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Research Article

ANTI-INFECTIVE POTENTIAL OF *MORINDA CITRIFOLIA* FRUIT EXTRACT

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ABSTRACT

Morinda citrifolia Linn (Rubiaceae), a plant with reported immunostimulant properties was selected for studying its anti-infective potential. The hydroalcoholic extracts of *M. citrifolia* fruits were evaluated in rats for their role in attenuation of physiological changes occurring due to the onset of *E. coli* induced sepsis. Results obtained from *in-vitro* studies indicated that the extract did not exert any anti-bacterial effect. However, the *in-vivo* studies revealed the ability of the extract to prevent alterations in four important physiological parameters namely - body temperature, pulse rate, breathing rate and WBC count. Our study indicates that the hydroalcoholic extract of *Morinda citrifolia* fruits is as effective as Gentamicin, a standard antibiotic used commonly for treatment of sepsis. This probably accounts for the widespread traditional usage of this plant as a febrifuge and anti-infective. Further studies need to be carried out to establish a correlation between its immunostimulant activity and anti-infective potential.

Keywords: *Morinda citrifolia,* Anti-infective, Sepsis, Macrophages, Immunostimulant

INTRODUCTION

Some of the major diseases that plague mankind – infection, cancer, autoimmunity and allergy involve the immune system[1]. Of these, the worldwide impact of infectious diseases is substantial. They are the second leading cause of death worldwide. Annually about 15 million deaths occur due to infections while millions more occur due to the complications associated with infection[2]. Infectious diseases also lead to compromised health and disability[3,4]. Against this background, understanding of the molecular pathogenesis of infection and mechanisms involved in host defense against infections can provide an insight into the treatment modalities that can be used for prevention and treatment of diseases that affect millions worldwide. Several natural or synthetic agents such as levamisol, isoprenosine, Bacillus Calmette Guerin (BCG), glucans, cytokines such as interleukin-1 (IL-1) and IL-2 which have been used to stimulate host resistance against infections have met with limited success[5]. Antibiotics, once considered a boon are no longer in contention as therapeutic modalities for treating infections of bacterial origin. The major reason for this is their indiscriminate use leading to development of resistant bacterial strains[6]. This has necessitated the search for alternative modalities for the treatment of infections. A number of studies are currently underway to determine the usefulness of plant based immunostimulants as therapeutic agents/adjuvants in treating a variety of diseases whose etiology involves the immune system. These agents have been reported to target the immune system and strengthen the barriers to infection[7]. They act via activation of the cells and organs of the immune system and secretion of cytokines[8].

Morinda citrifolia Linn. (family: *Rubiaceae*) fruits have been traditionally used for over 2000 years by ancient Polynesians as well Indians in the treatment of a wide array of diseases and have been purported to have anti-bacterial, anti-viral, anti-fungal, anti-tumor, anthelmintic, analgesic, hypotensive, anti-inflammatory and immune-enhancing effects[9-13]. Phytochemical investigations carried out on the fruits have revealed the presence of carbohydrates, gums and mucilages, proteins, amino acids, fats and oils, anthraquinone glycosides, coumarin glycosides, flavonoids, alkaloids, tannins, phenolic compounds and citric acid[14]. Recent pharmacological investigations of the fractions obtained from *Morinda citrifolia* fruits have revealed an indirect anti-tumor activity via stimulation of immune cells [15-17]. Detailed *in-vitro* and *in-vivo* studies carried out by the authors revealed that *M. citrifolia* fruits stimulated important cells of the immune system namely the neutrophils, macrophages, T-cells and the B-cells[18,19]. A recent study carried out on Morinda juice has revealed the diuretic potential of the juice[20]. Based on accumulated evidence, it was considered worthwhile to explore the anti-infective potential of *Morinda citrifolia* fruits by evaluating their role in the treatment of *E.coli* induced sepsis in rats.

MATERIALS AND METHODS

Plant material and preparation of extract

Dried fruits of *Morinda citrifolia* were obtained from M/s Anju Phytochemicals Pvt. Limited, Bangalore, India and authenticated at Piramal Life Sciences Research Center (Mumbai, India). A voucher specimen (no. 4944) has been deposited in the herbarium of the institute. The dried fruits were pulverized in a hammer mill and the powder so obtained was used for the extraction procedure. Dried *Morinda citrifolia* fruit powder (50 g) was continuously extracted in a Soxhlet extractor (70-75°C) with 300 mL of 50% v/v ethanol till extraction was complete. The extract was filtered, cooled and evaporated to dryness under reduced pressure in a rotary evaporator (Rotavap; Equitron Roteva, Medica Instrument Mfg. Co.). The crude extract (MCE) was reconstituted in 0.1% sodium carboxy methyl cellulose solution to desired concentration and used in the animal study.

Animals

Adult Wistar rats of either sex, weighing 180–200 g were used. They were kept under standard environmental conditions and fed with commercial rat feed and water *ad libitum*. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of C.U. Shah College of Pharmacy (Mumbai, India).

Effect of MCE on E. coli induced sepsis

The effect of MCE on *E. coli* induced sepsis was carried out in two parts. In the first part, the *in- vitro* anti-bacterial activity of MCE and standard drug Gentamicin was tested against *E. coli* (ATCC 25922) grown on nutrient agar. In the second part, the effect of *E. coli* induced infection on clinical parameters in normal control rats, treatment rats as well as rats treated with Gentamicin was evaluated.

In-vitro anti-bacterial effect of MC-04 on E.coli

About 25 mL of sterile molten agar media was mixed with 0.1 mL of the test culture (count adjusted to 1 X 10^3 org/mL using the McFarland scale) and poured into each sterile petri plate[20]. The plates were allowed to set at room temperature for one hour. Two holes were made in the medium with sterile cork bores. The holes were filled with 0.1 mL of MCE (100 mg/mL) and Gentamicin injection (40 mg/mL) respectively. The plates were then incubated at 37°C for 24 h.

Effect of treatment with MCE on rat physiological parameters in the presence of E. coli induced sepsis

The rats were divided into three groups (6 per group). *E. coli* (ATCC 25922) was cultured in nutrient broth. The broth was centrifuged and the *E. coli* pellet was transferred to sterile saline. Group 1 animals received 0.1% sodium carboxy methyl cellulose solution for 15 days. Group 2 animals were dosed with MCE (200 mg/kg body weight) for 15 days. Group 3 animals received the standard drug Gentamicin intramuscularly (200 mg/rat) in 2 shots, one 12 h prior to and one just before *E.coli* challenge[21-25]. On day 15, 3 h after the last dose, all the rats were injected intraperitoneally with *E. coli* suspension in sterile saline (equivalent to 1 X 10^6 cfu/kg body weight, count adjusted using the McFarland scale)[26-28].

The vital body signs namely rectal temperature, pulse rate, breathing rate and WBC count were recorded in all three groups just before and 6 h after challenge with *E. coli.* The breathing rate was estimated by visual inspection. The rectal temperature and pulse rate were estimated by using the Biopac system MP35 (Biopac Systems Inc, U.S.A.). WBC count was recorded on Sysmex F-820 (Transasia, India).

Statistics

Values are expressed as mean values ± SEM. The statistical significance of differences between the mean values was analyzed by One-way Analysis of Variance (ANOVA) followed by Dunnett's test. The value of P lass than 0.05 was considered to be significant.

RESULTS

In-vitro anti-bacterial effect of MC-04 on E. coli

The anti-bacterial activity of MCE was tested *in-vitro* against *E. coli* (ATCC 25922) inoculated on nutrient agar. Absence of zone of inhibition indicated that MCE did not elicit any anti-bacterial activity against *E. coli in-vitro.* Under the same conditions, Gentamicin (40 mg/mL) was found to elicit zone of inhibition on the agar media due to its bactericidal action (Fig.1.).

Effect of treatment with MCE on rat physiological parameters in the presence of E. coli induced sepsis

When challenged with *E. coli* the control group rats demonstrated a significant increase ($P < 0.05$) in two parameters namely pulse rate and breath rate. In addition, the rectal temperature was observed to be greater than 38°C. These observations are indicative of the onset of *E. coli* induced sepsis. The treatment group (MCE – 200 mg/kg p.o.) did not elicit any change in rectal temperature, pulse rate and breath rate. However, it revealed a significant ($P < 0.05$) increase in the WBC count of the rats. Animals treated with the standard drug, Gentamicin did not reveal increase in any of parameters evaluated (Table 1).

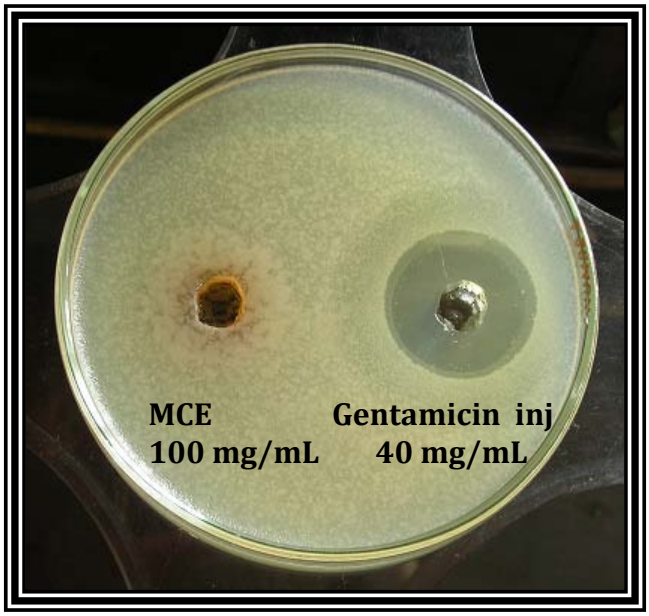

Fig. 1: Anti-bacterial effect of MC-04 and Gentamicin injection on *E. coli* incubated in agar media

Table 1: Effect of MCE on physiological parameters in rats suffering from E.coli induced sepsis

Group		Rectal Temperature	Pulse rate	Breath rate	WBC count
Normal control	Before	36.75 ± 0.24	295.83 ± 5.56	69.67± 2.85	5128 ± 192.55
	After	38.04* ± 0.15	389* ± 16.88	109.67* ± 8.28	5573 ± 159.01
Treatment group (MCE –	Before	36.73 ± 0.26	298 ± 5.09	65.67 ± 3.03	5500 ± 120.89
200 mg/kg))	After	37.05 ± 0.26	336.33 ± 5.29	70.83 ± 4.96	8301* ± 201.57
Standard (Gentamicin- 200	Before	36.57 ± 0.095	293 ± 4.97	74 ± 4.1	5103 ± 152.68
mg/rat)	After	36.62 ± 0.27	310.67 ± 8.2	65.67 ± 3.70	5268 ± 129.39

Values are expressed in mean ± S.E.M, n = 6, * $P < 0.05$, compared with normal control group (one way ANOVA followed by Dunnetts t test)

DISCUSSION

Sepsis is defined as the systemic response to infection and is manifested in rats by two or more of the following symptoms - temperature greater than 38°C or less than 36°C, increased pulse rate, increased breathing rate and WBC count greater than 12,000 cells/μL or less than 4,000 cells/μL[28]. As sepsis persists, there is a shift towards an anti-inflammatory immunosuppressive state.

A large number of CD4+ cells, B-cells and follicular dendritic cells undergo apoptosis leading to immunodeficiency. Enhancing the function of the innate and the adaptive immune system has been

found to reduce mortality in sepsis[21,29]. The beneficial effects of medicinal plants in countering *E. coli* induced sepsis has been amply demonstrated[21,22,30]. The protection offered by medicinal plants in *E. coli* induced sepsis has been attributed to the secretion of IL-1 and GM-CSF by activated macrophages leading to an increase in the number of neutrophils[31].

In the first study, absence of zone of inhibition indicated that the extract was devoid of anti-bacterial activity against *E. coli*. Therefore the probable mechanism of action *in-vivo* could be activation of macrophages to secrete cytokines which are responsible for recruiting neutrophils as well as enhancing their phagocytic activity[21]. This substantiates the assumption that the protection offered by MCE is due to its immunostimulant effect. The results of the second study indicated that control rats when exposed to *E. coli* showed adverse changes in physiological parameters which can be attributed to the onset of sepsis. However, in the treatment group, pretreatment with *Morinda citrifolia* prevented changes in rectal temperature, pulse rate and breath rate. The observed increase in the WBC count is indicative of the mechanism of activity of MCE and confirms the anti-infective potential of the plant. Pretreatment with *Morinda citrifolia* was found to be as effective as treatment with Gentamicin, a standard drug used commonly for treatment of sepsis. This is the first scientific study linking the immunostimulant properties of *Morinda citrifolia* to its therapeutic role as an anti-infective. Based on this study, *Morinda citrifolia* appears to be a promising candidate for alleviating a variety of diseases which arise due to improper functioning of the immune system.

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