

## CARDIOPROTECTIVE EFFECT OF ZANTHOXYLUM AMERICANUM MILL. ON ISOPROTERENOL-INDUCED CARDIOTOXICITY IN ALBINO WISTAR RATS

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### ABSTRACT

The role of traditional medicine in the solution of health problems is invaluable on a global level. Medicinal plants continue to provide valuable therapeutic agents, both in modern and in traditional medicine. With the associated side effects of the modern medicine, traditional medicines are gaining importance and are now being studied to find the scientific basis of their therapeutic actions. Isoproterenol (ISO) induced cardiotoxicity serves as a well standardized model for studying certain physiological and pathological events i.e, changes in lipids, enzymes and hormones during the course

of heart toxicity. Recently, herbal drugs, which are non-toxic and naturally occurring, are gaining much significance in the treatment of many diseases. *Zanthoxylum americanum* is a warehouse of assorted bioactive constituents or phytochemicals which find ample use in the pharmaceutical industry. The aim of present study is to evaluate the cardioprotective effect of *Zanthoxylum americanum* against ISO-induced oxidative stress. Adding up to this heart markers, lipid profile, electrolytes and heart histopathology were examined to assess the protective effect of *Bark* in ISO-induced cardiotoxicity. Our results showed protective effect against ISO-induced hepatic injury. Further studies are needed to evaluate the identification of bioactive constituents from the *Zanthoxylum americanum* for its cardioprotective activity.

**KEYWORDS:** *Zanthoxylum americanum*, cardioprotective, isoproterenol, wistar rats.

### INTRODUCTION

The heart consists of many different cell types. Broadly, they can be classified as parenchymal cells and non-parenchymal cells (NPCs). The cells form the structural basis of

the heart and make up the majority of the mass of the heart (Godoy et al., 2013). The NPCs include other various cell types such as Kupffer cells, sinusoidal endothelial cells, stellate cells, periportal fibroblasts and hepatic dendritic cells. Isoproterenol (4-[1-hydroxy-2-(propan-2-ylamino)ethyl]benzene-1,2-diol) is a synthetic catecholamine and beta-adrenergic agonist which causes severe oxidative stress in resulting infarct like necrosis (Klahret al, 1973). ISO-induced cardiotoxicity also serves as a well standardized model for studying certain physiological and pathological events i.e., changes in lipids, enzymes and hormones during the course of toxicity. It also alters the membrane permeability, heart tissue integrity, Ca<sup>2+</sup> overload and insufficient oxygen utilization. The heart is particularly sensitive to oxygen and the integrity of the heart cell depends on an adequate oxygen supply. It has been reported to exhibit many metabolic and morphological aberrations in the heart tissue of experimental animals similar to that of human heart toxicity by a multiple step mechanism (Ganesan et al, 2007). The primary disturbance of ISO-induced toxicity has been reported to enhance adenylate cyclase activity resulting in increased cAMP formation, which in turn leads to increased lipid accumulation in the heart (Gøtzsche, 1983). It is also well known to generate free radicals and to stimulate lipid peroxidation in the cell damage, has received radical and terminate the chain reaction before vital molecules are damaged. The present study is designed to evaluate the alterations in the heart tissue lipid profile, marker enzymes, tissue electrolytes and histopathological studies. This revealed the protective role of *Zanthoxylum americanum* against ISO-induced cardiotoxicity in wistar rats.

## MATERIALS AND METHODS

**Chemicals:** ISO was purchased from Sigma-Aldrich (USA). All other chemicals were of analytical grade and were supplied by Sisco Research Laboratories (Mumbai, India).

All the rat heart tissues (kept in liquid nitrogen (-196<sup>0</sup> C) and maintained at the same temperature) were collected from Department of Biochemistry, S.K. University. Heart tissue were homogenized and used for the studies of heart markers, lipid profile and electrolytes.

1. Control rats
2. ISO administered (85mg/kgbw, s.c., on 39<sup>th</sup> & 40<sup>th</sup> day)
3. *Zanthoxylum americanum* treated rats (300mg/kgbw orally)
4. *Zanthoxylum americanum* pretreated + ISO administered rats

### 5. Standard Drug Administrated

Heart tissue homogenate cholesterol and triglycerides were estimated using Accurex enzymatic diagnostic kit and HDL was estimated by using Autozyme cholesterol diagnostic kit. The homogenate was centrifuged at 2500g and the clear supernatant solution was used for the estimation of heart tissue marker enzymes.

**Total Cholesterol (TC)**(Allain *et al*, 1974):Cholesterol standard and water blank were also treated in a similar manner. After incubation, absorbance was read at 510nm and values are expressed as mg/dL.

**Triglycerides (TG)**(Foosati *et al*, 1982):Triglyceride standard and water blank were also treated in a similar manner. After incubation, absorbance of the standard and heart tissue homogenate was read at 510nm against and values are expressed as mg/dL.

**High Density Lipoprotein cholesterol (HDL)**(Assmann, 1983):The colour developed was read at 510nm against a blank and a standard (50 mg%) was run simultaneously. Values were expressed in mg/dL.

### Very Low Density Lipoprotein (VLDL) and Low Density Lipoprotein (LDL)

VLDL & LDL were calculated using the Friedewald *et al* (1972) formula as follows.

$$\text{VLDL} = \text{TG}/5$$

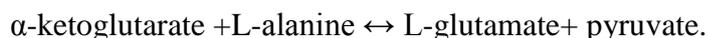
$$\text{LDL} = \text{Total Cholesterol} - \text{TG}/5 - \text{HDL}$$

**Aspartate aminotransferase (AST)**(Bayoumi *et al*, 1976).



The activity of glutamate oxaloacetate transaminase is expressed as IU/L (@ 340nm) of heart homogenate.

**Alanine aminotransferase(ALT)**(Bayoumi *et al*,1976)



The activity of glutamate oxaloacetate transaminase is expressed as IU/L (@ 340nm) of heart homogenate.

**Alkaline phosphatase (ALP):** This is based on the kinetic method and the absorbance was taken at 405nm` The activity of ALP was expressed as a U/L.

**Sodium:** Heart tissue homogenate sample was analyzed for sodium by magnesium uranyl acetate using the method of Trinder (1951).

**Potassium:** The heart tissue homogenate sample was analyzed for potassium by the method of Jacobs and Hoffmann (1931).

**Calcium:** Heart tissue homogenate calcium was estimated by OCPC method as described by Dycus and Lewis, (1957).

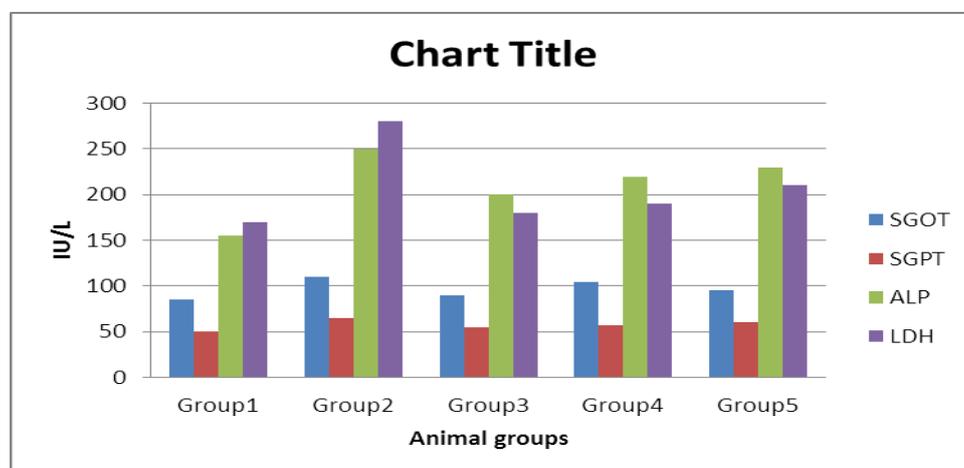
**Histopathological studies:** The heart tissues were cut in to small pieces and preserved in 10% buffer formalin for histomorphological examination (Raghuramulu *et al*, 1983).

**Statistical analysis:** All the results were expressed as mean  $\pm$  SD of a six individual observations. Duncan's Multiple Range (DMR) test was performed to know the level of significance among all the experimental groups.

## RESULTS AND DISCUSSION

### Effect of *Zanthoxylum Americanum* Extract on heart marker enzymes

AST, ALT and ALP are decreased in their activities in ISO administered rats when compared to control rats. Pretreatment with *Zanthoxylum americanum* showed significant increase in their activities when compared to ISO administered rats and were maintained near to normal levels. These observations are in agreement with earlier findings of Shreesh *et al*, 2008 (Fig. 1).

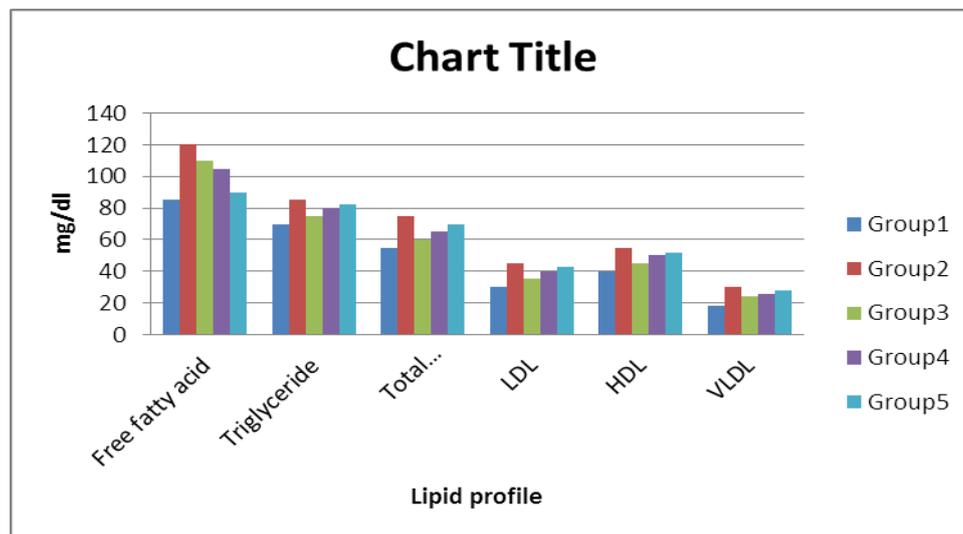


**Fig. 1: Effect of *Zanthoxylum Americanum* Extract On Heart Marker Enzymes.**

### Effect of Zanthoxylum Americanum Extract on heart tissue lipid profile

In heart tissue the levels of TG, TC, LDL and VLDL were significantly increased where as HDL decreased significantly in the ISO administered rats as compared to normal control rats.

Rats pretreated with Zanthoxylum Americanum Extract showed a significant decrease in the levels of heart tissue TG, TC, LDL and VLDL and a significance decrease in the level of HDL as compared to ISO administered rats (Libby et al, 2000) (Fig. 2).



**Fig 2: Effect of Zanthoxylum Americanum Extract on heart tissue lipid profile.**

### Effect of Zanthoxylum Americanum Extract on heart tissue electrolytes (Na, K+ & Ca<sup>2+</sup>)

ISO administered rats showed a significant increase in heart tissue levels of sodium and a significant decrease in potassium and calcium when compared to control rats. The rats pretreated with ZANTHOXYLUM AMERICANUM EXTRACT showed a significant decrease in sodium and a significant increase in potassium and calcium levels when compared to ISO administered rats. Electrolytes and minerals play a major role in metabolism, as well as most cellular activities. They hold fluids in compartments of the body and maintain normal acid-base balance (Damodara et al, 2007) (Fig. 3 & 4).

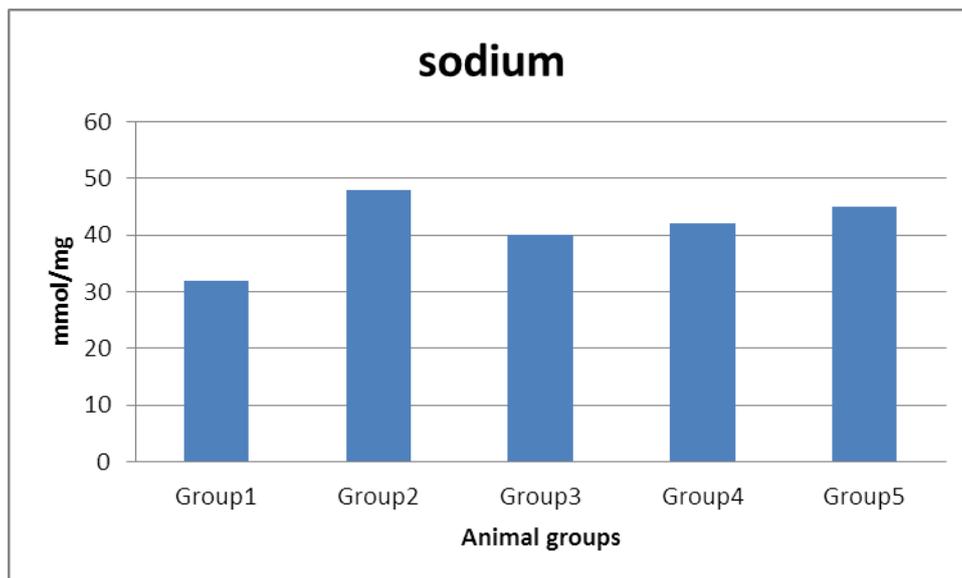


Fig. 3: Effect of *Zanthoxylum americanum* on sodium of control and experimental rats.

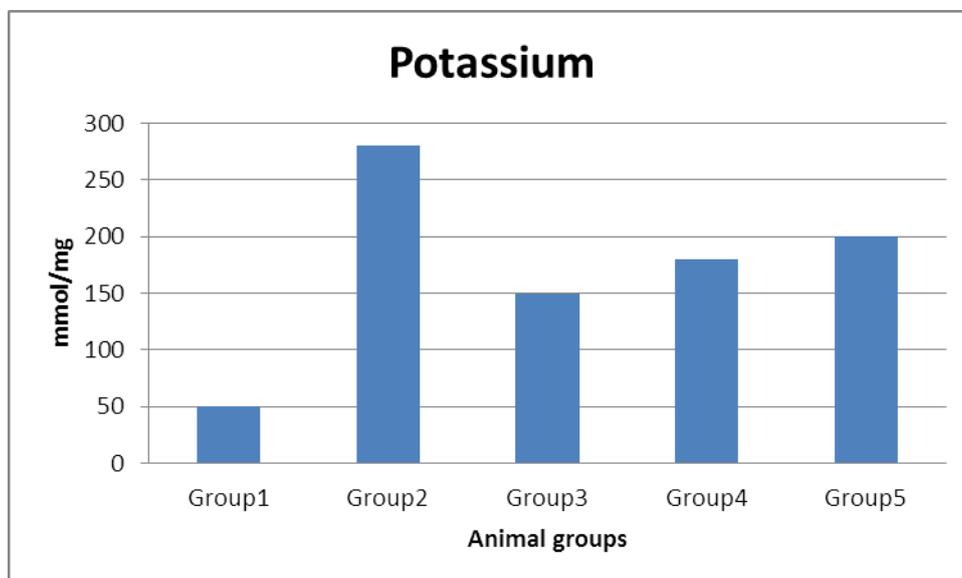
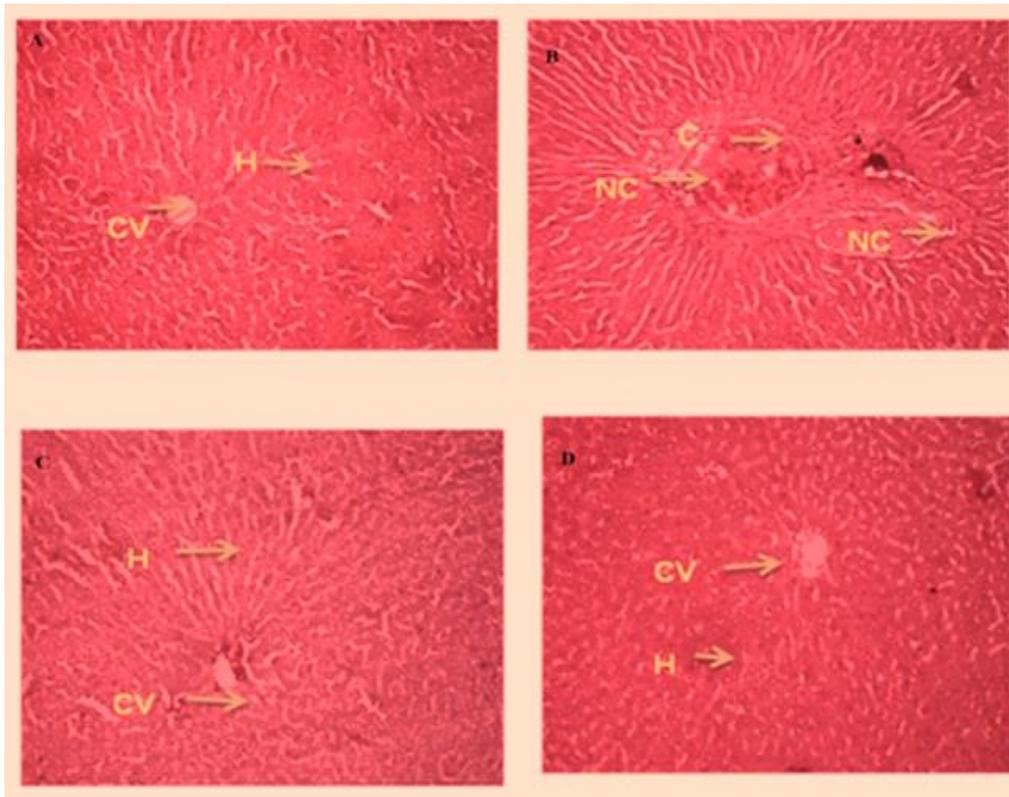


Fig. 4: Effect of *Zanthoxylum americanum* on potassium of control and experimental rats.

#### Histological studies of heart tissue of control and experimental rats

The histological observations of the heart sections are in agreement with the biochemical changes. Control rats showed normal architecture, *Zanthoxylum americanum* treated group showed near normal architecture with slight sinusoidal dilatations, ISO administered group showed inflammatory necrosis and fibrosis, pretreated showed near normal hepatic architecture. Restoration of normal architecture in pretreated group indicates the cardioprotective effect of *Zanthoxylum americanum*.



**A- Control group, B- ISO group (85 mg/kgbw), C- *Zanthoxylum americanum* + ISO (300 mg/kgbw), and D- ZANTHOXYLUM AMERICANUM EXTRACT treated.**

## CONCLUSION

The present study revealed the effects of ISO in ISO-induced cardiotoxicity on heart tissue and also protective effect of ethanol extract of *Zanthoxylum americanum* bark in these conditions in albino wistar rats. In order to evaluate the protective effect of ethanolic extract of *Zanthoxylum americanum* bark on marker enzymes, lipid profile, electrolytes such as  $K^+$ ,  $Ca^{2+}$  and  $Na^+$  and histology of heart tissue were studied. Hyperlipidemia is one of the major factors responsible for the occurrence of cardiotoxicity. It is postulated that ISO induces MI by its lipolytic action and increasing circulating lipids and lipoprotein levels as well as hepatic and cardiac lipids.

In the present studies also the TC, TG and lipoproteins such as VLDL and LDL were increased where as HDL was decreased. *Zanthoxylum americanum* pretreatment for a period of 40 days significantly ameliorated these lipid levels, which might be due to the presence of saponins, flavonoids, terpenes and glycosides. Rats pretreated with *Zanthoxylum Americanum* Extract exhibited decrease in the positive inotropic effect induced by ISO administration. *Zanthoxylum americanum* increased heart tissue  $K^+$  and  $Ca^{2+}$  levels and reduced  $Na^+$  levels

compared to ISO administered rats. This could be due to presence of alkaloids, saponins and Ca<sup>2+</sup> antagonist in Zanthoxylum Americanum Extract. Decreased levels of heart tissue marker enzyme in ISO induced cardiotoxicity can be attributed to the damage of heart structural integrity, because these are cytoplasmic in location and are released into circulation after cellular damage. Even mild changes in their levels may have led to the leakage of these enzymes from heart tissue into the blood stream, but Zanthoxylum Americanum Extract administration might have minimized the effect of ISO and would have prevented the damage, there by maintaining the values at near normal in pretreated rats when compared with ISO administered rats. ALT (in cytoplasm) and AST (in cell cytoplasm and mitochondria) occur in much higher concentration in heart than elsewhere and consequently their decrease reflects hepatic damage more specifically.

Zanthoxylum Americanum Extract pretreated rats showed near and normal architecture where as ISO administered rats showed necrotic architecture compared to control rats. ISO administered rats showed sinusoids adjacent to the terminal hepatic veins are dilated, and cardiocytes show nuclear vacuolation. In the case of extract pretreated rats near-normal hepatic architecture is seen, which is attributed to protective effect of Zanthoxylum Americanum Extract. The above mentioned cardioprotective effects of Zanthoxylum Americanum Extract are mainly attributed to its phytochemical constituents and active principles.

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