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Evaluating Antibacterial Characteristics of Opium

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Abstract: The antimicrobial effect of opium has been described in traditional medicine from the past. In this experimental study, opium solution was prepared in different concentration (250, 125, 62.5, 31.25, 15.6, 7.8 & 4.9 mg/mL). Its antibacterial activity against *Salmonella Typhimurium, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Staphylococcus epidermis, Enterococcus fecalis, Staphylococcus aureus, Citrobacter diversus* and *bacillus cereus* was evaluated with agar well diffusion method. Opium showed in vitro dose-dependent antibacterial effects on used bacteria as it did not exhibit any *in vitro* inhibition of the growth of test organisms in low concentrations. As concentration increased, antibacterial effect was appeared as in concentration of 250 µg/ well growth of all bacteria was inhibited. Our findings showed that opium has antibacterial effects.

Key words: Opium · Antibacterial · MIC

INTRODUCTION

The use of herbs and medicinal plants as the first medicines is a universal phenomenon. Every culture on earth, through written or oral tradition, has relied on the vast variety of natural chemicals found in plants for their therapeutic properties [1]. In last centuries, drugs were substances extracted from plants which had a particular therapeutic action. Thus, medicinal plants may be defined as any plant that could have medicinal use such as fox glove, opium poppy and garlic [1]. Numerous antimicrobials are present in plant tissues. In traditional medicine, opium has been considered as a remedy for many disorders [2]. This belief along with use of opium as recreation can lead to addiction [3]. From past, opium was considered as an antibacterial agent in traditional medicine. So in this study we assayed the *in vitro* antibacterial activity of opium against a range of bacteria.

MATERIALS AND METHODS

A stock solution of opium was made diluted with sterile distilled water to produce final concentrations of 250, 125, 62.5, 31.25, 15.6, 7.8 & 4.9 mg/mL. The opium solutions were preservative-free and sterile with no bacterial growth in the control culture.

The bacterial strains used to assess the antibacterial properties of opium included *Salmonella Typhimurium* (PTCC 1679), *Proteus mirabilis* (PTCC 2601), *Pseudomonas aeruginosa* (Research center of Ilam Medical University), *Klebsiella pneumoniae* (Research center of Ilam Medical University), *Escherichia coli* (Research center of Ilam Medical University), *Staphylococcus epidermis* (PTCC 2405), *Enterococcus fecalis* (PTCC 2321), *Staphylococcus aureus* (PTCC 1885), *Citrobacter diversus* (Research center of Ilam Medical University and, *bacillus cereus* (Research center of Ilam Medical University) (Table 1). Bacteria were obtained from

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Table 1: Zones of inhibition (mm) produced by opium in experimental concentrations against tested bacteria

	250	125	62.5	31.25	15.6	7.8	4.9	Erythromycin	Chloramphenicol
Citrobacter diversus	18	13	8	4	-	-	-	-	4
Bacillus cereus	15	7	5	-	-	-	-	18	-
Salmonella Typhimurium	15	11	7	-	-	-	-	5	-
Proteus mirabilis	16	13	10	6	-	-	-	-	5
Pseudomonas aeruginosa	16	11	6	4	-	-	-	4	-
Klebsiella pneumoniae	11	4	-	-	-	-	-	-	-
Escherichia coli	16	8	6	4	-	-	-	10	12
Staphylococcus epidermis	14	10	5	-	-	-	-	6	6
Enterococcus fecalis	11	5	3	-	-	-	-	8	6
Staphylococcus aureus	13	11	6	-	-	-	-	16	14

pasture institute, Faculty of Veterinary Medicine and Research Center of Ilam University of Medical Science, separately. The organisms were maintained on nutrient agar (Hi Media, India) slope at 4°C and sub-cultured before use. The bacteria studied are clinically important ones causing several infections and it is essential to overcome them through some active therapeutic agents.

In vitro antibacterial activity of the opium solution was studied by the agar well diffusion method [2]. Mueller Hinton agar (Hi Media, India) was used as the bacteriological medium. The solution was diluted in dimethylsulphoxide (DMSO) at the concentrations of 4.9, 7.8, 15.6, 31.75, 62.5, 125 and 250 mg/mL. The antibacterial activity was evaluated at two replicate for different concentrations 1.25, 2.5, 5, 10, 156, 312 and 625 µg/ well. Fresh bacterial cultures was grown for 18 h (to the end of the exponential growth phase) in trypticase soy broth and diluted by sterile 0.9% saline solution to the required density. Mueller Hinton agar was melted and cooled to 48-50°C and a standardized inoculum (1.5×10^8 C FU/mL, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile Petri dishes to give a solid plate. Wells were prepared in the seeded agar plates. The test compound (100 µl) was introduced in the well (8.5 mm). The plates were incubated overnight at 37°C. The antimicrobial spectrum of the extract was determined for the bacterial species in terms of zone size around each well. The diameters of zone of inhibition produced by the agent were compared with those produced by the commercial control antibiotics, erythromycin (5 mg/ml) and chloramphenicol (5 mg/mL) (Table 1). For each bacterial strain controls were maintained where pure solvents were used instead of the solution. The control zones were subtracted from the test zones and the resulting zone diameter was shown in Table 1. The experiment was performed three times to minimize the error and the mean values were presented. Each experiment was repeated three times and each dilution series assay was performed in duplicate for all strains in each experiment.

The MIC was determined by the agar well diffusion technique. A two-fold dilution series was prepared to achieve a decreasing concentration range of 10 to 0.625% (v/v). A 50 ml volume of each dilution was added aseptically into the wells of Mueller Hinton agar plates that were already seeded with the standardized inoculums (10^6 CFU/ml) of the test bacteria. Sterile DMSO, without oil, was used as negative control. All doses were done in triplicate. The agar plates were kept at 37°C for 24 h. The lowest concentration of opium solution showing a clear zone of inhibition was considered as the MIC.

RESULTS AND DISCUSSION

Opium solution did not exhibit any *in vitro* inhibition on the growth of test organisms in low concentrations. As concentration increased, antibacterial effect appeared as at concentration of 250 µg/ well growth of all bacteria was inhibited. *K. pneumoniae* and *E. fecalis* were more resistant in comparison to other strains. Some degree of resistance in tested of bacteria to chloramphenicol and erythromycin was recorded. The results of the zones of inhibition (mm) were shown in Table 1. The MIC results of tested bacteria were shown in Table 2. The lowest MIC belonged to *E. coli* and *P. mirabilis* and the highest amount was recorded for *K. pneumoniae*.

Our results have shown that opium had in vitro dose-dependent antibacterial effects on used bacteria. Opium contains at least 20 alkaloids and by some claims to 50. Observed effects in this study could be attributed to canadine and coptisin [5].

Antibacterial effects of opiates that had been showed in different studies [6] are in agreement with our findings. Antibacterial and antifungal effects of bupivacaine and morphine are reported, but they are dose dependent. For bupivacaine doses less than 2.5 µg/ well could not inhibit bacterial growth. For morphine the situation was more complex. Morphine did not show antibacterial effects at 2 mg/kg, the dose that is clinically used but was effective in

Table 2: The MIC values of selected bacteria

Bacteria	MIC
Citrobacter diversus	62.5
Bacillus cereus	125
Salmonella Typhimurium	125
Proteus mirabilis	31.25
Pseudomonas aeruginosa	62.5
Klebsiella pneumoniae	250
Escherichia coli	31.25
Staphylococcus epidermis	125
Enterococcus fecalis	125
Staphylococcus aureus	62.5

this dose in combination with bu pivacaine [7]. It had been suggested that this effect is related to interaction with membranes [8, 9]. Some strains are resistant, as *P. aeruginosa* is resistant to lidocaine and procaine [10] and also bupivacaine. In this study a similar pattern was seen for *Klebsiella pneumoniae* that was the most resistant to opium in comparison to other strains.

Our findings showed that opium has antibacterial effects and validate this hypothesis in traditional medicine.

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