

**EVALUATION OF CONCOMITANT TREATMENT OF SIMVASTATIN AND ZINGIBER OFFICINALE IN DOXORUBICIN INDUCED CARDIOTOXICITY IN WISTAR RATS**

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**ABSTRACT**

The present study was aimed to evaluate the combined effects of simvastatin (SIM) and ethanol extract of *Zingiber officinale* (ZO) in doxorubicin (DOX) induced cardiotoxicity in Wistar rats. DOX 10 mg/kg i.p single dose to causes cardiac damage and increases the levels of cardiac biomarker enzymes viz. ALT, AST, LDH and CKMB. In addition, a significant rise in HR, ST- segment and alterations in ECG patterns were observed in DOX treated group. SIM (1.8 mg/kg & 3.6 mg/kg) and ZO (200 mg/kg & 400 mg/kg) alone and in combination were given to rats as pretreatment for 30 days orally. Pretreatment with SIM and ZO alone significantly ( $P < 0.001$ ) reduced the elevated serum biomarker enzyme levels and ECG alterations in DOX induced cardiotoxic rats. But, combined pretreatment with SIM and ZO normalized the biochemical parameters and ECG changes in DOX induced cardiotoxic rats. The result obtained from the present study indicates concomitant pretreatment of SIM and ZO showed significant improvement than single treatment.

**KEY WORDS:** Cardiotoxicity, Simvastatin, *Zingiber officinale*, Doxorubicin.

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**INTRODUCTION**

Although clinical care is improved, public awareness is raised and health innovations were widely used. Cardiotoxicity remains the leading cause of death worldwide<sup>1</sup>. Cardiotoxicity is the acute condition of cardiac muscle damage inhibiting the normal function of the heart that occurs as a result of an overabundance of hydroxyl radicals (OH<sup>•</sup>) leads to oxidative stress causing death of cardiac muscle cells. More serious cases can result in congestive heart failure (CHF), heart attack, or death<sup>2</sup>.

Doxorubicin (DOX) is a broad spectrum anti-tumor antibiotic used to treat cancer patients. However, the potential usefulness of this drug is currently limited by the development of a dose-dependent cardiomyopathic process terminating in severe heart failure<sup>3</sup>. Although several mechanisms have been suggested to explain the pathogenesis of DOX-induced cardiotoxicity, hydroxyl radical induced oxidative stress appears to play an important role. Due to the presence of less developed antioxidant defence mechanism in heart, particularly

vulnerable to injury by anthracycline-induced reactive oxygen species<sup>4</sup>.

Many plant species and their constituents are used in Indian Ayurvedic Medicine for the treatment of cardiotoxicity<sup>5</sup>. The prophylactic and therapeutic effect of many herbal extracts, such as *Ocimum sanctum*<sup>6</sup>, *Zingiber officinale*<sup>7</sup>, *Allium sativum*<sup>8</sup> etc., have been reported in reducing cardiovascular disorders<sup>9</sup>. In traditional system *Zingiber officinale* (ZO) is used as cardiostimulant, anti-pyretic & anti-bacterial agent. The British herbal compendium reported its actions as carminative, anti-emetic, peripheral circulatory stimulant, anti-inflammatory<sup>10</sup> & hypolipidemic agent<sup>7</sup>. The rhizomes of ZO possess pungent principles (oleoresins) such as "gingerol", "zingerone" and "shogaol" are responsible for antioxidant and anti-platelet activity<sup>7</sup>. ZO rhizomes also consist of vit.C<sup>11</sup>, which is responsible for antioxidant activity. Previous studies on ZO provide clear evidence that the ZO ethanol extract pre-treatment enhances the antioxidant defence in

myocardial damage and exhibits cardioprotective property<sup>7</sup>.

Statins like simvastatin, a widely used group of hypocholesteremic drug, has shown a beneficial effect in reducing cardiovascular related morbidity and mortality in patients with or without coronary artery disease and with or without high cholesterol levels<sup>22</sup>. The primary mechanism of action as cardioprotective effect is likely through its effectiveness in inhibiting lipid peroxidation, preservation of antioxidant enzymes as well as scavenging of free radicals<sup>4</sup>. Along with therapeutic effects statins also known to possess dose dependent side effects like liver damage, rhabdomyolysis and kidney failure<sup>17</sup>.

It could be beneficial to use herbal supplements concurrently with conventional medicines to exhibit augmented protective efficacy than a single drug. The aim of present study is to explore whether concomitant pretreatment with simvastatin and ZO exerts better effect than simvastatin and ZO alone.

## MATERIALS AND METHODS

### Collection of plant material

The ZO plant rhizome was collected from the local market of Belgaum, Karnataka, India. Identified and authenticated by Dr. Harsha Taxonomist, Regional Medical Research Centre (ICMR), Belgaum, where the herbarium of the specimen is deposited (voucher no. RMRC-523).

### Preparation of extract

The fresh rhizomes of *Zingiber officinale* R. were peeled, chopped into tiny bits, air dried and ground with a mechanical grinder. The grounded material (500gm) was macerated in absolute ethanol for 72 hrs. The filtrate was evaporated under vacuum drier and residue obtained was stored at 4°C for further experimental study.

### Chemicals

Simvastatin was a generous gift from Get Well pharmaceuticals, India. Doxorubicin hydrochloride injection (ADRIIM, mfg. by Fresenius kabi, Dabur).

### Experimental animals

Healthy male Wistar rats weighing between 150-200 gm were procured from Sri Venkateswara enterprises Bangalore. They were housed in standard laboratory condition at room temperature along with 12 h light/dark cycle. The animals were provided with standard pelleted diet obtained commercially from the manufacturer (Amrut Laboratories, Sangli) and water ad libitum. After seven days of acclimatization period, they were randomly selected for different experimental groups. Ethical clearance was obtained from Institutional Animal Ethics Committee.

### Dose selection

i) Human dose of simvastatin 20 mg/kg and 40 mg/kg were converted into animal dose 1.8 mg/kg & 3.6 mg/kg respectively<sup>20</sup> and administered orally for 30 days.

ii) ZO at 2 different doses 200 mg/kg<sup>7</sup> and 400 mg/kg dissolved in 1% Tween 80 in normal saline and administered orally for 30 days.

iii) At the end of the treatment period, animals of all the groups excluding group I were administered with DOX (10mg/kg, single dose i.p).

### Experimental protocol

Male Wistar rats were divided into eight groups of six each.

Group I (Normal control) - Normal saline (5 ml/kg b.w).

Group II (Disease control) - Normal saline + DOX.

Group III - 1.8 mg/kg b.w of Simvastatin + DOX.

Group IV - 3.6 mg/kg b.w of Simvastatin + DOX.

Group V - 200 mg/kg b.w of ZO + DOX.

Group VI - 400 mg/kg b.w of ZO + DOX.

Group VII - 1.8 mg/kg b.w of Simvastatin + 400 mg/kg b.w of ZO + DOX.

Group VIII - 3.6 mg/kg b.w of Simvastatin + 200 mg/kg b.w of ZO + DOX.

### Biochemical analysis

After 72 hrs of the DOX injection animals were anaesthetized with Thiopentone sodium (50 mg/kg). The blood was collected from by puncturing retro-orbital sinus. Serum was separated by centrifugation. Serum Creatine kinase isoenzyme (CKMB), Lactate dehydrogenase (LDH), Serum alanine aminotransferase (ALT) and Serum aspartate aminotransferase (AST) were measured kinetically at 340 nm according to standard methods by using commercially available diagnostic kits from ERBA, Germany.

### Examination of electrocardiogram (ECG)

Cardio physiology parameters were estimated by using ECG. 72 hrs after DOX injection Lead II ECGs of all animals were recorded using Biopac Student Lab PRO 3.7 software (model no. MP-35) make BIOPAC systems, Inc.42 Aero Camino, Goleta, CA93117. For each ECG tracing QT interval, QRS complex and heart rate were measured.

### Statistical analysis

Results were expressed as Mean  $\pm$  S.E.M. the statistical significance of any difference in each parameter among the groups was evaluated by one-way ANOVA, using Dunnett's multiple comparison test as post hoc test.

### RESULTS

Acute administration of DOX (10 mg/kg i.p single dose) induces cardiotoxicity and showed significant increase in the levels of serum cardio biomarker enzymes viz., LDH,

CKMB, ALT, AST when compared to normal rats ( $P < 0.0001$ ). The increased concentration of serum enzymes is a well accepted quantitative index of myocardial damage caused by DOX treatment. Pretreatment with SIM and ZO alone significantly ( $P < 0.001$ ) reduced the elevated serum enzyme levels when compared to DOX treated rats. But, concomitant pretreatment with SIM and ZO normalized the activities of these enzymes in DOX induced cardiotoxic rats. (**Table 1**)

### ECG alterations

DOX treated group showed significant changes in the repolarization phase of the ECG: Significant prolongation of QT interval and elevation of ST segment, with no significant effect on QRS complex as compared to normal group. In addition a significant ( $P < 0.0001$ ) increase in heart rate of DOX treated rats was observed as compared to normal group. Pretreatment with SIM and ZO alone significantly reduced the ECG alterations when compared to DOX induced cardiotoxic rats. Concomitant pretreatment with SIM and ZO significantly normalized these changes when compared to DOX induced group. Hence SIM and ZO combined pretreatment significantly restored the changes in ECG parameters induced by DOX. (**Table 2**)

For all the biochemical and ECG parameters studied, pretreatment with SIM (1.8 mg/kg & 3.6 mg/kg) alone and ZO (200 mg/kg & 400 mg/kg) alone showed significant decrease in all the parameters compared to DOX treated rats. But, the normalization of all the above parameters was observed in combined (ZO & SIM) pretreated rats.

### DISCUSSION

DOX induced myocardial damage have been well established in patients and in experimental animal models<sup>15</sup>. In the present study, development of oxidative cardiac injury due to single dose (10mg/kg) of DOX was confirmed by the significant increase in serum cardiac biomarker enzymes such as ALT, AST, LDH and CK-MB and alterations in ECG. The existing experimental evidence suggests that DOX oxidative stress is due to the generation of hydroxyl radicals in the heart tissue<sup>12</sup>. The generated reactive oxygen species such as superoxide radicals and hydroxyl radicals are potential to cause damage to various intracellular components<sup>13</sup>. Heart tissue is particularly susceptible to free radical injury. Further, DOX also has high affinity for the phospholipid component of mitochondrial membrane in cardio myocyte, leading to accumulation of DOX in the heart tissue<sup>14</sup>. It is well known that anti oxidants could attenuate DOX induced cardiotoxicity by eliminating free radicals<sup>16</sup>.

The previous studies on ZO provide a clear evidence that the pretreatment enhances the anti oxidant defense in myocardial damage and exhibits cardioprotective property<sup>7</sup> and it also has been proved that statins (SIM) provides protection from DOX induced cardiac damage. The mechanism of simvastatin involves 'inhibition of lipid peroxidation, tissue fibrosis, preservation of anti oxidant enzymes and scavenging of free radicals'<sup>21</sup>.

A significant reduction in the serum biomarker enzymes were observed in the myocardial damaged rats pretreated with SIM and ZO alone. But, the normalization of marker enzymes and ECG pattern were observed in concomitant (ZO & SIM) pre-treated rats.

It is observed that combined pretreatment with SIM and ZO showed the highest significant effect than SIM and ZO alone treatment. It is also observed that both the combination pretreatments (SIM-1.8 mg/kg + ZO-400 mg/kg) and (SIM-3.6 mg/kg + ZO-200 mg/kg) showed dose dependent protective action against DOX treatment. Concomitant treatment could be a beneficial therapy i.e. of increase dose of ZO with lower dose of SIM (400 mg/kg b.w + 1.8 mg/kg) than a combination of lower amount of ZO with higher amount of SIM (200 mg/kg b.w + 3.6 mg/kg) in order to prevent the dose dependent side effects of statins like liver damage<sup>18</sup> and rhabdomyolysis<sup>19</sup>.

### CONCLUSION

The present results suggest that concomitant pretreatment with SIM and ZO restored the levels of cardiac marker enzymes and ECG pattern in DOX induced cardiotoxic rats. The advantages of combination therapy of statins with ZO are the enhanced cardio protective effect, reduced dosage of statins and decreased risk of adverse effects. In this study we demonstrated that concomitant treatment of SIM and ZO significantly ameliorated DOX induced cardiotoxicity in rats as compared to SIM and ZO alone.

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Table 1: Effect of various treatments on serum biological marker enzymes

GROUPS	ALT (IU/L)	AST (IU/L)	LDH (IU/L)	CK-MB (IU/L)
I-Normal control	22.37±1.194	31.05±0.77	109.3±2.87	82.49±1.87
II-Disease control	54.36±2.573 <sup>###</sup>	96.59±2.26 <sup>###</sup>	218.8±4.58 <sup>###</sup>	256.5±4.13 <sup>###</sup>
III-(SIM-1.8mg/kg + DOX)	40.40±2.18*	52.05±2.23 <sup>***</sup>	140.1±2.15 <sup>***</sup>	119.2±4.44 <sup>***</sup>
IV-(SIM-3.6mg/kg + DOX)	36.92±2.56 <sup>**</sup>	57.29±2.48 <sup>***</sup>	121.08±2.95 <sup>***</sup>	96.47±2.82 <sup>***</sup>
V-(ZO-200mg/kg + DOX)	32.35±1.29 <sup>***</sup>	36.51±1.41 <sup>***</sup>	129.4±3.78 <sup>***</sup>	109.2±2.51 <sup>***</sup>
VI-(ZO-400mg/kg + DOX)	27.31±1.61 <sup>***</sup>	34.29±1.55 <sup>***</sup>	123.4±2.68 <sup>***</sup>	94.27±2.4 <sup>***</sup>
VII-(SIM-1.8mg/kg + ZO-400mg/kg + DOX)	25.21±1.25 <sup>***</sup>	31.55±1.40 <sup>***</sup>	115.4±3.49 <sup>***</sup>	88.22±2.25 <sup>***</sup>
VII-(SIM-3.6mg/kg + ZO-200mg/kg + DOX)	27.29±2.18 <sup>***</sup>	34.44±1.76 <sup>***</sup>	118.5±2.85 <sup>***</sup>	87.46±2.69 <sup>***</sup>

Compared with Normal control: # P&lt;0.01, ## P&lt;0.001, ### P&lt;0.0001.

Compared with Disease control: \*P&lt;0.01, \*\*P&lt;0.001, \*\*\*P&lt;0.0001.

**Table 2: Effect of various treatments on ECG parameters**

Groups	HEART RATE (BPM)	QRS COMPLEX (sec)	QT INTERVAL (sec)
I – Normal control	352.5±18.45	0.055±0.0018	0.090±0.0012
II – Disease control	420.7±14.97 <sup>###</sup>	0.048±0.0028 <sup>###</sup>	0.110±0.0012 <sup>###</sup>
III- (SIM 1.8mg/kg + DOX)	384.3±15.71 <sup>**</sup>	0.050±0.0012 <sup>*</sup>	0.095±0.0015 <sup>***</sup>
IV- (SIM-3.6mg/kg + DOX)	364.3±16.40 <sup>***</sup>	0.055±0.0028 <sup>*</sup>	0.090±0.0015 <sup>***</sup>
V- (ZO-200mg/kg + DOX)	374±15.41 <sup>***</sup>	0.048±0.0021 <sup>*</sup>	0.100±0.00015 <sup>**</sup>
VI- (ZO-400mg/kg + DOX)	363.2±16.03 <sup>***</sup>	0.050±0.0028 <sup>*</sup>	0.095±0.0015 <sup>***</sup>
VII- (SIM-1.8mg/kg + ZO-400mg/kg+DOX)	332.2±16.61 <sup>***</sup>	0.050±0.0015 <sup>*</sup>	0.095±0.0025 <sup>***</sup>
VIII-(SIM-3.6mg/kg+ ZO-200mg/kg+ DOX)	325.2±16.21 <sup>***</sup>	0.055±0.0028 <sup>*</sup>	0.094±0.0015 <sup>***</sup>

Compared with Normal control: # P<0.01, ## P<0.001, ### P<0.0001.  
 Compared with Disease control: \*P<0.01, \*\*P<0.001, \*\*\*P<0.0001.

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