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Effect of Aqueous Extract of Turmeric on Pathogenic Bacteria Isolated from Semen in a Sample of Iraqi Infertile Men

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Abstract

Background Contamination of semen with sexually and none sexually transmitted bacteria plays an important role in male infertility, contaminating bacteria should be eradicated by antibiotics, but most of bacteria become resistant to available antibiotics. Therefore most researchers search to find other antibacterial agents.

Objective To evaluate the frequency of bacteria in semen of infertile men and investigate the antibacterial activity of aqueous extract of turmeric (AET) on certain isolated bacteria from semen of infertile men compared with that of doxycycline as a standard antibiotic.

Methods Forty two semen samples obtained from infertile men attending The Higher Institute of Infertility Treatment and Assisted Reproductive Technology were evaluated bacteriologically using standard bacterial culture. Then investigated the antibacterial activity of AET on certain isolated bacteria from semen of infertile men compared with doxycycline using disc diffusion method.

Results Out of 42 semen samples of infertile men, 35(83.03%) were infected with different bacterial species (spp.) accompained with highest incidence rate. The overall frequency of *N.gonorrhea* and *S.epidermides* was 8(19%) and for remaining bacterial isolated from semen *(S.aureus, G.vaginalis* and *E.coli)* were 6(14.28%). Comparable antibacterial activity of ATE and doxycycline was found to be evident against most isolated bacteria ($P<0.001$) in mean inhibition zones of AET between Gram's positive and Gram's negative bacteria (23.35±0.68 and 8.35±1.52, respectively).

Conclusion Most of isolated bacteria from semen of infertile males had high frequency, which were generally accepted as pathogenic bacteria. Antibacterial activity of AET was comparable with doxycycline against most isolated bacteria and it was found more effective on Gram's positive than Gram's negative bacteria.

Keywords Turmeric, pathogenic bacteria, semen, infertile men

Introduction

The presence and profound influence of microorganisms in semen is evidence that pathological microorganisms play a significant role in male infertility [(1)]. Genital tract infection and inflammation have been associated with 8-35% of infertility cases [(2)]. Some possible pathomechanisms of the development of infertility linked with infection are considered: direct effect on sperm function (motility, morphology, etc), deterioration of spermatogenesis, autoimmune processes induced by inflammation and dysfunction of accessory sex glands [(3,4)].

Among the most common microorganisms involved in sexually transmitted infection that interfere with male fertility, are *N. gonorrhea* which has been reported in the colonization of human sperm and *G.vaginalis* that may assume a pathogenic role by extension to prostate. Less frequently, male infertility is due to none

sexually transmitted epdidmo-orchitis mostly caused by *E.coli* [5-7].

Opportunistic microorganisms including *S.aureus*, *S.fecalis* and *diphtheroids species* (spp.) responsible for semen contaminations generally originate from the urinary tract of patient or can be transmitted by the partner via sexual intercourse causing classical infection of urogenital tract and subclinical reproductive tract infection and could contribute to the deterioration of sperm quality of infertile men [3,8,9], therefore the presence of urogenital tract infection and inflammation must be eradicated by antibiotics and anti-inflammation treatment [2], but the continuous evolution of bacterial resistance to currently available antibiotic has necessitated the search for novel and effective antimicrobial component [10].

Curcuma longa commonly known as turmeric is widely used as spice and coloring agent and is well known for its medicinal properties such as antioxidant, anti-inflammatory, anti-platelet, cholesterol, antibacterial and antifungal [11]. More recently, alcoholic extract of turmeric was used for *in vitro* sperm activation of infertile patients [12].

However, due to limited information on its effective and practical application to bacteriospermia in infertile men, this study was designed to found the frequency of isolated bacteria from semen of infertile men and to investigate the effect of aqueous extract of turmeric on certain isolated bacteria from semen samples of infertile male.

Methods

Study population:

A total of 42 selected semen samples with ≥10 round cells /HPF were collected from infertile men attending Institute of Embryo Research and Infertility Treatment for examination from March to November 2010. The patients had no clinical signs or symptoms of infection of lower genital tract and negative history for receiving antibiotic treatment. Semen samples were collected by masturbation into sterile wide-mouth plastic container after 3-5 days of abstinence. Each infertile man was instructed to wash genitilia before masturbation.

Microbial Examination:

Collected semen samples were cultured after liquefaction both aerobically and anaerobically. For aerobic culture one drop of 0.1ml was inoculated into blood agar, MacConkey agar and sabrouaud dextrose agar (HiMedia laboratories, india) while the anaerobic culture was done on chocolate agar under 5% CO_2. All isolated microorganisms were identified by bacteriological feature of bacterial colonies and biochemical test and regarded as a cause of infection if the colony count was $\geq 10^3$ bacteria /ml of semen. Skin contaminant (*e.g coagulase-negative Staphylococcus, Streptococcus spp*. and *Corynebacterium spp.* were reported only if there were no more than two different species in the ejaculates.

Preparation of aqueous extract of turmeric:

Turmeric was purchased and cleaned from dust, it was crushed into powder using electrical mixer. Turmeric powder 1g was dissolved in 1L of D.W in a clean glass beaker, and a mixture was stirred for 4 hours at room temperature. Then, the mixture was filtrated using filter paper to collect aqueous extract of sterile turmeric in another glass beaker then it was filtrated by filter with 45 micrometer and 22 micrometer. Aqueous extract of turmeric with concentration 1mg/ml was considered as stock solution for other dose 25µg.

Sensitivity test:

Antibacterial activity of turmeric was tested by using disc diffusion method, 25µg concentration was chosen for this test. Sterile 6mm diameter filter paper discs were impregnated with these concentrations to prepare the turmeric discs. Certain isolated bacteria from semen of infertile men were tested, few colonies of overnight culture of every type of bacteria were mixed with normal saline, the turbidity of this suspension was adjusted to match a 0.5 Macfarland turbidity standard. Mueller-Hinton agar (HiMedia labrotories, India) surface was inoculated with

that bacterial suspension then turmeric disc and antibiotic disc with 30µg of doxycycline for all those bacteria were added on surface of plate using flamed forceps. The plate was incubated at 37°C. After 24 hours, the inhibition zones were measured in mm using a ruler

Statistical analysis:

Statistical package for social sciences (ssps) version 16 (13), and Microsoft office excel 2007 were used to analyze the results of this study using descriptive table and ANOVA test.

Results

Frequency of isolated bacteria from semen of infertile men

The frequency and type of organisms isolated from semen of infertile men are shown in table (1). Out of total number of 42 patients semen samples 35(83.03%) yielded bacterial growth with *N.gonorrhoea, S.epidermedis, S aureus G.vaginalis and E.coli,* as pure culture, having the highest incidence rate of 6 (14.29%) for both *N.gonorrhoea* and *S. epidermedis* 5(11.90%) for *S.aureus*,4(9.52%) for *G.vaginalis* and 3(7.14%) for *E.coli*. Those microorganisms were also detected as mixed culture therefore the overall their incidence rate were 8 (19%) for both *N.gonorrhoea* and *S. epidermedis* and 6(14.29%) for remaining spp. of bacteria (*S aureus, G.vaginalis and E.coli)* respectively.

Table 1. Frequency of isolated bacteria from semen of infertile men

Isolated bacteria	Frequency	%
N. Gonorrhea	6	14.29
S. Epidermedis	6	14.29
S. Aureus	5	11.90
G. Vaginalis	4	9.52
E. Coli	3	7.14
S. Fecalis	2	4.76
Corynebacterium spp.	3	7.14
N. Gonorrhea + E. Coli	1	2.38
N. Gonorrhea + S. Aureus	1	2.38
G. Vaginalis + E. Coli	2	4.76
Corynebacterium spp. + S. Epidermedis	2	4.76
Total	35	83.03

The results of antibacterial activity of (AET) showed zone of inhibition in millimeter for five of the highest incidence rates of bacteria in semen samples *(N.gonorrhoea, G.vaginalis, E.coli,* as Gram's negative and *S. aureus and S. epidermedis)* as Gram's positve in comparison with standard antibiotic (doxycycline) are presented in table 2 (a+ b).

From table 2 (a) no significant differences were shown in mean inhibition zones of AET concentration 25µg/ml against all isolated bacteria except for *Staph. epidermedis* (p=0.025) (23.50±1.00) as compared to mean inhibition zones of doxycycline (10.57±4.37).

From table 2(b), it was observed that AET had highly significant difference (P<0.001) in mean inhibition zones between group of Gram's positive and group of Gram's negative bacteria (23.35±0.68 and 8.35±1.52. respectively).

Table 2. Anti bacterial activity of turmeric on Gram's positive and Gram's negative bacteria compared with doxycycline

A. Comparison between different type of bacteria.

Antibacterial agent	Gram's positive		Gram's negative			p-value
	S. aureus	*S. epidermidis*	*N. gonorrhea*	*G. vaginalis*	*E. coli*	
Turmeric 25 µg/ml Doxycycline	23.16±0.98 15.40±4.11	23.50±1.00 10.57±4.37	9.87±2.29 19.00±4.14	9.50±3.18 10.33±5.36	5.16±2.53 9.16±5.83	<0.001 0.533
p-value	0.076	0.025	0.068	0.980	0.550	

B. Comparison between Gram's positive and Gram's negative bacteria.

Antibacterial agent	Gram's positive	Gram's negative	p-value
Turmeric 25 µg/ml Doxycycline	23.35±0.68 12.58±3.03	8.35±1.52 13.68±3.07	<0.001 0.805
p-value	0.005	0.134	

Discussion

In the current study table 1 shows that 35 (83.03%) out of 42 semen samples of infertile men yielded bacterial growth as pure and mixed culture. This result is in accordance with other study that found that bacterial growth from semen culture was 79% of total number of the study [14]. This high percentage of infected infertile patients may be due to stringent patient selection in the present study. Moreover it was shown from this table, that there were five spp. of bacteria resulted from semen culture had the highest incidence rate of *N. gonorrhoea and S epidermidis* 6(14.29%), *S. aureus* 5(11.90%), *G. vaginalis* 4(9.52%) and *E.coli* 3(7.14%) and the overall frequency of *N. gonorrhoea and S epidermidis* was 8(19%) and 6(14.29%) datively for remaining spp. of bacteria.This finding is in agreement with other studies which reported that those spp. had the highest frequency [1,15].

It is generally accepted that those five spp. of bacteria are regarded as pathogens because *N. gonorrhoea and G.vaginalis* are well recognized etiological agent of sexually transmitted diseases and, are related to male infertility [16]. Other common bacteria generally that were isolated from semen, *S. aureus and E. coli* were the main organisms with most negative influence on sperm quality [15,17], and *S.epidermidis* may play an important role in sperm impairment due to infertility [18]. Therefore those pathogenic bacteria should be eradicated by antibiotics or other antimicrobial agents from semen especially before using assistant reproductive technique.

Many researchers have studied antibacterial activity of various extracts of turmeric on different bacteria as food or clinical but not from semen samples isolates or standard strains [10,19,20]. Most of isolated bacteria, in the current study, were generally accepted as pathogenic bacteria because they showed negative influence toward reproductive potential and sperm quality leading to infertility [15-18]. Moreover those bacteria were found to have the highest incidence rate; therefore in vitro antibacterial activity of AET was investigated in comparison with standard antibiotic (doxycycline) against *S. aureus and S. epidermides* as Gram's positive bacteria and *N. gonorrhoea G. vaginalis and E. coli* as Gram's negative bacteria. Table 2a shows comparable antibacterial activity of AET to doxycycline against all isolated bacteria except for *S. epidermidis* which was more sensitive to turmeric (P=0.025). This finding is in agreement with another study [20], which reported that

AET shows comparable activity to standard antibiotic involving doxycycline.

The mechanism of action of doxycycline appears to be carried out by inhibiting protein synthesis by binding to 30S ribosom subunit and blocking the aminoacyl transfer RNA from entering the acceptor site on ribosom [21]. On the other hand, curcumin is a polyphenolic and hydrophobic agent which is considered a major active component of turmeric [22-25]. Curcumin to which have been attributed most of medical effect of turmeric such as antibacterial, anti-inflammatory and antioxidant activities [26,27], and hypothesis of mechanism of antibacterial action has proposed different workers which involve: hydrophobic and hydrogen bonding of phenolic compounds to membrane protein followed by partition in the lipid bilayer; perturbation of membrane permeability consequent to its expansion and increased fluidity causing the inhibition of membrane embedded enzyme; membrane disruption; destruction of electrons transport systems and cell wall perturbation [28]. So we can conclude there was relatively similar effectiveness of AET with doxycycline because both of them bind and acts on membrane protein of bacterial cell.

From Table 2b, it was observed that AET had more effectiveness against Gram's positive bacteria as compared to Gram's negative bacteria ($P<0.001$). This result is in accordance with other studies who reported that AET is more effective against Gram's positive bacteria compared to Gram's negative bacteria [10,19,20,29]. On the other hand it was found that the curcumin is soluble in ethanol and acetone, and alcohol was found to be better solvent for extraction of antimicrobially active substances compared to water [30,31]. Moreover it was found the curcumin was active against *S. aureus and S.epidermidis* whereas did not show antibacterial activity on *P.aeruginosa E.coli*, but *N.gonorrhoea and G.vaginalis* are Gram's negative bacteria [32], therefore, the antibacterial activity of AET on those bacteria may be similar to *E.coli and P.aeurginosa*. From the above results the reason of varying degrees of sensitivity between Gram's positive and Gram's negative bacteria may be related to low curcumin content in AET and may be due to the intrinsic tolerance of Gram's negative to curcumin which is responsible of antibacterial activity of turmeric.

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