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Therapeutic Effect of *Zanthoxylum armatum* Fruit on Glycoproteins, Biochemical Changes and Electrolytes in Isoproterenol Induced Cardiotoxic Rats



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ABSTRACT

Considerable numbers of myocardial infarction cases are asymptomatic or associated with minor and atypical symptoms and are found unintentionally during routine checkups that reveal the existence of risk factors. *Zanthoxylum armatum* (*Z. armatum*) fruits are used as age-old herbal remedies. Their pharmacological and therapeutic actions are not exploited in detail. Hence, the present study was aimed to evaluate the preventive effect of *Z. armatum* fruit on glycoproteins, biochemical changes and electrolytes in isoproterenol hydrochloride (ISO) induced myocardial infarction (MI). MI was induced by subcutaneous injection of ISO (20 mg/100mg) to male albino Wistar rats for two consecutive days at an interval of 24hr. Rats were treated with *Z. armatum* fruit (200mg and 400mg/kg) for a period of 30 days and ISO was injected on the 28th and 29th day. At the end of experiment i.e on 30th day biochemical changes, glycoproteins and electrolytes were monitored from control and experimental groups. ISO induced rats showed a significant increase in the levels of glycoproteins, blood glucose, serum urea, uric acid, creatinine, iron and a decrease in plasma protein. It also exhibited alteration in the levels of electrolytes (Na⁺, K⁺ and Ca²⁺) in serum. Pretreatment with *Z. armatum* fruit positively altered the ISO induced alteration in electrolytes and other biochemical parameters. Thus, the results of our study demonstrate that *Z. armatum* fruit had reduced the extent of myocardial damage induced by ISO and maintained the biochemical parameters at normal.

INTRODUCTION

Cardiovascular disease (CVD) remains the main source of death in both developed and developing countries which incorporate high blood pressure, coronary heart disease, congestive heart failure and stroke (Joseph et al., 2017). It is anticipated that CVD particularly MI will be the essential reason for death in India by the year 2020 (Dominguez et al., 2014). MI occurs when the blood supply to a part of the heart is interrupted, causing death of heart tissue (Rajdurai and Stanely, 2007).

High dose of ISO, a β -adrenergic agonist, has been reported to induce MI in experimental animals which result in hyperglycemia, hyperlipidemia an increase in cardiac enzymes, biochemical modifications and generation of oxidative stress (Prabhu et al., 2006b; Hakimog˘lu et al., 2007). ISO induced oxidative stress is a known standard method and the contribution of free radicals in the pathophysiology of diseases are managed by supplementation with antioxidants (Miller and Rice-Evans, 1997).

The antioxidant activities are higher in medicinal plants with minimum side effects and thus these herbal drugs are recommended widely (Miliauskas et al., 2004). The world health organization (WHO) has expected that 80% of individuals in developing countries depend on the traditional medicine, often plant drugs, for their most vital health care needs. Medicinal plants play a vital role in the discovery of novel drugs used in modern medicine. *Z. armatum* DC is a medicinal plant fit into the family of Rutaceae which is commonly known as Indian prickly ash, Nepal pepper (or) Toothache tree. It has many chemical constituents which were reported to have several biological activities (Cebrián-Torrejón et al., 2012; Pachon et al., 2007).

We have previously reported the effect of *Z. armatum* fruit on cardiac marker enzymes, endogenous antioxidant levels, mitochondrial and lysosomal marker enzymes and membrane bound enzymes in ISO induced MI. So, from the above observation, the present study was extended to evaluate the therapeutic effect of *Z. armatum* fruit on glycoproteins and biochemical changes along with electrolyte levels in ISO induced MI in rats. It will help to gain more insight into the pharmacological mechanisms of *Z. armatum* fruit against MI.

MATERIALS AND METHODS

Collection of plant material and preparation of extract

The *Z. armatum* fruit was collected from Kolli hills, India. The taxonomic identity of the plant was confirmed from the ABS Botanical Conservation, Research and Training Centre, Salem, Tamilnadu, India. (Voucher Specimen No: AUT/ECP/101). Extract was prepared by the maceration procedure from dried fruits using 50% ethanol for 5 days. Extract was concentrated using rotary evaporator. Isoproterenol hydrochloride was purchased from Sigma Chemical Co., St. Louis, MO, USA. All the other chemicals and reagents used were of analytical grade.

Animals

Male Wistar albino rats (*Rattus norvegicus*) weighing about 100–120 g were obtained from animal house of PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India. They were housed in polypropylene cages under a 12:12 hr light and dark cycle at around 37°C. The rats had free access to tap water and food. They were fed on a standard pellet diet (AVM Cattle and Poultry Feeds, Coimbatore) and water *ad libitum*. The clearance of the ethical committee for experimentation on animals was obtained before the start of the experiment (Proposal No: 158/PO/bc/99/CPCSEA). The experiment was carried out according to the guidelines of the Committee approved by the Animal Ethical Committee of PSG Institute of Medical Sciences and Research, Coimbatore.

Induction of MI

ISO hydrochloride was used to induce MI in rats. Animals were injected subcutaneously with freshly prepared ISO hydrochloride in sterile normal saline at a dose of 20 mg/100g body weight.

Experimental design

Animals were divided into six groups of six rats in each group and the grouping of animal is shown in table-1.

Table 1: Treatment schedule

Groups	Diet/Treatment
GI- Normal control rats	Standard rat pellet for 30 days
GII - <i>Z.armatum</i> fruit treated rats	Hydroethanolic extract of <i>Z. armatum</i> fruit 400mg/kg body weight for 30 days (oral intragastric tube).
GIII – ISO treated rats	ISO (20mg/100g body weight) injected subcutaneously twice at an interval of 24hr on 28 th and 29 th day.
GIV- Rats pretreated with <i>Z. armatum</i> fruit (low concentration) + ISO	Hydroethanolic extract of <i>Z. armatum</i> fruit (200mg/kg body weight for 30 days) + ISO (20mg/100g body weight) subcutaneously twice at an interval of 24hr on 28 th and 29 th day.
GV- Rats pretreated with <i>Z.armatum</i> fruit (high concentration) + ISO	Hydroethanolic extract of <i>Z. armatum</i> fruit (400mg/kg body weight for 30 days) + ISO (20mg/100g body weight) subcutaneously twice at an interval of 24hr on 28 th and 29 th day.
GVI - Rats pretreated with standard drug + ISO	Standard drug verapamil (1mg/ kg body weight for 30 days) + ISO subcutaneously twice at an interval of 24hr on 28 th and 29 th day.

At the end of the experimental period i.e., 12 hr after the second dose of ISO injection, all the rats were scarified by cervical dislocation under mild chloroform anesthesia. Blood was collected and serum was separated after centrifugation at 2500 rpm. The heart tissue was excised immediately and thoroughly washed with ice-cold physiological saline. Serum was used for various biochemical estimations.

Assay of glycoproteins in serum

Estimation of hexose

Protein bound hexoses were estimated by the method of Dubois and Gilles, (1956). To 0.1 ml of serum sample, 5 ml of 95% ethanol was added, mixed and then centrifuged. The precipitate was dissolved in 1 ml of 0.1N NaOH. Subsequently, 1 ml of distilled water and to all the tubes, 8.5 ml of orcinol-sulphuric acid reagent was added and kept in a water bath for exactly 15 min at 90°C. The tubes were cooled under tap water, and the colour developed was measured at 540 nm in a UV-Visible- Spectrophotometer against a blank. The values were expressed as mg/dl protein.

Estimation of hexosamine

Hexosamine was estimated by the method of Wagner, (1979). To 1 ml of serum, 2.5 ml of 3 N HCl was added and kept for 6 hr in a boiling water bath and then neutralized with 6 N NaOH. To 0.8 ml of the neutralized sample, 0.6 ml of acetylacetone reagent was added. The tubes were heated in a boiling water bath for 30 min. 2ml of Ehrlich's reagent was added after cooling and mixed well. Blank contained 0.8ml of double distilled water. The colour developed was measured at 540 nm in a UV-Visible-Spectrophotometer. The values were expressed as mg/dl.

Estimation of fucose

Fucose was estimated by the method of Dische and Shettle, (1948). Two tubes, each containing 0.1 ml of sample (labeled as control and test) were taken. 5 ml of 95% ethanol was added, mixed well and then centrifuged. The precipitate was dissolved in 1ml of 0.1 N NaOH. 1 ml of double distilled water served as the blank. A series of standards in 1ml volume were also set up along with the test. All the tubes were kept in cold condition, and 4.5 ml of H₂SO₄-water mixture was added. The tubes were kept in boiling water bath for 3 min and cooled. To this, 1ml of cysteine reagent was added to all the tubes except control and kept for 60 min at room temperature. The colour developed was measured at 396 nm and 430 nm in a UV-Visible- Spectrophotometer against blank. The fucose content was calculated from the difference in the readings obtained at 396 nm and 430 nm and subtracting the values obtained without cysteine. The values were expressed as mg/dl.

Estimation of blood glucose

The level of blood glucose was determined by Trinder, (1969). 0.1 ml of blood was added to 1 ml of 0.05 M sodium hydroxide. Then 0.1 ml of 10% zinc sulphate was added and mixed well and centrifuged. To 0.2 ml of the supernatant, 4.0 ml of the enzyme-dye reagent was added. The tubes were placed in a water bath at 37°C for 45 min. The colour developed was read at 430 nm. The values were expressed as mg/dl.

Estimation of blood urea

The level of urea was determined by the method of Geyer and Dabich (1971). 0.2 ml of sample was deproteinised with 2.8 ml of TCA. To 2 ml of the supernatant obtained by

centrifugation, 1 ml of DAM-TSC reagent and 1.5 ml of acid ferric reagent were added and the solution was heated in a boiling water bath for 15 minutes. Aliquots of standard urea and blank containing 2 ml water were also treated in a similar manner. After cooling, the color developed was read at 520 nm. The values were expressed as mg/dl.

Estimation of uric acid

The level of uric acid was determined by the method of Caraway (1963). 0.1 ml of the sample was taken and to this 2.9 ml of water was added followed by 0.6 ml each of phosphotungstic acid and sodium carbonate. A blank was set up with 3.0 ml water. Standard were also treated in the same manner. Serum uric acid is oxidized to allantoin and carbon dioxide by phosphotungstic acid reagent in alkaline solution. Phosphotungstic acid is reduced in this reaction to tungsten blue, which is measured at 640 nm after 10 minutes. The result was expressed as mg/dl in serum.

Estimation of serum creatinine

Creatinine was estimated by the method of Slot (1965). To 3 ml of deproteinized supernatant (0.1 ml of serum + 3.9 ml 10% TCA), 2 ml of alkaline picrate solution was added. Blank containing 3 ml of water and aliquots of standard in 3 ml of water were also treated in a similar manner. After 30 minutes the colour was measured at 520 nm. The values were expressed as mg/dl.

Estimation of serum iron

Serum iron was determined by the method of Ramsay, (1969a). Equal volume of serum, sodium sulphite and dipyrindyl reagent were mixed in centrifuge tubes. The tubes were heated in a boiling water bath for 5 min. The contents were cooled and 12 ml of chloroform was added to each tube. The tubes were closed and mixed vigorously for 30 sec and centrifuged at 1000 ×g. The intensity was measured at 520 nm in a UV-Visible-Spectrophotometer. The values were expressed as µg/dl for serum.

Estimation of plasma iron binding capacity

Plasma iron binding capacity was determined by the method of Ramsay, (1969b). Four ml of ferric chloride was added to 2 ml of plasma. 400 mg of magnesium carbonate was added after standing for 5 min and mixed well. The mixture was incubated for one hour at room

temperature with frequent shaking. The contents were centrifuged, and 4 ml of supernatant was taken and the plasma iron binding capacity was determined as described in the previous method. The values were expressed as $\mu\text{g}/\text{dl}$ serum.

Estimation of serum protein

Serum protein was estimated by Lowery et al., (1951). 0.5 ml of serum was precipitated with 0.5 ml of 10% TCA, centrifuged for 10 min and the precipitate was dissolved in 1.0 ml of 0.1 N NaOH. 0.1 ml of aliquot was taken and made up to 1.0 ml with distilled water. Then, 4.5 ml of alkaline copper reagent was added and allowed to stand at room temperature for 10 min. After incubation, 0.5 ml of Folin's-Ciocalteau reagent was added and the blue colour developed was read at 620 nm after 20 min. The values were expressed as g/dl serum.

Estimation of A/G ratio

Serum albumin was measured by direct spectrophotometric determination of albumin in serum by dye binding method, using bromocresol green dye (Doumas et al., 1971; Corcoran and Durna, 1977). After mixing; the solution was left at room temperature for 10 minutes. This absorbance was read at 630nm against a reagent blank. Albumin standard was similarly treated. The albumin concentration was expressed in g/L. Serum total globulin was estimated by subtracting albumin from the total serum protein. The value for Globulin/Albumin ratio was obtained by dividing albumin value by globulin value.

Estimation of heart glycogen

The glycogen content in the heart was estimated by the method of Morales et al., (1973). A known amount of the tissue was subjected to alkali digestion in a boiling water bath for 20 min after the addition of 5 ml of 30% potassium hydroxide. The tubes were cooled, and 3 ml of absolute ethanol and a drop of ammonium acetate were added and placed in a freezer overnight to precipitate the glycogen. The precipitated glycogen was collected after centrifugation at $3,000\times g$. The precipitate was washed thrice with alcohol and dissolved in 3 ml of double distilled water. Aliquots were taken and made up to 1 ml with double distilled water. 4 ml of anthrone reagent was added to the tubes and kept in ice bath and heated in a boiling water bath for 20 min. The green colour developed was measured at 640 nm in a UV-Visible spectrophotometer. The levels were expressed as mg/gm tissue.

Estimation of serum electrolyte

The concentration of Na⁺ and K⁺ ions in serum was estimated using commercial kits purchased from Lab-Care Diagnostics Private Limited, India. The level of Ca²⁺ ion in serum was measured by the O-cresolphthalein complexone method using a reagent kit purchased from Span Diagnostic Limited, India. In this method, *o*-cresolphthalein complexone binds to calcium tightly in alkaline solution to form a purple/red complex at pH 10-12. The intensity of the color, measured at 575 nm, is directly proportional to calcium concentration in the sample. The result was expressed as m mol/ L.

Statistical analysis

The results were articulated as mean \pm standard deviation. Statistical analysis was carried between the experimental groups using one way analysis of variance (ANOVA) employing statistical package for social science (SPSS Version 16.0). Post hoc testing was performed for inter-group comparisons using Fisher's least significant difference (LSD) tests. The level of significance was set as (p<0.05).

RESULTS



Examination of the blood can give an early clue of information of cardiovascular health risk and may serve as a valuable tool for monitoring the biological system. The present study revealed that ISO induced rats showed a significant increase in the glycoprotein components such as hexose, hexosamine and fucose in the serum when compared to the normal control rats (Table 2). Pretreatment with the hydroethanolic extract of *Z. armatum* fruit (200 and 400 mg/kg) for 30 days, significantly decreased the level of glycoprotein components in the serum, when compared with ISO induced non treated rats. When comparing control rats and plant alone treated rats, there was no significant change.

Table 2: Effect of *Z. armatum* fruit on the level of glycoproteins in serum of normal and ISO induced MI rats.

GROUPS	Hexose (mg/dl)	Hexosamine (mg/dl)	Fucose (mg/dl)
G- I	128.27 ± 6.71	37.12 ± 2.17	34.24 ± 1.08
G- II	127.68 ± 9.23	36.91 ± 1.73	34.81 ± 1.89
G- III	234.24 ± 11.34 ^a	50.28 ± 7.27 ^a	52.57 ± 3.71 ^a
G- IV	167.28 ± 8.38 ^{a,b,c}	41.21 ± 5.7 ^{a,b,c}	41.73 ± 7.38 ^{a,b,c}
G- V	136.21 ± 12.71 ^{a,b}	39.14 ± 4.7 ^b	36.27 ± 3.27 ^b
G- VI	130.93 ± 13.71 ^b	38.17 ± 3.75 ^b	36.19 ± 2.27 ^b

Values are mean + SD of six samples in each group. a,b,c- significant at 5% level (p<0.05). Group comparison: a- GI vs GII, GIII, GIV, GV, GVI: b- GIII vs GIV, GV, GVI: c- GVI vs GIV, GV.

Glycoprotein components are involved in stabilizing the tissue by keeping up the structural stability of collagen fibrils in the myocardium. The appearance of an abnormal level of different proteins in the blood reflects cardio myocyte damage and help to distinguish myocardial necrosis (Alpert et al., 2000). A significant increase in glycoprotein levels in the serum of the ISO induced myocardial infarcted rats may be due to the secretion of glycol-conjugates from the cell membrane into the circulation which is a physiological change to a pathological process (Padmanabhan et al., 2008). Pretreatment with *Z. armatum* fruit brought about decreased levels of glycoprotein components and providing conformation on the membrane stabilizing effect of *Z. armatum* fruit.

Table 3 illustrates the levels of blood glucose, urea, uric acid, creatinine, serum iron and plasma iron binding capacity and the level of total protein and A/G ratio was decreased in ISO induced rats when compared to normal rats. Administration of hydroethanolic extract of *Z. armatum* fruit (200 and 400 mg/kg) and standard drug verapamil for 30 days to ISO induced rats significantly brought down the biochemical parameters. The level of TP and A/G ratio was increased in ISO induced rats when compared to ISO alone treated rats (Table 3). There was no significant difference between standard drug treated rats and *Z. armatum* fruit (400 mg/kg) treated rats. When comparing control rats and plant alone treated rats there was no significant difference.

Table 3: Effect of *Z. armatum* fruit on the levels of biochemical parameters in normal and ISO induced MI rats.

GROUPS	Blood glucose (mg/dl)	Blood urea (mg/dl)	Serum UA (mg/dl)	Creatinine (mg/dl)	Serum Iron (µg/dl)	Plasma Iron binding capacity (µg/dl)	TP (g/dl)	A/G ratio
G I	59.74 ±7.3	24.28 ±3.8	2.91 ±1.94	0.27 ±0.08	40.17 ±2.74	30.12 ±2.31	6.7 ±1.28	2.8 ±0.03
G II	60.21 ±3.02	25.11 ±3.14	2.84 ± 1.65	0.26 ± 0.02	41.43 ± 3.71	30.64 ± 1.75	6.81 ±0.09	2.9 ±0.05
G III	110.74 ±4.31 ^a	49.37 ± 3.37 ^a	6.75 ±0.89 ^a	1.19 ±0.03 ^a	74.42 ±4.23 ^a	12.16 ± 1.32 ^a	2.91 ±0.08 ^a	0.91 ±0.04 ^a
G IV	71.27 ±3.27 ^{a,b,c}	35.43 ±2.9 ^{a,b,c}	3.91 ±0.8 ^{a,b,c}	0.6 ±0.15 ^{a,b,c}	64.28 ± 1.78 ^{a,b,c}	21.74 ±2.3 ^{a,b,c}	5.43 ± 0.04 ^{a,b,c}	1.81 ±0.07 ^{a,b,c}
G V	64.59 ±1.28 ^{a,b}	26.2 ±01.9 ^{a,b}	2.5 ±0.14 ^b	0.31 ±0.04 ^b	49.21 ±4.59 ^{a,b}	29.24 ±1.05 ^{b,c}	6.13 ± 0.01 ^{a,b,c}	2.75 ±0.06 ^b
G VI	63.24 ±3.28 ^b	26.57 ±1.84 ^{a,b}	2.79 ±0.05 ^b	0.34 ±0.07 ^b	47.16 ±5.23 ^{a,b}	31.88 ±0.09 ^b	6.62 ± 0.03 ^{b,c}	2.94 ±0.01 ^b

Values are mean + SD of six samples in each group. a,b,c- significant at 5% level (p<0.05). Group comparison: a- GI vs GII, GIII, GIV, GV, GVI: b- GIII vs GIV, GV, GVI: c- GVI vs GIV, GV.

Blood glucose level is increased in ISO treated rats due to the generation of ROS and subsequent oxidative stress (Smith and Romijn, 2006). Urea is the final product of protein metabolism. Elevated levels of urea indicate a renal response to systemic hypoperfusion with respect to decreased cardiac output in decompensated heart failure (Cheesman and Slater, 1993). Rats have been induced of myocardial infarction by ISO resulted in increased blood

glucose and blood urea level, probably related to low cardiac output because of ventricular dysfunction (Kasper et al., 2015).

Uric acid is thought to be a marker of risk for CVD (Nadkar and Jain, 2008). The level of serum uric acid is increased in ISO induced rats. In hypoxia tissue, ATP depletion happens and the enzyme xanthine oxidase catalyzes the conversion of hypoxanthine to xanthine, uric acid and superoxide (Battelli et al., 2014; Hayden and Tyagi, 2004). This could be one of the reasons for the elevated level of serum uric acid in ISO induced rats. *Z. armatum* fruit has phenolic acids which could decrease hypoxia by regulating the cardiac rhythm and reducing oxidative damage.

Serum creatinine concentration significantly increased in view of direct leakage of the low molecular mass of creatinine from myocardial cells of the infarcted zone (Saka et al., 2012). A decreased level of serum total protein in ISO injected rats could be due to increased free radical production by ISO. Free radical generation and lipid peroxidation process are associated with ISO induced cardiac damage. ISO injection produces an excessive generation of free radicals (Pingitore et al., 2015; Gliozzi et al., 2015). This could be one of the reasons for the decreased level of serum total protein. The capacity of *Z. armatum* fruit to scavenge free radicals, and inhibit lipid peroxidation may help to increase serum protein level and A/G ratio in ISO induced myocardial infarcted rats.

Figure 1 demonstrates the levels of glycogen in heart tissue of normal and ISO induced rats. The level of glycogen in heart was diminished in the ISO induced rats when compared to normal rats. Pretreatment with the hydroethanolic extract of *Z. armatum* fruit for a period of 30 days significantly increased the activities of heart glycogen close to normal rats.

Glycogen is an essential source for the production of ATP. The observed decrease in glycogen of heart could be due to enhanced glycogenolysis and lipolysis. ISO administration followed by β receptor binding initiated phosphorylase kinase leading to glycogenolysis and lipolysis. This might be one of the reasons for the decreased level of heart glycogen in ISO induced rats (Lippi et al., 2013).

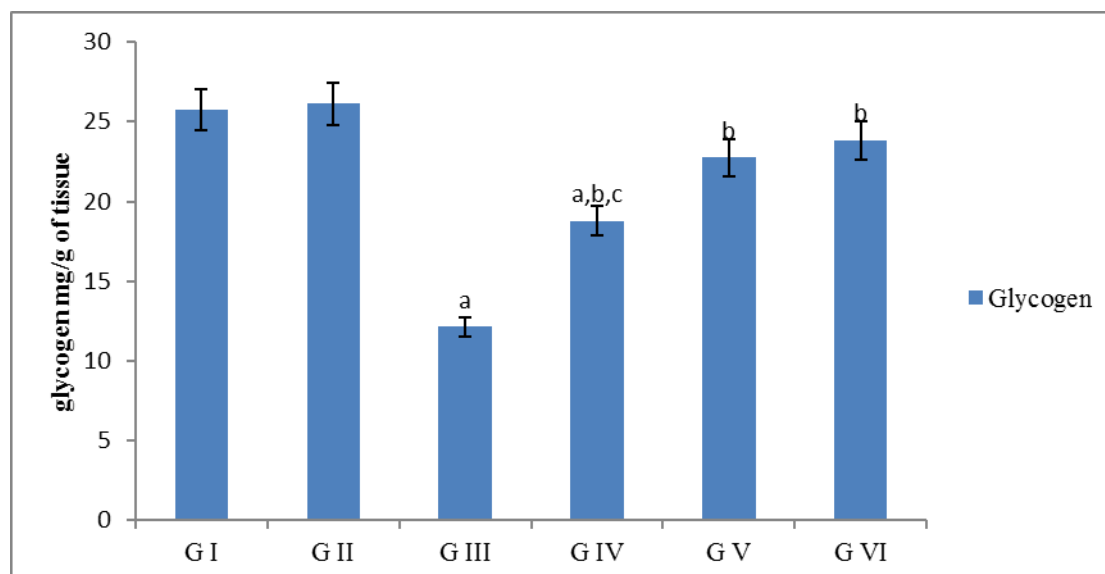


Figure 1: Effect of hydroethanolic extract of *Z. armatum* fruit on tissue glycogen levels in normal and ISO induced MI rats.

Values are mean + SD of six samples in each group. a,b,c- significant at 5% level ($p < 0.05$). Group comparison: a- GI vs GII, GIII, GIV, GV, GVI: b- GIII vs GIV, GV, GVI: c- GVI vs GIV, GV.

Table 4 shows the Na^+ , Ca^{2+} and K^+ ion levels in the serum of normal and ISO induced rats. The Na^+ and Ca^{2+} ion levels were increased significantly with subsequent decrease in K^+ ion level in ISO induced rats when compared to normal control rats. Pretreatment with hydroethanolic extract of *Z. armatum* fruit for a period of 30 days significantly decreased the levels of Na^+ and Ca^{2+} ions and significantly increased K^+ ion level in ISO induced rats when compared to ISO induced nontreated rats (Akhigbe et al., 2008). The changes in the levels of sodium and potassium in isoproterenol-induced rats may be attributable to loss of cellular integrity, inhibition of the Na^+/K^+ adenosine triphosphatase function as a result of energy depletion and changes in the ratio of intracellular-to-extracellular volume (Constantinides et al., 2001).

Table 4: Effect of *Z. armatum* fruit on the levels of electrolytes in serum of normal and ISO induced MI rats.

GROUPS	Na ⁺ (mMol/L)	K ⁺ (mMol/L)	Ca ²⁺ (mMol/L)
GROUP I	156.21 ± 1.7	5.97 ± 0.57	9.19 ± 1.08
GROUP II	154.21 ± 1.92	6.1 ± 1.04	9.27 ± 2.25
GROUP III	193.73 ± 9.31 ^a	3.24 ± 3.37 ^a	16.65 ± 3.79 ^a
GROUP IV	129.61 ± 3.28 ^{a,b,c}	5.09 ± 0.14 ^b	12.43 ± 1.38 ^{a,b,c}
GROUP V	153.79 ± 5.71 ^b	5.83 ± 1.28 ^b	10.43 ± 4.27 ^b
GROUP VI	150.97 ± 6.71 ^b	5.91 ± 2.08 ^b	9.71 ± 0.27 ^b

Values are mean + SD of six samples in each group. a,b,c- significant at 5% level (p<0.05). Group comparison: a- GI vs GII, GIII, GIV, GV, GVI: b- GIII vs GIV, GV, GVI: c- GVI vs GIV, GV.

CONCLUSION

From all the biochemical parameters studied, *Z. armatum* fruit of higher concentration (400 mg/kg) showed a significant effect than the lower dose (200mg/kg). These observations highlight that administration of *Z. armatum* fruit to ISO induced rats possesses significant cardioprotection by reducing the changes in the biochemical parameters. Hence, *Z. armatum* fruit is one of the useful phyto-nutrient for protecting the heart from necrosis during myocardial infarction.

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CONFLICT OF INTEREST

There is no conflict of interests.

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