Effect of *Nigella sativa* extract on biochemical constituents and its antibacterial activity on normal and *Pseudomonas* infected rats

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Abstract: *Nigella sativa* has assumed a great importance in folk medicine due to its use as an antibacterial agent. An alcoholic extract of *Nigella sativa* was used in this study to detect its antibacterial effect against Gram negative bacterium (*Pseudomonas aeruginosa*) by agar gel diffusion techniques as well as its effect on biochemical constituents of non-infected and infected male rats with a virulent strain of *Pseudomonas aeruginosa* (proved to be serum resistant) by using a bacterial assay. Satisfactory results were obtained by oral administration of the alcoholic extract in doses of 25 and 125mg/ 100g.b.wt. for 30 successive days to overcome bacterial infections. Bacteriological examination and biochemical analysis were used for detection of the used strain in either treated or non-treated infected male rats to evaluate the use of *Nigella sativa* extract as a bactericidal agent.
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Key Words: *Nigella sativa*, *Pseudomonas*, biochemical constituent, antibacterial activity.

1. Introduction:

Nigella sativa has assumed great due to its use as an antihelmintic, antispasmotic and antibacterial agent (**Bedvian, 1936; Perrot and Pans, 1971; Bellakholer, 1978; Hanafy and Hatem, 1991; Salami *et al*., 1992 and Akhtar *et al*., 1996**).

The acute toxicity of *Nigella sativa's* constituents was estimated and recorded (**Sigh Murya *et al*., 1983)**, but its antibacterial activity and its role on biochemical changes in infected and non-infected rats with *Pseudomonas aeruginosa* require further study. So, the present work was conducted to reveal the biochemical and antimicrobial effects of *Nigella sativa*.

2.Material and Methods:

***Nigella sativa* extracts**:

Five hundred gram of *Nigella sativa* seeds were extracted until exhaustion by percolation using 95% ethyl alcohol, and evaporated under reduced pressure until dryness using a Rotavapor, and then kept in refrigerator until used.

Laboratory animals:

Twenty five mature male rats, 25 mature mice and ten rabbits were used. They were fed on ordinary rations and water *ad-libitum*.

***In vitro* sensitivity test**:

The bactericidal activity of *Nigella sativa* was detected by the test diffusion technique according to **Fingold and Martin (1982).**

The following different dilutions of *Nigella sativa* extract: 25, 31.25, 62.5, 125 and 250 mg/ml were used and each added to 5 discs (each 8mm in diameter). All plates were incubated aerobically overnight at 37˚C and the results were recorded by taking a mean of the diameter of the inhibition zones.

Detection of pathogenicity on tested organisms:

To ensure the virulence of the tested strain of *Pseudomonas aeruginosa* before induction of infection in male rats, a bactericidal assay was applied.

Virulence of *Pseudomonas aeruginosa* in mice:

Bacteria were grown overnight in tryptic soya broth at 18˚C to obtain broth culture of $1.5x10^9$ C.F.U. using a McFarland nephelometer. Ten fold dilutions were used to inject mice I/P in groups, with 5 mice in each group. One group of mice was used as a control. Mortality rate was recorded after 72 hours (**Liu, 1966**).

Bactericidal assay:

Using the guidelines of Taylor (1983), 10ml samples of a 9-24 hour brain heart infusion broth culture was used to inoculate 10ml of sterile brain heart infusion growth wormed to 37˚C, then incubated for 2 hours at 37˚C to produced bacteria. The bacterial suspension was centrifuged for 20 minutes at 3000x*g* and bacterial pellets were suspended in 10ml gelatin veronal buffer containing 0.15M $CaCl_2$ and 0.5M $MgCl_2$, pH7.4. Reaction mixtures of 250 ml containing 25ml of bacterial suspension ($1x10^7$ C.F.U./ml) estimated by using McFarland nephelometer standards, and 225ml of undiluted bovine serum were incubated at 37˚ C for 3 hours.

Fifty ml samples collected initially at "0" and at the end of 6 hours incubation were placed in 9ml gelatin veronal buffer.

The numbers of viable bacteria were determined by plating 10 fold dilutions of these samples on blood agar plate. After incubation the numbers of colonies were counted. Isolates of *Pseudomonas aeruginosa* were classified as serum resistant when the number of colonies was greater than that "0" hour sample.

Bacteriological examination:

Re-isolation of the infected organism from all tested male rats (treated or not-treated) was done by examination of blood samples collected from living rats or the internal organs of dead rats. All samples were culture on blood agar medium and *Pseudomonas aeruginosa* and incubation aerobically at 37˚C for 24 hours. Suspected growing colonies were studied for morphological appearance, haemolytic activity and colonial characters and biochemically according to Konemann *et al*. (1992). The blood samples were left to clot and the serum was separated for biochemical analysis and a RID test.

Radial immunodiffusion (RID test:

Radial immunodiffusion (RID) is a technique that is routinely used for measuring the concentration of various soluble antigens (usually protein) in biological fluid (Mancini and Vearman, 1964).

Biochemical analysis:

Normal male rats and rats infected with a strain of *Pseudomonas aeruginosa* were used in this assay **(Liu, 1966**). A broth suspension of $3x10^8$ per ml *Pseudomonas aeruginosa*, was prepared using a McFarland nephelometer (**Finegold and Martin, 1982).**

To induce a bacterial infection, each rat was injected s/c with 1ml of a virulent strain of *Pseudomonas aeruginosa*. Six groups of five mature rats (180-200gm.b.wt.) were used in this experiment. The first group was kept as a control; the second and third groups were administrated *Nigella sativa* extract at doses of 25 and 125mg/100g.b.wt. The fourth group was challenged with a virulent strain of *Pseudomonas aeruginosa* and kept as a control positive. The fifth and sixth groups were infected by the same virulent strain and treated with a *Nigella sativa* extract at doses of 25 and 125mg/100g.b.wt. orally for 30 consecutive days. Blood samples from all used rats were collected for bacteriological examination and biochemical analysis.

The activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (AP) were determined by the method described by (**Reitman and Frankel,1957 and Roy,1970**). Urea and creatinine levels in serum were estimated as explained by (**Koplan,1965 and Husdain and Rapoport ,1968**).

Statistical analysis:

Statistical analysis of the obtained data was carried using student "t" test as explained by (**Sndecor, 1969**).

3.Results:

***In vitro* sensitivity test**:

The antimicrobial activity of *Nigella sativa* extract was detected by the presence of a detectable wide zone of inhibition in the dilutions of 250 and 125mg/ml and decreased gradually with an increase of the dilution of used extract (**Table 1).**

Pathogenicity test:

Virulence:

Pseudomonas aeruginosa when injected I/P in rabbits produced a mortality rate reaching 100%. All rabbits inoculated with the strain which died during the experimental period showed weight loss, reduction in daily weight gain, dehydration, and a dull and severe liquid to watery diarrheoa; these were observed before their death.

Bactericidal assay:

The serum resistance of *Pseudomonas aeruginosa* strain was determined. Bovine serum was used to test the serum resistance. Isolates of *Pseudomonas aeruginosa* were classified as serum resistant when the obtained member of viable *Pseudomonas* in 6 hour samples was greater than that in the 0 hour sample.

The effect of tested extract on serum enzyme activities of normal and infected rats showed significant increase of AST, ALT and AP, while the level of creatinine was significantly increased in serum of normal rats but there was a significant decrease in the serum of the infected group when compared with negative and positive controls respectively. Insignificant changes in urea levels of both normal and infected groups were found **(Table 2**)

RID test:

All serum samples showed no zone of inhibition in agarose gel diffusion when compared with control samples which indicate the absence of *Pseudomonas aeruginosa* in sera of male rats treated with *Nigella sativa* extract.

Bacteriological examination of rats:

Pseudomonas aeruginosa was isolated from infected non-treated male rats when cultured on specific media with the same morphological and biochemical characteristics, but there was no isolation of *Pseudomonas aeruginosa* from male rats treated with *Nigella sativa* extract with the two doses used.

Table (1): Antimicrobial activity of *Nigella sativa*

Micoorganism	Conc. Mg/ml	Zone of inhibition (m.m)
Pseudomonas aeruginosa	25	-
	31.25	+
	62.5	++
	125	+++
	250	++++

Table (2): Effect of oral administration of *Nigella sativa* extract in serum enzymatic activity and biochemical constituents in serum of infected and non infected rats (n-5)

Group	Dose	AST	ALT	AP	Urea	Creatinine
Control -ve	____	192±1.2	34.8±0.2	28.7±0.09	38.82±1.8	1.47±0.06
Nigella sative extract	25	213.2±1.48	43.4±1.03	38.0±1.2	39.02±1.7	1.77±0.11
	125	233±1.24	57.99±0.9	46.6±3.3	43.5±2.7	2.0±0.9
Infection group		163±1.25	27.8±0.26	19.2±0.88	55.2±4.5	4.88±0.12
Nigella sative extract after infection	25	193±1.22	29.2±2.2	29.85±0.91	51.2±2.6	3.3±0.4
	125	222.3±1.1	48.8±1.22	38.05±1.2	47.27±1.1	2.55±0.06

4. Discussion:

Nigella sativa has assumed great importance as an antibacterial agent (**Akhtar *et al.*, 1996; Salami *et al.*, 1997 and Hanan *et al.*, 1998).**

It is worthy to mention that the stain of *Pseudomonas aeruginosa* used in this study was isolated from bull semen with a record of low fertilising capacity. This observation is hand-to-hand with that mentioned by others (**Lukacevic *et al.*, 1962; Naidu *et al.*, 1982 and Jovicin *et al.*, 1992**) who isolated *Pseudomonas aeruginosa* from bulls with a low fertilising capacity. *Pseudomonas aeruginosa* was found to be one of the most important microorganisms which has a significant role in the capacity of fertilisation in males.

Virulence of microorganism is mainly defined as the relative capacity of an organism to resist available defense mechanism (**Sparling, 1983**) and the ability of an organism to resist the bactericidal effect of serum is among the most important virulence factors. In this study an *in vitro* assay **(Taylor, 1983)** was used to determine the serum resistance of a *Pseudomonas aeruginosa* isolate which was found was to be serum resistant. **Wilson (1968**) and **Taylor (1983)** suggested that bacterial activity of serum against Gram-negative bacteria is mainly meditated by antibody and complement. In addition, **Pluschke *et al.* (1983**) also found that the bacterial surface structures which may be important for serum resistance, include smooth lipopolysaccharides, acidic capsule polysaccharide and outer membrane proteins.

The antimicrobial susceptibility of *Pseudomonas aeruginosa* was determined on the extract of *Nigella sativa* using the test diffusion technique (**Finegold & Martin, 1982**) and the results indicate that the seed extract has good antibacterial activity with therapeutic potential for the treatment of some Gram –ve bacteria infection (**Ferdous *et al.*, 1993**). Oral administration of *Nigella sativa* in doses of 25 and 125mg/100g.b.wt respectively to normal and infected mature rats for 30 successive days significantly increased ALT, AST, AP and the level of creatinine. This effect is in agreement with **Tennekoon *et al.* (1991) and Hanan *et al.* (1998**) who used a *Nigella sativa* extract in the treatment of rats infected by *Pseudomonas aeruginosa* and recorded a significant increase in ALT, AST and AP and a decrease in the level of creatinine and urea when compared with the positive group.

The effect of the tested extract on serum enzymatic activity and the level of creatinine and urea of rats before and after infection by *Pseudomonas aeruginosa* may be attributed to the influence on the liver and kidney as the plant contain volatile oils which may irritate renal tissue and increase the level of creatinine.

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