

ORIGINAL RESEARCH ARTICLE

**Protective Role of *Terminalia arjuna* Seed Extract Formulations Against Cardiotoxicity Induced by Mercuric Chloride in Wister Rats**

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**ABSTRACT**

Among the toxicants, heavy metals are the major environmental pollution with especially mercury and its compounds, it is also induced cardiotoxicity in animal. After heavy metal intoxication, mercuric chloride being scaterly accumulated in blood cells and heart tissue. In the present experimental studies, the animals were divided into four groups each group consisting of six rats containing of 150-180gm range of body weight. Oral administration of sub-lethal dose of mercuric chloride (1.30mg/kg body weight of the animal) was given to the treated group animals daily for 45 days. Mercury chloride has promoted cardiac tissue damage and enhances the cardiac biomarker enzymes activity. The cardiac biomarker enzymes are significantly increased in the level of alkaline phasphatase (ALP), alanine transferase (ALT), aspartate transaminase (AST), creative phosphokinase (CPK), and lactate dehydrogenase (LDH) activities. And an enhanced level of serum total cholesterol (TC) and lipid peroxidase (LPO) content in heart tissue were also noticed in mercury intoxicated rat. In additional, a significant rice in R-R interval, P wave, PR interval, QRS complex and ST interval-segment alterations were also noticed in electrocardiogram (ECG) spectrum in mercury chloride intoxicated animals. The changes in biochemical, bio-enzymological and electrophysiological activities supporting the occurrence of cardiotoxicity in mercuric chloride intoxicated rats. During the recovery period, the administration of *T. arjuna* (TA) seed extract (5 mg/kg animal body weight daily) was dosed to mercuric chloride intoxicated animals for another 45 days. Treatment with TA significantly reduced the elevated serum biomarker enzyme levels and ECG spectrum fluctuations in mercury chloride induced cardio toxicity rats.

**Key words:** Mercuric chloride, Cardiac toxicity, *Terminalia arjuna*, ALP, ALT, AST, CPK, LDH, CPK, TC, LPO and ECG.

**INTRODUCTION**

Cardiovascular disease is the number one disease in human beings. It causes death mainly affected to south Asian country peoples. Mercury chloride is one of the heavy metal in environmental and industrial pollution <sup>[1]</sup>. Hence mercury and its compounds are widely used in industries and their hazards to animals have been well documented <sup>[2, 3, 4, 5, 6]</sup>. It causes cardio toxicity in animals when it has been intoxicated in chronic condition of exposure in heart tissue. Heavy metal exposure has been linked to increased incidence of cardiovascular diseases in animals <sup>[7, 8, 9]</sup>. Mercury and its compounds are still widely used in an industry like the production of parameters, thermometers, hydrometers, pyrometers, dry cell batteries, lamps, pesticides, plastics, caustic soda and other laboratory uses <sup>[10]</sup>. Recently, more

attention has been given to the toxic effect of mercury on the cardiovascular system and the association with hypertension, carotid atherosclerosis, myocardial infarction, and coronary heart disease <sup>[11]</sup>. However, mercury chloride accumulation and its toxicity have caused to cardiac dysfunction leading to heart failure during chronic period.

In India, the various types of herbal plants and their formulations are widely used in medicinal practices for the treatment of various diseases <sup>[12, 13]</sup>. Among the various herbal plants, TA is used as one of the medicinal plant in Ayurvedic system of medicine for querying the heart diseases. It ayurvedic formulations have been used for the treatment of cardiac disorders mainly in human beings <sup>[14, 15]</sup>. And *Terminalis arjuna* (TA) seed

extract as known to possess antioxidant activities, as they are rich in various antioxidant molecules such as tannins, arjunic acid, arjunolic acid, arjungenin, arjunoglycosides, flavonoids, ellagic acid, gallic acid and etc. Hence, the extract of TA seed formulations has been pharmacologically used for long years as a potential cardio protective agent [16]. Those herbal medicines having additional antioxidant properties may therefore, have protective role in cardiovascular disease or damages [17]. Ancient physician's used the powdered bark of TA for alleviating the cardiovascular disease and wound healing [18, 19]. But only few authors are used the seed extract against the cardiovascular diseases. With this point of view, the present experimental study has been designed to investigate the efficacy of TA seed extract on mercury intoxicated rat to prove its cardio protective potential by the way of conducting the biochemical, bioenzymological and ECG spectrum functional activities.

## MATERIALS AND METHODS

### Collection of plant material

The seed of TA was collected from the plant which is located in and around Chidambaram town, Cuddalore district, Tamilnadu, India.

### Preparation of extract

The collected TA seeds were cleaned and dried in shade at room temperature for 30 days. The dried seeds were powdered by a mechanical blender and passed through a 20-mesh sieve. The seed powder (500g) was successively extracted with Methanol solvent using a Soxhlet apparatus. The extraction was carried out for 24 hrs at room temperature with mild shaking. The extracts were filtered and powdered at 35°C, and the weight of the separated filterate or formulations was recorded and percentage yield was calculated. The separated formulations were used for this experimental study.

### Chemicals

Mercury chloride (HgCl<sub>2</sub>) and all chemicals of ALP, ALT, AST, CPK, LDH, and TC, were purchased from Hi-Media laboratories Private Ltd. Mumbai, India.

### Animal

Normal adult female rats, *Rattus norvegicus*, of the Wister strain weighing ranging from 160-180g were used in this experimental study. The selected animals were divided at random into four groups (each group consisting of six rats). And they were housed in polypropolined cage (6

rats/cage) and acclimatized to laboratory conditions for 2 weeks prior to the experimentation at constant temperature of 22 ± 3 °C, with 12-12 h light-dark cycle (8.00-20.00h light: 20.00-8.00 h dark) at a humidity of 50±10%. They were supplied with an adequate amount of rat feed (pellet diet) and water ad *libitum* regularly. The principle of laboratory animal care [20] was followed during the entire experimental work schedule and our University Ethics Committee also approved the experimental protocol (IAEC- No 816/2011) of RMMCH, Annamalai University.

### Experimental design

Twenty four healthy adult male Wistar strain rats were procured from the animal house of RMMCH, Annamalai University and divided into four groups on the basis of body weights of the animals. The treatment schedule of each group was as follows:

Group I	Control group	Animals were subjected to control groups' feed rat pellet diet and an adequate amount of potable water.
Group II	Mercuric-chloride treated group	Initially, rats were supplied with a normal diet and adequate amount of water for the entire experimental period and along with this the animals were subjected to forceful oral administration of Mercuric chloride (1.30 mg/kg body wt. of animal) orally administered for 45 days (as single dose/ day) of experimentation.
Group III	Mercuric-chloride followed by TA treated group	Initially, rats were supplied with a normal diet and adequate amount of water for the entire experimental period and along with this the animals were intoxicated with sub-lethal dose of mercuric chloride for 45 days. These rats were then dosed with TA (5mg/kg body wt. of animal) for another 45 days.
Group IV	TA alone administration group	Rats were supplied with a normal diet and adequate amount of water for the entire experimental period and along with this the animals subjected to oral administration of TA alone (5 mg/kg body wt. of the animals) for 45 days

### Measurement of ECG Spectrum analysis

At the end of experimental period the rats were anaesthetized with light anaesthetic using the intramuscular injection of ketamine hydrochloride (24 mg /kg body weight) and using the suitable recording system. ECG spectrum heart rates were measured through the computerized ECG instrument which is available in the Department of Biotechnology, SASTRA University, Tanjore, Tamilnadu, India.

### Animals sacrificed for serum and organ collection

The whole experimental design was continued for 90 days. After completing the experimental

schedule the animals were sacrificed and then their blood and heart tissue was collected. The blood sample was taken from the tail vein and serum was trapped and then used for various biochemical and bio-enzymatic analyses. Blood sample were collected in dry test tube and allowed to coagulation at an ambient temperature for 30 min. Serum was separated by centrifugation at 2000rpm for 10 min<sup>[21]</sup>. After collecting the blood from the animals they were sacrificed under light ether anesthesia with cervical dislocation and heart tissues were isolated from the animals in a cold room and it was used for biochemical assay.

### Bio-enzymological and Biochemical analysis

AST and ALT activity was assayed by using the diagnostic kit based on the method of Reitman and Frankel<sup>[22]</sup>. An alkaline phosphate was estimated by King and Armstrong method<sup>[23]</sup>. The activity of lactate dehydrogenase was assayed by the method of King<sup>[24]</sup>. Creatinephospho kinase (CPK) activity in serum was determined by the method of Okinaka *et al.*,<sup>[25]</sup>. The level of TC was estimated by the method of Allain *et al.*,<sup>[26]</sup>. The concentration of TBARS in the heart tissue was estimated by adopting the method of Nichans and Samuelsen<sup>[27]</sup>.

### Histopathological studies

At the end of the experimental study, all the rats were sacrificed by cervical decapitation and the hearts were dissected out, washed in ice cold saline. The histopathological observation of cardiac tissue was carried out through adopting the method of Gurr<sup>[28]</sup>.

### Statistical analysis

Values are given as mean  $\pm$  S.D. for six rats in each group. The data for various biochemical parameters were analyzed using analysis of 't'-test and the group means was compared by Duncan's multiple range test<sup>[29]</sup>. Values are considered statistically significant at 5% level of confidence limit ( $p < 0.05$ ).

## RESULTS

(Figure a-d) shows the electrocardiographic pattern of control and experimental animals ECG activities. Normal control rats showed a normal ECG spectrum pattern which is mentioned in figure a, where as animals treated with mercuric chloride alone showed significant elevation of P wave, QRS complex and R-R interval at the end of 45th day. In addition there was an increase in heart rate, prolongation of QT interval and cardiac cycles compared to normal control animals. During the recovery period, post treatment of TA administered rats exhibited near normal ECG spectrum pattern such as significant ( $p < 0.001$ ) restoration of P wave, QRS complex and R-R interval, whereas heart rate, QT interval and cardiac cycle were maintained near to normal values. The data of the experimental animals such as P wave, QRS complex, QT interval, R-R interval, heart rate and cardiac cycle are shown in (Table-1).

### Effect of Mercuric chloride and followed by TA on serum marker enzymes

At sub-lethal dose of mercuric chloride intoxicated rats exhibited significantly enhanced the levels of serum marker enzymes AST, ALT, ALP, LDH, TC, CPK and LPO activity compared to control rats. It indicates that the mercury toxicity can promote the cellular injury in the target organs. The post-treatment of TA for another 45 days reversed the bio-enzymological activities near to normal level. The TA alone administrated no changes compared to normal level (Tables 2 & 3).

### Heart tissue on histopathology

Figure - (A to D) illustrates the histopathological assessments of different cardiac segments of experimental animals. Mercuric chloride treatment caused the disorganization of normal radiating pattern of cell plates in the heart tissue. During the recovery treatment TA before mercuric chloride intoxication reduced such changes and kept the organ almost similar to that of normal heart tissue.

**Table 1: Productive effect of Terminalia arjuna seed extract patterns mercuric chloride induced cardiotoxicity in intoxicated rats**

Treatment	Heart rate R-R interval (sec)	P wave (mV)	PR interval (sec)	QRS complex (sec)	QT interval (sec)
Control	63.16 $\pm$ 1.27	0.010 $\pm$ 0.003	0.010 $\pm$ 0.002	0.072 $\pm$ 0.002	0.100 $\pm$ 0.005
HgCl <sub>2</sub>	57.70 $\pm$ 0.86	0.020 $\pm$ 0.007	0.020 $\pm$ 0.007	0.048 $\pm$ 0.007	0.156 $\pm$ 0.008
HgCl <sub>2</sub> +TA	72.12 $\pm$ 0.54	0.008 $\pm$ 0.002	0.010 $\pm$ 0.003	0.040 $\pm$ 0.004	0.072 $\pm$ 0.007
TA	75.00 $\pm$ 0.21	0.030 $\pm$ 0.004	0.004 $\pm$ 0.004	0.032 $\pm$ 0.002	0.080 $\pm$ 0.005

**Table 2: Level of alanine transferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in the serum and lipid peroxidates (LPO) content of rat treated with mercuric chloride followed by TA seed extract treatment**

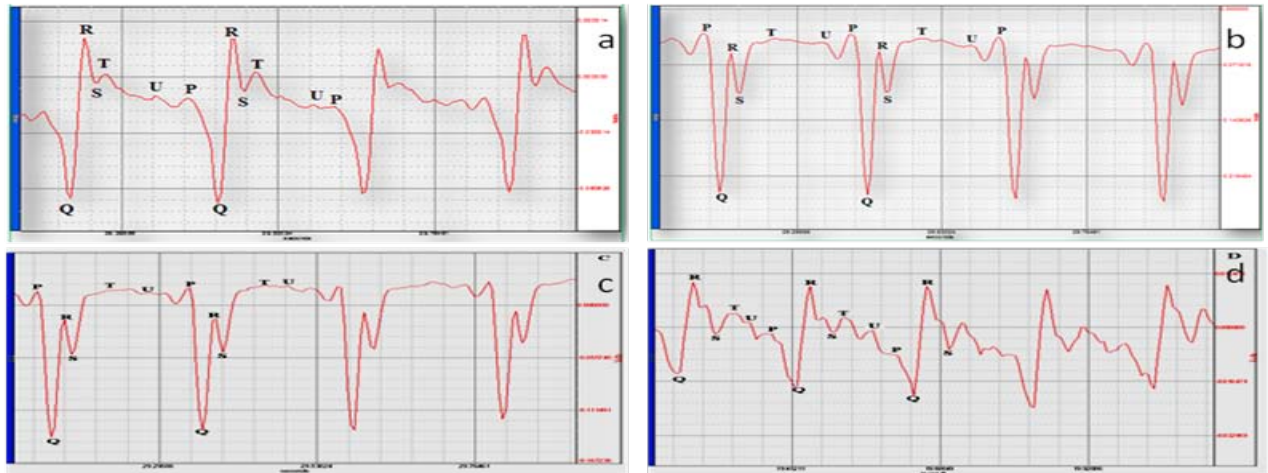
Organs	Control	HgCl <sub>2</sub> + treated	HgCl <sub>2</sub> + extract (TA)	Extract alone (TA)
ALT(U/L)	33.50 $\pm$ 2.89	73.58 $\pm$ 2.66	40.73 $\pm$ 2.27	30.72 $\pm$ 2.31
AST(U/L)	43.11 $\pm$ 2.80	138.25 $\pm$ 1.84	64.55 $\pm$ 2.34	41.62 $\pm$ 2.22
ALP(U/L)	251.44 $\pm$ 2.95	310.39 $\pm$ 2.29	276.15 $\pm$ 2.64	248.91 $\pm$ 2.75
LPO(mole of DA/mg)	0.72 $\pm$ 0.02	2.88 $\pm$ 0.10	1.65 $\pm$ 0.65	0.71 $\pm$ 1.70

Mean  $\pm$  S.D. of six individual observations, Significance ( $P < 0.05$ ) Group I compared with group II Significance ( $P < 0.05$ ) Group II compared with group III.

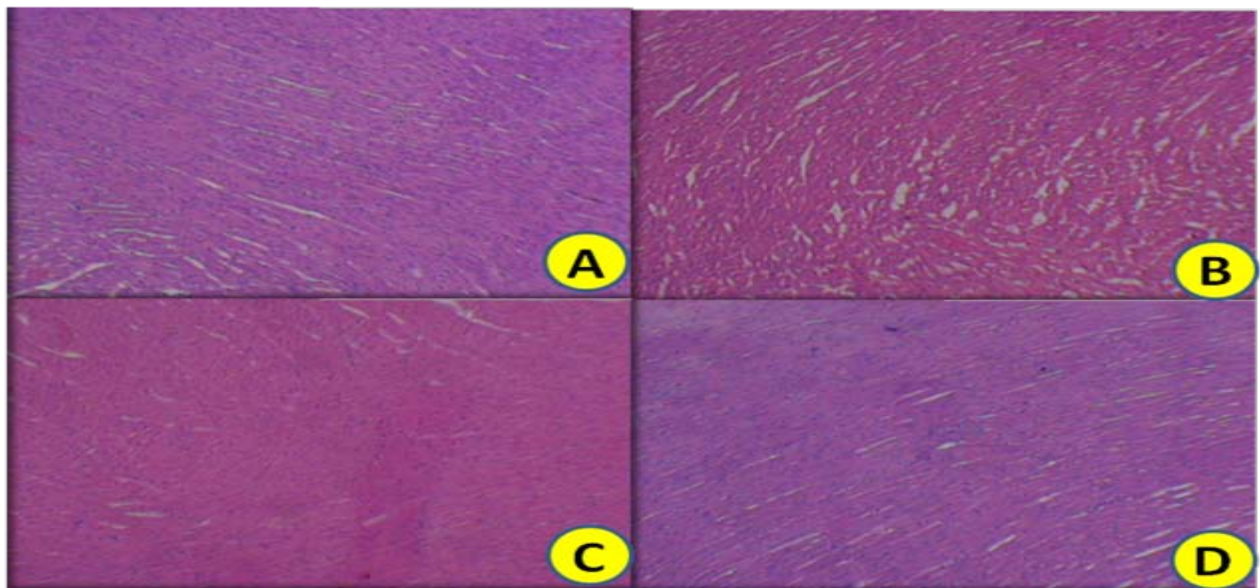
**Table 3: Level of lactate dehydrogenase (LDH), creative phosphokinase (CPK) and total cholesterol (TC) in the serum of rat treated with mercuric chloride followed by TA seed extract treatment**

Organs	Control	HgCl <sub>2</sub> + treated	HgCl <sub>2</sub> + Extract (TA)	Extract alone (TA)
LDH (U/I)	56.27 $\pm$ 3.38	183.33 $\pm$ 3.78	67.15 $\pm$ 3.20	52.46 $\pm$ 3.08
CPK (IU/L <sup>-1</sup> )	129.40 $\pm$ 2.63	203.22 $\pm$ 2.96	138.00 $\pm$ 2.20	125.62 $\pm$ 2.10
TC (U/I)	75.93 $\pm$ 2.80	141.00 $\pm$ 2.53	73.31 $\pm$ 1.91	72.51 $\pm$ 2.13

Mean  $\pm$  S.D. of six individual observations, Significance ( $P < 0.05$ ) Group I compared with group II Significance ( $P < 0.05$ ) Group II compared with group III.



**Fig 1: (a-d) shows the alteration of ECG spectrum patterns of control (a), mercuric chloride (b), mercuric chloride followed by TA (c), and alone TA (d)**



**Fig 2: Shows the alteration of histopathology patterns of control (A), mercuric chloride (B), mercuric chloride followed by TA (C), and alone (D)**

## DISCUSSION

The heart is made up of a specialized muscle. It is very helpful to make the heart that contracts regularly and continuously pumping blood to the entire body. The pumping action of the heart is mainly depending upon by a flow of electricity through the heart muscles that repeats itself in a cycle. If this electrical activity is disrupted by toxicants it can affect the heart's ability to pump the blood properly to the various organs. The electrical activity of the heart can be detected with

the help of 'electrocardiogram' (also called an ECG). A death is described as sudden when it occurs unexpectedly, spontaneously and/or even dramatically.

Most sudden deaths are due to a heart condition and are then called sudden cardiac death (SCD). The actual mechanism of death is most commonly a serious disturbance of the heart's rhythm known as a 'ventricular arrhythmia' (a disturbance in the heart rhythm in the ventricles) or 'ventricular

tachycardia' (a rapid heart rate in the ventricles). This can disrupt the ability of the ventricles to pump blood effectively to the body and can cause a loss of all blood pressure. This is known as a cardiac arrest. If this problem is not resolved in about two minutes, and if no-one is available to begin resuscitation, the brain and heart become significantly damaged and death follows quickly. Heart disease is the most common cause of an unexpected sudden death in all age groups. Xenobiotic chemicals are also causes cardiotoxicity in animals. Among these heavy metals are also promoting the cardio-toxicity especially mercury and its compounds.

In the present experimental studies, the levels of cardiotoxicity enzymatic indices were drastically increased in the serum of rat when dosed with mercuric chloride for 45 days. The histoarchitecture of the heart tissue of mercury intoxicated rat was completely damaged and causes the leakage of enhanced level of bio-marker enzymes. At sub-lethal dose of mercuric chloride induced myocardial infarction, the release of cellular cardiac enzymes is correlated with changes in plasma membrane integrity and/or permeability. This might be due to damage in cardiac tissue which is rendering it leakage<sup>[30]</sup>. This hypothesis is supported by the results of our study, as we have observed a significant increase in serum level of cardiotoxicity enzymatic indices (ALP, ALT, AST, CPK, LDH, and TC) after mercuric chloride treatment. These effects could be secondary events following mercury-induced lipid peroxidation (LPO) of cardiac membranes, with a consequent increase in enzyme leakage from cardiac muscle cells. The similar types of results were also observed by Nasa et al., (1997)<sup>[31]</sup> in rats when treated with isoproterenol. And they are suggested that due to the chemical treatment the cardiac myocardial cell are damaged. In the present experimental study, the cell membrane become permeable due to the mercury toxicity, which result in the leakage of marker enzymes alanine transaminase (ALT), aspartate aminotransferase,(AST), and lactate dehydrogenase(LDH), creative phosphokinase (CPK) into blood, this accounts for the increased activity of these enzyme in mercuric induced myocardial ischemic rats. Normally if the myocardial membrane becomes permeable or may rupture, due to stress, thereby resulting in the leakage of these enzymes into the blood stream thus increases their concentration in the serum<sup>[32]</sup>.

During the recovery period, treatment of administration of *Terminalia arjuna* (TA) seed extract on mercuric intoxicated rat showed significantly reduction in elevated serum cardio marker enzyme activities. Hence, the post treatment of TA on mercuric intoxicated animals the increased levels of serum marker enzymes were decreased to reach near to normal level of control. This reduction in enzyme levels could be due to its action on maintaining membrane integrity thereby restricting the leakage of these enzymes from the cardiac tissue of mercury intoxicated rats. In the present study, we found that TA protected myocardium from mercuric chloride induced myocardial functional and structural injury through restoration of marker enzymes. Similar type of results reported by number of authors in animals induced myocardial toxicity with the help of ISO treatment and withdrawal effect of myocardial toxicity with the help of N-acetylcysteine, S-allylcysteine and  $\alpha$ -tocopherol resulted in stabilization of the cardiac mitochondrial membrane and lysosomal enzymes<sup>[33, 34]</sup>.

*Terminalia arjuna* (TA) seed extract formulations prevented the increase in serum ALP, ALT, AST, CPK, LDH, TC and LPO content induced by mercuric chloride. Our bioenzymological and biochemical findings were supported by the improvement in histological architecture of heart tissues in TA seed extract formulation treated group (Fig. A, B, C, D), suggesting that the TA seed formulations may have a potential protective effect against mercuric chloride-induced cardiac damage. In this context, it has been reported that the oral administration of TA significantly decreased mercuric chloride -induced cardiac injury, as evidenced by significant reductions in serum cardiotoxicity enzymatic activities. Similar type of results were also observed in doxorubicin and cyclophosphamide induced in cardiotoxicity in intoxicated rat when again treated with alpha-lipoic acid and dl- $\alpha$ -lipoic acid respectively<sup>[35, 36]</sup>. The protective effect of TA seed extract could be attributed to its antioxidant properties, which should reduce myocardial damage and consequently decrease the release of cardiac enzymes. Administration of TA after mercuric treatment in rats restored all the biochemical and bioenzymological parameters altered by this cardiac toxicity to near normal levels in the cardiac tissue. TA formulation alone treatment also decreased the activity of serum ALP, ALT,



AST, LDH, CPK TC, and LPO content maintained the level of cardioprotective role in animals. The TA seed extract formulations are a photochemical derived from dietary compound and beneficial activity for human health, because it possesses the strong antioxidant activities, and free radical scavenging activities.

The significant alteration of ECG spectrum patterns in mercury chloride administered rats as compared to normal control animal rats, the result finding were elevation of ST segment reduction in P waves, QT complex and decrease in heart rate. Moreover, ECG spectrum changes are an indicator of the severity of mercuric induced myocardial damage<sup>[37]</sup>. The present experimental data clearly supporting our biochemical, bio-enzymological and histopathological alterations promoted by mercury toxicity in intoxicated rat serum. The present results suggest that concomitant post-treatment with TA formulations restored the levels of cardiac marker enzymes and electrocardiogram patterns in mercury chloride induced cardio toxicity rats. Hence, we could conclude that correction and protection of the oxidative stress and cardiotoxicity in the experimental animals have been found by applying the TA seed extract formulations. Further complex studies are needed to fully characterize the responsible of TA seed extract and elucidate their possible mode of action and mechanism that is in progress.

### CONCLUSION

These findings might be rational to understand the beneficial effects TA on cardioprotection against myocardial injury. TA seed extract formulation was found to be most effective in the functional recovery of the heart and restoration of biochemical, bio-enzymological and electrophysiological alterations.

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