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The Antibacterial Effect of Qutran (wood Tar) from Olive Trees on Pathogenic Bacteria

Abdulrahman Al-Asmari[1*], Yunus Siddiqui[1], Mozaffarul Islam[1], Mohammed Al-Ghamdi[1], Abdulrahman El-Elaiwai[2]

***Corresponding author:**

Abdulrahman Al-Asmari

[1]Research Centre, [2]Urology, Prince Sultan Military Medical City, Riyadh, Saudi Arabia

Abstract

Amongst the folklore medicine in Saudi Arabia, the tar preparation which is locally known as Qutran comes from various trees and has been used for a number of ailments in humans as well as in animals. This product is commercially produced and marketed in local shops selling herbs or herbal products but the source of this product is not mentioned. Three Gram positive and two Gram negative bacteria were exposed to various concentrations of Qutran and the growth inhibition was determined by the disk and agar diffusion method and enumeration of colony forming units by micro-dilution procedure. Both the Gram positive and Gram negative microorganisms showed a great sensitivity in terms of reduction in colony forming unit counts (CFU). Disk and agar diffusion assays showed a phenomenal inhibition of bacterial growth to Gram positive but lesser inhibition of bacterial growth in Gram negative bacteria. It was observed by the enumeration of CFU counts that the Qutran exerts more growth inhibition (killing effect) on Gram positive microorganisms than onto Gram negative bacteria. This effect could be taken as action on the mucopeptide content of Gram positive bacteria than its presence in the trilaminar cell walls of Gram negative bacteria. The minimal biocidal activity (MBC) of Qutran towards the Gram positive microorganisms tested was determined as 0.625mg/ml, while the Gram negative microorganisms showed no growth above 1.25mg/ml.

Keywords: Qutran, antibacterial effect, minimum bactericidal concentration, Gram positive, Gram negative bacteria

Introduction

It has been a challenging task for the clinicians to treat the present day infections with the most potent medicines either the sulfa drugs, antibiotics or the structural analogues. The abuse or misuse of antibiotics has created a phenomenal problem that new strains of microorganisms are being evolved and thus there is a constant demand for the super antibiotics or chemical compounds against which the microorganisms do not manifest any resistance. Herbal medicines have been in use for centuries but because of a poor dissemination of knowledge, their use has been confined to either local folklore or regional remedies. So far, there has not been any report that the microorganisms have developed resistance against any herbal extract, which was used to combat the infection.

The local inhabitants of the southern region (Asir) in the Kingdom of Saudi Arabia are known to use a popular remedy in the form of Qutran which they apply to get relief from oral aches and pain. We are told that they are also using this material to treat camels and other cattle from scabies, where the skin starts losing hair.

Qutran is an Arabic word translated in English as “Tar”. Two types of tars are known; one is from fossil fuel and contains high content of benzene known to be very toxic and even carcinogenic. The tar obtained from wood especially from Pine trees either by heating or destructive hydrolysis under pyrolysis, contain very heavy oil. The heating of pine wood causes tar and pitch to drip away from the wood and leave behind charcoal. The tar made from the Birch-bark is normally known as “Russian oil" and known to be used in leather protection. The by-products of wood tar are turpentine and charcoal. It is available as tar water which is used in medicaments, cosmetics and food. Wood tar is also used as a flavoring agent in candies like TervaLeijona liquorish, alcohol (TervaViina) and as a spice for meat. There is a saying in Finland that if the vodka, sauna and tar cannot cure considers that the disease is fatal. They use the wood tar in their regional medicine because of its microbiocidal properties. It produces nice scent when used in saunas by mixing tar with water and heated to turn into steam. Tar is an important component in cigarettes and anti-dandruff agent in shampoos [1]. It was mainly used in the preservation of boats or ships made of wood and Navy used to use this material indiscriminately. Lately its use has been curtailed because the ships are no longer built of wood but made of iron and steel. This material is still used in the preservation of wood in docks and floating platforms. Producing tar from wood was known in ancient Greece, and has probably been used in Scandinavia since the Iron Age.

Tar-like products can also be produced from other forms of organic matter such as peat. A tar-like substance can be produced from corn stalks by heating in a microwave oven. Mineral products resembling tar can be produced from fossil hydrocarbons including petroleum that is normally called asphalt. The antimicrobial effect

of botanical tar (coming from the trees) has been reported earlier [2, 3, 4]. The presence of some alkylphenanthrenes in wood tar pitch was reported by Jonsson [5] but Lopez *et al.* [6] have reported the structural analysis of tar obtained after pyrolysis of wood. Essential oils from medicinal and herbal plants have also been studied for their growth inhibitory property on bacteria and viruses [7, 8, 9]. We studied the antibacterial effect of Qutran obtained from the dried olive trees which is true representative of tar rather using the commercially available product.

Materials And Methods

The Qutran preparation was brought in the Research Centre at Prince Sultan Military Medical City (PSMMC) formerly known as Riyadh Military Hospital in Riyadh, the Kingdom of Saudi Arabia from the city of Abha in Asir region. A stock solution of Qutran was prepared in dimethyl sulfoxide (DMSO) at a concentration of 200mg/ml (weight/volume) and stored in dark at room temperature. Further dilutions of Qutran were made in either physiological saline or phosphate buffer saline (PBS) at pH 7.4 and used in the antibacterial experiments.

Microbial cultures of *Staphylococcus aureus*(*S. aureus,* ATCC 25923)*, Escherichia coli* (*E. coli,* ATCC 25922), *Enterococcus faecalis*(*E. faecalis,* ATCC 29212) and *Pseudomonas aeruginosa*(*P. aeruginosa, ATCC 27853)* were purchased from Microbiologicals, Inc. MD, USA. The *S. aureus* (MRSA positive strain #12498) was obtained from the Department of Laboratory Medicine, (PSMMC) in Riyadh, Saudi Arabia. Stock cultures of these microorganisms were prepared in Brain Heart Infusion (BHI, Scharlau Laboratory, Barcelona, Spain) broth containing 50% glycerol and the aliquots were kept frozen at -40^{0}C for future use.

Colony Forming Unit (CFU) counts

Bacterial cultures were grown in BHI broth for 24h and centrifuged at 2000xg for 10 minutes to get the growing bacteria in the pellet, which was suspended in fresh broth. The optical density of these cultures was adjusted to 0.5 McFarland Standard. One log dilutions of the bacterial cultures were prepared in sterile physiological saline and equal volumes were mixed with various dilutions of Qutran taken in micro centrifuge tubes. Reaction tubes were vortexed to mix the content and incubated for 6 and 24h in a 37^0 C incubator. The control cultures were incubated with the diluent only that contained the highest concentration of DMSO (2.5%). All of these tests were conducted in triplicates.

After the prescribed incubation time of 6 and 24h, an aliquot from control and each concentration was removed and log dilutions were made in physiological saline. The 100µl inoculum of different dilutions were used to inoculate the BHI agar plates in triplicate and spread with the help of L-shaped glass rod and a plate rotator (plate turn table). Plates were incubated and the CFU were counted after 48h of incubation.

Disk and Agar diffusion

The disk diffusion assay was based on the procedure of Kirby-Bauer [10]. Six millimeter disks were cut from filter paper (Trans-Blot filter paper, Bio-Rad Scientific, CA. USA) using a paper punch and sterilized for 15minutes at 121^{0}C. Container with filter disks was left in drying chamber and then used in assay. After spreading 24h old bacterial inoculum, disks soaked with various concentrations of Qutran were placed and plates were incubated for a maximum of 48h. Zone of growth inhibitions were recorded at 24 and 48h.

Pour plates method was used in the agar diffusion procedure, with a slight modification of Barry and Brown method [11]. Molten agar was kept at 45^{0}C in a water bath and 100µl of inoculum were added in the tubes containing 15ml of agar. Plates were poured and once the agar was solidified, they were left at the refrigeration temperature for 2-3hours. A dedicated agar punch was used to cut holes and then filled with different concentrations of Qutran. Middle well (control) received only the saline containing the highest concentration of DMSO used with the test reagent. After incubation for 48h, the zones of growth inhibition were recorded.

Results

Effect of Qutran on Gram Positive Bacteria (Quantitation of CFU through the microdilution procedure)

Exposure of *S. aureus* (MRSA positive), *S. aureus* (MRSA negative) and *E. faecalis* cultures to various concentrations of Qutran revealed the antibacterial effect of Qutran in 6 and 24h of incubation. Within 24h of incubation, no bacterial colonies were observed with 0.3mg of Qutran/ml. Relatively it was highly bactericidal on *E. faecalis* where the minimum bactericidal concentration (MBC) of Qutran was observed to be < 0.3125 mg/ml. The Qutran preparations showed almost identical bactericidal effect on *S. aureus* and MRSA strains (Fig1. A-C).

Effect of Qutran on Gram negative bacteria (CFU counts)

E. coli and *P. aeroginosa* were exposed to Qutran dilutions and the growth was checked by the dilution methods of counting colonies after 6 and 24h of exposure. No growth was observed after 6h of incubation with 2.5mg of Qutran but at lower concentration decent number of CFU were recorded. Figure 2A and B, shows the bactericidal effect of Qutran on *E. coli* and *P. aeruginosa* cultures.

Agar and Disk Diffusion

Table 1 and 2 demonstrate the numerical values of the disk and agar diffusion procedure respectively. The zones of bacterial growth inhibition are pronounced in both the procedures. Gram positive organisms show larger zones of growth inhibition compared to Gram negative organisms. The concentration of

Qutran less than 500µg/well was not effective in showing the zones of growth inhibition with Gram negative bacteria.

Discussion

Our results using three different end points (CFU counts, disk, and agar diffusion procedures) consistently show the bactericidal effect of Qutran on pathogenic bacteria and these three end points show concordance in this study. Its medicinal use is limited to either in production of tar-based shampoos or in folklore medicine. Little use has been in making candies and some use in Sauna baths. There are some studies in finding out the components in pine-wood tar [5, 6]. Although it is suggested that it is highly antibacterial but not many references are available. Brockow*et al.* [12] reported the effect of tar solution (liquor carbonis detergents) on the reduction of *S. aureus* colonized on human skin. The growth inhibitory effect of bamboo charcoal obtained by pyrolysis in absence of air and made composite with silver on *S. aureus* and *P.aeruginosa* was reported by Yang *et al.* [13]. To the best of the author's knowledge no study has been reported so far where the anti-bacterial role of the Qutran preparation originating from olive trees was documented. The close studies that we could find was the antifungal effect of coal tar gel on *Malassezia furfur* [14] and antibacterial effect of the various extracts of tar by organic solvents [15]. The bacteriostatic effect of wood tar was also reported byVeijola and Mustakallio over five decades ago [16]. Skin diseases including psoriasis have been treated successfully with pine tar [17, 18, 19] while it was also proved useful on wound healing [20].

The northern region of Saudi Arabia has a multitude of herbal plants used in the folklore medicine. The word of mouth made us to explore the anti-bacterial effect of Qutran preparation which the people have been using for the treatment of cariogenicity and also on animals to treat the scabies in sheep and camels. Sometimes they use Qutran to rub on dentine to get relief from pain and also when the tooth needs to be extracted. The local inhabitants were requested to provide various samples of Qutran but we were able to receive only one, which we have used in this study. Three different end points like the colony forming unit counts, agar and disk diffusion procedures were chosen to elucidate the antibacterial effect of Qutran preparation [21, 22]. The antibacterial effect of Qutran on three Gram positive and two Gram negative bacteria has been described in this study. By using the most common pathogens, it is fully acknowledged that the preparation has certainly a good potential to be used as a medicament for antibacterial therapy and in particular on Gram positive bacteria. The Gram negative bacteria like *E. coli* and *P. aeruginosa* were less sensitive to the bactericidal dose of Qutran. Research is underway to know its effect on yeast and possibly on viruses but at the same time its safety, toxicity and adverse effects *in vivo* needs to be addressed. The biocompatibility of Qutran also has a premium concern that needs to be analyzed as soon as possible although it is possible mutagenicity and clastogenicity has been ruled out in one study from pine-tar resins [23].

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References

[1]. Roelofzen JH, Aben KK, van der Valk PG, van Houtum JL, van de Kerkhof PC, Kiemeney LA. Coal tar in dermatology. J Dermatologic Treatment. 2007; 18:329-334.

[2]. Chanthachum S and Beuchaf R. Inhibitory effect of Kiam (Cotylelobiumlanceotatumcriah) wood extract on Gram positive food-borne pathogens and spoilage microorganisms. Food Microbiology. 1997; 14:603-608.

[3]. Mohan D, Shi J, Nicholas DD, Pittman CU Jr, Steele PH, Cooper JE. Fungicidal values of bio-oils and their lignin-rich fractions obtained from wood/bark fast pyrolysis. Chemosphere. 2006; 71:456-465.

[4]. Kartal SN, Terzi E, Holmeyr J, Imamura Y. Efficacy of tar oil recovered during slow pyrolysis of macadamia nut shells. International bio-deterioration and Biodegradation. 2011; 65:369-373.

[5]. Jonsson R. Separation and identification of some naturally occurring alkylphenanthrenes. Talanta. 1968; 15:425-431.

[6]. Lopez D, Acelas N, Mondragon F. Average structural analysis of tar obtained from pyrolysis of wood. Bioresour Technol. 2010; 101:2458-2465.

[7]. Siddiqui YM, Ettayebi M, Haddad AM, Al-Ahdal MN. Effect of essential oils on the enveloped viruses: antiviral activity of oregano and clove oils on herpes simplex virus type-I and Newcastle disease virus. Med Sci Res. 1996; 24:185-186.

[8]. Minami M, Kita M, Nakayua T, Yamamoto T, Kuriyama H, Imanishi J. The inhibitory effect of essential oils on Herpes simplex type-1 replication in vitro. MicrobiolImmunol. 2003; 47:681-684.

[9]. Vukovic N, Sukdolak S, Solujic S, Niciforovic N. Antimicrobial activity of the essential oil obtained from roots and chemical composition of the volatile constituents from the roots, stems and leaves of balata nigra from Serbia. J Medical Food. 2009; 12:435-441.

[10]. Drew DL, Barry AL, O'Toole R, Sherris JC. Reliability of the Kirby-Bauer disc diffusion method for detecting methicillin-resistant strains of Staphylococcus aureus. Applied Microbiology. 1972; 24:240-247.

[11]. Barry AL and Brown SD. Fluconazole disk diffusion procedure for determining susceptibility of Candida species. J ClinMicrobiol. 1996; 34:2154-2157.

[12]. Brockow K, Grabenhorst P, Abeck D, Traupe B, Ring J, Hoppe U, Wolf F. Effect of gentian violet, corticosteroid and tar preparations in Staphylococcus-aureus-colonized atopic eczema. Dermatology. 1999; 199(3):231-236.

[13]. Yang F-C, Wu K-H, Liu M-J, Lin W-P, Hu M-K. Evaluation of the antibacterial efficacy of bamboo charcoal/silver biological protective material. Materials Chemistry Physics. 2009; 113:474-479.

[14]. Nenoff P, Haustein UF, Fiedler A., The antifungal effect of a coal tar gel on Malassezia furfur in vitro. Dermatology. 1995; 191:311-314.

[15]. Kizil G, Yavuz M, Aytekin C. Antimicrobial activity of the resins obtained from the roots and stems of *Cedruslibani*and *Abiescilicica*.PriklBiokhimMikrobiol. 2002; 38:166-168.

[16]. Veijola V and Mustakallio E. The bacteriostatic effect of the wood tar, Ann Med ExpBiolFenn. 1963; 41:407-414.

[17]. Merk HF, Mukhtar H, Kaufmann I, Das M, Bickers DR. 1987. Human hair follicle benzo [a] pyrene and benzo [a]pyrene 7,8-diol metabolism: effect of exposure to coal tar-containing shampoo. J Invest Dermatology. 1987; 88:71-76.

[18]. Schmid MH and Korting HC. Coal tar, pine tar and sulfonated shale oil preparations: comparative activity, efficacy and safety. Dermatology. 1996; 193:1-5.

[19]. Faure P and Antognarelli C. Treatment of Psoriasis with pine-tar, past and present. Rev Hist Pharm. 1996; 44:352-355.

[20]. Stone OJ and Anthony JA. The effect of tar on wound healing. Arch Environ Health. 1970; 20:603-604.

[21]. Rodríguez-Tudela JL and Barchies F. Method for the determination of minimum inhibitory concentration (MIC) by broth dilution of fermentative yeasts. ClinMicrobiol. 2003; 9:1-8.

[22]. Hsu DI, Hidayat LK, Quist R, Hindler J. Comparison of method-specific vancomycin minimum inhibitory concentration values and their predictability for treatment outcome of methicillin-resistant Staphylococcus aureus (MRSA) infections. Int J AntimicrobAgents. 2008; 32:378-385.

[23]. Athanasiou K and Lillis D. Absence of mutagenic and clastogenicity action of pine-tar resin in the Salmonella/microsomal and CHO culture systems. Mutation Research 1982; 103: 229-232.