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Exploration of the therapeutic potential effect of *Sepia officinalis* in animal model of sepsis induced by cecal ligation and puncture

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ABSTRACT

Objective: The present investigation explored the therapeutic potential effect of *Sepia officinalis* body tissue (SOBT) and *Sepia officinalis* polysaccharide (SOP) extracts, in animal model of sepsis [induced by cecal ligation and puncture (CLP)].
Materials and methods: Experimental animals were divided into 4 groups, Group 1: Sham control rats. Group 2: Septic rats. Group 3: Septic rats treated with methanolic extract of *Sepia officinalis* body tissue (SOBT) (500 mg/kg body weight) for 2 days. Group 4: Septic rats treated with *Sepia officinalis* polysaccharide (SOP) extract (200 mg/kg body weight) for 2 days.
Results: The antioxidant activity of SOBT and SOP was proven by DPPH test. CLP-induced liver and kidney toxicities showed by an increase in the ALAT, ASAT, γGT, ALP, creatinine, BUN and uric acid concentrations in serum. Moreover, CLP-induced oxidative stress in liver and kidney evidenced by the increase of MDA levels, decrease in GSH concentrations and decrease in the enzymatic antioxidants (SOD, CAT, GST). In addition, CLP caused decrease in CYP1A2 content in liver.
Conclusions: Our findings demonstrate the therapeutic efficacy of SOBT and SOP in liver and kidney disorders. Therefore this study suggests that SOBT and SOP could be a potential therapeutic agents for sepsis treatment.

Introduction

Sepsis is a severe clinical syndrome characterized as a systemic inflammatory response to infection [1]. Sepsis progresses from systemic inflammatory response syndrome to multiple organ dysfunction which ultimately leads to death [2]. Surgery may be one of the most common causes of sepsis [3]. In addition, false surgical procedure can cause contamination which leads to sepsis accompanied by bacterial infection [4]. The presence of microbial pathogens in the bloodstream triggers systemic inflammation and can lead to sepsis, which often overcomes the most powerful antibiotic therapies and causes multiorgan system failure, septic shock and death [5]. Sepsis research relies heavily on animal models [6]. Although it is difficult to create a supportive treatment environment similar to that applied in intensive care units (ICU) in animal models, experimental models are of great importance in understanding the physiopathology of septic shock and finding new therapeutic approaches prior to clinical trials [7]. The cecal ligation and puncture (CLP) sepsis model is highly stable, repetitive, and applicable, thus it is currently regarded as the "golden standard" for sepsis-related studies [8]. This model, by virtue of cecal ligation and perforation, leads to the pollution of the abdominal cavity by bacteria-carrying intestinal contents, gives rise to generalized peritonitis, and induces a wide range of systemic inflammatory responses [9]. The CLP model used in the present study creates a model similar to the clinical features of septic shock and resembles human sepsis, as the CLP model displays hypodynamic and hypometabolic phases following the hypermetabolic phase [10]. Over the last 10–15 yr, there has been a wealth of studies which describe oxidative stress (OXS) in patients with sepsis, with evidence of reactive oxygen species (ROS) production, associated damage, and antioxidant depletion [11]. Mitochondrial oxidative stress has a role in sepsis-induced organ dysfunction [12]. Despite recent advances in antibiotic therapy and intensive care, sepsis remains a widespread problem in critically ill patients [13]. As a result of the continuous evolution of microbial

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pathogens towards antibiotic-resistance, there have been demands for the development of new and effective antimicrobial compounds [14]. Marine invertebrates are constantly exposed to high concentrations of bacteria, fungi and viruses, many of which may be pathogenic and their survival depends on efficient antimicrobial mechanisms to protect themselves against microbial infections [15].

Data from our previous study revealed the antibactrial activities of the *Sepia officinalis* body tissue (SOBT) and *Sepia officinalis* polysaccharide (SOP) extracts against the most common types of bacteria in CLP-septic rats [16]. Continuing to study the therapeutic role of SOBT and SOP in CLP sepsis model in rats, the present study carried out to provide opportunities to elucidate the different mechanisms that may be involved in the *in vivo* antiseptic effects of SOP and SOBT.

Materials and methods

Sample collection

Fresh cuttlefish (*Sepia officinalis*) were purchased directly from a fishmonger. The animals were transported to the laboratory in an ice box containing ice cubes and a few pinches of table salt. The animals were immediately washed under running tap water. Then, dissected to separate the body tissue from its bone.

Preparation of Sepia officinalis *extracts*

Preparation of methanolic extract of Sepia officinalis *body tissue (SOBT)*

The body tissues of the *Sepia officinalis* were cut into small pieces, homogenized with a mixer. Ten gram of the homogenate was extracted with methanol (15 ml) at room temperature for 48 h. The methanolic extract was centrifuged for supernatant collection. The supernatant was concentrated under vacuum in a rotary evaporator at low temperature [17].

Preparation of Sepia officinalis *polysaccharide (SOP) extract from cuttlebone*

The extraction of polysaccharide was prepared according to the method described by Okutani and Morikawa [18]. The air dried cuttlebones were pulverized and washed with acetone. The powder (10 g) was extracted with hot 10 mM EDTA solution (170 ml) for 48 h and filtered using Whatman No. 1 filter paper. Then saturated barium hydroxide solution was added to the filtrate and standing over night. The obtained precipitate was collected on Whatman No. 1 filter paper and washed with water. The precipitate was dissolved in 10 mM EDTA solution and was dialyzed against deionized water using dialysis membrane cutoff (8 KD) for 48 h. The dialysate was freeze-dried and stored until use.

Evaluation of the free radical scavenging activity

The free radical scavenging activity of the SOBT, SOP extracts and ascorbic acid (standard antioxidant) was analyzed by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [19].

Experimental animals

The experimental animals used in this study were male Wistar rats (*Rattus norvegicus*) weighing 150–160 ± 5 g. The animals were obtained from the National Research Center (NRC, Dokki, Giza). Animals were grouped and housed in polyacrylic cages (five animals per cage) in the well-ventilated animal house of the Department of Zoology, Faculty of Science, Cairo University. Animals were given food and water ad libitum. Rats were maintained in a friendly environment with a 12 h/12 h light-dark cycle at room temperature (22 °C–25 °C). Rats were acclimatized to laboratory conditions for 7 days before commencement of the experiment. Experimental protocols and procedures were approved by the Institutional Animal Care and Use Committee (IACUC) (CUFS/S/PHY/17/14) of the Faculty of Science, Cairo University, Egypt.

Induction of sepsis by cecal ligation and puncture (CLP) surgery

CLP surgery was performed as described by Liu et al. [20] (2015). After animal anesthetization by sodium pentobarbital (30 mg/kg body weight), a midline laparotomy was performed in the surgical area after disinfection with betadine. The cecum was then identified, exteriorized through the abdominal incision, and ligated just to the ileocecal valve to avoid intestinal obstruction. After this, cecum was punctured with 18-gauge needle to make two pores and then pressing on cecum for releasing faeces (which are the source of the bacterial infection). The cecum was returened into the abdominal cavity and the abdominal incision was closed using a 4-0 silk thread. Sham-operated rats underwent the same procedures, but the cecum neither ligated nor punctured.

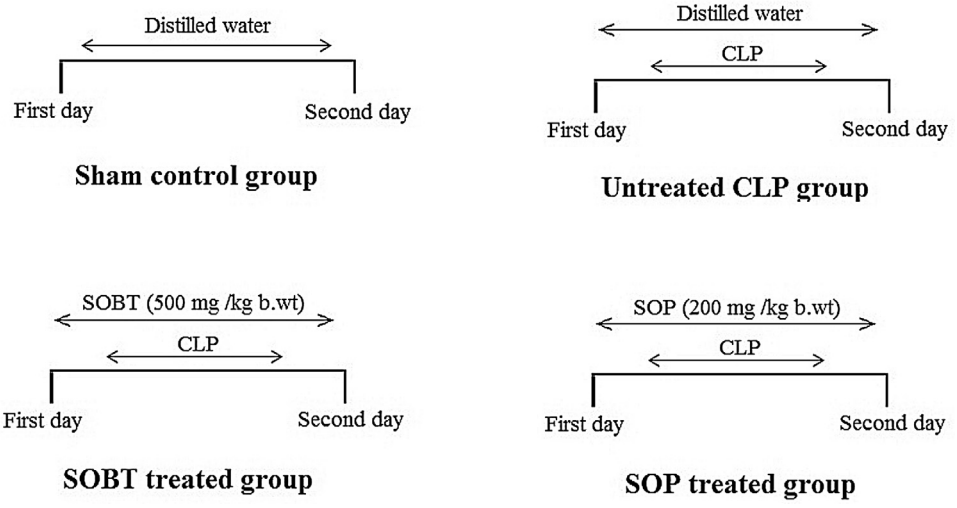

Fig. 1. Diagram of the experimental design of the treatment.

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