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Protective effects of ethanolic peel and pulp extracts of *Citrus macroptera* fruit against isoproterenol-induced myocardial infarction in rats

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ABSTRACT

Increases in the incidence of cardiovascular disease (CVD) have aroused strong interest in identifying antioxidants from natural sources for use in preventive medicine. *Citrus macroptera* (*C. macroptera*), commonly known as “Satkara”, is an important herbal and medicinal plant reputed for its antioxidant, nutritious and therapeutic uses. The aim of the present study was to investigate the cardio-protective effects of ethanol extracts of *C. macroptera* peel and pulp against isoproterenol (ISO)-induced myocardial infarction (MI) in rats. Male albino Wistar rats (n = 36) were pre-treated with peel and pulp extracts (500 mg/kg) for 45 days. They received a challenge with ISO (85 mg/kg) on the 44th and 45th days. Our findings indicated that subcutaneous injection of ISO induced severe myocardial injuries associated with oxidative stress, as confirmed by elevated lipid peroxidation (LPO) and decreased cellular reduced glutathione (GSH) and anti-peroxidative enzymes, including glutathione peroxidase, glutathione reductase and glutathione-S-transferase, compared with levels observed in control animals. Pre-treatment with *C. macroptera* peel and pulp extracts prior to ISO administration however, significantly improved many of the investigated biochemical parameters, i.e., cardiac troponin I, cardiac marker enzymes, lipid profile and oxidative stress markers. The fruit peel extract showed stronger cardio-protective effects than the pulp extract. The biochemical findings were further confirmed by histopathological examinations. Overall, the increased endogenous antioxidant enzyme activity against heightened oxidative stress in the myocardium is strongly suggestive of the cardio-protective potential of *C. macroptera*.

1. Introduction

Despite improved clinical care, the availability of modern medicines and greater health awareness [1], the World Health Organization (WHO) has predicted that cardiovascular disease (primarily myocardial infarction) will be a major cause of death worldwide by the year 2020 [2,3]. Myocardial infarction (MI), a common presentation of ischemic heart disease (IHD), occurs when cardiac ischemia surpasses a clinical threshold, resulting in irreversible myocardial damage. IHD is an acute condition leading to necrosis of the myocardium as a result of an imbalance between myocardial metabolic demands and the coronary supply of oxygen

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and nutrients [2,4,5]. MI leads to free radical generation in the heart, which contributes to further toxic reactions and eventually cardiac cell death [6].

Reactive oxygen species (ROS) such as free radicals, oxygen ions, and peroxides are generated during aerobic metabolism as by-products and are tightly controlled by antioxidants [7]. However, excess production of ROS or depletion of antioxidants can lead to a state of oxidative stress that can inflict damage to lipids, proteins, and DNA [8]. Following MI, ROS production is usually increased, which can lead to further damage to the myocardium. The first line of cellular defense against oxidative injury in the heart as well as most tissues includes antioxidant enzymes [9]. Dietary antioxidants can prevent the deleterious effects of ROS by restoring the balance between production and clearance of ROS by mechanisms such as scavenging ROS or enhancing endogenous antioxidant enzyme activity [10].

Natural products have high global demands because of their purported superiority in terms of both safety and efficacy against oxidative stress-induced cardiovascular disease, including MI [11]. The human body has myriad endogenous defense mechanisms that feature cellular antioxidants, such as glutathione, bilirubin and a free radical scavenging enzyme system that includes superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase, all of which work together against oxidative stress. The levels of these enzymes can be reduced because of enhanced lipid peroxidation. Therefore, the dynamic balance between ROS and antioxidants is important [12].

Citrus fruits have been a natural boon to mankind for years. Earlier studies have reported that almost all species of citrus fruits have significant antioxidant properties and are effective against stress-induced ulcer, cancer and other chronic diseases [13,14]. *C. macroptera* (Var. anamnesis) is a citrus fruit from the Rutaceae family. In Bengali, the fruit is known as "Satkara", and in English, it is called "wild orange" (Fig. 1). This semi-wild fruit is widely distributed, particularly along the hill tracks of the Sylhet division of Bangladesh and in some regions of India. In Bangladesh, people usually use the rind of the fruit in chicken, meat dishes and pickle preparations for extra flavor [15,16]. Additionally, the fruit is widely used in the treatment of hypertension, to soothe stomach pain and in alimentary disorders in Assam, India [17,18]. In addition, *C. macroptera* is used as an herbal medicine for treating various fatal disorders in Northern India [19].

The phytochemical composition of *C. macroptera* was previously reported to contain water (90.40%), β-carotene (22.00 mg/100 g), thiamine (0.08 mg/100 g), riboflavin (0.01 mg/100 g) and (sodium 3.50, potassium 89.00, calcium 25.00, magnesium 10.00, iron 0.15, zinc 0.21, copper 0.07 and phosphorus 12.00 mg/100 g) [20]. Recently, the antioxidant properties of the fruit's peel and pulp were analyzed and reported to contain 620.91 and 291.06 mg of polyphenols (gallic acid), 508.33 and 145.02 mg of flavonoids (catechin), 585.99 and 526.08 mg of tannin (tannic acid), 56.26 and 120.83 mg of ascorbic acids per 100 g, respectively [16]. *C. macroptera* also confers strong protection against lipid peroxidation in rat liver and kidney tissues because of its strong antioxidant properties [15].

Isoproterenol (ISO) is a synthetic catecholamine and β-adrenergic agonist that causes severe stress to the myocardium, resulting in an infarct-like necrosis of the heart muscle if administered in high doses [2]. It has been reported that pathophysiological and morphological changes of ISO-induced cardiac dysfunctions in laboratory animals are comparable to those in humans suffering from MI [4]. Studies have shown that hypoxia is the major cause of ISO-induced cardiac damage because of myocardial hyperactivity, coronary hypotension and excessive generation of highly cytotoxic free radicals resulting from the auto-oxidation of catecholamines [21]. Following oxidation, catecholamines form quinoid compounds, which stimulate the production of superoxide anions and subsequently hydrogen peroxide. Hydrogen peroxide becomes a highly reactive hydroxyl radical in the presence of iron, causing oxidative damage to preserved lipids, proteins and DNA, ultimately affecting the infarcted myocardium [22].

Although Uddin *et al.* [23] proposed the cardio-protective potential of *C. macroptera* fruit, this potential not been experimentally confirmed using an appropriate animal- or cell-based model. The present study was undertaken to investigate the cardio-protective effects of both fruit peel and pulp extracts of *C. macroptera* against experimentally induced MI in rats and to compare the effects of the two extracts. The possible mechanisms that may underlie the therapeutic efficacies were also investigated by studying the alterations in cardiac marker enzymes, troponin I, lipid metabolism, lipid peroxide reactions and the antioxidant defense system. Additionally, histopathological examinations of heart tissues were conducted to confirm the biochemical observations.

2. Materials and methods

2.1. Experimental animals

The experiment was carried out using adult male albino Wistar rats (n = 36) aged between 5 and 7 weeks and weighing between 110 and 140 g. The animals were bred and maintained in an institutional animal house facility under standard conditions of ventilation, temperature (28 ± 2 °C), humidity (40–70%) and light/dark conditions (12/12 h). The rats were individually housed in polypropylene cages with soft wood-chip bedding and access to standard food and water *ad libitum*. The experiments were

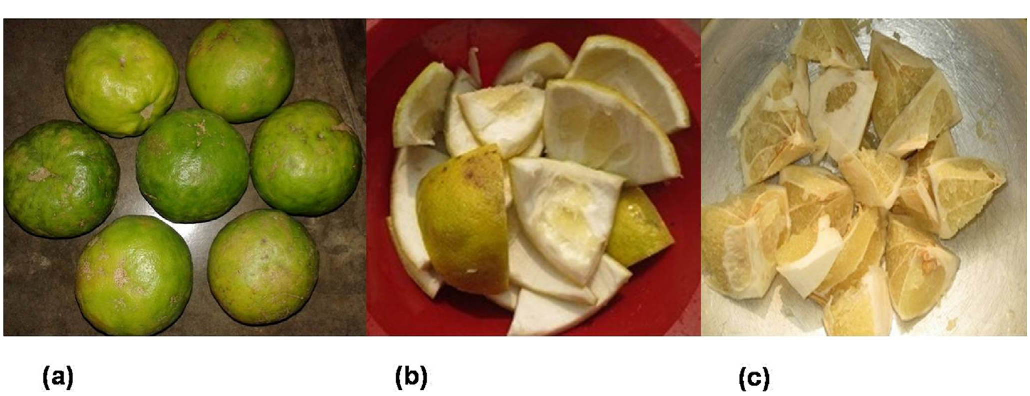

Fig. 1. *C. macroptera* (a) whole fruit, (b) peel, and (c) pulp.