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Hypoglycemic and antioxidant activities of *Caesalpinia ferrea* Martius leaf extract in streptozotocin-induced diabetic rats

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ABSTRACT

Objective: To evaluate the antidiabetic and antioxidant effects of aqueous ethanolic extract of *Caesalpinia ferrea* (*C. ferrea*) leaf in normal and streptozotocin (STZ) induced diabetic rats.

Methods: Male Sprague-Dawley rats divided into 6 groups of 6 rats each were assigned into diabetic and non-diabetic groups. Diabetes was induced in rats by single intraperitoneal administration of STZ (65 mg/kg body weight). *C. ferrea* extract at the doses of 250 and 500 mg/kg body weight was orally administered to both diabetic and non-diabetic animals for a period of 30 days. After completion of experimental duration serum, liver and pancreas were used for evaluating biochemical and histopathological changes.

Results: Oral administration of *C. ferrea* leaf extract significantly reduced elevated serum glucose, α-amylase, liver function levels and significantly increased serum insulin, total protein and body weight as well as improved lipid profile due to diabetes. Furthermore, the treatment resulted in a marked increase in glutathione peroxidase, superoxide dismutase, catalase and reduced glutathione, and diminished levels of lipid peroxidation in liver and pancreas of diabetic rats. Histopathological studies demonstrated the reduction in the pancreas and liver damage and confirmed the biochemical findings.

Conclusions: From the present study, it can be concluded that the *C. ferrea* leaf extract effectively improved hyperglycaemia while inhibiting the progression of oxidative stress in STZ-induced diabetic rats. Hence, it can be used in the management of diabetes mellitus.

1. Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by abnormal metabolism of carbohydrates, proteins, and fats resulting from inadequate pancreatic insulin secretion with or without concurrent impairment of insulin action [1]. According to the American diabetes association, the chronic hyperglycemia is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels [2]. DM is considered the most prevalent disease in the world affecting 25% of the population. It afflicts 150 million people and is predicted to rise to 300 million by 2025 [3]. It is likely to be the fifth leading cause of death worldwide [4].

Previous studies have demonstrated that DM exhibits enhanced oxidative stress and highly reactive oxygen species (ROS) production in pancreatic islets due to persistent and chronic hyperglycemia, thereby depletes the activity of the

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antioxidative defense system, and thus promotes free radical generation [5]. Oxygen free radicals have been suggested to be a contributory factor in complications of DM [6]. It seems to be an oxidative stress-related disorder and the antioxidants may be useful in preventing it [7]. Therefore supplementation of therapeutics with antioxidants may have a chemoprotective role in diabetes [8].

Many plant extracts and their products have been shown to have significant antioxidant effect in treating many kinds of diseases [9]. The use of medicinal plants for the treatment of human diseases has increased considerably worldwide [10]. Ethnopharmacological evidence has shown that the use of plants is also helpful in prophylaxis or treatment of diabetes. Given that, herbal medicine possesses significant efficacy, low incidence of side effects, low cost and relative safety [11], while synthetic anti-diabetic agents can produce serious side effects, as hypoglycemic coma and disturbances of the liver and kidneys [12].

The little studied genus *Caesalpinia* contains more than 500 species of worldwide distribution [13]. Previous studies of species of this genus reported remarkable biological activities such as antimicrobial (*Caesalpinia bonducella*) [14], antidiabetic (*C. bonducella*) [15], antimalarial (*Caesalpinia pluviosa*) [16], and anti-inflammatory (*Caesalpinia sappan*) [17]. To date, less than 30 species of this genus have been studied for their phytoconstituents. The metabolites described include predominantly flavonoid derivatives, steroids, triterpenoids, and cassane diterpenes [18].

Caesalpinia ferrea (*C. ferrea*) Martius (Leguminosae), popularly known as “pau-ferro” or “jucá”, is a large tree belonging to the Fabaceae family. It is found mainly in the north and northeast of Brazil. In folk medicine, the tea of the stem bark of *C. ferrea* has been used for the treatment of diabetes. In view of its ethnomedicinal importance, the Brazilian Ministry of Health has included this species on the national list of medicinal plants important to the health system [19].

The pharmacological properties of *C. ferrea* fruits or stem barks include antiulcerogenic [20], anti-inflammatory [21], analgesic [22], antibacterial [23], antihypertensive [24], antidiabetic [19], and cancer chemopreventive [25] activities. Recently a unique chalcone trimer (pauferrol A) and two chalcone dimers (pauferrol B and pauferrol C), were isolated from the stems of *C. ferrea*. These chalcones exhibited potent inhibitory activities against topoisomerase II [26,27]. The leaves contain three formerly unknown di-O-glycosyl-C-glucosyl flavones which were isolated, purified and identified namely: Isovitexin 2″-O-β-[xylopyranosyl-(1‴→2″)-O-β-xylopyranosyl]; Vitexin 2″-O-β-[xylopyranosyl-(1‴→2″)-O-β-xylopyranosyl]; Orientin 2″-O-β-[xylopyranosyl-(1‴→2″)-O-β- xylopyranosyl [28]. However, there is no experimental evidence proving biological activities of *C. ferrea* leaf up till now. Therefore, the present study was aimed to evaluate the possible hypoglycemic properties of *C. ferrea* leaf in streptozotocin (STZ) induced diabetic rats.

2. Materials and methods

2.1. Chemicals

STZ, reduced glutathione, 5,5’-dithiobis (2-nitrobenzoic acid) (DTNB), 1-chloro 2,4-dinitrobenzene (CDNB) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Metaphosphoric acid (MPA) and nitroblue tetrazolium were purchased from Fluka (Switzerland), and pyrogallol from Merck (Germany). All chemicals were of analytical grade.

2.2. Plant material

Leaves of *C. ferrea* were collected from a tree cultivated in the Zoological Garden, Cairo, Egypt, in May 2012. The plant was identified by Prof. Salwa Quashti, National Research Centre (NRC), Cairo, Egypt. A voucher specimen (C253) has been deposited at the herbarium of the NRC.

2.3. Plant extraction and isolation

Leaves (2.5 kg), dried in the shadow, were crushed and exhaustively extracted with 70% (v/v) aqueous EtOH under reflux (three times, each extraction for 8 h with 2 L). The obtained eluent was dried under vacuum at 55–60 °C to give 200 g aqueous ethanolic extract that was used in the present study.

2.4. Phytochemical screening

This aqueous ethanol extract of *C. ferrea* was screened for the presence of various phytoconstituents such as steroids, alkaloids, glycosides, flavonoids, carbohydrates, amino acids, saponins, terpenoids, tannins, and phenolic compounds as described by Dawang & Datup and Mythili & Ravindhran [29,30].

2.5. Determination of the scavenging of DPPH radical

The quantitative DPPH assay was carried out according to the method of Kedare and Singh [31]. The extract was dissolved in a concentration of 1 mg/mL in ethanol. From this stock solution, concentrations of regular dilution were prepared. Then 500 μL of sample, 375 μL ethanol and 125 μL of 1 mmol/L prepared DPPH solution were placed together. The test was performed in triplicate. All samples were incubated in sequence for 30 min in the dark at room temperature and their absorbance was measured at a wavelength of 517 nm on UV–vis spectrophotometer (Shimadzu, Duisburg, Germany). Ascorbic acid was used as positive control. Percentage of radical scavenging activity (RSA) was calculated as follows:

$$RSA\% = [(Abs\ of\ control - Abs\ of\ sample)/Abs\ of\ blank] \times 100$$

2.6. Acute toxicity study

The mean lethal dose (LD_{50}) of the aqueous ethanolic extract of *C. ferrea* leaf was determined in rats (weighing 180–200 g) using the method described by Chinedu *et al*. [32].

2.7. Experimental animals

Male Sprague-Dawley rats weighting 180–200 g were purchased from the Animal House of National Research Centre, Egypt. Animals were acclimated for a period of 7 days in our

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