INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

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STUDY THE INHIBITORY EFFECT OF *THUJA OCCIDENTALIS* AGANIST *PSEUDOMONAS AERUGINOSA* ISOLATED FROM SURGICAL WOUNDS *IN VITRO* AND *IN VIVO*

Bushra jasim Mohammed
Genetic Engineering and Biotechnology Institute for Postgraduate Studies/ Baghdad University, Iraq

ABSTRACT
Pseudomonas aeruginosa, conceders as a major bacterial cause of morbidity and mortality in patients who are suffered from wounds and burns, it's characterized by highly antibiotic resistance. The research was designed to study the inhibitory effect of Thuja *occidentalis* against growth of *P. aeruginosa* which was isolated from surgical wounds *in vitro* and *in vivo*. *P. aeruginosa* isolated from thirty five swabs were collected from patients who suffered from surgical wounds infection. The results of determining inhibitory effect of *Thuja occ* against *P. aeruginosa* using optical density technique (O.D) showed that alcohol and aqueous extracts of *Thuja occ* were effective against *P. aeruginosa* in different concentrations and had the best effect at (50%) concentration which reach to (O.D 0.043) in aqueous extract and (O.D 0.050) in alcohol extract while the least effect was observe at (10%) concentration with O.D value reached to (1.611) in aqueous extract and (1.725) in alcohol extract. The result of determining inhibitory effect according to well diffusion method showed that *Thuja occ* had the best effect at (50%) concentration with inhibition zone reached to (21) millimeter in aqueous extract and (18mm) in alcohol extract while the less effect was observe at (10%) concentration which reached to (13mm) in aqueous extracts and (10mm) in alcohol extract. The outcomes of in *vivo* study clarified that *P. aeruginosa* caused clinical gross pathological effect in rat liver tissues when applied topically (0.1 ml) of (1.5x 10^{8} cfu \ml) *P. aeruginosa* on their induced wounds, such as irregular arrangement and degeneration of hepatocytes with hemorrhage, sclerosing bile duct that surrounded with lymphocytes and fibroblast, as well the blood culture gave positive result for *P. aeruginosa*. That effect was decreased when applied topically (0.1) ml *P. aeruginosa* and *Thuja occ* aqueous extract (5 mg/kg) on their wounds as challenge dose which the sections showed decrease the lesions, normal appearance hypatocyte, slight congestion in central vein and a few filtration of inflammatory cell in the portal area. Whereas the results revealed that the comet assay wasn't showed any positive outcomes which mean that *P. aeruginosa* infection in rats wasn't induced DNA breakage. The data reflect the ability of *Thuja occ* to reduce *P. aeruginosa* infections in vitro and certain clinical pathological changes in rat blood and livers, with promising encourage to use it as biotherapeutic agents against this bacteria.

KEYWORDS - *Thuja occidentalis* , *Pseudomonas aeruginosa* , *In vitro* , *In vitro,* Optical density technique, Well diffusion method, Histopathological assay , Comet assay**.**

INTRODUCTION

Which does not accept the controversial there are many side effects from the use of chemical drugs in spite of efficiency in getting rid of pathogens, especially dangerous ones[1], and since diseases and methods of treatment were discovered by ancient civilizations and until now scientists are still trying to reach to normal, non toxic and highly effective treatments derived from plants [2], so that the use of medicinal plants in the treatment and prevention of diseases is one of the most promising future aspirations and despite check them out at the present time but research is under way to select effective chemical materials from medicinal plant extracts and this encouraged us to highlight on *Thuja Occidentalis* which called tree of life in most countries of the world because the large pharmacological properties and it contains several active substances like d.α-pinine, d- α –thujone ,1-Fenchone,1-broneol acetic,formic andisovaleric-acid, terpineol, sabinene, camphene, camphor valerianic acid,occidol-β-sitosserol,quercetin rhodoxanthine, tanine resins, mucilage and vitamin C [3].Therefore *Thuja occ* help in the treatment of many diseases, including skin diseases and remove warts, rashes, prostate, sores, hemorrhoids, gonorrhea, influenza and symptoms of cold, insomnia, fever and headaches, dental pain, general pain, muscle aches and numerous other diseases, addition to many other uses as a antibacterial ,antifungal and antioxidant [4]. So due to these properties we aimed to study comprehensive effect of *Thuja occ* against bacteria of wound infections such as *P. aeruginosa*, which is a major bacterial agent of morbidity and mortality in compromised patients who are debilitated by wounds or burns [5]. On the other hand antibiotic therapy has a limited success due to the increasing occurrence of resistant strains[6], therefore, in the present study used rat wounds model to evaluate the therapeutic efficacy of *Thuja occ* extract against *P. aeruginosa* which isolated from surgical wounds *in vitro* and *in vivo.*

MATERIALS & METHODS

Thirty five swabs were collected from patients who suffered from surgical wounds infection. Diagnosis of bacterial isolates was performed according to[7] and routine susceptibility testing of some antibacterial agents from (Oxoid- England) Trimethoprim- Sulphametho xazole (30µg), Azithromcin (15µg), Cefotaxim (30µg), Chloramphenicol (30µg), Ciprofloxacin (5µg) Linco mycin (2µg), Gentamycin (10µg), Tetracycline (30µg), Ampicillin (10µg), and Amikacin (30µg) was performed by disk diffusion method on Mullor-Hinton agar (Difco-USA) as described in [8].

Performance *of Thuja occ* extracts

Alcohol and aqueous (thuja tea) extracts of Thuja *occ* were performed according to[9] and the dilutions of extract performed by adding autoclaved distil water to prepare the concentrations (10%, 20%, 30, 40%, 50%).

***In vitro* study**

Determining inhibitory effect of *Thuja occ* against *P. aeruginosa*

The antibacterial activity of alcohol and aqueous extracts of *Thuja occ* against *P. aeruginosa* was evaluated by agar well diffusion method according to [10,11] and measured the optical density ($OD._{600}$) nm of bacterial concentration by using the spectrophotometer according to [12, 13].

***In vivo* study**

Laboratory animals

Fifteen albino male rats were obtained from biotechnology research center, their age between (8-12) weeks and weighting (250 to 300) gm dieted basal diet and hosted in plastic cages of (80 x 60 x 40 cm) size, in three groups of five rats per cage, at temperature (25-28°C) according to Vodopich and Moor (1992). Rats were anesthetized with an IM injection of ketamine (50 mg/kg) and xylazine (5 mg/kg) from (Sigma/USA) according to[14]. Under anesthesia, the back and flank of both sides of the body were shaved, rats were returned to their cages for (24) hours to allow any edema caused by the shaving procedure to recede, after that. Then under anesthesia, skin cut was induced at length (3 cm) and depth (0.5 cm). Rats were divided as following:-

- Group A (control A): Rats of this group applied topically 0.5 ml by *Thuja occ* aqueous extract (5mg/ml) was onto the surface of each wound.
- Group B (Control B): Rats of this group applied topically by ($1.5x\ 10^8$ cfu \ml) *P. aeruginosa*
- Group C (Challenge dose group) : Rats of this group applied topically by ($1.5x\ 10^8$ cfu \ml) *P. aeruginosa* then thuja aqueous extracts (5mg/ml) were applied approximately half an hour after wound inflictions. The treatment groups received topical application twice a day for tow weeks. Blood sample was taken after (7) days post infection via tail vein and detection for *P. aeruginosa* in blood culture, then the animal sacrificed, livers were removed and divided into two parts, the first to estimate DNA damage by comet assay and the second for histopathological examination.

Comet assay

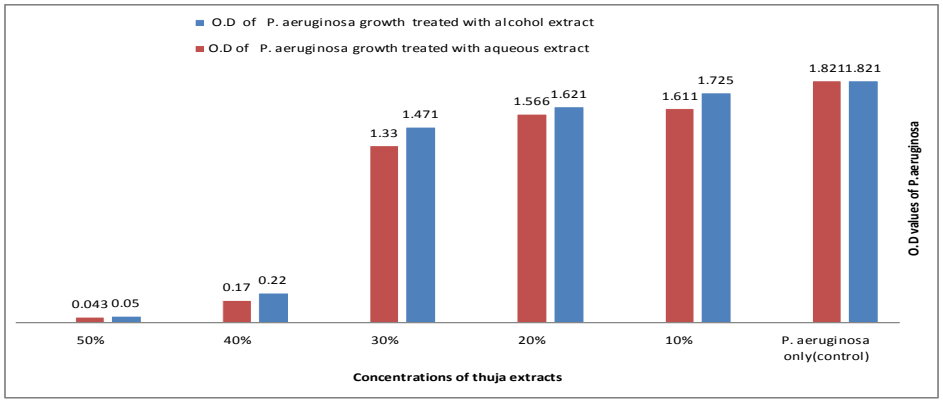
Comet assay was carried out by using chemicals and reagents from (Sigma/USA) according to [15], briefly: pieces of livers were milled, suspended in iced homogenizing buffer at (PH7.5) containing NaCl and Na_2EDTAcentrifuged at (800 X g) for (7 minutes), the precipitates resuspended in iced homogenizing buffer. Nuclei slides put into lysing solution containing (2.5) M NaCl, (100 mM) EDTA and (10 mM) Trizma base in dH_2O.After (60 min), slides were placed into electrophoresis buffer (pH 13) (300 mM NaOH /1 mM EDTA) for 10 min. Electrophoresis was carried out in dark at (0°C) for (15 min) with power supply (~0.75 V/cm) and (250 mA). Slides were neutralized with buffer containing (0.4 M) Tris at pH (7.5), and stained with Ethidium Bromide and visualized by fluorescence microscopy.

Histopathological examination

Parts of livers were fixed in (10%) formaldehyde (Sigma/USA) for (24 hr), then subjected to histological examination according to [16], under supervision of histopathologist by using hematoxyline –eosin stain (Sigma/USA).

RESULTS & DISCUSSION

The result showed that *P. aeruginosa* in current investigate was associated with frequent adverse effects and resistance to antibiotics like (Trimethoprim-Sulphamethoxazole, Azithromcin, Cefotaxim, Chloramphenicol, Ciprofloxacin, Lincomycin, Gentamycin, Tetracycline, Ampicillin, and Amikacin therefore alternative methods to control this bacteria are needed[17].



*The less value indicate good inhibition activity

FIGURE 1. The O.D value of *P. aeruginosa* growth treated with aqueous and alcoholic *Thuja occ* extract

The finding of determining inhibitory effect of *Thuja occ* against *P. aeruginosa* by using optical density technique revealed that alcohol and aqueous extracts of *Thuja occ* were effective against *P. aeruginosa* in different concentration and had the best effect at (50%) concentration which reach to (0.043) in aqueous extract and (0.050) in alcohol extract while the less effect was observe at (10%) concentration with O.D value reached to (1.611) in aqueous extracts and (1.725) in alcohol extract and as shown in figure (1).

The results of determining inhibitory effect according to well diffusion method showed that the aqueous and alcohol extracts of *Thuja occ* were effective against *P. aeruginosa* in different concentration and had best effect at (50%) concentration which reached to (21mm) in aqueous extract and (18mm) in alcohol extract while the less effect was observe at (10%) concentration which reached to (13mm) in aqueous extracts and (10mm) in alcohol extract and as shown in figure (2).

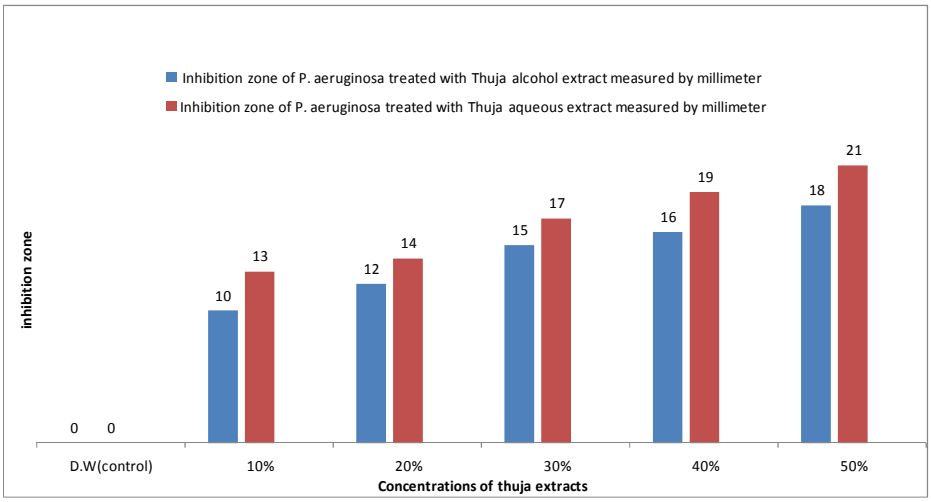


FIGURE 2. Inhibition zone of *P. aeruginosa* treated with treated with aqueous and alcoholic *Thuja occ* extract measured by millimeter

The results of comet assay revealed that *P. aeruginosa* infection in rats couldn't induce DNA breakage, so the comet assay didn't showed any positive outcomes under fluorescence microscopy, DNA didn't migrated by electrophoresis and showed as circular shape which indicated the absence of DNA damage. Infections were found in all skin sections and as presence of green pus with bad odor, also appearance of lesion in organs like degenerative changes in addition to the occurrence of an areas of congestion and the presence of necrotic foci surrounded by hemorrhagic areas as well as the blood culture were contain gram-negative rod-shaped bacteria suspected to be *P. aeruginosa* colonization. Histo-pathological examination showed various changes in liver sections according to treatment material. Sections from control - groupA which treated with *Thuja occ* extracts only showed normal appearance in hypatocyte , normal central vein and normal sinusoids as illustrated in figure (3), and the blood culture show no *P. aeruginosa* colonization.

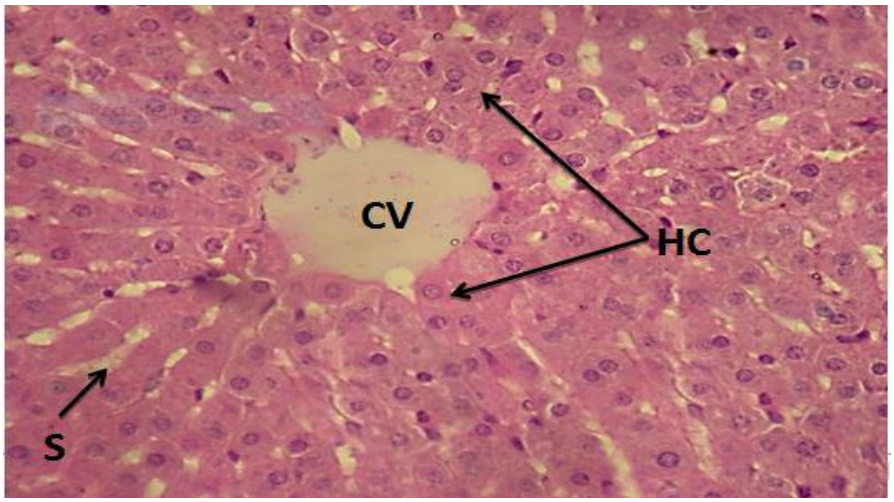


FIGURE 3: liver of control A group showing normal central vein (CV), normal hepatocytes (HC) and normal sinusoids (S). H&E X400.

Liver sections from positive control (B) which treated with *P. aeruginosa* only, showed irregular arrangement and degeneration of hepatocytes with hemorrhage, sclerosing bile duct that surrounded with lymphocytes and fibroblast as shown in figure (4).

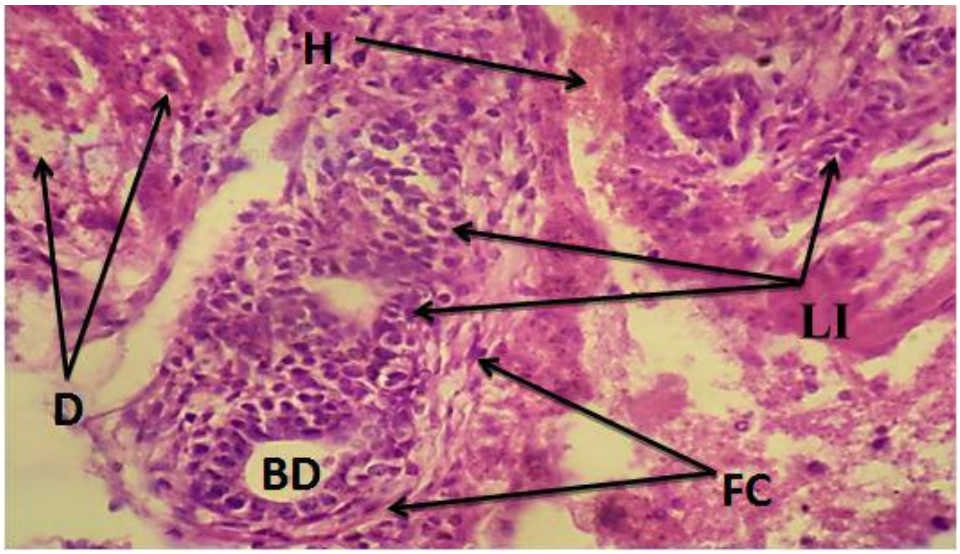


FIGURE 4: liver of control B group showing degeneration (D) of hepatocytes with hemorrhage (H), sclerosing bile duct (BD) surrounded with lymphocytes infiltration (LI) and fibroblast (FC), H&E X400

In liver sections from animals of group C - challenge dose which applied *P. aeruginosa* topically on their wounds showed normal appearance in hypatocyte and decreased in the lesion, it was noticed as a slight congestion in central vein and a few filtration of inflammatory cell around it and in the portal area, but the rest of the liver tissue looks healthy as illustrated in figure (5).

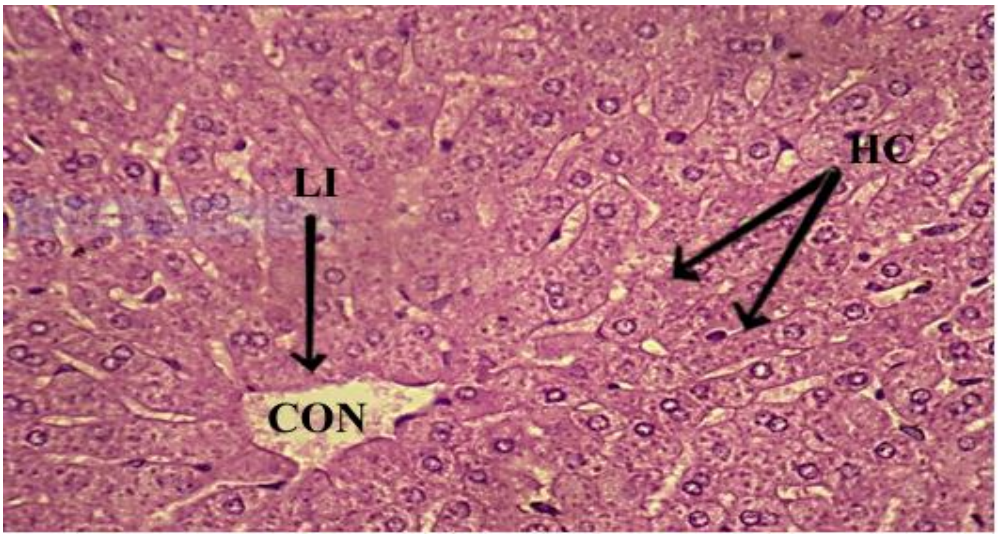


FIGURE 5: liver of group C showing normal hypatocyte(HC) slight congestion in central vein (CON) and a few lymphocytes infiltration of (LI) (H). H&E X400.

DISCUSSION

The outcomes showed that *thuja extract*s have a high antagonistic activity on *P. aeruginosa* with promising inhibitory spectrum and this may due to the active materials of the plant like thujen and its essential oils are potentially useful sources of antimicrobial compounds [18]. In addition to the high tannin content which have been identified as bacterial inhibitor by binding with bacterial cell walls and leads to lack of necessary substrate for microbial growth and protease production. Also[19] suggested that some Thuja species contains large amounts of three substances (alpha, beta and gamma thujaplicin) that in low concentration would serve as chelators for some pathogenic pacteria. Furthermore this activity is strongly dependent on the kind of extract, the present work showed that the aqueous extract had the best activity than alcohol *extract* which accordance with that claimed by [20] who confirmed such result when studied the composition and antimicrobial activity of the essential oil of some plants. Histopathological sections from rats of

control A and challenge dose C groups showed normal appearance and the tissue looks healthy this may be due to the antiseptic effect of *thuja* which protect the wounds from *P. aeruginosa* infections and capable to prevent the adherence, replication, colonization of this bacteria[21], while all rats of positive control B were died between days 7 and 9 and had the similar pathological changes. This results closely related with observation by[22] when she study the inhibitory effect of *Thuja occ* against *C. albicans* and *Staphylococcus aureus*, not only that, *thuja* have biotherapeutic potential for prophylaxis and therapy for fungal infection as mention in the study of [23] who noticed *thuja* protect mice from *Candida albicans* , also the observation by[24] who found that the *Thuja occ* can used as antibacterial agent when they studied antibacterial proprieties of *Thuja occ* leaves , such finding was confirmed by [25] when their study the effect Bio-control of clinical fungal isolates associated with fungal keratitis using medicinal plant extract.

CONCLUSION

From present outcomes, we conclude that the use of *thuja* in burn cases would be a valid alternative to other treatments and can be easily prepared in any laboratory with minimum equipment. They are inexpensive and easy to apply. Optimal management of burn patients is enormously expensive, and in our country, it is a great problem, *Thuja* treatment would mean a significant reduction in costs and inconvenience to the patient because it would ensure quality in burns care in a cost-effective manner and the studies should be aimed at opening new possibilities of thujen synthesis to increase their production on an industrial scale.

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