Biomedicine & Pharmacotherapy 90 (2017) 171–178

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Effect of aquo-alchoholic extract of *Glycyrrhiza glabra* against *Pseudomonas aeruginosa* in Mice Lung Infection Model

Ankita Singh Chakotiya[a], Ankit Tanwar[a], Pranay Srivastava[a], Alka Narula[c], Rakesh Kumar Sharma[b,*]

[a] *Division of CBRN Defence, Institute of Nuclear Medicine and Allied Sciences, Delhi – 110054, India*
[b] *Defence Food Research Laboratory, Mysuru – 570011, India*
[c] *Department of Biotechnology, Jamia Hamdard, Delhi – 110062, India*

ARTICLE INFO

Article history:
Received 6 November 2016
Received in revised form 16 March 2017
Accepted 21 March 2017

Keywords:
Pseudomonas aeruginosa
Lung infection
Glycyrrhiza glabra
Liquid chromatography mass spectrometry

ABSTRACT

The prevalence of lung infection caused by *Pseudomonas aeruginosa* strains that are classified as multi-drug resistant has increased considerably and is mainly attributed to relative insufficiency of potent chemotherapeutic modalities. The present study was conducted to evaluate the antimicrobial activity of aquo-alcoholic extract of *Glycyrrhiza glabra* against the *P. aeruginosa* causing lung infection in Swiss albino mice. The study involves evaluation of lethal dose of *P. aeruginosa* in Swiss albino mice and analysis of disease manifestation that includes bacteremia, hypothermia, reduction in body weight and other parameters for 48 h of infection. Physical manifestations of infected mice showed a significant decline in body temperature that is 29 ± 0.57 °C (at 48th h) from 38.81 ± 0.33 °C (0 h) and 30% weight loss was observed at the end of the study. Further the efficacy of *G. glabra* extract against lung infection induced with the calculated lethal dose was evaluated by employing bacteremia, histopathology and radiological analysis. Bacterial burden showed that 2.30 ± 0.02 Log10 CFU/mL at day 7, a significant decline in the bacterial load as compared to day 1 when the bacterial burden was found to be 3.32 ± 0.1 Log10 CFU/mL. Histopathological results showed more diffuse and patchy accumulation of inflammatory cells within the alveolar space also the infiltrates were noted in all the lung section of infected mice. In treated animal group improved lung histology was seen with the exudates were less seen in D1 dose (20 mg/kg) and disappeared in D2 dose (80 mg/kg). The study clearly declares that the *G. glabra* extract is effective against lung infection caused by *P. aeruginosa* at dose of 80 mg/kg. The LCMS results revealed that the extract contains Glycyrrhizin, Stigmasterol and Ergosterol, Licochalcone and Glabridin. The current study expected to further exploit the biomedical properties of this extract in the preparation of a potent regimen against such threatening pathogen.

1. Introduction

Medical care facilities or Intensive care units (ICU) are the most common site to acquire respiratory infections by immunosuppressed patients [1]. Nosocomial pneumonia is the most common respiratory infection acquired by immunocompromised patients, including burn, cancer, human immunodeficiency virus (HIV) positive and cystic fibrosis patients etc. Also it represents a major cause of morbidity and mortality in patients especially underlying in severe condition in ICU [1–5]. Various bacterial pathogens are responsible to cause nosocomial contamination in medical care facilities, leading to unwanted secondary infections like pneumonia. The predominant pathogens responsible for this infection are *Staphylococcus aureus, Pseudomonas aeruginosa* and Enterobacteriaceae [6]. *P. aeruginosa* Gram-negative bacteria, in particular,

Abbreviations: ICU, intensive care unit; HIV, human immunodeficiency virus; XDR, extensively drug-resistant; NCDC, National Centre for Disease Control; INMAS, Institute of Nuclear Medicine and Allied Sciences; LCMS, liquid chromatography mass spectrometry; IAEC, Institutional Animals Ethics Committee; CPCSEA, Committee for the Purpose of Control And Supervision of Experiments on Animals; INSA, Indian National Science Academy; LD, lethal dose; CFU, colony forming unit; EDTA, ethylenediaminetetracetic acid; ANOVA, analysis of variance; RT, retention time; HPLC, high performance liquid chromatography; MIC, minimum inhibitory concentration; CXR, chest X-ray.

* Corresponding author.
E-mail address: rksharmadrl@yahoo.com (R.K. Sharma).

http://dx.doi.org/10.1016/j.biopha.2017.03.055
0753-3322/

presenting a critical challenge as associated with a high mortality rate [7]. Moreover, the bacterium is the most frequently isolated multi-drug resistant pathogen from the biological sample of immunocompromised patients admitted in hospitals. *P. aeruginosa* rarely causes pneumonia outside of hospital [8]. Pathogenesis is caused by inhalation/aspiration of aerosols containing the bacteria followed by tracheobronchial colonization. *P. aeruginosa* can cause acute pneumonia once its cargo gets increased in the upper respiratory tract and passes upto lower respiratory region [7]. Successive colonization of pathogen deep into the lungs resulting into clinical manifestation of pneumonia. Additional to this the *P. aeruginosa* exhibit exceptional ability to possess mechanisms of antibiotic resistance, including membrane impermeability, efflux pump systems and changes in drug targets and is posing an gigantic therapeutic trouble [9,10]. Emergence of XDR (Extensively Drug-Resistant) strains of *P. aeruginosa* required continued efforts to establish new therapeutics and medical countermeasures [11]. Several approaches are undertaken to discover novel therapeutic agents from herbals that are being utilized conventionally as the medicament of different ailments. In our previous studies, we have identified potent herbals against multi-drug resistant *P. aeruginosa* on the basis of *in silico* herbal informatics based ethnopharmacological approach [12]. Further *in vitro* antimicrobial efficacy testing has suggested *Glycyrrhiza glabra* as one of the potent lead [13]. *G. glabra* is a traditional therapeutic herb grows in various parts of the world. The antimicrobial activity of *G. glabra* has been both investigated and utilized medicinally since ages. Also, the herbal have been reported with its antibacterial activity on skin, respiratory and urinary system [14]. *G. glabra* has also been used in traditional medicine in Unani and Ayurveda [15]. Bioactive constituents of *G. glabra* are reported to be biodynamic as they show antitussive, mucolytic, expectorant, antiulcer, antimutagenic, antimicrobial, immunostimulant as well as a flavoring effect [16]. The herbal was found to be lethal against *P. aeruginosa* as it causes membrane disruption and suppress the efflux activity and biofilm formation at *in vitro* level [17].

In order to develop novel therapeutic countermeasures for the lung infection caused by *P. aeruginosa,* an improved understanding of lethal dose of the pathogen to cause infection on suitable model are needed and further, effect of herbal on the infection was evaluated. Thus, the aim of the present study is to determine the lethal dose of infection *i.e. P. aeruginosa,* in mice model and the antimicrobial activity of *G. glabra* at *in vivo* level in mice lungs infection model.

2. Materials and methods

2.1. Isolation and identification of test organism

The *P. aeruginosa* was isolated from a clinical sample of biological fluid in Department of Microbiology, National Centre for Disease Control (NCDC), New Delhi, India. The clinical isolate was processed to identify on the basis of morphological, cultural, and biochemical characteristics, as described in previous studies [13]. The isolated sample was immediately enriched in sterile nutrient broth (at 37 °C for 48 h). The antibiotic susceptibility test was carried out in our previous studies, to determine the drug susceptibility pattern [13].

2.2. Inoculum preparation

A loopful of culture was taken from lawn of *P. aeruginosa* grown on Mueller Hinton Agar plate, inoculated into fresh sterile nutrient broth and incubated at 37 °C for 17–18 h. The turbidity was adjusted to 0.5 McFarland standards by standard procedure. This suspension was used as inoculum.

2.3. Plant material

The shade-dried plant materials (1.0 kg) were procured from a reputed supplier of medicinal herbs in New Delhi, India. Fresh and dried stems of *G. glabra* Linn. (mulethi) was collected from Punjab, Northern India. The plant material was authenticated by botanist and pharmacognosist, with a specimen deposited in the repository at INMAS (Institute of Nuclear Medicine and Allied Sciences).

2.4. Preparation of herbal extract

The aqua-alcoholic stem extract of *G. glabra* was prepared by using hot continuous percolation method. The dried plant material was pulverized the powdered plant material was transferred to a Soxhlet apparatus and extracted with aqua-alcoholic solvent system. The pooled filtrate was filtered through Whatman Paper No.1 and concentrated by the solvent evaporation in a rotary evaporator (EYELA SB-1200, Hyderabad, India) at ~ 65 ± 2 °C, 160 to 180 rpm and reduced pressure of 40 Torr. The residual solvent was removed using vacuum oven and the yield was estimated. The crude extract was spray dried to amorphous powder (light brown colored). Standardization and quality control of the herbal extract was carried out as per World Health Organization norms [17]. The percentage yield, moisture, quantitative anlaysis of phytoconstituents was performed in our previous study [17].

2.5. Liquid chromatography mass spectrometry analysis

The crude extract was dissolved in methanol and analyzed on Acquity TQD QBB 1152 using Thermo Scientific™ Accucore™ RP-MS analytical column (100 × 2.1 mm, 2.6 μm) with auto-injector (SIL-10 AD VP) and diode array detector (SPD M–10 A). Elution was carried out with the mobile phase containing 0.1% formic acid in water at a flow rate of 0.40 mL/min. LC–MS analysis was performed using a LCQ MS mass spectrometer (Thermo-Finnigan, India). The cone gas and desolvation gas flow rate were set at 30 and 650 L/h. The desolvation temperature was 350 °C. The positive and negative ions were scanned in the range of 150–1500 Da (Da). The comparative analysis of the obtained peaks with respect to mass spectral data of reference compound(s) was used to identify known compounds in the crude extract.

2.6. Animals and conditions

Six to seven-week-old, male, Swiss albino mice (weight, 20 to 25 g) were reared in the animal house of the INMAS, Delhi (India) were used for this experiment. Animals were fed normal mouse chow and drinking water and were housed in standard micro-isolator polycarbonate cages in a room maintained under standard laboratory conditions, *i.e.* controlled temperature (20 to 23 °C) and a 12 h light/dark cycle. Institutional Animals Ethics Committee (IAEC) of INMAS (Reg. No. 8/GO/a/99/CPCSEA) have granted the permission for the use of animals, all the experiments were performed in accordance with the guidelines for the care and use of laboratory animals, laid down by the Indian National Science Academy (INSA) [18]. The treated animals were kept at isolated facilities of NCDC at BSL-II level.

2.7. Determination of lethal dose (LD_{50}) and pathogenesis study

LD_{50} (lethal dose at 50%) of *P. aeruginosa* was determined in Swiss mice challenged *via* intratracheal route. To conclude LD_{50},