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Cardioprotective and Antioxidant activity of Onion (*Allium cepa*) Leaves Extract in Doxorubicin Induced Cardiotoxicity in Rats.

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ABSTRACT

The study evaluated the cardioprotective and antioxidant activity of onion (*Allium cepa*) leaves extract in Doxorubicin (DOX) induced cardiotoxicity in rats. In this study twenty four male Albino Wistar rats weighing between 200-250g were used. The animals were treated as follows. Group 1: animals served as control and received saline (0.9%) 10ml/kg/p.o, Group 2: animals received doxorubicin 10mg/kg, i.v once 48 h before sacrifice. Group 3: animals received vitamin E (4mg/kg/p.o.) for 14 days followed by Doxorubicin, Group 4: animals received aqueous *Allium cepa* leaves extract of (200mg/kg/day/p.o.) for 14 days followed by Doxorubicin. In each group, body wt of rats were taken before and after Doxorubicin administration. After 48 hrs of doxorubicin administration blood was collected for serum CK-MB and LDH estimation. Isolated hearts were dried and weighed. In the heart tissues superoxide dismutase (SOD) & catalase (CAT), glutathione (GSH) and malondialdehyde (MDA) were estimated. Results showed that the mean heart weight/body weight (HW/BW) ratio in group 2 was significantly ($p < 0.001$) decreased, CKMB ($p < 0.01$), LDH ($p < 0.05$) increased, GSH ($p < 0.01$) decreased, MDA ($p < 0.01$) increased, SOD and CAT ($p < 0.01$) enzymes were decreased as compared to group 1. Group 3, 4 has shown significant ($p < 0.001$) increase in (HW/BW) ratio, decrease in CKMB and LDH levels ($p < 0.01$), GSH levels were significantly ($p < 0.01$) increased, MDA levels significantly decreased ($p < 0.01$), SOD and CAT enzymes levels significantly ($p < 0.01$) increased as compared to group 2. It may be concluded that the aqueous extract of *A. cepa* leaves possesses cardioprotective and antioxidant activity in doxorubicin induced cardiotoxicity in rats.

Key Words: *Allium cepa*, Cardiotoxicity, Doxorubicin, Vitamin E.

INTRODUCTION

Cardiotoxicity is a well-known side effect of several cytotoxic drugs, especially of the anthracyclines and can lead to long term morbidity. The mechanism of anthracycline induced cardiotoxicity seems to involve the formation of free radicals leading to oxidative stress. Free radicals generation and lipid peroxidation in myocardial cells have been suggested to be responsible for cardiotoxicity induced by DOX [1]. The clinical use of DOX is limited by dose dependence cardiotoxicity which may lead to severe and irreversible form of cardiomyopathy with congestive heart failure and high mortality is one of the factors that limit its use [2]. The cardiomyopathies represent a variety of diseases affecting the myocardium in either a diffuse or multifocal manner that frequently results in heart failure. The term congestive heart failure is used for the chronic form of heart failure in which the patient has evidence of congestion of peripheral circulation and of lungs. It is end result of various forms of serious heart diseases [3].

Doxorubicin is an anthracycline antibiotic having antitumour action and produced by the fungus *Streptococcus peuceitius* var. *caesioides*. Doxorubicin produces clinically useful responses in a variety of human cancers. However, the toxicity of doxorubicin has limited its usefulness. This side effect is mainly due to the doxorubicin-mediated free radical formation [4]. It is capable of causing breaks in DNA strands by activating topoisomerase II and generating quinone type of free radicals. It produces cardiotoxicity as a unique adverse effect [5]. Tissues with less developed antioxidant defense mechanism such as the heart are highly susceptible to injury by anthracycline-induced oxygen radicals [6]. Many investigators have described the role of reactive oxygen species including hydroxyl radical in DOX-induced cardiotoxicity [7-9].

Vitamin E refers to a group of eight fat-soluble compounds that include both tocopherols and tocotrienols [10]. Vitamin E is one of the most extensively studied antioxidants as a protective agent against doxorubicin cardiotoxicity. It has been reported that Vitamin E act as peroxy radical trapping radical chain breaking antioxidant along with free radical scavenging property [11]. Herbal approach for protection against cardiotoxicity is achieved by the use of natural drug Onion (*Allium cepa*) leaves extract. The preliminary phytochemical screening of *Allium cepa* extract showed the presence of flavonoids, alkaloids, tannins and anthocyanidins. Of all the healthy compounds contained in onions, two stand out: sulfur and quercetin both being strong antioxidants. It is also a rich source of vitamins (viz vitamin C, folic acid, vitamin E), minerals, amino acids, essential oil with many sulphurous components, allin and allicin [12].

Research shows that onions may help guard against many chronic diseases. That's probably because onions contain generous amounts of the flavonoid quercetin. Studies have shown that quercetin protects against cataracts, cardiovascular disease, and cancer. In addition, onions contain a variety of other naturally occurring chemicals known as organosulfur compounds that have been linked to lowering blood pressure and cholesterol levels. Onions contain 25 active compounds that appear to inhibit the growth of cancerous cells allin being the main constituent. Onion has been found to help combat heart disease, inhibit strokes, lower blood pressure and cholesterol, and stimulate the immune system. The potassium salts and the flavonoids that are present in onion perform an anti-inflammatory action [13].

Taking into consideration the antioxidant potential of flavonoids and anthocyanidins present in the fresh onion leaves extracts the present study was conducted to evaluate the cardioprotective and antioxidant potential of *Allium cepa* against doxorubicin-induced cardiotoxicity in rats.

MATERIALS AND METHODS

Drugs and chemicals used

Doxorubicin powder injection was gifted as doxorubicin hydrochloride by Serum Institute of India Ltd, Pune. The α -tocopherol used in the form of pediatric oral suspension. All the solvents, chemicals used were of analytical grade and chemicals required for sensitive biochemical assays were purchased from Merck. CK-MB kit was purchased from Aspen Laboratories, Rapid Diagnostic Pvt Ltd and LDH kit from Crest Biosystems Goa, India. All drug solutions were freshly prepared before each experiment. *Allium cepa* extract was dissolved in distilled water and administered orally.

Collection of plant material

Leaves of *Allium cepa* were purchased locally and authenticated by Dr. Dhabe, Dept. of Botany, Dr. Babasaheb Ambedkar Marathwada University (BAMU) Aurangabad. A voucher specimen no. 0536 has been deposited in the same department.

Preparation of Aqueous Extract

Allium cepa leaves were collected, dried under shade and coarsely powdered. The powder obtained (1000 g) was defatted using petroleum ether (60-80°C). The marc was extracted with methanol by doing soxhlet for 65-72hrs. The filtrate was air dried and concentrated. The dried marc was treated with 2M HCl in 1:2 ratio [1 Extract: 2 (2mM HCl)] for enrichment of flavanoids and water solubility of extract, fractionated with sufficient ethyl acetate, solvent was recovered and the marc was concentrated under reduced pressure. Appropriate dose of the extracts was made in distilled water [14].

Animals

Twenty four (24) Male albino rats (Wistar strain) weighing between 200-250 g, were procured from Wockhardt Ltd, Aurangabad. Animals were housed under standard laboratory conditions of temperature $25 \pm 1^\circ\text{C}$ with free access to food (Amrut rat and mice feed, Sangli, India.) and water. The experiments were performed during the light cycle (12-12 h). The experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethics Committee, Y.B. Chavan College of Pharmacy, Aurangabad.

Experimental protocol

The animals were divided in following experimental groups, each group comprising of six animals.

Group I: Animals served as vehicle control and received saline (10 ml/kg, p.o).

Group II: Animals served as doxorubicin treated (10mg/kg, i.v; once 48 hr before sacrifice).

Group III: Animals served as standard drug treated and received vitamin E (4mg/kg/day, p.o) for 14 days) as a pre-treatment followed by doxorubicin administration (10mg/kg, i.v; once 48 hr before sacrifice).

Group IV: Animals received aqueous leaves extract of *Allium cepa* (400mg/kg/day, p.o) for 14 days as a pre-treatment followed by doxorubicin administration (10mg/kg, i.v; once 48 hr before sacrifice).

Cardiotoxicity Assessment Parameters**Preparation of serum:**

Blood was collected from retro-orbital plexus from the inner canthus of the eye (under light ether anesthesia) using glass capillary tubes. Serum was separated using R-24 research centrifuge (Remi Instruments Ltd., Mumbai) at 3000 rpm for 15 min. and used for estimation of CK-MB [15], LDH [16].

The animals were sacrificed under ether anaesthesia by cutting the carotid artery. The hearts were quickly removed, rinsed in ice cold saline, dried on a filter paper, and weighed. A 10 % homogenate was prepared in 0.15 M KCl for the estimation of tissue malondialdehyde according to Ohkawa et al [17]. The homogenate for tissue glutathione was prepared in 0.02 M EDTA and measured according to Lindsay & Sedlak [18], Ellman [19].

Preparation of Post Mitochondrial Supernatant (PMS)

The tissues were homogenized in chilled potassium phosphate buffer (50mM, pH 7.4) using a Remi homogenizer. The homogenate was centrifuged in a refrigerated centrifuge at (10,500 rpm) for 20 minutes at 4°C to obtain the PMS, which was used for various biochemical analyses. The post mitochondrial supernatant (PMS) was used for the estimation of enzymes such as Catalase according to Clairborne [20] and Superoxide Dismutase according to Marklund [21]. Protein estimation in the PMS was done according to Lowry et al [22].

Statistics

The mean \pm SEM values were calculated for each group. One-way ANOVA followed by Dunnett's multiple comparison tests were used for statistical analysis. Values of $p < 0.05$ was considered statistically significant. The entire statistical analysis was performed using statistical package, GraphPad InStat Version 3 (GraphPad Software Inc., USA)

RESULTS

The mean heart weight/body weight ratio in doxorubicin (10 mg/kg) treated group was significantly ($p < 0.01$) decreased as compared to control group. Pre-treatment of Vitamin E (4 mg/kg) and *Allium cepa* (200 mg/kg) extracts followed by DOX treatment group 3 and 4 has shown the significant ($p < 0.01$) increase in heart weight/body weight ratio as compared to that of doxorubicin treated group 2. In DOX treated group a significant increase in the levels of cardiac markers i.e CK-MB ($p < 0.01$) and LDH ($p < 0.05$) levels were observed as compared to control group 1. The CK-MB levels in Vitamin E and *Allium cepa* extract treated groups showed a significant ($p < 0.01$) decrease as compared to DOX treated group, whereas LDH levels in Vitamin E and *Allium cepa* extract treated group showed a significant decrease ($p < 0.01$) and ($p < 0.05$) respectively as compared to DOX treated group. Results also showed that the DOX treated group significantly decreased the levels of GSH as compared to control group. Vitamin E (4 mg/kg) and *Allium cepa* extract (200mg/kg) treated groups showed a significant ($p < 0.01$) increase in GSH levels compared to that of DOX treated group. (Table 1)

The DOX treated group showed significant increase in the levels of MDA as compared to control group. Vitamin E and *Allium cepa* extract treated groups showed a significant ($p < 0.01$) decrease in MDA levels compared to that of DOX treated group 2. (Table 2)

Table.1: Effect of *Allium cepa* extract on Heart wt./body wt. ratio, Creatine kinase, Lactate dehydrogenase and tissue glutathione in Doxorubicin induced Cardiotoxicity.

Treatment given (n=5)	Heart wt./body wt. ratio (x 10 ⁻³)	CK-MB (U/L)	LDH (U/L)	Tissue GSH (μmol/gm tissue)
Control	4.502 ± 0.09	2368.66 ± 173.72	3439.176 ± 605.11	1.148 ± 0.2689
Doxorubicin	2.988 ± 0.15**	5173.63 ± 424.0**	6786.06 ± 491.21*	0.292 ± 0.11**
Vitamin E	4.32 ± 0.102**	2767.236 ± 447.04**	2421.818 ± 471.5**	1.539 ± 0.115**
<i>Allium cepa</i> extract	4.05 ± 0.1835**	2683.638 ± 487.38**	3668.65 ± 1275*	1.37 ± 0.136**

No. of samples (N) = 5. The observations are Mean ± SEM and ** $p < 0.01$ and * $p < 0.05$ as compared to DOX (One Way - ANOVA followed by Dunnett's test.)

Table.2: Effect of *Allium cepa* leaves extract on tissue MDA, Superoxide Dismutase and Catalase in Doxorubicin induced Cardiotoxicity.

Treatment given (n=5)	Tissue MDA (μmol/gm tissue)	SOD (U/mg protein)	Catalase (nmols of H ₂ O ₂ consumed/min/mg protein)
Control	2.403 ± 0.22	83.06 ± 7.978	36.38 ± 1.98
Doxorubicin	7.57 ± 0.67**	65.82 ± 7.048**	12.911 ± 3.45**
Vitamin E	4.518 ± 0.89**	131.386 ± 14.848**	33.09 ± 5.08**
<i>Allium cepa</i> extract	3.31 ± 0.15**	120.628 ± 5.572**	31.815 ± 1.937**

The observations are Mean ± SEM and ** $p < 0.01$ as compared to DOX (One Way - ANOVA followed by Dunnett's test).

DISCUSSION

The clinical use of DOX is limited by dose dependence cardiotoxicity which may lead to severe and irreversible form of cardiomyopathy with congestive heart failure and high mortality is one of the factors that limit its use [2]. Many investigators have described the role of reactive oxygen species including hydroxyl radical in DOX induced cardiotoxicity [7-9]. A relationship between DOX induced cardiotoxicity and oxidative stress has been confirmed in many experimental models. Repeated administration of DOX beyond a certain dose has been shown to cause cardiomyopathic changes in patients [23] and in variety of animal models [24]. The most common hypothesis is the formation of free radicals and superoxide [25-28]. The proposed mechanism for the cytotoxic effect of DOX is the production of reactive oxygen species (ROS) during its intracellular metabolism [27]. Cardiac cells are more susceptible to free radical damage because of their highly oxidative metabolism and relatively poor antioxidant defenses that is low levels of free radicals detoxifying enzymes/molecules like superoxide dismutase & GSH. Furthermore DOX also has high affinity for the phospholipid component of mitochondrial membrane in cardiac myocytes, leading to accumulation of DOX in cardiac tissue [28]. The DOX induced cardiotoxicity is secondary evident following lipid peroxidation of cardiac membrane leads to increase in leakage of LDH and CPK from cardiac myocytes into plasma [29]. In response to acute and chronic coronary artery occlusion in dog model, myocardium showed twofold to threefold increase in CK-MB activity in both the ischemic and non-ischemic myocardium. In contrast, individual with normal cardiac tissue had low percentage of CK-MB.

Our findings are consistent with above mentioned studies and *Allium cepa* leaves extract were found to inhibit the DOX-induced CK-MB release in serum. It is widely reported that DOX-induced free-radical generation triggers membrane peroxidation and disruption of cardiac myocytes, which can lead to increased release of CKMB in the serum [30]. Our study shows that Vitamin E and *Allium cepa* led to decrease in CKMB release in doses used. Also the administration of DOX induced cardotoxicity manifested significant increase in serum LDH levels. The results are consistent with previous studies [31, 32]. The LDH levels are significantly decreased in Vitamin E treated group and less significantly decreased in *Allium cepa* leaves extract treated group. GSH may play an important role in protecting the heart from peroxidative attack [27].

DOX significantly decreased the level of tissue GSH in accordance with the previous studies [33]. Decrease in the levels of GSH represents its increased utilization by myocardial cells due to oxidative stress. Treatment with Vitamin E & *Allium cepa* has significantly restored the GSH levels, this effect could be attributed either to increased biogenesis of GSH or the reduction in oxidative stress levels leading to decreased generation of toxic free-radical species.

The protective activity further supported by increased myocardial antioxidant enzyme activity and decrease extent of lipid peroxidation. The decrease in antioxidant enzymes and lipid peroxidation are known to cause cellular damage and responsible for reactive oxygen species (ROS) induced organ damage. The antioxidant enzymes such as SOD and CAT constitute the major supportive team of defense against free radicals. The equilibrium between these enzymes is an important process for the effective removal of ROS in intracellular organelles [34]. In present study, a significant decrease in levels of SOD and CAT enzymes in DOX treated group was observed. Vitamin E & *Allium cepa* leaves extract treatment significantly reversed the changes in antioxidant levels induced by DOX. A decrease in the activity of SOD can result in the decreased removal of superoxide ion, which can be harmful to the organs. Moreover, the enhanced SOD activity in the Vitamin E & *Allium cepa* leaves extract treated group might be involved in the scavenging of O_2^- generated from DOX. Increased levels of MDA and decreased levels of GSH, SOD and CAT were observed in heart tissue in DOX treated animals. Vitamin E & *Allium cepa* leaves extract efficiently counteracted the DOX induced cardiac tissue damage by significantly decreasing the MDA levels and increasing the GSH, SOD and CAT activities. The observed elevated CAT levels in DOX treated animals support the above hypothesis that this increase is possibly required to overcome excessive oxidative stress [35]. Cardioprotective activity of *Allium cepa* was further supported by increased myocardial antioxidant enzyme activity and decreased extent of lipid peroxidation. The most abundant ROS generated in living cells are superoxide anion and its derivatives, particularly highly reacting and damaging hydroxyl radical, which induces peroxidation of cell membrane lipids [36]. Lipid peroxidation is known to cause cellular damage and is primarily responsible for ROS-induced organ damage [37]. Present study shows that DOX has considerably increased the MDA levels, which was significantly prevented by *Allium cepa* extract treatment.

Doxorubicin also causes decrease in heart weight to body weight ratio, which indicates loss of myofibrils and cytoplasmic vacuolization in myocytes [38, 39]. The present study also showed the average heart weight to body weight ratio in DOX group was significantly decreased when compared with control group and *Allium cepa* extract treated groups which showed an increase in heart weight to body weight ratio as compared with DOX group.

CONCLUSION

Thus from above observations and results it was concluded that the *Allium cepa* leaves extract possesses antioxidant and cardioprotective activity which might be due to flavonoids and anthocyanidins and further studies showing isolation and characterization of active phytoconstituents may be needed.

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