GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

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THE ANTIBACTERIAL AND ANTIOXIDANT CHARACTERISTIC OF PARSLEY IN SEMINAL FLUIDS OF INFERTILE MEN

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ABSTRACT
Twenty two samples of seminal fluid of infertile men were studied to detect the antibacterial and the antioxidant characteristic of parsley oil (*Petroselinum crispum*). The study revealed that parsley oil at various concentrations has inhibitory growth effect against *E. coli, P. mirabilis, klebsiella sp, and S. aureus*, and the more effective concentrate was $7x\ 10^{-4}$g/ml. The effect of four parsley oil concentrations ($7x10^{-4}$, $7x\ 10^{-6}$, $7x\ 10^{-7}$, $7x10^{-8}$g/ml) was significant ($P < 0.05$) for increasing the number of motile spermatozoa as compared with control, whereas the concentration of $7x10^{-5}$ g/ml was outperformed other concentrations concerning increasing the number of motile spermatozoa as compared with control ($P< 0.01$).

KEYWORD: Seminal fluid, Parsley, Antibacterial, Antioxidant, Sperm motility.

INTRODUCTION

Parsley has been reported to have a number of possible medicated attribute including, antimicrobial, antioxidant (Jagadeesan *et al*., 2014). There is a lack of information about the antibacterial properties of Parsley. Based on higher content of compounds with antimicrobial activity, a strong inhibitory effect of essential oils of dill and parsley grown in summer time could assist in preserving foods although different nutrient content of food may influence microbial resistance (Vokk *et al*., 2011). Plant extracts and essential oils have been used as alternatives to antibiotic due to their antimicrobial activities and the favorable effect on the animal intestinal system (AL-Kassie, 2009; Sunder *et al*., 2013).

Bacteriospermia is commonly found in semen sample from infertile men, even in the absence of a clinically apparent male accessory gland infection, and the cause-effect relationship between bacterial infections and semen contamination and male infertility is still being debated (Jakop *et al*., 2006). Genital tract infection is the most important cause of male infertility affecting not only sperm cell function, but also the whole spermatogenesis (Henkel and Schill, 1998; Elena *et al*., 2009). Results of numerous investigations point out to the antioxidant properties of parsley. For example, the flavonoid apigenin, one of the components of parsley plant, was shown to express strong antioxidant effects by increasing the activities of antioxidant enzymes and related to that, decreasing the oxidative damage to tissue (Sanocka *et al*., 2004). The ethanolic extracts of parsley, brahmi and lettuce are protective against D-galactose induced oxidative stress in testes and epididymis (Jovanka *et al*., 2010). Both parsley oil and *panax ginseng* extract induced a protective action against Zearalenone (ZEN)-induced alteration in the reproductive performance and the combined treatment may be useful than the single treatment (Rahul *et al*., 2009). Results revealed that feeding diets containing different levels of parsley resulted an in significant increase in semen volume, sperm concentration, live and normal morphology sperm, semen quality factor, sperm motility, of local Iraqi ganders (Hassan *et al*., 2006).

MATERIALS & METHODS

The parsley oil (*Petroselinum Crispum*), was obtained from the market, twenty-two seminal fluids of infertile men were used in this study. The dry weight of Parsley oil in one milliliter was calculated by drying 0.25 ml in a clean Petri Dish, then the (Total weight after drying + The weight of Petri Dish) – (The weight of Petri Dish) equal to 5.5517-5.3757 = 0.176 in 0.25 ml or $7x\ 10^{-1}$ g in one milliliter. Bacteriological study was carried out; semen was cultured after liquefaction on blood agar, MacConkey's Agar, and chocolate agar, by using calibrated loop (0.001ml). The plates were incubated at 37ºC for 24-48 hours. All positive cultures were subcultured and identified using standard biochemical test. Susceptibility test was performed by preparing a bacterial suspension in a tube saline from the pure colony of each isolate of a concentration 10^8 CFU ml-1, according to the McFarland scale (Burt and Reinders, 2003). Inoculate the plate containing Muller-Hinton agar, by dipping a sterile swab in to the bacterial suspension. Streak the swab over the surface of the medium three times. Sterile filter paper discs were impregnated with 10 micro liter of each concentration of Parsley oil ($7x10^{-4}$ g/ml, $7x\ 10^{-5}$ g/ml, $7x\ 10^{-6}$ g/ml, $7x\ 10^{-7}$ g/ml, $7x10^{-8}$g/ml) and fix on the inoculated plate using a sterile forceps. After overnight incubation, the diameter of each zone (including the diameter of the disc) should be measured and recorded in mm. To evaluate the effect of parsley oil on spermatozoa In vitro, for each semen sample under test, two rows of 6 wells were arranged in a serological plate, in the first row place 90 micro liter of Earls Balanced Solution (EBS)

which is frequently used in Spermatozoa preparation for Assisted Reproductive Technology (ART). Afterwards 10 micro liter of Parsley oil that has $7x10^{-3}$ g transfer to the first well of the 1st row and mix well, transfer 10 micro liter from the first well to the next and mix well, continue successive transfer till the fifth well, The sixth well serve as control and have only (EBS) solution, this will give final dilutions of $7x10^{-4}$g/ml, $7x10^{-5}$g/ml, $7x10^{-6}$g/ml, $7x10^{-7}$g/ml and $7x10^{-8}$g/ml. Transfer 10 micro liter from each dilution and control to each 6 well of the corresponding second row that have 10 micro liter of seminal fluid. Mix the plate well and incubate for 30 min at 37ºC. After incubation, transfer 10 micro liter of a mixture from each of dilution and control on a slid and covered by a cover slip , then the mean number of motile sperm were obtained by reading 25 Microscopic fields.

Statistical Analysis

Data were analyzed by using student's t-test, in order to determine whether significance difference existed between the number of motile sperms in the samples treated by parsley and control samples, and a *P* value <0.05 was considered as statistically significant.

RESULTS & DISCUSSION

The result of bacteriological culture of 22 semen samples was positive in 68 % of the total and the most isolated organisms were *staphylococcus aureus* 53% of 15 positive samples, followed by 20% each of the *Escherichia coli and Proteus mirabilis, and Klebsiella sp.*7%, (Table-1). These findings are consistent with the result of research that indicates that *Staphylococcus aureus* was obviously the most prevalent organism implicated in primary infertility in the semen of males, while *Escherichia coli* was the most prevalent Gram negative organism isolated (Momoh *et al*., 2011). Parsley oil had inhibitory growth effect at various concentrations against Gram positive and Gram negative bacteria, it was effective against *E .coli, P. mirabilis, klebsiella sp, and S. aureus* respectively, and the more effective doze was $7x\ 10^{-4}$g/ml (Table- 2). This result agrees with Vokk *et al*. (2011). Results revealed there was a significant increase in the number of motile spermatozoa in 22 seminal fluid samples after treated with parsley, the most effective doze was $7x10^{-5}$ g/ml ($P<0.01$)and the others four dozes were significant at ($P<0.05$) (Table-3). This study clearly confirmed that the Parsley have inhibitory growth effect against (*E. coli, P. mirabilis, klebsiella sp, and S. aureus*), and activator effect on spermatozoa in Vitro.

The study also showed that *Escherichia coli* and *Staphylococcus aureus* were the most Gram negative and Gram positive organisms isolated from the semen of infertile men, respectively. On the basis of above results, it is necessary to do a study in vivo to know more about antibacterial and sperm activation characteristics of Parsley.

TABLE 1: Bacteria isolated from 15 positive cultures of seminal fluids samples (%) .

Isolated bacteria	Semen samples (%)
E.coli	3 (20)
S. aureuse	8(53)
Klebsiella spp.	1(7)
P. mirabilis	3(20)

TABLE 2: The inhibitory zone of Parsley oil at various doze, in gm /ml on some microorganism isolated from infertile semen sample

Type of Microorganism	The concentration of Parsley oil				
	$7x10^{-4}$ g/ml	$7x10^{-5}$ g/ml	$7x10^{-6}$ g/ml	$7x10^{-7}$ g/ml	$7x10^{-8}$ g/ml
E.coli	12(z)	7	5	5	R
S.aureus	7	R	R	R	R
Klebsiella spp.	7	7	6	R	R
P.mirabilis	7	9	7	R	R

R: Resistant (No zone)

(Z): zone of inhibition in mm

TABLE 3: Mean±SE of motile sperms in seminal fluids of treated and control groups (twenty two Infertile men)

Doze of Parsley	No. seminal fluid samples	No. sperm /control mean ±SE	No. sperm/ treatment mean ±SE	P value
$7x10^{-4}$ g/m	4	Mean 2.0 ±0.41	Mean 16.0 ±4.42	<0.05
$7x10^{-5}$ g/ml	8	Mean 6.25 ±3.55	Mean 18.3 ±6.80	<0.01
$7x10^{-6}$ g/ml	3	Mean2.33 ± 0.33	Mean 10.33 ±1.76	<0.05
$7x10^{-7}$ g/ml	4	Mean 2.75 ±1.44	Mean 7.75 ±1.44	<0.05
$7x10^{-8}$ g/ml	3	Mean 5.0 ±2.08	Mean 5.0 ±2.08	<0.05

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