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Prophylactic Effect of Costus and Selenium Nanoparticles in Isoproterenol Induced Myocardial Infarction in Rats

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ABSTRACT

Background: Myocardial infarction (MI) is a serious condition caused by an imbalance between blood supply and the demand of the myocardium leading to cardiac arrhythmia, heart failure, cardiogenic shock, and myocardial fibrosis.
Objective: This study aimed to investigate the protective effect of Costus and Selenium Nanoparticles (SeNPs) to ameliorate the myocardial infarct damage that has been induced by isoproterenol (ISO).
Material and methods: Six groups of weight matched rats (n=7) were used. G1 (control) received saline (1ml/rat) orally, G2 rats were orally given Costus 500 ml/kg body weight (b.wt), G3 rats were intraperitoneally injected with SeNPs (30 µgm/kg b.wt), G4 rats were injected subcutaneously with ISO (85 mg/kg b.wt) once daily at last two consecutive days, G5 where rats were pretreated with Costus prior to ISO injection and G6 where rats were pretreated with SeNPs prior to ISO injection. **Results:** ISO-treated group exhibited an elevation in serum creatine kinase (CK-MB) & aspartate aminotransferase (AST), and C- reactive protein (CRP) as well as disturbances in the levels of glucose & insulin. Also, there was significant increment in total cholesterol, and triglyceride with a noticeable decline in HDL. Additionally, changes in serum electrolytes and the parameters suggestive of oxidative damage. Rats either pretreated with Costus or SeNPs manifested significant decrease in CKMB & AST levels. Besides, a significant reduction in fasting blood glucose and insulin. This treatment reversed the hyperlipidemia. **Conclusion:** Serum electrolytes including Na, K, Ca^{+2} & Mg were significantly improved. Moreover, the previous treatment imposed anti-inflammatory effects by the mitigation of serum CRP and reduced the oxidative stress. Thus, Costus & SeNPs act as promising cardioprotective modalities to alleviate the unfavorable outcome of myocardial infarction.
Keywords: Isoproterenol, Myocardial infarction, Costus, Selenium nanoparticles.

INTRODUCTION

Globally, cardiovascular diseases (CVDs) are the leading cause of death. The incidence and mortality of CVDs continue to increase in spite of the availability of advanced treatment. CVDs are caused by pathological disorders such as cardiomyopathy, atherosclerosis, coronary heart disease (CHD) and myocardial infarction (MI) [(1)]. MI is an extremely dangerous condition that takes place due to a serious unbalance between the blood supply and demand for the myocardium. The mechanical, electrical, structural, and biochemical functions of the heart are all impacted by MI [(2)]. Damage to the myocardium is caused by oxidative stress, which is brought on by an increase in free radical production in ischemic tissue and a decrease in antioxidant levels [(3)]. There were several experimental protocols available for developing cardiomyopathy in animal models. Catecholamines cause complex structural and biochemical changes leading to myocardial infarction, cellular damage, and necrosis [(4)]. ISO (isoproterenol hydrochloride) is a catecholamine, cardiotoxic substance due to its ability to destroy myocardial cell & induce MI in experimental animals. ISO produce extremely cytotoxic free radicals, which cause cardiac membrane perioxidation, resulting in damage and destruction [(5)]. ISO is a model that is standardized and is frequently utilized for inducing MI in rats in order to examine the therapeutic benefits of several medicines and investigate the effects of a variety of possible cardioprotective bioactive compounds [(6)].

Many medications are used to treat myocardial infarctions, despite they are not devoid of adverse effects and have a limited effect on annual costs and survival. Thus, numerous studies have concentrated on developing novel therapeutic strategies to avoid MI. Traditional medicine is gaining popularity around the world. Many herbal plants extracts and products have been used for a long-time and are widely used worldwide. Recently, they have been utilized to treat a number of fatal disorders [(7)].

Costus is a member of the Zingiberaceae family of medicinal plants. Alkaloids, Tanning, terpenoids, flavonoids, glycosides, sapogenins, steroids & furan derivatives were all present in costus. They may exhibit therapeutic effects in humans and animals. It is widely used in folkloric medicine to treat ailments such as cough, inflammation, rheumatism, diarrhea, arthritis, hepatic disorders, epileptic attack, haemorrhoids and also had served as an antidote for poison. Costus has anti-inflammatory, antioxidant, antifungal, antitumor, antiulcer, antibacterial, and immunostimulant properties [(8)]. Nanotechnology has shown to be a promising technique for a variety of applications. SeNPs have distinct properties such as high surface area, low toxicity and improved bioactivity. So, they have gained a great attention [(9)]. They guard cells against oxidative stress and death. Additionally, it demonstrated anti-apoptotic, antioxidant, and anti-inflammatory effects. Cardiovascular disease is one of the disorders associated to selenium, as inadequate selenium consumption has been linked to cardiomyopathy [(10)]. Therefore, the aim of this investigation was to explore

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the potential protective effects of Costus and SeNPs against myocardial damage caused by ISO.

MATERIALS AND METHODS

Animals: For this experiment, 42 male albino rats were used weighing (180-200 g/b.wt). Rats were supplied by the Nuclear Research Center, Atomic Energy Authority, Egypt. They were kept in cages in a standard environment at a certain temperature 25-28 C, humidity 45-64%, 12-hour light and dark cycle. Rats left 7 days for acclimatization prior starting the experiment. They were fed a regular diet with free water access.

Ethical approval:

The experimental animal's Local Ethics Committee of Ain Shams University, Egypt gave its approval to the study protocol (REC-FS, Ino. 00033).

Induction of myocardial infarction (MI)

Isoproterenol at a dosage of 85 mg/kg b.wt. dissolved in saline was subcutaneously injected once daily for 2 days to cause MI in rats [(11)].

Chemicals: SeNano capsules were purchased from the Chinese company Shanghai Stone Nano- Technology Port Co. Ltd. Se particles were in the form of an orange powder coated in capsules each one contains 0.225 g of powder including Nano-Se (45µg). The size of Se particles diameter ranged from 60 to 80 nm. ISO was purchased from Sigma Chemical Co. (St Louis, MO, USA). Costus was purchased from Emtnan Health Shop Company.

Experimental design

Rats were divided into six groups of seven each where they received treatment for 30 days. Group I (control) received saline (1ml/rat), Group II (Costus) received Costus orally at a dose of 500 mg/kg b.wt through a gastric feeding tube [(12)]. Group III (SeNPs) rats received selenium nanoparticles intraperitoneally 30µ gm/kg b.wt (5days/week) [(13)]. Group IV (ISO group) received isoproterenol at a dose of 85 mg/kg b.wt subcutaneously once daily for 2 consecutive days for MI induction. Group V (Costus + ISO) received Costus 500 mg /kg b.wt (5days/ week) orally for 30 day prior to ISO injection at 29th & 30Th of the experiment. Group VI (SeNPs + ISO) rats were injected intraperitoneally with SeNPs 30µ gm/ kg b.wt prior to ISO injection.

Blood sampling

The rats were fasted overnight and anesthetized with diethyl ether, blood samples were collected. Following that, the animals were sacrificed and then dissected to harvest their hearts. The serum were separated and used for estimation of cardiac enzymes, CRP, glucose, insulin, lipid profile and electrolytes.

Tissue homogenate preparation

Hearts from experimental groups were immediately removed and saline-rinsed (NaCl 0.9%) to remove blood at the end of the treatment. The heart's tissues were homogenized in suitable buffer using Teflon homogenizer. The supernatant from the centrifuged homogenate was used to estimate the biochemical parameters.

Biochemical analysis

The activities of Aspartate Aminotransferase (AST) and Creatine kinase (CK-MB) were assessed utilizing commercially available ELISA (enzyme-linked immunosorbent assay) kits in accordance to the manufacturer's instructions.

C reactive protein (CRP) was also measured in sera by Immunoturbidimetry assay using kit purchased from (Roche Diagnostics Gmbs, Mannheim, Germany).

A glucose test kit (Spectrum-Diagnostics, Cairo, Egypt) was used to measure the level of glucose using the glucose oxidase technique.

Insulin levels were measured using the radioimmunoassay (RIA) technique with rat-specific kits (Diagnostics Products Corporation (DPC), Los Angleles, CA90045-6900, USA) according to the kit's protocol.

Calculating the homeostasis model assessment as an indicator of insulin resistance (HOMA-IR) HOMA-IR = insulin (IU/mL) x glucose (mg/dL)/22.5 [(14)].

Enzymatic colorimetric techniques were used on a spectrophotometer (Milton Roy Spectronic 1201) to assess the levels of serum triglycerides, high density lipoprotein (HDL), and total cholesterol (TC) with commercial kits (Biodiagnostic reagent kits). Meanwhile, low-density lipoprotein cholesterol (LDL-C) was calculated according to **Friedwald *et al.*** [(15)] by the following equation. LDL-C (mg/dl) = TC - (TG/5+HDL-C).

Commercial kits (Spectrum-Diagnostics, Cairo, Egypt) were used to assess serum electrolyte values (potassium, sodium, calcium, and magnesium).

The method developed by **Ohkawa *et al.*** [(16)] was used to measure Malondialdehyde (MDA), a stable byproduct of lipid peroxidation, in the cardiac homogenate. The activities of catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), and nitric oxide (NO) in the myocardium were determined in accordance with the procedures described by **Aebi** [(17)], **Sun *et al.*** [(18)], **Beutler *et al.*** [(19)] and **Green *et al.*** [(20)] respectively.

Statistical analysis:

The statistical differences between groups were achieved with SPSS software package (version 20). Values were presented as Mean ± SD. The comparison between groups was carried out using (AVOVA) one way analysis of variance. P value < 0.05 was considered significant.

RESULTS

The levels of CK-MB & AST, inflammatory marker (CRP), glucose and insulin in the serum of experimental rats are shown in (table 1). Serum levels of CK-MB & AST are noticeably elevated in the ISO group when compared to the control group. However, pretreatment with Costus and SeNPs significantly decreased this elevation to be nearly reached to control value. Interestingly, the SeNPs displayed a more reduction in their levels than Costus.

Table (1): Protective effect of Costus and Selenium NPs on the levels of cardiac enzymes (CK-MB, AST), inflammatory marker (CRP) and the levels of glucose and insulin in isoproterenol-induced myocardial infarction in rats

Parameters/ Groups	CK-MB (U/L)	AST (U/L)	CRP (mg/l)	Glucose mg/dI	Insulin IU/ml	HOMA-IR
Control	289.14^{c}±6.34	28.64^{c}± 0.93	2.26^{d}±0.17	90.7^{c}± 1.7	0.37^{c}± 0.022	13.52^{c}±0.97
Costus	288.79^{c}± 5.94	29.24^{c}±0.84	1.91^{d}±0.15	89.14^{c}± 1.11	0.39 ^{c} ± 0.027	14.15^{c} ±0.99
SeNPs	288 ^{c}±6.01	30.0^{c}± 1.11	1.60^{d}±0.12	88.5^{c}±1.66	0.43 ^{c} ± 0.022	15.4^{cs}±0.79
Isoproterenol	394.43^{a}± 7.91	96.19^{a}± 1.86	13.75^{a}± 0.29	150.3^{a}±2.77	0.73^{a} ± 0.023	44.21^{a}±1.62
Costus + ISO	333.29^{b}±5.32	52.36^{b}±1.97	5.79^{b}±0.11	118.7^{b}±2.85	0.52^{b} ± 0.019	24.81^{b}±0.9
SeNPs +ISO	316.29^{b}± 6.06	49.57^{b} ±1.77	3.66^{c}± 0.17	111.14^{b}± 2.61	0.49^{b} ± 0.017	21.82^{b}±0.96

CK-MB: Creatine Kinase-MB; AST: Aspartate transaminase (AST); CRP: C reactive protein; Homa–IR Insulin resistance.

The values of the table represent the mean ± SE significant at ($p<0.05$). Means with dissimilar superscript letters are significant, whereas those with the same superscript letters are not significant. An assessment of the inflammatory status after the treatment of ISO was done by evaluating the inflammatory marker CRP in the serum. As regards CRP level in serum, it was noticeably higher in the ISO group compared to the control group. But a considerable drop in its level was seen in the Costus- & SeNPs- treated group, suggestive of anti-inflammatory activity of Costus & SeNPs. The SeNPs had a more potent impact on reducing the level of CRP compared to Costus.

The levels of serum glucose, insulin, and the extent of HOMA-IR significantly increased in ISO group. Our data revealed that pretreatment with Costus & SeNPs demonstrated that their levels decreased significantly in comparison with the ISO group, while the SeNPs displayed a more significant anti-hyperinsulinemic and anti-hyperglycemic activity than Costus.

Subcutaneous injection of ISO led to a substantial increase in TC, LDL, and TG levels and a significant decrease in HDL when compared to control. Pretreating the ISO group with either Costus or SeNPs caused significant decline in TG, LDL, and TC levels as well as a hike in HDL levels. Furthermore, SeNPs had a more powerful effect than Costus on reducing dyslipidemia in rats (Table 2).

Table (2): Protective effect of Costus and Selenium NPs on the lipid contents in isoproterenol induced myocardial infarction in rats

Groups/parameters	TC (mg/dI)	HDL (mg/dI)	LDL (mg/dI)	TG (mg/dI)
Control	93.14^{c}±2.83	42.55^{a}±0.59	25.38^{c}± 1.94	126.0^{c}± 2.76
Costus	92.28^{c}±2.77	42.97^{a}± 0.64	24.8^{c}±1.7	123.23^{c}±2.8
SeNPs	91.57^{c}±2.47	43.24^{a}±0.7	24.04^{c}±1.43	121.42^{c}± 5.2
Isoproterenol (ISO)	152.61^{a}± 4.12	36.3^{c}±0.67	77.97^{a}±2.63	191.71^{a}±5.5
Costus + ISO	104.83^{b}±4.01	38.31^{b}±0.77	38.59^{b}± 3.1	140. 0^{b}±3.55
SeNPs + ISO	98.71^{b}± 3.52	40.17^{b}±0.7	32.14$_{b}$±2.59	132.0^{bc} ±3.05

TC: Total cholesterol; HDL: High density lipoprotein; LDL: Low density lipoprotein; TG: Triglycerides

The values of the table represent the mean ± SE significant at ($p<0.05$). Means with dissimilar superscript letters are significant, whereas those with the same superscript letters are not significant. Na & Ca^{2+} levels significantly increased with a corresponding considerable decrease in K and Mg levels in the ISO group. Conversely, pretreatment with Costus and SeNPs resulted in a notable reduction in Na and Ca^{2+} levels with an elevation in K and Mg levels as compared to ISO group (Table 3).

Table (3): Protective effect of Costus and Selenium NPs on the level of serum electrolytes in isoproterenol induced myocardial infarction in rats

Groups/parameters	Na (mEq/L)	K (mEq/L)	Ca^{2+} (mmol/L)	Mg (mg/dl)
Control	147.0^{b}±2.44	6.21^{a}±0.25	8.69^{c}± 0.36	1.83^{a}±0.1
Costus	146.0^{b}± 2.49	5.96^{ab}±0.24	8.31^{c}±0.27	1.89^{a}±0.09
SeNPs	146.0^{b}±2.19	5.92^{ab}±0.27	8.0^{c}±0.26	1.9^{a}± 0.11
Isoproterenol (ISO)	175.14^{a}± 2.98	3.78^{c}± 0.17	12.22^{a}± 0.41	0.75^{c}± 0.06
Costus +ISO	155.29^{b}± 3.53	5.27^{ab}± 0.18	10.3^{b}±0.46	1.15^{b}±0.09
SeNPs + lSO	151.71^{b}±3.36	5.77^{ab}±0.18	9.4^{b}±0.30	1.32^{b}± 0.13

Na: sodium; K: potassium; Ca^{2}+: calcium; Mg: Magnesium

The values of the table represent the mean ± SE significant at ($p< 0.05$). Means with dissimilar superscript letters are significant, whereas those with the same superscript letters are not significant. Malondialdehyde (MDA) & nitric oxide (NO) in the heart were markedly elevated in the ISO group compared to the control group, while cardiac catalase (CAT), super oxide dismutase (SOD), and reduced glutathione (GSH) were decreased significantly. In contrast, pretreatment of rats with Costus or SeNPs resulted in significant protection against ISO-induced cardiac damage with an apparent reduction in MDA & NO with a significant elevation in GSH, SOD, and CAT levels relative to infarcted group (Table 4).

Table (4): Protective effect of costus and selenium NPs on the level of oxidants & antioxidants in isoproterenol induced myocardial infarction in rats

Groups/parameters	MDA (nmol/g tissue)	SOD (µ/g tissue)	CAT (µ/g tissue	GSH (mg/g tissue)	NO (Mmol/g tissue)
Control	35.64^{bc}±1.49	173^{a}±2.89	61.86^{a}±1.56	16.05^{b}±0.56	40.55^{c}±0.66
Costus	34.66^{c}±1.43	175.88^{a}±3.56	62.14^{a}±1.33	17.64^{a}±0.39	39.51^{c}±0.68
SeNPs	35.36^{bc}±1.32	177.16^{a}±3.12	63.71^{a}±2.1	18.78^{a}±0.49	40.07^{c}±0.92
Isoproterenol (ISO)	58.23^{a}±1.11	76.05^{c}±4.04	36.43^{c}±1.65	8.95^{d}±0.33	70.73^{a}±2.18
Costus +ISO	40.07^{b}±1.45	158.57^{b}±2.78	49^{b}±2.43	12.63^{c}±0.23	54.06^{b}±1.99
SeNPs + ISO	38.51^{bc}±2.1	165.16^{b}±2.34	54.29^{b}±1.95	13.82^{c}±0.59	49.59^{b}±2.41

MDA: Malondialdehyde; SOD: Superoxide dismutase; CAT: Catalase; GSH: Reduced glutathione: NO: Nitric oxide

DISCUSSION

Among all forms of cardiovascular diseases, myocardial infarction (MI) is one of the leading causes of mortality and morbidity. It was induced by isoproterenol, which is a common model for studying the cardio-protective effect of different medications [(21)].

The cardiac enzymes AST & CK-MB are important measures for both early and late phases of cardiac damage. Our findings demonstrated that ISO treatment caused significantly elevation in serum cardiac marker levels CK-MB & AST. These findings confirm the onset of myocardial infarction and releasing the cardiac enzymes into the blood stream [(22)]. On the other hand, pretreatment of Costus and SeNPs prevented the ISO-mediated elevation of cardiac enzymes indicating cardio-protective effects of Costus & SeNPs. The bioactive compounds in the Costus have the ability to mitigate the harmful effects of ISO and it may be responsible for this cardioprotection. This finding is consistent with previous studies that revealed that certain antioxidant metabolites found in the Costus can lessen tissue oxidative damage [(23)]. Similar findings, suggested that Selenium could be a promising candidate to ameliorate the cardio-toxicity. This may be due to maintaining membrane integrity, decreasing enzyme leakage and enhancing free radical scavenging [(24)].

Inflammatory markers are crucial prognostic factors in cardiovascular patients and are useful tool for early diagnosis of ischemia and myocardial lesions CRP is the most promising predictive factor. It was considerably increased in ISO-treated group. The findings of other researchers support this result [(25)]. Rats pretreated with Costus and SeNPs decreased its level that could have contributed to their anti-inflammatory property.

Furthermore, the MI caused by ISO in rats indicated a notable increase in blood glucose, insulin and insulin resistance (HOMA-IR) in relation to control group. This is in agreement with findings from a prior study which indicated that the hyperglycemia is an expected condition after acute myocardial infarction. There is a higher release of glucose intolerance in the early hours of (AMI). The infarction's size increased, due to the rapidly rising serum glucose levels [(26)].

In comparison with the ISO group, Costus and SeNPs ameliorated the blood glucose, insulin & HOMA-IR resulting in significant decrease in their levels. Costus may have a hypoglycemic effect by enhancing insulin production, causing the beta cells of the islet of Langerhans to release insulin and increase the sensitivity of cell receptors to insulin [(27)]. Moreover, the ability of Se to decrease blood glucose explains that Se is an insulin mimic [(28)].

Dyslipidemia is an important risk factor which is a strong predictor for cardiovascular diseases. The current study revealed that ISO injection significantly increased serum lipid contents (TC, LDL, and TG) while concurrently reducing HDL as compared to control. The alterations in the lipid contents could be caused by ISO that induced lipid metabolic disturbances and change the characteristics of cardiac cell membranes. Cholesterol levels increasing as a result that ISO could be linked to a decrease in its use and an increase in biosynthesis. Triglyceride levels may have increased after ISO treatment because the activity of lipoprotein lipase has decreased and the hormone-sensitive lipase activity increased, which reduced the uptake of TGs from the circulation [(29)]. In contrast, TC, LDL, & TG were significantly reduced by Costus and SeNPs, while HDL was raised. The ability of Costus to decrease cholesterol levels associated with diosgenin, which is a steroid saponin compound that can prevent the synthesis of cholesterol in the body. Furthermore, the antihyperlipidemic properties of flavonoids and polyphenols in Costus inhibits the lipase enzymes needed for lipid absorption [(30)]. Also, our findings showed that SeNPs produced marked amelioration of the lipids. SeNPs might scavenge free radicals, which in turn lowers serum cholesterol & triglycerides. Thus, it can be suggested that the decrease in TG and TC levels may be caused by HDL-c, which may accelerate the outflow of TG and TC into hepatic tissue for catabolism [(31)].

Limited studies have been accomplished on the effect of ISO-induced MI on the electrolytes. Consequently, the current investigation was to assess ISO's impact on the electrolytes. Electrolyte imbalance could be linked to heart metabolic disorders. Our observation revealed that Na^+ and Ca^{2+} concentrations increased significantly as a result of ISO-induced MI, but K^+& Mg^{2+} levels were decreased. Pretreatment with Costus and SeNPs ameliorated the electrolytes level in ISO-treated group because of their antioxidant activities. **Tousson's** findings [(32)] support our results.

Cytotoxic free radical generation in myocardial cells has been associated to heart damage and myocardial cell death induced by ISO. Lack of antioxidants and excessive free radical generation can result in the production of lipid peroxides, which could lead to tissue damage. As evidenced by a substantial increase in MDA and NO levels in cardiac tissue & a corresponding decline in CAT, SOD, and GSH tissue levels. Our study revealed that ISO-induced MI was linked to oxidative stress. Our findings are consistent with prior research that found an association between oxidative stress and ISO-induced cardiotoxicity [(33)]. Oxidative stress was caused by ISO attributable to an imbalance between the body's antioxidant defences and production of reactive oxygen species (ROS).

Conversely, pre-treatment with Cost us & SeNPs lowered intracellular free-radical production and thereby mitigated oxidative damages. Thus, they could restore the equilibrium between ROS and antioxidant enzymes. The antioxidant components of Costus (phenols, flavonoids, and terpenoids) may be responsible for its ability to protect the membrane bilayer from lipid peroxidation. Consequently, it may be possible to improve membrane stability and restore biochemical profiles that have been changed resulting from ISO [(34)]. Lipid peroxidation was reduced by Costus by increasing the activity of glutathione reductase. The terpenoids & glycosides contents of Costus may be responsible for its cardioprotective effect. Concerning, the effect of SeNPs on lipid peroxidation our findings revealed that SeNPs decreased MDA and NO levels in cardiac tissue while increasing GSH levels and antioxidant enzymes (CAT and SOD). These findings verify the ameliorative effects of SeNPs, which may be attributed to its ability to reduce free radical generation and so mitigate the oxidative stress. Other researchers have found that SeNPs have antioxidant properties. It has the ability to protect against oxidative stress by improving antioxidant defence mechanisms and free radical scavenging capacity [(35)].

CONCLUSION

The findings of this study justified the valuable cardioprotective effect of Costus & SeNPs against isoproterenol-induced myocardial infarction in rats, but SeNPs exerted greater effects than Costus in limiting myocardial damage extension & minimizing the risk of its complications.

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Author contribution: Authors contributed equally in the study.

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