CARDIOPROTECTIVE EFFECT OF *CHEILOCOSTUS SPECIOSUS* (J. KÖENIG) C. SPECHT. AGAINST ISOPROTERENOL INDUCED MYOCARDIAL TOXICITY IN RATS

Abdulrahman A I Al-Yahya

College of Applied Medical Sciences, Shaqra University, Shaqra (Saudi Arabia)

ABSTRACT

This study evaluated the cardioprotective effect of *Cheilocostus speciosus* (J. Köenig) C. Specht. *(*Syn, *Costus speciosus* (Köenig ex Retz. Sm.) (Family Costaceae) during isoproterenol induced cardiac toxicity in rats. A methanolic extract of the rhizomes was prepared by maceration. Rats were administered the methanolic extract at two different doses of 200 mg/kg or 400 mg/kg orally and captopril (30 mg/kg) was used as standard drug. All the drugs were once daily for two weeks. At the end of treatment, two doses of isoproterenol (150 mg/kg, s.c) were administered to rats. Blood was withdrawn to estimate creatinine kinase-MB (CK-MB) activities. The heart tissue was subjected to histological examinations to determine the extent of damage. Administration of isoproterenol to control animals causes an elevation in serum CK-MB activity and the damage to myocardium was supported by histological examinations. C. *speciosus* at both doses prevented the increase in the biomarker activity as well as reduced the myocardial damage as observed in histological examination. However, it was relatively less effective compared to captopril in preventing the myocardial damage. *C. speciosus* showed dose-dependent cardioprotection and effectively prevented the myocardial dysfunction during isoproterenol induced cardiotoxicity in rats.

Key-words: Acute myocardial injury, *Cheilocostus speciosus* extract, captopril, isoproterenol, flavonoids, methanolic extract.

INTRODUCTION

Cheilocostus speciosus (J. Köenig) C. Specht. belongs to the family Costaceae (Zingiberaceae). The plant is reported to possess several pharmacological activities that includes anti-inflammatory, antiangiogenic, antioxidant, antibacterial and antifungal activities due to the presence of susquiterpines (Duraipandiyan *et al.,* 2012; Al-Attas *et al.,* 2015; Selim and Al Jaouni, 2016), antihyperglycemic activity through inhibitory effect on α-glucosidase and glycation (Perera *et al.,* 2016), anticholineesterase (Bhattacharya *et al.,* 1972), antipyretic, anti-inflammatory and analgesic activities (Srivastava *et al.,* 2013). It also shows antidiuretic, larvicidal, antistress, estrogenic, astringent, aphrodisiac, purgative, anthelminthic, expectorant activities (Pawar and Pawar, 2012). It also has anti-fertility and anabolic properties also. The plant is so widely used that it is on the verge of extinction.

Of all these activities of *C. speciosus*, the most widely studied effect is its antioxidant effect. There are several reports that indicate that the rhizomes of this plant possess strong antioxidant effect that can attenuate the Fenton reaction-mediated oxidation of biological lipid substrates (Pai Kotebagilu *et al.,* 2015). It was rated as third plant with potent antioxidant effect among eighteen commonly used antioxidant plants (Lee *et al.,* 2015). It is also reported to reduce hydrogen peroxide induced oxidative damage on biological substrates (Pai Kotebagilu *et al.,* 2014) and also prevent development of cancer due to its antioxidant action (Baskar *et al.,* 2012). The antioxidant effect of this plant is directly related to its phenolic contents (Vijayalakshmi and Sarada, 2008; Nehete *et al.,* 2010).

It is well known from several studies that plants having phenolic compounds and exhibiting antioxidant effects protects heart from different types of damage such as ischemia reperfusion and isoproterenol induced cardiac injury (Panda *et al.,* 2016; Wang *et al.,* 2017; Cao *et al.,* 2017; Garjani *et al.,* 2017; Rasines-Perea *and* Tissedre, 2017). Furthermore, the rhizomes of *C. speciosus* known as *Ru-rta* are believed to regulate blood pressure and correct heart problems (Clifford, 2001). Hence, the present study was carried out to evaluate the effect of methanolic extract of rhizomes of *C. speciosus* on myocardial damage induced by isoproterenol in rats.

MATERIALS AND METHODS

Preparation of the extract

The rhizomes of *C. speciosus* were purchased from a local market in Riyadh (Saudi Arabia). It was authenticated by Prof A M Sadaby (College of Applied Medical Sciences, Shaqra University, Saudi Arabia) and a voucher specimen (CAMS/rh03/09-2016) has been preserved in the college for future reference. The rhizomes were

finely powdered and extracted using methanol in a closed glass jar for 72 h initially. The extraction of the solid residue (marc) was further done twice for 48 h each. The extract obtained was evaporated under reduced pressure. The final extract obtained after evaporation was stored in a refrigerator till use.

Preliminary Chemical Analysis

The preliminary chemical analysis revealed the presence of carbohydrates, saponins, flavovoids and amino acids.

Experimental Animals

Laboratory bred male Wistar rats (180–220 g) were housed in a controlled environment at a temperature of 25 ± 2 °C under 12:12 hr light dark cycle. The animals were maintained under standard conditions in an animal house and all the experimental procedures were approved by the university scientific committee.

Dose Selection

The methanolic extract of *C. speciosus* was administered at two doses of 200 mg/kg and 400 mg/kg orally. The doses were selected after doing preliminary experiments in our laboratory and from previous reports (Ali *et al.,* 2014). Captopril was administered at a dose of 30 mg/kg orally (Milanez *et al.,* 1997). The extract and captopril were suspended in distilled water using 2% acacia. The control animals received only vehicle, which as 2% acacia in water.

Isoproterenol Induced Myocardial Damage in Rats

Animals were divided into five groups of six animals each.

Group I had normal animals and they received only vehicle (2 ml/kg, *p.o*)

Group II animals were treated with vehicle (2 ml/kg, *p.o*) for two weeks and they received isoproterenol (ISO).

Groups III and IV received orally *C. speciosus* extract- 200 mg/kg and 400 mg/kg, respectively for two weeks and followed by administration of ISO.

Group V was treated with captopril at a dose of 30 mg/kg orally for two weeks followed by ISO.

All treatment was given daily for 2 weeks. At the end of the treatment period, ISO (150 mg /kg, s.c) was administered to all the animals (except group I) for two consecutive days (Asdaq *et al.,* 2008). Forty eight hours after the first dose of ISO, animals were anesthetized using ether and blood was withdrawn and the serum was used for the estimation of creatinine kinase-MB (CK-MB) using commercially available kits. Thereafter, all the animals were sacrificed, and the hearts were used for histological examinations using H&E stain. The myocardial damage was determined by giving scores depending on the intensity as follows (Karthikeyan *et al.,* 2007); no changes—score 00; mild—score 01 (focal myocytes damage or small multifocal degeneration with slight degree of inflammatory process); moderate—score 02 (extensive myofibrillar degeneration and/or diffuse inflammatory process); marked—score 03 (necrosis with diffuse inflammatory process).

Statistical Analysis

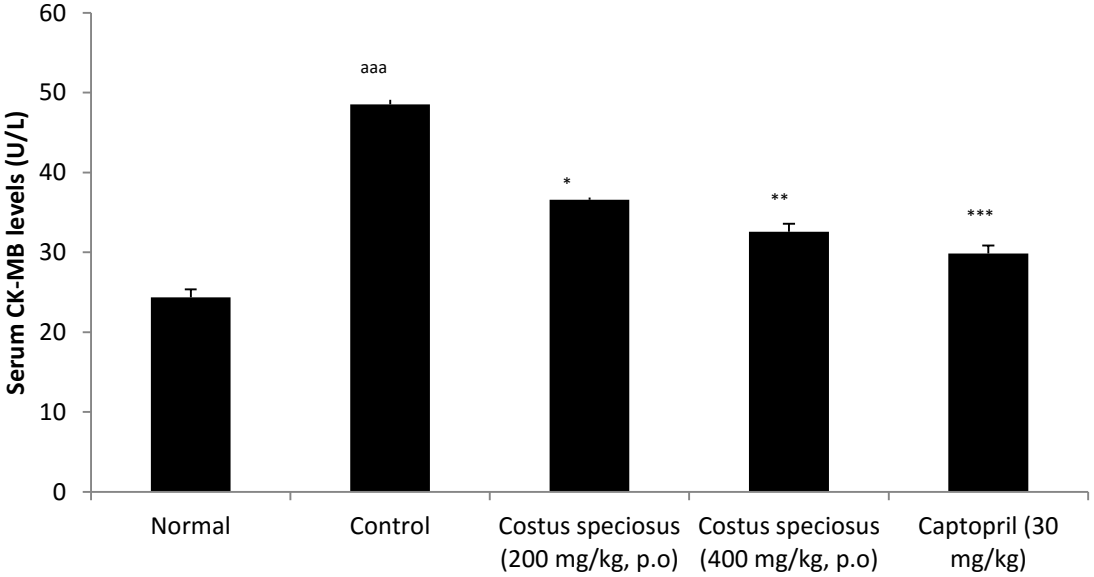
Results are given as mean ± SE. Statistical significance was determined through one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests. For comparison of histological scores, Kruskal-Wallis test with Dunn post test was used. $P < 0.05$ indicated statistically significant difference.

RESULTS

Effect on serum CK-MB levels: Administration of ISO to rats induced myocardial damage that was confirmed by an increase in serum CK-MB levels compared to normal animals. The methanolic extract of *C. speciosus* significantly reduced the serum CK-MB levels in a dose dependent manner when compared to ISO administered control group. As expected, captorpil (30 mg/kg) also reduced the serum CK-MB levels when compared to ISO control group ($p<0.001$) indicating cardioprotective effect (Fig **1**).

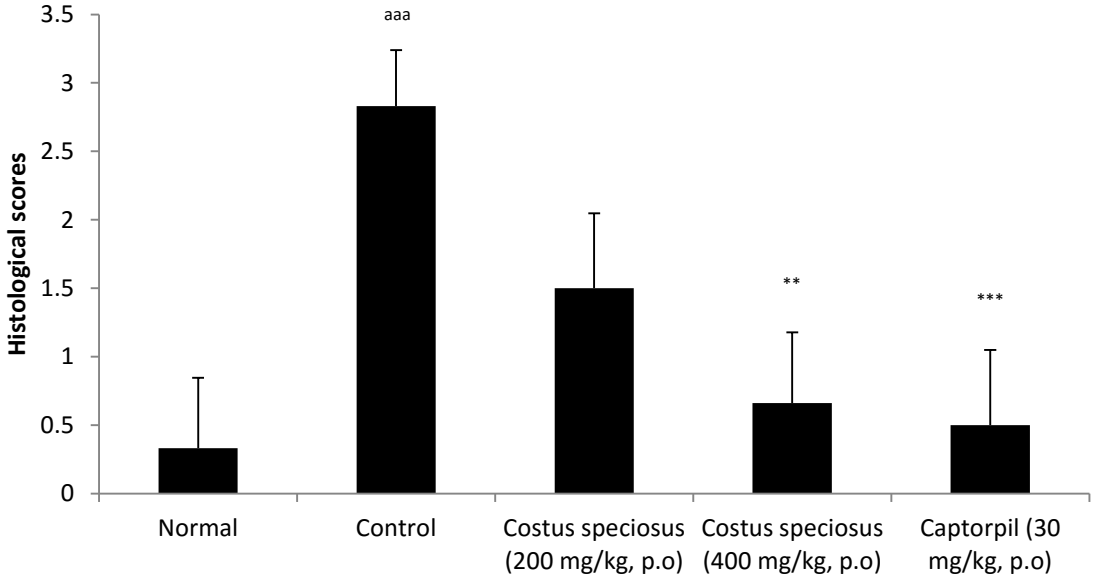
Effect on histological scores: Heart damage as indicated by loss of cellular architecture, nuclear duplication, increased infiltration of leucocytes and prominent hyperchromasia was observed in control animals treated with ISO (Fig **3**). The histological scores that were given based on the severity of damage was significantly less in *C. speciosus* (400 mg/kg, *p.o*) and captopril (30 mg/kg, *p.o*) treated groups compared to ISO treated control while the lower dose of *C. speciosus* (200 mg/kg, *p.o*) did not show any significant reduction in severity scores (Fig **2**). Sections of heart tissue from animals treated with lower dose of *C. speciosus* (200 mg/kg, *p.o*) showed mild to

moderate damage ranging from loss of cellular architecture to nuclear duplication and increased infiltration of leucocytes (Fig **4**) while those treated with higher dose of *C. speciosus* (400 mg/kg, *p.o*) showed no or very mild damage such as some loss of cellular architecture (Fig **5**). Captopril (30 mg/kg, *p.o*) almost prevented the ISO induced myocardial damage with sections from animals showing almost no damage (Fig **6**).



All values are mean ± SE, n=6, [aaa,] $p<0.001$ compared to normal animals, [***]$p<0.001$ compared to ISO treated control.

Fig. 1. Effect of *C. speciosus* extract and captopril on serum CK-MB levels



All values are mean ± SE, n=6, [aaa] $p<0.001$ compared to normal animals, [**]$p<0.01$, [***]$p<0.001$ compared to ISO treated control.

Fig. 2. Effect of *C. speciosus* extract and captopril on histological scores.

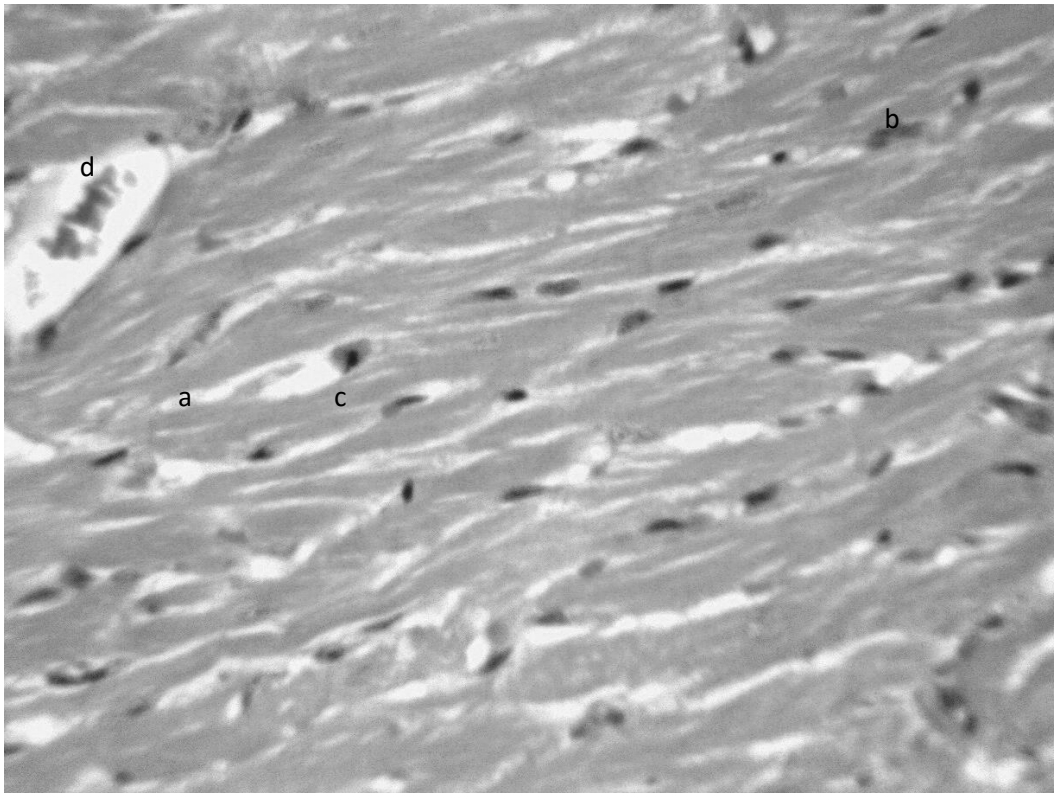


Fig. 3. Microscopic section of heart from isoproterenol (ISO) control animals (H&E stained, ×400) showing severe damage to heart tissue. There is loss of cellular architecture (a), nuclear duplication (b) , increased infiltration of leucocytes (c) and prominent hyperchromasia (d).

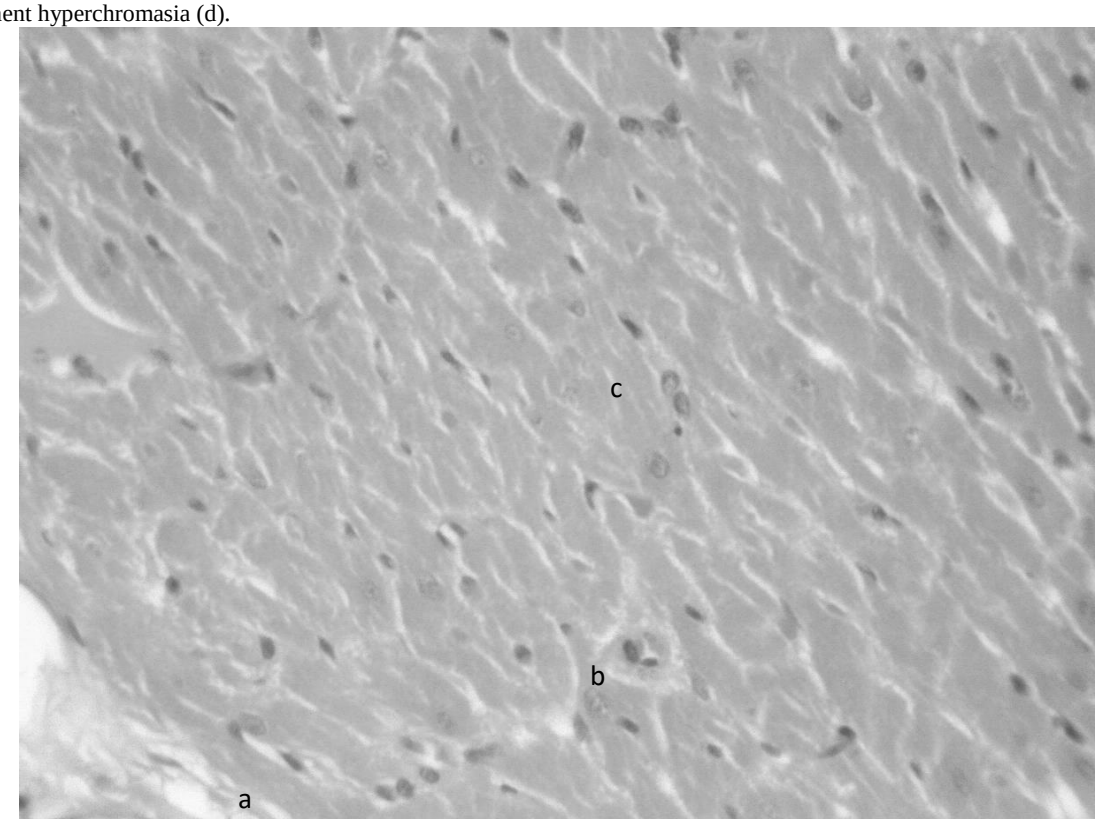


Fig. 4. Microscopic section of heart from *C. speciosus* (200 mg/kg, *p.o)* + isoproterenol (ISO) treated animals (H&E stained, ×400). . There is loss of cellular architecture (a), nuclear duplication (b) and increased infiltration of leucocytes (c).

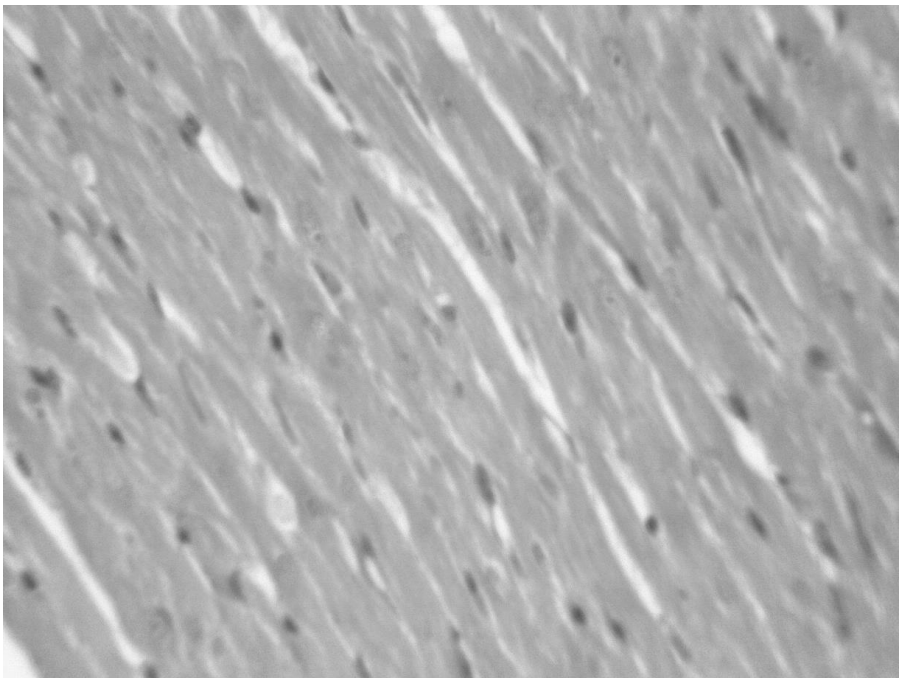


Fig. 5. Microscopic section of heart from *C. speciosus* (400 mg/kg, *p.o)* + isoproterenol (ISO) treated animals (H&E stained, ×400) showing almost normal cyto-architechture.

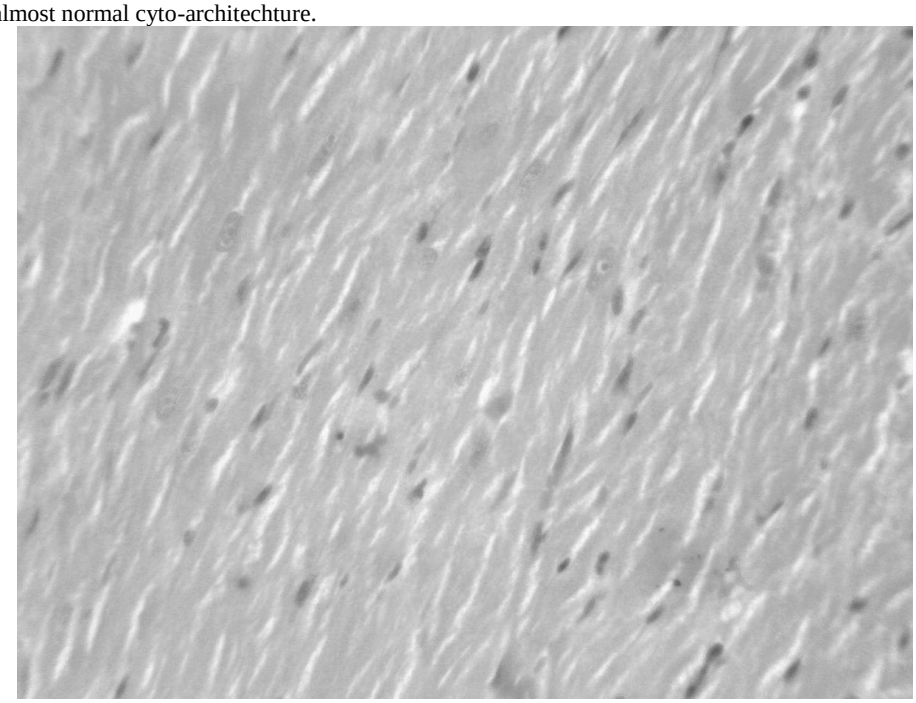


Fig. 6. Microscopic section of heart from captopril (30 mg/kg, *p.o)* + isoproterenol (ISO) treated animals (H&E stained, ×400) showing almost normal cyto-architechture.

DISCUSSION

The present study was undertaken to determine the effect of methanolic extract of *C. speciosus* rhizomes on the ISO induced myocardial infarction in rats. The results of the study suggest that *C. speciosus* rhizomes possess cardioprotective activity during myocardial damage induced by ISO in rats.

As mentioned earlier, *C. speciosus* is so widely used traditionally that it is on the verge of extinction if its cultivation is not increased. As mentioned earlier, the rhizomes of *C. speciosus* is the most widely part of the plant, hence, this was used in the present study. The doses of the extraction for administration to rats were dose-dependent study reported in earlier literature (Ali *et al.,* 2014) and also by preliminary studies carried out in our laboratory. Administration of higher dose such as 500 mg/kg or 1000 mg/kg orally produced toxic effects in the animals. The animals started to show weakness after 4-5 days of drug administration and continuing the administration for 3-4 days more resulted in deaths of some animals. Hence, doses more than 400 mg/kg orally were not used. On the other hand, administration of doses less than 200 mg/kg such as 100 mg/kg or 50 mg/kg did not produce any significant effect.

The rhizomes of C. *speciosus* are reported to contain a number of chemical constituents that includes saponins such as tigogenin, diosgenin, sapogenin along with steroids and alkaloids (Muniyandi *et al.,* 2013). It is also known to contain aliphatic hydroxyl ketones, triterpenes, starch mucilage, oxa-acids, fatty acids, abscisic acid and corticosteroids (Rajesh *et al.,* 2009). An important constituent with respect to the antioxidant effect of *C. speciosus* rhizomes are flavonoids, that are a subclass of polyphenols, which are further divided into subclasses such as flavones, flavonols, isoflavones, anthocyanins, flavanols, proanthocyanidins and other plant phenolics (Nehete *et al.,* 2010; Chang *et al.,* 2012). As mentioned earlier (Vijayalakshmi and Sarada, 2008; Nehete *et al.,* 2010), these polyphenols are responsible for the antioxidant effect of *C. speciosus*.

ISO induces myocardial through reduction of endogenous antioxidants such as superoxide dismutase (SOD) and catalase which leads to myocardial damage due to oxidative stress (Yang *et al.,* 2011). Once the myocardium is damages due to oxidative stress, it causes release of CK-MB present in the myocytes to the plasma leading to an increase in serum CK-MB levels. An increase in levels of CK-MB is an indication of myocardial damage. In the present study, ISO administration led to an increase in serum CK-MB levels indicating damage to myocardium. Administration of methanolic extract of *C. speciosus* rhizomes and captorpil attenuated the increase in serum CK-MB levels suggesting cardioprotective effect. The cardioprotective effect was further confirmed by histological studies, wherein changes in cellular architecture inflammation and necrosis were taken as parameters for determination of myocardial damage. The results of the histological studies supported the biochemical findings.

The exact mechanism of action for cardioprotective effect of *C. speciosus* rhizomes cannot be explained with the present data. However, we speculate that the powerful antioxidant effect of the plant may contribute at least in part to its cardioprotective action.

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(*Accepted for publication June 2017*)