Experimental and Toxicologic Pathology 63 (2011) 535–540

Contents lists available at ScienceDirect

Experimental and Toxicologic Pathology

journal homepage: www.elsevier.de/etp

Cardioprotective effects of aqueous extract of *Oxalis corniculata* in experimental myocardial infarction

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ARTICLE INFO

Article history:
Received 7 March 2010
Received in revised form 1 April 2010
Accepted 7 April 2010

Keywords:
Isoproterenol
Creatine phosphokinase
Glucose-6-phosphate dehydrogenase
Oxidative stress
DPPH
Histopathology

ABSTRACT

The present study evaluated the protective potential of aqueous extract of *Oxalis corniculata* (OCE) against isoproterenol (ISO) induced myocardial infarction in rats. Myocardial infarction in rats was induced by isoproterenol (200 mg/kg) at an interval of 24 h for 2 days. OCE was given to rats as pretreatment for 30 days orally using an intragastric tube. Isoproterenol caused a significant increase in the activity of cardiac injury marker enzymes like creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) and increased the concentration of serum lipids. OCE pretreatment significantly reduced the concentration of CPK, LDH, serum total cholesterol, LDL cholesterol and triglycerides. OCE also reduced the activity of lipogenic enzyme, glucose-6-phosphate dehydrogenase in ISO administered rats. Oxidative stress produced by isoproterenol was significantly lowered by the administration of OCE which was evident from increased activities of antioxidant enzymes (catalase and superoxide dismutase) and reduced concentration of lipid peroxidation products (TBARS and conjugated dienes). Concentration of vitamin C, protein sulfhydryl groups and reduced glutathione (GSH) was also high in OCE pretreated rats. Histopathology of heart of ISO administered rat pretreated with OCE showed normal myocardium with very little evidence of inflammatory infiltration. Results of our *in vitro* findings also confirmed that OCE exhibits significant antioxidant and radical scavenging activity against DPPH, superoxide and nitric oxide radicals. These findings provided evidence that *O. corniculata* was found to be protecting the myocardium against ischemic insult and the protective effect could attribute to its antioxidative and antihyperlipidemic activities.

1. Introduction

Myocardial infarction is the acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand (Boudina et al., 2002). It is the most dreaded sequel among ischemic heart diseases invariably followed by several biochemical alterations such as hyperlipidemia, lipid peroxidation, free radical damage, thrombosis etc. leading to qualitative and quantitative alteration of myocardium. The model of isoproterenol (ISO) induced myocardial ischemia is considered as one of the most widely used experimental model to study the beneficial effects of many drugs and cardiac function (Grimm et al., 1998). Isoproterenol (ISO) is a synthetic β-adrenergic agonist that causes severe stress in the myocardium, resulting in infarct like necrosis of heart muscle. It is also well known to generate free radicals and stimulate lipid peroxidation, which may be a causative factor for irreversible damage to the myocardial membrane (Chatelain et al., 1987). Thus, increased production of reactive oxygen species (ROS) may be a unifying mechanism in ischemic injury progression, and administration of antioxidants may be protective against ISO induced cardiac damage.

Recently, there has been a growing interest in establishing the therapeutic potentials of medicinal plants against various diseases. The use of plant extracts for medicinal purposes seems to be more natural, less expensive and without side effects. The biological activities of these plants are due to the presence of various biologically active compounds like vitamins, flavonoids and polyphenols. Hyperlipidemia and oxidative stress has been implicated in the pathogenesis of myocardial infarction also. Therefore therapeutic interventions having hypolipidemic and antioxidant activity may exert beneficial effects against various cardiovascular diseases including ischemic heart diseases (Bandyopadhyay et al., 2004; Young and Woodside, 2001).

Oxalis corniculata (Family: *Oxalidaceae*) is a widely used medicinal plant with lot of therapeutic properties. The leaves of this plant are quite edible with a tangy taste of lemon. The entire plant is rich in vitamin C and β-carotene (Lee Allen Peterson, 1977). Since the

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doi:10.1016/j.etp.2010.04.004

plant is rich in vitamin C, it is used as an antiscorbutic in the treatment of scurvy. The plant is used as multi therapeutic agents as antihelmintic, anti-inflammatory, astringent, depurative, diuretic, stomachic and styptic and hepatoprotective (Chetri et al., 2008). It is also used in the treatment of influenza, fever, urinary tract infections, enteritis, diarrhea and traumatic injuries.

The medicinal properties of *O. corniculata* is not exploited properly. The present study highlights the *in vitro* free radical scavenging activities and the possible ability of aqueous extract of *O. corniculata* against ISO induced alterations in myocardial enzymes, antioxidant status, serum lipid profile and cardiac pathology in rats.

2. Materials and methods

2.1. Chemicals

Isoproterenol, DPPH, Nitro blue tetrazolium (NBT), phenazine methosulfate (PMS), Griess reagent, ascorbic acid and butylated hydroxyl toluene (BHT) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used were of standard analytical grade.

2.2. Collection and preparation of plant extract

O. corniculata plant was collected from the local areas of Kottayam district, Kerala, India and authenticated by taxonomist from Tropical Botanical Garden Research Institute (TBGRI), Palode, Thiruvananthapuram, India. One voucher specimen was stored in our herbarium for future reference. After collection, plant was washed thoroughly with tap water. The leaves were separated manually, shade dried and ground into fine powder. The leaf powder was extracted with water (5:1, v/w) at room temperature ($30 \pm 1\,^{\circ}C$) under stirring for 8 h and the extraction process was repeated till the solvent became colorless. Extract was then filtered through Whatman No. 1 filter paper followed by lyophilization (Hetosic, Model CD 2.5) to obtain the dry extract which was stored at $4\,^{\circ}C$ until use.

2.3. In vitro antioxidant activity of aqueous extract of O. corniculata

Total phenolic content (TPC) of OCE was extimated using Folin–Ciocalteau reagent (Singleton and Rossi, 1965). The antioxidant activity of OCE was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH method (Shimada et al., 1992). Butylated hydroxyl toluene (BHT) was used as positive control. Superoxide radical scavenging activity was done according to the method of Liu et al. (1997). Superoxide anions were generated in a non-enzymatic phenazine methosulfate–nicotinamide adenine dinucleotide (PMS–NADH) system through the reaction of PMS, NADH and oxygen. Ascorbic acid was used as positive control. Nitric oxide radical scavenging activity of OCE extract was done according to the method of Sreejayan and Rao (1997). Curcumin was used as positive control.

2.4. Animals and diets

Male albino rats (Sprague–Dawley) weighing about 160–180 g were used for the experiment. The animals were housed in polypropylene cages and given standard rat chow (Sai Feeds, Bangalore, India) and tap water *ad libitum*. Temperature of the animal laboratory was maintained at $25 \pm 3\,^{\circ}C$ with alternate 12 h periods of light and dark. Food intake and body weight were recorded daily and weekly respectively. The guidelines for care and use of laboratory animals published by the US National Institutes of Health (NIH Publication no.85-23, revised 1996) were followed throughout the experimental period. Rats were divided into 4 groups with 6 rats each that were treated as follows:

- Group I: Control rats.
- Group II: Rats pretreated with OCE alone.
- Group III: Control rats with ISO.
- Group IV: OCE pretreated rats with ISO.

Aqueous extract of *O. corniculata* (250 mg/kg body weight) was administered orally to the rats of group II and IV for 30 days. Control rats received the same volume of distilled water. The rats of group III and IV received subcutaneous injection of isoproterenol at a dose of 200 mg/kg body weight, twice at an interval of 24 h (Anurag and Rajamohan, 2003). At the end of the experimental period (12 h after the second dose of isoproterenol injection) rats were sacrificed by under deep ether anesthesia. Blood samples and heart was collected in ice-cold containers for the estimation of various biochemical parameters.

2.5. Biochemical analysis

Activity of creatine phosphokinase (CPK) and lactate dehydrogenase was done according to the method of King (1965). Serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvic transaminase (SGPT) were measured by the method of Reitman and Frankel (1957). Total cholesterol (Abell et al., 1952), triglycerides (Van Handel and Zilver Smith, 1957), HDL and LDL (Warnic and Albers, 1978) were analyzed in all groups. Estimation of glucose-6-phosphate dehydrogenase (G-6PDH) was done using the method of Kornberg and Horecker (1955). Thiobarbituric acid reactive substances (TBARS) (Ohkawa et al., 1979), conjugated dienes (CD) (Rao and Recknagel, 1968), catalase (Takahara et al., 1960), superoxide dismutase (SOD) (Kakkar et al., 1984), reduced glutathione (GSH) and protein sulfhydryl groups (Sedlak and Lindsay, 1968) was analyzed in the heart. Vitamin C in serum was estimated according to the method of Jagota and Dani (1982). Protein content was estimated by the method of Lowry et al. (1951).

2.6. Histopathological analysis of heart

The heart tissues were rapidly dissected out and fixed by immersion at room temperature in 10% formalin solution. For the histological examinations, paraffin embedded tissue sections of heart (5 μm) were stained with hematoxylin–eosin (H&E). The tissue samples were then examined and photographed under a light microscope for observation of structural abnormality.

2.7. Statistical analysis

The SPSS statistical program was employed for statistical evaluation. The results were evaluated using analysis of variance (ANOVA) utilizing the *F*-test. The results were presented as the mean value ± SD for the control and experimental rats. Differences among the means for the groups were assessed using the Duncan's Multiple Range Test to determine which mean values were significantly different at $p < 0.05$.

3. Results

Total phenolic content (TPC) of OCE was estimated to be 120 ± 1.34 mg gallic acid equivalents/g plant extract. Fig. 1 shows the DPPH radical scavenging activity of OCE. The estimated EC_{50} value of OCE was found to 8.18 μg/ml and that of standard compound BHT was 3.84 μg/ml and is inversely related to antioxidant capacity. The estimated EC_{50} values stands for the concentration