibition by length (2.7 Cm) , (1.8 Cm) , (0 Cm) respectively (Image 5)

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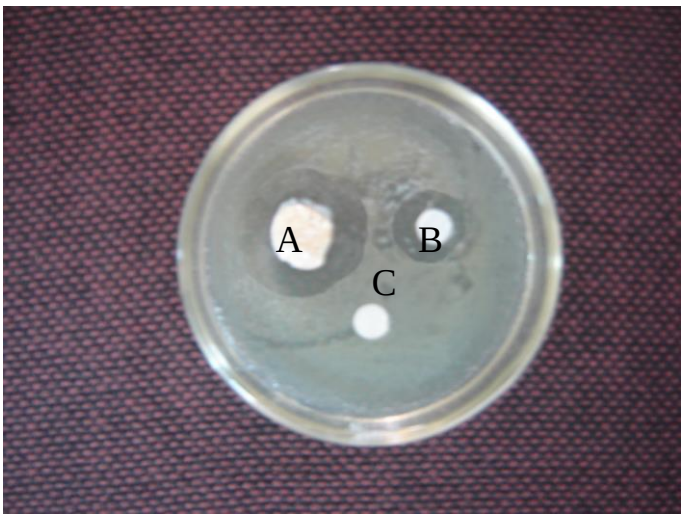

Image (5) Zone of inhibition of mixture , antibiotics , saline against *Pseudomonas aeruginosa* (A) Mixture (B) Antibiotics (C) Saline

Therefore the two Phases of results confirmed the curative properties of honey , Nigella sativa oil and propolis for wounds .

Pseudomonas aeruginosa constitule environmental contaminants both for burned wounds and other trauma from any source , there by

causing sepsis, specifically , the repeated occurrence of *Pseudomonas aeruginosa* as a pathogen in burns because it occupies multiple ecological niches in nature by virtue of its minimal growth requirements and its ability to produce a large number of extracellular protective and toxic substances , such as slime glycolipoprotein, haemolysin, fibrinolysin . lecithinase , elastase , Dnase and Phospholipase may contribute to the pathogenicity of this opportunistic microorganism (14) (15) .

Their occurrence in burns and other wounds continues to contribute to extended treatment times . increased costs and delayed healing rates , causing skin grafts to fail , and to increased risks of septicaemia (16) .

Many studies showed that burn provides a highly nutritions surface for organisms to colonize and the incidence of serious infection varies with the size and depth of the burn , it is characterized by rapid separation of the eschar from the underlying tissue and a heavy exudate of purulent material from the burn wound (17)

Our study was in accordance with many studies that using honey as adjuvant method for accelerating wound healing since ancient times particularly in the treatment of burns. It was observed that honey treatment led to a reduction in inflammation, scarring and Contractures , and the antioxidant effect of honey in the mopping up free radicals , to gether with its antibacterial action , low pH , high viscosity and hygroscopic effect , all seem to play a combined role in the efficacy of honey in burns treatment ,and it plays a role in the initial phase by limiting liquid peroxidation and alter stage by controlling infection and promoting healthy granulation . (18) (19) (20) , and without side effect of antibiotics (21).

The acidity of honey (typically below PH = 4) may also assist in the antibacterial action of macrophages , as an acid PH inside the Vacuole is involved in killing ingested bacteria (22).

The high glucose levels that honey provides would be used by the infecting bacteria in preference to amino acids from the serum and dead cells , and thus would give rise to lactic acid instead of ammonia and the amines and sulphul compounds that are the cause of malodour in wound . (22)(23)(24)(25)(26) . in addition to contain vitamins and trace elements (vitamin C content of honey) (23).

Our study was in accordance with (27) it is believed that natural preparation will not have an adverse effect on the body compared to synthetic preparations .

Nigella sativa Lipid fraction is an oily fraction comprised of polyunsaturated fatty acids , saturated fatty acids , glyceryl esters , volatile oil and sterols (28) (29).

Finally Nigella sativa oil is active against standard strains of *Pseudomonas aeruginosa* and may be used therapeutically in susceptible

cases , this was in accordance with (30) (31) , using the oil as flavoring agent in food and as antiseptic agent , provides an effective and affordable therapy ,with no side – effects for treating infections through improving immune system function by either balancing an overactive immune system or by enhancing an immune system suppressed by illness or stress preferably topically administering an effective amount of a composition comprising the Nigella sativa lipid fraction .

Propolis is known to have a wide spectrum of therapeutic effects , the most important among them are flavonoids various phenolics and aromatic compounds , this is adietetic product of the highest purity , containing all biologically active substances (32)

It can be used in large doses without harmful side effects because it does not contain any solvents (33).

In our study the activities against *Pseudomonas aeruginosa* were confirmed this would indicate broad-spectrum activity , this accordance with(34), that showed modern science proved that propolis has strong antibacterial effect on certain gram-positive and gram-negative microorganisms so it is possible that propolis may be a good condidate to be used in preventing growth of microorganisms, it can be used safely also this accordance with (35).

Another studies showed the chemical composition of propolis is quite complicated more than 300 compounds have been identified in propolis the contents depend on the collecting location time and plant source , consequently , biological activities of propolis gathered from different phytogeographical areas and time peroids vary greatly . these components have multiple effects on bacteria , fungi and viruses (36) .

Propolis contains some minerals such as Mg , Ca, I , K, Na, Cu , Zn , Mn, and F as well as some vitamins like B_1 , B_2 , B_6 , C and E and a number of fatty acids (37)(38).

They (39) showed that why using of propolis in treatment of wounds, inhibits bacterial growth by preventing cell division , thus resulting in the formation of pseudo – multicellular . In addition propolis disorganized the cytoplasm , the cytoplasmic membrane and the cell wall caused apartial bacteriolysis and inhibited protein synthesis .

Our study confirmed using of mixture is atypical treatment for the wound this accordance with (40) (41).

Conclusions :

The God stated

In the name of God, the merciful , the compassionate

((**And yours lord inspired the bees, take your dwelling in the hills in the tree and intrellises**))

Bee / (68)

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