

VALENCENE, A SESQUITERPENE EXHIBITS ANTI-LIPID PEROXIDATION AND ANTIOXIDANT EFFECTS IN THE PLASMA OF ISOPROTERENOL-INDUCED MYOCARDIAL INFARCTED RATS

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ABSTRACT

Aim: *The present study was aimed to evaluate the effects of valencene, a sesquiterpene on plasma lipid peroxidation and antioxidants in isoproterenol-induced myocardial infarcted rats.*

Methods: *Rats were induced myocardial infarction by isoproterenol (100 mg/kg body weight) on the 1st and 2nd day and then on the 4th-day valencene treatment was given to rats orally, daily, for 14 days.*

Results: *Isoproterenol-induced myocardial rats showed an increase in the levels of serum myoglobin, plasma thiobarbituric acid reactive substances, and lipid hydroperoxides and a significant decrease in the levels of plasma non-enzymatic antioxidants such as reduced glutathione, vitamin- C, and vitamin-E. Oral treatment with valencene (12 mg/kg/body weight) daily for 14 days significantly reduced serum myoglobin and plasma lipid peroxidation and significantly increased non-enzymatic antioxidants.*

Conclusion: *Thus this study reveals the anti-lipid peroxidation and antioxidant effects of valencene in isoproterenol-induced myocardial infarcted rats.*

Keywords: *Myocardial infarction, Valencene, Lipid peroxidation, Isoproterenol; Non-enzymatic antioxidants.*

1. INTRODUCTION

Myocardial infarction (MI) is a vital pathological feature resulting in high levels of mortality and morbidity. Hence, it attracts continuing attention from basic and clinical researchers, and practicing physicians. Reduction in mortality rate and prevention of MI is of utmost importance. A MI requires abrupt therapeutic consideration. Treatment aims to safeguard as much heart muscle as possible and to avoid further complications. MI is the acute condition of necrosis of the myocardium that occurs as a result of an imbalance between coronary blood supply and myocardial demand. Isoproterenol-induced MI is a simple and universally accepted model to examine the defensive function of many drugs [1, 2, 3]. This model of MI is the outcome of isoproterenol's severe inotropic and chronotropic effects. Further, following isoproterenol injection, reperfusion is feasible. Isoproterenol, an inducer of MI, in its overdose can cause severe stress in the myocardial tissue, thereby

making MI [4] and this model mimics human MI [5-7]. The myocardial injury caused by isoproterenol is due to excessive free radical production [8]. An imbalance between the oxidants and antioxidants is one of the main mechanisms of isoproterenol-induced MI [9]. An increase in oxidative stress in MI can cause suppression of endogenous antioxidants, development of heart failure, and augmented death risk [10]. Further, oxidative stress advances cardiomyocyte death and this is well documented in ischemic hearts [11]. In isoproterenol-induced myocardial infarcted rats, plasma lipid peroxidation (LPO) products such as thiobarbituric acid reactive substances and lipid hydroperoxides are augmented and plasma non-enzymatic antioxidants (vitamin-C, vitamin-E, and reduced glutathione) are lessened [3, 12]. In MI, LPO is increased and the antioxidant system is lessened.

Many synthetic drugs are used for the treatment of myocardial infarction. However, they cannot meet the demands due to side effects. Recently, there has been an upsurge of interest to investigate the cardioprotective potential of natural products. Natural products have lesser side effects than synthetic drugs. Sesquiterpenes are a class of terpenes having three isoprene units. Valencene, a sesquiterpene is a heavy citrus aroma component of citrus fruits and is present in grapefruit, nectarines, tangerines, and Valencia orange. It is a carbobicyclic compound and sesquiterpene that is 1, 2, 3, 4, 4a, 5, 6, 7-octa hydro naphthalene which is substituted by a prop-1-en-2-yl group at position 3 and by methyl groups at positions 4a and 5 (Fig.1). Valencene exhibits anti-septic, antioxidant, anti-allergic and anti-diabetic activities [13-15]. Therapeutic interventions which lessen oxidative stress may attenuate MI. Further, pharmacological enhancement of myocardial antioxidants can reduce increased oxidative stress. However, valencene has not yet been investigated to determine its cardioprotective effects. Therefore, this study was conducted to assess the effects of valencene on plasma LPO products and non-enzymatic antioxidants in isoproterenol-induced myocardial infarcted rats.

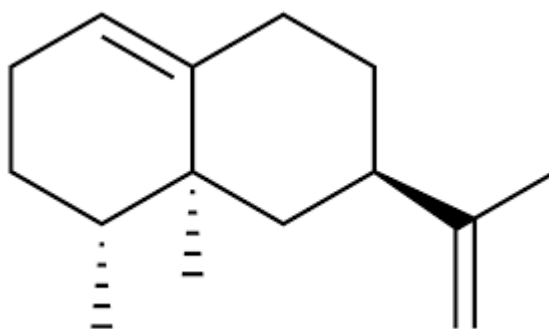


Fig.1. Structure of valencene

2. EXPERIMENTAL

Animals: The whole experiment was performed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India and approved by the Institutional Animal Ethical Committee. All animals received humane care and the study protocols comply with the institution's guidelines. This study was conducted in healthy male albino Wistar rats (*Rattus norvegicus*) weighing 180-

210g, obtained from the Central Animal House, Biogen Laboratory Animal Facility, Bengaluru, Karnataka. They were housed in polypropylene cages lined with husk, renewed every 24 h under a 12:12 h light-dark cycle at around 22° C, and had free access to tap water and food. The rats were fed on a standard pellet diet (Pranav Agro Industries Limited, Maharashtra, India).

Experimental myocardial infarction: For the induction of MI, rats were injected subcutaneously with 2 ml of isoproterenol hydrochloride (100 mg/kg body weight) dissolved in saline twice at a gap of one day (1st and 2nd days) [16, 17].

Dose-dependent effects of valencene: A pilot study was performed to know the dose-dependent effects of valencene with 3 different doses (3 mg, 6 mg, and 12 mg/kg body weight) in isoproterenol (100 mg/kg body weight) induced myocardial infarcted rats. Valencene (3, 6, and 12 mg/kg body weight) treatment was given to isoproterenol-induced myocardial infarcted rats orally, daily, for 14 days and the effect of valencene on the cardiac diagnostic marker, serum myoglobin was evaluated. The level of serum myoglobin in myocardial infarcted rats treated with valencene (3 mg, 6 mg, and 12 mg/kg body weight) (Groups 6, 7, and 8), daily for 14 days showed dose-dependent significant ($P < 0.05$) effects in attenuating serum myoglobin (Groups 6, 7, 8). We observed the highest protection with 14 days of valencene (12 mg/kg body weight) (Group 8) (**Fig. 2**). Hence, the dose of 12 mg/kg body weight of valencene and 14 days period of treatment was preferred for further biochemical parameters. Oral administration of valencene (3 mg, 6 mg, and 12 mg/kg body weight) to normal rats (Groups 2, 3, and 4) did not show any significant change in the serum myoglobin levels as compared to normal control rats (Group 1).

Animal treatment and sacrifice: After one week of acclimatization, 24 male albino Wistar rats were divided into 4 groups (n= 6 rats). Group 1: Normal control rats were administered 2 ml of saline orally, daily, using an intragastric tube for 14 days; Group 2: Normal rats were administered 2 ml of valencene (12 mg/kg body weight) dissolved in saline orally, daily, using an intragastric tube for 14 days; Group 3: Rats were induced MI by using isoproterenol (100 mg/kg body weight) on 1st and 2nd days; Group 4: Isoproterenol induced myocardial infarcted rats were treated with 2 ml of valencene (12 mg/kg body weight) from 4th day (i.e. one day after the 2nd injection of isoproterenol), orally, daily, using an intragastric tube for 14 days. On the 15th day morning, all the rats were anesthetized using pentobarbital sodium (60 mg/kg body weight). Then, all the rats were sacrificed by cervical decapitation. Blood was collected in two tubes, one containing ethylene diamine tetraacetic acid for the separation of plasma and another without anticoagulant for the serum. Then plasma and serum were separated.

Estimation of serum cardiac diagnostic marker: Serum myoglobin was estimated by an enzyme-linked fluorescent immunoassay (Biomerieux, France).

Determination of LPO products in the plasma: The plasma LPO product, thiobarbituric acid reactive substances were estimated by the method of Yagi [18]. Plasma (0.5 ml) was

added to 4 ml of 0.083 N sulphuric acid (H₂SO₄). Then, 0.5 ml of 10% phosphotungstic acid was added and mixed. After allowing it to stand at room temperature for 5 min, the mixture was centrifuged at 3,000 x g for 10 min. The supernatant was discarded and the sediment was mixed with 2.0 ml of H₂SO₄ and 0.3 ml of 10% phosphotungstic acid. Then the mixture was shaken well for 10 min and centrifuged at 3,000 x g, and the sediment was suspended in 4.0 ml of distilled water and 1.0 ml of thiobarbituric acid reagent was mixed. The reaction mixture was heated for one h at 95°C. Five ml of n-butanol was mixed after cooling and the mixture was shaken vigorously and centrifuged for 15 minutes. The intensity of the color in the butanol layer was measured at 530 nm. Along with the test samples standard malondialdehyde solution (1-5 nmoles) in 4.0 ml volume and blank having 4.0 ml distilled water were processed. Further, another LPO product, lipid hydroperoxides in the plasma was estimated by a standard procedure [19]. To 0.2 ml of plasma, 1.8 ml of the Fox reagent was mixed and incubated for 30 min at room temperature and the color developed was measured at 560 nm.

Estimation of non-enzymatic antioxidants in the plasma: The reduced glutathione level in the plasma was estimated by the method of Ellman [20]. 0.2 ml of plasma was taken and precipitated with 2.0 ml of 5% trichloroacetic acid (TCA). One ml of the clear supernatant was taken after centrifugation and to this, 0.5 ml of Ellman's reagent and 3.0 ml of phosphate buffer were added. The yellow color developed was read at 412 nm. The vitamin-C level in the plasma was estimated by the method of Omaye et al [21]. To 1.5 ml of 6% TCA, 0.5 ml of plasma was mixed and centrifuged at 3,500 x g for 20 min. To 0.5 ml of supernatant, 0.5 ml of dinitro phenyl hydrazine reagent was added and the test tubes were allowed to stand at room temperature for 3 h, then they were removed and placed in ice-cold water. Thereafter, 2.5 ml of 85% H₂SO₄ was added to all the test tubes and allowed to stand for 30 min and the color developed was read at 530 nm. The vitamin-E level in the plasma was estimated by the procedure of Baker et al. [22]. Two ml of petroleum ether and 1.5 ml of ethanol were added to 0.5 ml of plasma mixed well and centrifuged. The supernatant was then evaporated to dryness at 80°C and 0.2 ml of 2, 2'-dipyridyl solution and 0.2 ml of ferric chloride were added, mixed well, and kept in dark for 5 min. Then, 2 ml of butanol was added and the red color developed was read at 520 nm.

Statistical analysis: The statistical analysis was performed by SPSS software (Version 12.0). Groups of data were compared by using one-way ANOVA followed by Duncan's Multiple Range Test. All the data were expressed as mean ± S.D (six rats per group). An experiment-wise *p*-value of < 0.05 was deemed to be statistically considerable throughout this study.

3. RESULTS AND DISCUSSION

A considerable (*P*<0.05) amplification of myoglobin in the serum was detected in isoproterenol-induced myocardial infarcted rats (Group-5). A considerable (*P*<0.05) dose-dependent reduction in this serum heart diagnostic marker was noted in myocardial infarcted rats treated with valencene (3 mg/kg b.w) (Group 6), valencene (6 mg/kg b.w) (Group 7), and

valencene (12 mg/kg b.w) (Group-8) as compared to isoproterenol control rats (Group 5). But, 12 mg/kg b.w dose of valencene (Group 8) revealed the maximum effect. Also, valencene (3, 6, and 12 mg/kg b.w) treatment in normal rats did not reveal any considerable effect (Groups 2, 3, and 4) as compared to group 1 rats (**Fig. 2**).

Isoproterenol administration to rats (Group 3) considerably ($P<0.05$) amplified plasma LPO products such as thiobarbituric acid reactive substances and lipid hydroperoxides as compared to normal control rats (Group 1). However, myocardial infarcted rats treated with valencene (Group 4) considerably ($P<0.05$) lessened these LPO products in the plasma as compared to isoproterenol alone -induced myocardial infarcted rats (Group 3) (**Figures 3& 4**).

Significantly ($P<0.05$) decreased levels of non-enzymatic antioxidants such as reduced glutathione, vitamin-C, and vitamin-E were observed in the plasma of isoproterenol-induced myocardial infarcted rats (Group 3) compared to the normal control rats (Group 1). Isoproterenol-induced myocardial infarcted rats treated with valencene significantly ($P<0.05$) increased these non- antioxidant systems in the plasma of isoproterenol-induced myocardial infarcted rats (Group 4) as compared to isoproterenol alone -induced myocardial infarcted rats (Group 3) (**Figures 5, 6 and 7**).

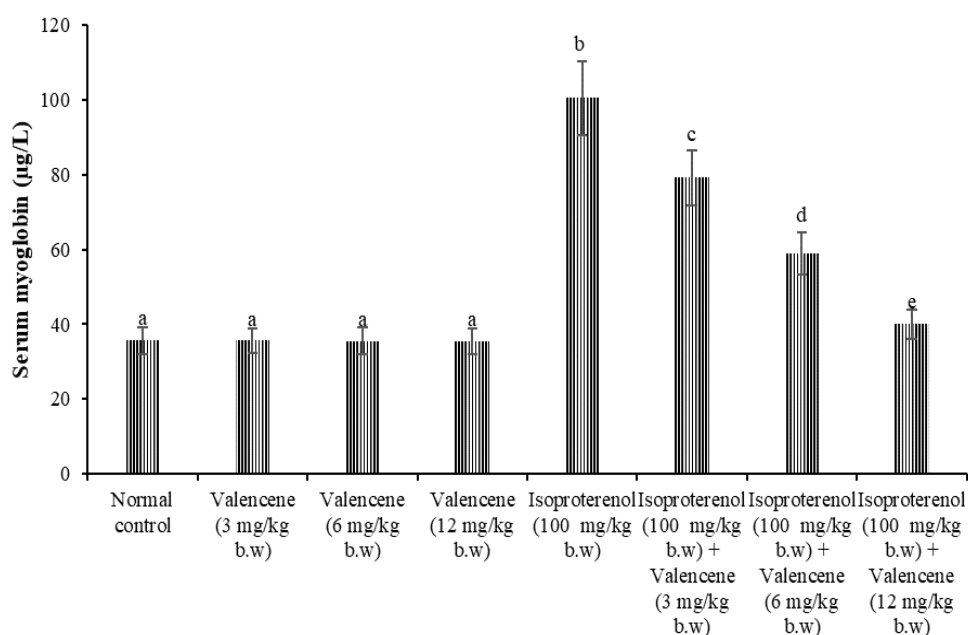


Fig.2 Dose-dependent effects of valencene on serum myoglobin

All columns are mean \pm standard deviation for 6 rats in each group; Columns that have a different alphabet (a, b, c, d, e) differ significantly ($P<0.05$) from each other; Duncan's Multiple Range Test.

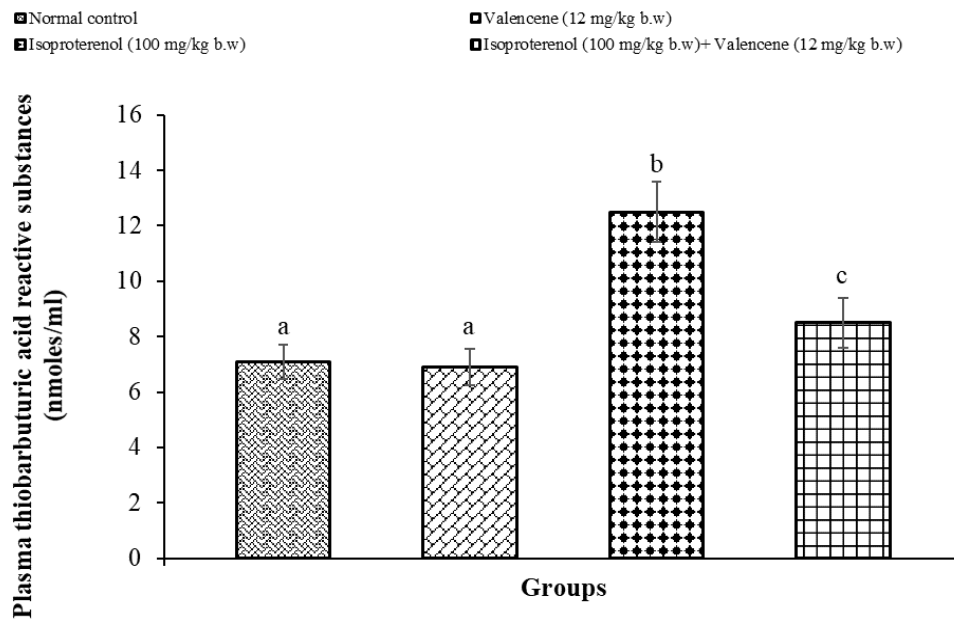


Fig 3. Effect of valencene on plasma thiobarbituric acid reactive substances
 All columns are mean ± standard deviation for 6 rats in each group; Columns that have a different alphabet (a, b, c) differ significantly ($P<0.05$) from each other; Duncan's Multiple Range Test.

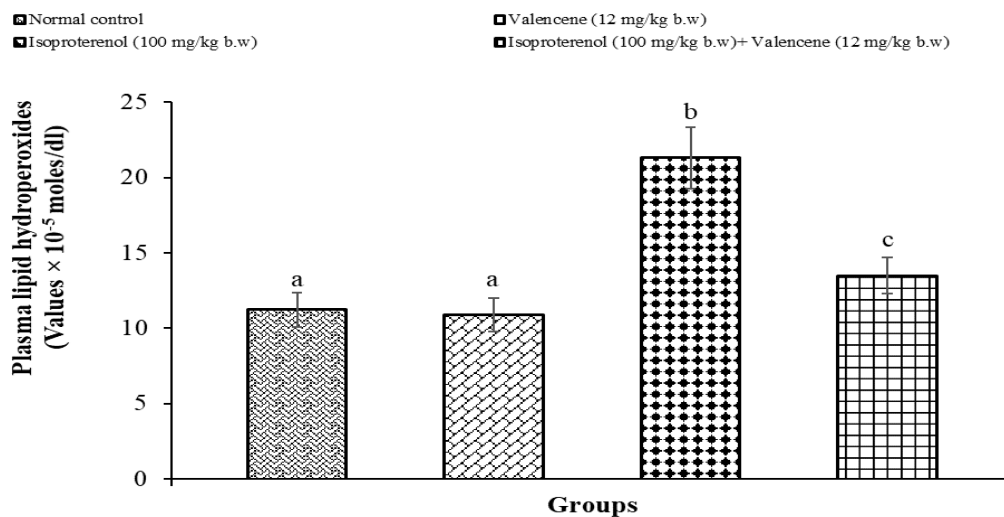


Fig 4. Effect of valencene on plasma lipid hydroperoxides
 All columns are mean ± standard deviation for 6 rats in each group; Columns that have a different alphabet (a, b, c) differ significantly ($P<0.05$) from each other; Duncan's Multiple Range Test.

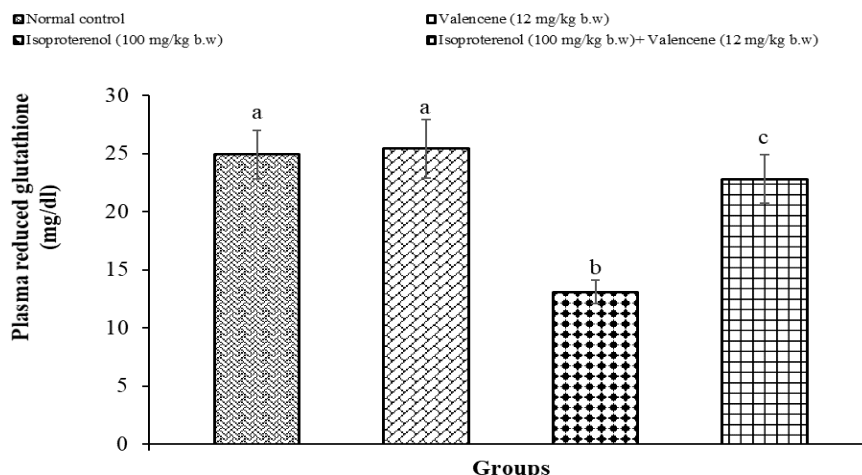


Fig 5. Effect of valencene on plasma reduced glutathione

All columns are mean ± standard deviation for 6 rats in each group; Columns that have a different alphabet (a, b, c) differ significantly ($P<0.05$) from each other; Duncan’s Multiple Range Test.

We evaluated the cardioprotective role of valencene in MI for the first time. Assessment of cardiac diagnostic markers during MI is an unavoidable procedure. The finding of serum myoglobin in clinical diagnosis is valuable for the diagnosis of MI and myocardial infarct size. Myoglobin is a small cytosolic protein. It is present in myocytes. Further, myoglobin is one of the earliest markers released into the circulation after the onset of myocardial necrosis. It is released into the circulation as soon as one hour after coronary occlusion [23]. Isoproterenol-induced cardiac damage releases myoglobin from the heart into circulation. Three doses (3, 6, and 12 mg/kg b.w) of valencene therapy to myocardial infarcted rats orally, daily, for two weeks dose-dependently reduced this cardiac diagnostic marker, by its cardioprotective effects.

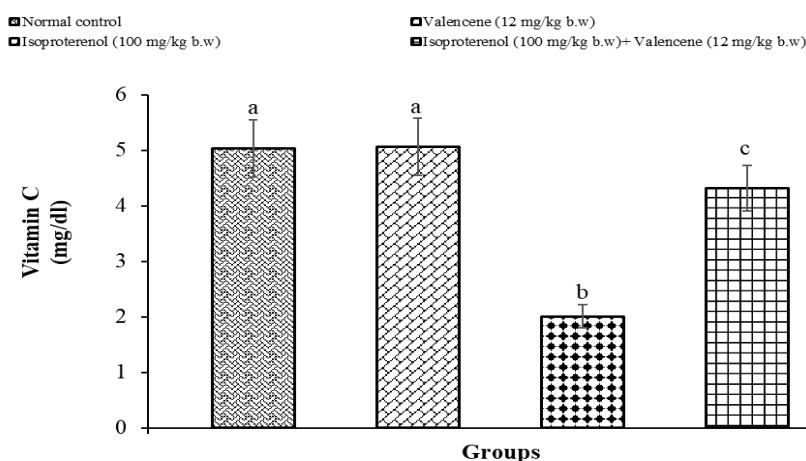


Fig 6. Effect of valencene on plasma vitamin C

All columns are mean ± standard deviation for 6 rats in each group; Columns that have a different alphabet (a, b, c) differ significantly ($P<0.05$) from each other; Duncan’s Multiple Range Test.

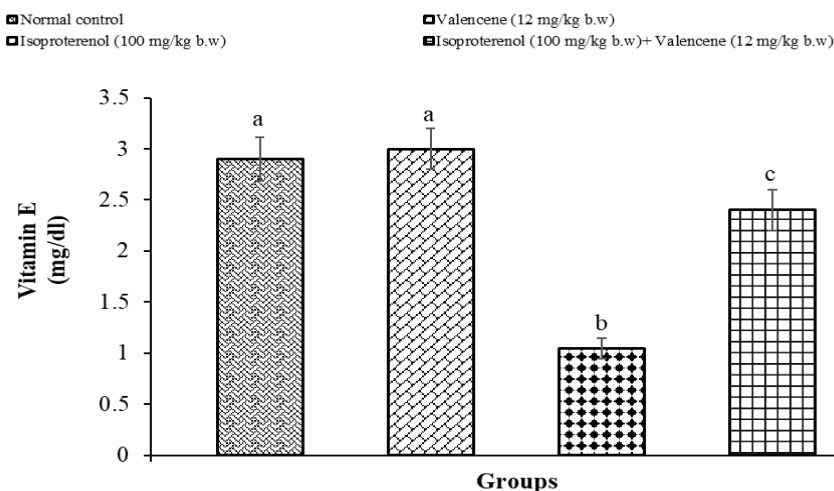


Fig 7. Effect of valencene on plasma vitamin E

All columns are mean \pm standard deviation for 6 rats in each group; Columns that have a different alphabet (a, b, c) differ significantly ($P < 0.05$) from each other; Duncan's Multiple Range Test.

Plasma biochemical parameters are highly useful in the diagnosis, progress, and cure of MI. Oxidative stress burden is considered as the origin and initiative mechanism of isoproterenol-induced MI in animals [3, 11]. LPO is the process of reactive oxygen species (ROS) induced damage of polyunsaturated fatty acids and is a complex free radical chain reaction. Also, LPO is a crucial incident in MI. The circulatory LPO products are a sign of oxidative stress in tissues. It is strongly allied with the occurrence and progress of MI. A lot of scientific studies established that isoproterenol can up-regulate the concentration of free radicals and down-regulate the concentration of the antioxidant system [3, 11]. These accumulated free radicals can further lead to LPO and cardiomyocyte damage. A considerably increased level of thiobarbituric acid reactive substances and lipid hydroperoxides in the plasma signifies oxidative stress in the heart of myocardial infarcted rats [24]. But; isoproterenol-induced myocardial infarcted rats treated with valencene (12 mg/kg b.w) considerably reduced the rise of thiobarbituric acid reactive substances and lipid hydroperoxides in the plasma and reduced oxidative stress, by its anti-lipid peroxidation property.

Antioxidant therapy is a potent approach to the treatment of myocardial injury. Cells are also provided with various non-enzymatic antioxidants (vitamin-C, vitamin-E, and reduced glutathione) as a defense mechanism against toxic ROS. Heart failure after MI is closely linked with the antioxidant deficit. A non-enzymatic antioxidant, reduced glutathione reacts with singlet oxygen, superoxide anion, and hydroxyl radicals and acts directly as a free radical scavenger. It stabilizes membrane structure. Vitamin C and vitamin E are free radical scavenger non-enzymatic antioxidants. Vitamin-C is a quencher of free radicals and a powerful quencher of singlet oxygen and it ceases LPO. Further, intake of this vitamin

decreases the risk of CVD's such as MI. Vitamin E quenches both singlet oxygen and peroxides. In this study, a significant decrease in reduced glutathione levels in the plasma was observed in the isoproterenol-induced myocardial infarcted rats revealing that depletion of reduced glutathione resulted in enhanced LPO and excessive LPO caused increased reduced glutathione consumption. Further, the depletion of reduced glutathione causes cardiac dysfunction. We also observed a drastic decrease in the plasma vitamin-C, and vitamin-E levels in the isoproterenol-induced myocardial infarcted rats. The decrease in the plasma vitamins of myocardial infarcted rats might be due to increased free radicals and LPO [3]. Valencene therapy increased plasma-reduced glutathione, vitamin-C, and vitamin-E in isoproterenol-induced myocardial infarcted rats, by its antioxidant effects.

4. CONCLUSION

In conclusion, our study demonstrates the involvement of LPO and antioxidants in the occurrence and progress of MI. Valencene lessened plasma LPO and improved non-enzymatic antioxidants. Thus, it exhibits cardioprotection via anti-lipid peroxidation and antioxidant effects in isoproterenol-induced myocardial infarcted rats. These experimental findings with valencene may offer a way for the development of anti-MI therapy for human MI.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

5. REFERENCES

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