Original Article

Adhatoda vasica Possesses Anti-microbial Activity and Effectively Ameliorates Ischemia Reperfusion Injury Induced by Doxorubicin through Controlling Oxidative Stress

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ABSTRACT

Background and Objectives: Myocardial ischemia is one of the leading causes deaths among cardiac pathological conditions. Doxorubicin belongs to anthracycline class of drug which is effective against a wide variety of neoplasms. However, doxorubicin-induced cardiotoxicity limits the clinical utility of the drug. One of the major causes of myocardial ischemia is oxidative stress. *Adhatoda vasica* have been shown to possess strong anti-inflammatory during ischemia reperfusion injury induced by doxorubicin in zebrafish model. **Materials and Methods:** Cardiac ischemia was induced in Zebrafish using doxorubicin. *Adhatoda vasica* was further supplemented and the antioxidants enzymes were analysed. Zebrafish was also infected with MRSA and the anti-microbial activity of *A. vasica* was analysed. **Results:** Supplementation with *Adhatoda vasica* extract to doxorubicin reduces the oxidative stress induced by doxorubicin through improvement of catalase, superoxide dismutase and GSH activities and subsequently reduces lipid peroxidation. Doxorubicin mediated inflammatory response in cardiac tissue is neutralized by supplementation with *Adhatoda vasica* plant extract and improves the cardiac tissue architecture. *A. vasica* plant extract is effective against inhibiting the growth of MRSA which has been shown to cause serious complications during cardiac surgery and cardiac prosthetic implantation. *A. vasica* plant extract causes a dose-dependent suppression of virulence gens such as *spA* and m*ecA*. **Conclusion:** Therefore, *A. vasica* extract may be useful in controlling microbial infections during cardiac complications. In conclusion, the present study indicates that *A. vasica* can be useful during cardiac complications.

Keywords: Doxorubicin, Cardiac ischemia, Antioxidant, MRSA, *Adhatoda vasica*.

Submission Date: 08-06-2022;
Revision Date: 19-07-2022;
Accepted Date: 22-08-2022.

DOI: 10.5530/ijper.56.4.199

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INTRODUCTION

Doxorubicin is an anti-neoplastic drug. The drug has been shown to be effective against a broad spectrum of cancers ranging from solid tumors to leukemia. The drug also causes a number of adverse side-effects such as mild nausea, cardiotoxicity, reproductive toxicity in male and females,[1,2] developmental defects in embryos,[3] etc. The drug has been shown to affect glycose metabolism.[4] Relatively, doxorubicin-induced cardiomyopathy remains one of the life-threatening side-effects of the drug compared to other side-effects of the drug. A number of mechanisms have been put forward to explain the cardiotoxicity of the drug. Activation of innate immunity, premature senescence of cardiomyocytes, impaired cardiac repair, alterations in cellular iron homeostasis, ROS etc., are some of the contributing factors for the development of doxorubicin-induced cardiomyopathy.[5,6] Oxidative stress is one of the widely explored and accepted mechanisms of doxorubicin-induced cardiomyopathy. Similarly, oxidative stress also plays important role in the development of ischemia reperfusion injury.[7] Oxidative stress subsequently causes lipid peroxidation.[8]

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Adhatoda vasica otherwise called as Malabar nut tree is a plant widely found in South-East Asia especially, all over the Southern part of India. It has been widely used in Chinese and Indian medicines. Extracts from various part of the plant has been used for the treatment of a variety of diseases such as leprosy, skin diseases, piles, asthma etc.[9] Vasicine is one of the chemical compound from the plant which belongs to quinazoline alkaloid class. Vasicine has been demonstrated to be active against tuberculosis.[10] Vasicinone is another compound found in *Adhatoda vasica* which also is classified as quinazoline alkaloid. Both vasicine and vasicinone contribute to the anti-oxidant activities of the plant extract.[11] In addition, vasicol and vasicinolone are also found in the plant extract.[12] The plant extract has been shown to possess anti-inflammatory property.[13] The occurrence of Methicillin-resistant *Staphylococcus aureus* (MRSA) in surgical units possess a greater threat to the patients undergoing cardiac surgery.[14] Endocarditis is one of the life threatening complications caused by Methicillin-resistant *Staphylococcus aureus* infection.[13]

Reactive oxygen species is one of the major reasons for the ischemia reperfusion injury and cardiac related complications induced by doxorubicin.[13] Supplementing plant extracts to nullify the adverse effects of the drug is being actively explored. Therefore, in the current work we evaluated the effect of *Adhatoda vasica* extract since it possesses strong anti-oxidant activities. Doxorubicin treated zebrafish were used as the animal model to evaluate the curative potential of the *Adhatoda vasica* extract. Further in the current work, the anti-microbial activity of *A. vasica* plant extract was estimated both *in vivo* and *in vitro*.

MATERIALS AND METHODS

Collection and Maintenance of Animals

The zebrafish (*Danio rerio*) were acclimatized in 35L of dechlorinated water and oxygen bubbling at temperature 26 ± 1°C throughout the study. The care and husbandry of zebrafish was carried out according to the standard guidelines for maintenance. Fishes were treated with the hydroalcoholic extract of dried leaves of *A. vasica.*

Preparation of Plant Extract

Hydroalcoholic extract was prepared by dissolving 20 g of the plant powder in 50 ml of Sterile Milli Q water and 50ml of ethanol (100%), stirred for 1hr and subjected to filtration using Whatman filter paper No.1. The filtrate was dried at 40-50°C in hot air oven and stored at room temperature for further analysis.

Determination of LC_{50}

Zebra fishes weighed 0.7-0.8g were measured and separated in various tanks and optimized to the concentrations of hydroalcoholic extract of *A. vasica* 2µg/ml to 10µg/ml and then 10 µg/ml to 50 µg/ml. Finally, the doses tried was 5,10,15 and 20 µg/ml and observed for 7 days under treatment for dose effect.

Treatment with Doxorubicin

After confirming the safe doses of the *A. vasica*, the fishes were injected with doxorubicin and treated with the fixed doses of the *A. vasica*. After the treatment period the fishes were euthanized and used for further analysis.

Catalase Assay

This method was adapted from Goth with slight modifications. 0.2 ml of cardiac tissue extract was added with 1.0 ml substrate and incubated (65 pmol per ml hydrogen peroxide in 60 mmol/l sodium-potassium phosphate buffer, pH 7.4) at 37°C for 1 min. Cardiac tissue catalase activity is linear up to 100 kU/l. Upon exceeding 100 kU/l the tissue extract was diluted using phosphate buffer (2 to 10-fold) and the whole assay was repeated. One unit catalase may be defined as it ability to decompose 1 pmol of hydrogen peroxide in l min under the given condition.[15]

GSH Assay

Reduced glutathione level was estimated by the following method.[16] Cardiac tissue was homogenate and (500µL of 0.1M potassium phosphate buffer (pH7.4)) was precipitated by adding 4% sulfosalicylic acid (500µL). The mixture was incubated at 4°C for around 1 hr, followed by centrifugation at 1200g for 20min. The supernatant (33 µl) was transferred to tubes containing 900µL of 0.1M potassium phosphate buffer (pH7.4) and 66µL of 100mM dithiobis (2-nitrobenzoic acid) (DTNB). Yellow coloured product was formed when GSH reacts with DTNB which is recorded by reading the absorbance at 412 nm within 10min.A blank was kept which contains all the reagents devoid of glutathione solution and the extract were also included.

Superoxide Radical Scavenging Assay

The 0- 1.2 mg/ml of sample was added with 0.2 mL NBT (0.08 mM), 0.4 mL NADH (0.25 mM), and 0.2 mL PMS (0.06 mM). The mixture was incubated at room temperature for 10 min in complete darkness. Post-incubation the absorbance was taken at 560 nm.[17]

TBARS Assay

TBARS assay was carried out using the TBARS assay kit (Cayman Chemical 10009055) following the manufacturer's protocol. All the reagents were allowed to reach room temperature before the assay. 100 µl each of tissue extract sample and SDS was added and mixed well followed by addition of 4 ml of color reagent (530 mg of TBA in 50 ml of diluted TBA acetic acid solution with 50 ml diluted TBA sodium hydroxide solution). The mixture was boiled for 1 hr and incubated in ice for 10 min. The mixture was centrifuged at 1600g for 10 min at 4°C. The absorbance of each of the sample was measured at 530 nm and 550 nm. Using the standard curve, the concentration of malondialdehyde was estimated.

Antimicrobial Activity

To test the antimicrobial activity of *Adhatoda vasica* 5 µl of respective bacterial suspension (0.5 OD_{600}) was administered by intramuscular injection. Fishes were orally fed for 7 days with different doses such as 10mg/Kg, 15mg/Kg, and 20mg/Kg. The Control group was received 5µL sterile phosphate buffered saline (PBS). After the 8th day fish were euthanized. Microbial population was checked from muscle tissue (site of infection) via spread plate method. DNA was isolated for performing polymerase chain reaction (PCR).

Polymerase Chain Reaction

Two sets of primers were used for studying the infection. mecA gene and Spa gene were analysed for evaluating the infection. Following are the details of the primers used. mecA forward: 5' CAGGTACTGCTATCCACCCTC 3'mecA reverse: 5' TGAGTTCTGCAGTACCGGAT; spA forward: 5' GAAGACGGCAACGGAGTACA; spA reverse 5' GCGACGACGTCCAGCTAATA. PCR was performed for 35 cycles with each cycle consisting of denaturation at 95°C for 1 min, annealing at 52.2°C for mecA and 54.1°C for Spa gene for 1 min and annealing at 72°C for 1 min. Final extension was carried out for 5 min with storage at 4°C.

Histopathology

Heart tissues samples were recovered from fixative. Fixed tissue was washed with saline solution. Dehydration of the tissue was done using 70% alcohol followed by 90% graded alcohol. The tissue was cleared using clearing agent. Paraffin wax was used as impregnating agent followed by trimming to remove excessive paraffin. A 30 mm iron bock was used to position the block at the correct angle and in position for sectioning. The tissue sections were made with 5-8 µm thickness using microtome. The sections were fixed to the slides preconditioned with egg albumin. Staining of the slides was done with Haematoxylin and Eosin. The stained sections were observed under light microscope and microphotographs were taken for pathological observations.

Statistical Analysis

The results obtained were subjected to statistical analysis using GraphPad Prism version 8.0. The data represented as mean ± standard deviation and analysed by one-way analysis of variance (ANOVA) with Tukey's multiple *t*-tests.

RESULTS

LC_{50} analysis of *Adhatoda vasica* treatment revealed non -toxic property of plant extract. So, evaluation of enzyme analysis was carried out.

Catalase Activity

Evaluating the catalase activity revealed that the treatment with doxorubicin impaired the free radical scavenging activity in the heart. On the other hand, treatment with the hydroalcoholic extract of *Adhatoda vasica* at concentrations between 5 and 20 µg/mL increased the catalase activity in a dose-dependent manner. (Figure 1) Extract of *Adhatoda vasica,* at a concentration as low as 5 µg/mL itself was sufficient to restore the catalase activity and further concentrations such as 10 µg/mL, 15µg/mL and 20 µg/mL gradually increased the activity of catalase. At 20 µg/mL concentration, the activity of catalase was doubled.

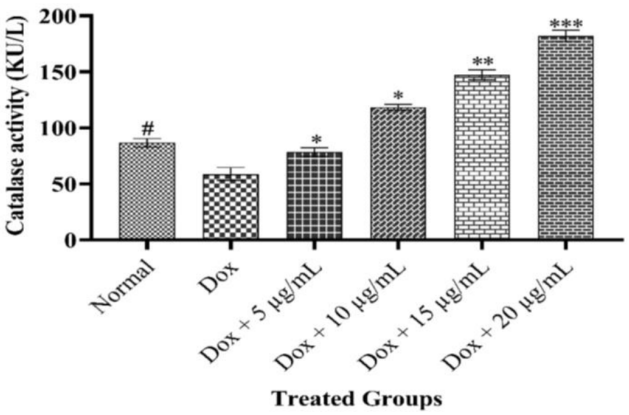

Figure 1: Catalase activity.

The total amount of catalase activity was analysed betwixt treated and untreated fish tissue sample. The Sample values are asseverated by using Mean (±Standard Deviation), One-way analysis of variance. The values are highly significant between treated and untreated samples. #p< 0.05 vs the control group, *p< 0.05 vs the DOX group.

GSH Activity

Estimation of reduced glutathione activity in cardiac tissue revealed that treatment with doxorubicin decreased the glutathione activity compared to the control. When hydroalcoholic extract of *Adhatoda vasica* was supplemented along with doxorubicin, the activity was improved (Figure 2). Furthermore, the activity increased with increasing concentrations of the extract. At a concentration of 20 µg/ml of the extract the GSH activity was more than the control implying that *Adhatoda vasica* was very much effective in stimulating the anti-oxidant activity.

Superoxide Radical Scavenging Assay

Doxorubicin increased the concentration of superoxide radicals compared to control in the cardiac tissue. As expected hydroalcoholic extract of *Adhatoda vasica* was effective in scavenging the superoxide radicals. 5 µg/ml of the extract slightly decreased the concentration of superoxide radicals. By contrast, at 10 µg/ml, 15 µg/ml and 20 µg/ml concentrations a stronger decline in superoxide radicals (Figure 3).

TBARS Assay

Doxorubicin has been shown to induce lipid peroxidation especially in the heart tissue. Our results are in agreement with earlier reports that doxorubicin promotes lipid peroxidation. Statistically significant amounts of lipid peroxidation were evidenced upon doxorubicin treatment. But doxorubicin's effect was effectively nullified by the activity of the hydroalcoholic extract of *Adhatoda vasica*. With increasing concentrations of the extract lipid peroxidation was reduced significantly. Especially, the supplementation of extract of *Adhatoda vasica* at a concentration of 20 µg/ml effectively compromised the lipid peroxidation effect induced by doxorubicin (Figure 4).

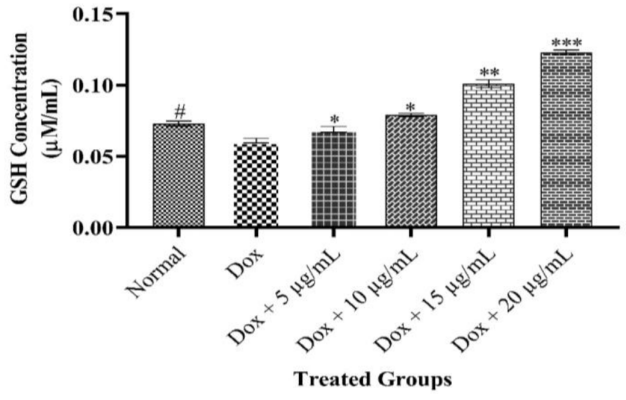

Figure 2: Reduced glutathione activity.

The total amount of glutathione (GSH) activity was analysed betwixt treated and untreated fish tissue sample. The Sample values are asseverated by using Mean (±Standard Deviation), one-way analysis of variance. The values are highly significant between treated and untreated samples (*P*-value < 0.0001). #p< 0.05 vs the control group, *p< 0.05 vs the DOX group.

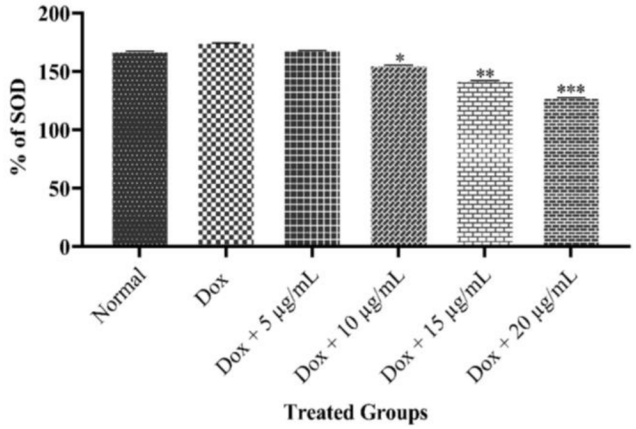

Figure 3: Superoxide Radical Scavenging Assay.

The total percentage of superoxide radical scavenging was analysed betwixt treated, untreated fish tissue sample. The Sample values are asseverated by using Mean (±Standard Deviation), one-way analysis of variance. The values are moderate significant between treated, untreated samples (*P*-value < 0.0001). Data are represented as the means ± SD. #p< 0.05 vs the control group, *p< 0.05 vs the DOX group.

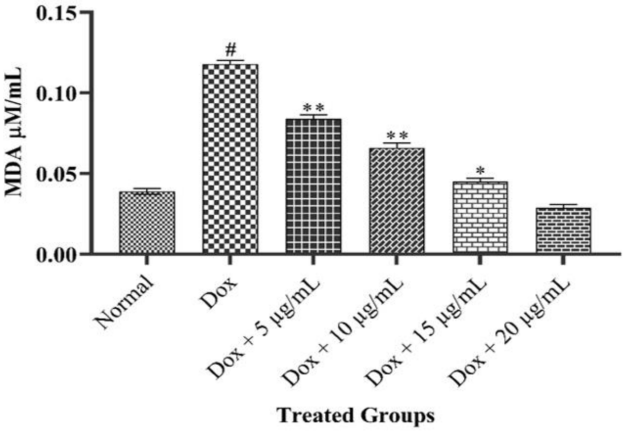

Figure 4: TBARS Assay.

The total amount of Lipid peroxidation was analysed betwixt treated, untreated fish tissue sample. The Sample values are asseverated by using one-way analysis of variance. The values are moderate significant between treated, untreated samples (*P*-value < 0.0001). Data are represented as the means ± SD. #p< 0.05 vs the control group, *p< 0.05 vs the DOX group.

Histopathology

Histopathological sections of the hearts of the doxorubicin treated animals displayed disarray of the tissue architecture with neutrophil infiltration into the cardiac muscles (Figure 5 Panel B) compared to the control (Figure 5 Panel A). On the other hand, recovery was evidenced in the tissues of animals treated with extract of *Adhatoda vasica*. Neutrophil infiltration was reduced in *Adhatoda vasica* treated animal groups suggesting the recovery of the tissues from the damage induced by doxorubicin.

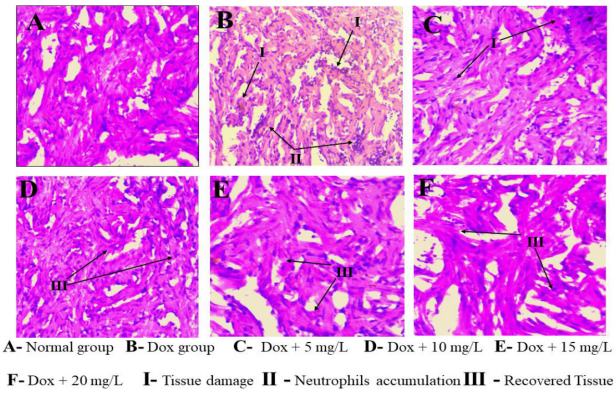

Figure 5: Histopathology analysis.

Dox induced groups showed tissue damage and high neutrophils accumulations. Treated (D, E, F) groups are trying to repairing tissues and reducing accumulations of neutrophils.

Table 1: Zone of inhibition of different microorganisms.

Microbes Names	ADV Extracts mg/mL (Zone of inhibition in mm)			10 µg Amx
	1 mg	1.5 mg	2 mg	
Klebsiella pneumoniae	11.5	13	14	12
Shigella dysenteriae	15	17.4	20	16.5
Methicillin Resistant *Staphylococcus aureus*	19.5	20.5	23	12.5
Streptococcus pneumoniae	17	19	21	10
Candida albicans	18	19	21.5	11

Anti-microbial Activity

In vitro anti-microbial activity of *Adhatoda vasica* was evaluated against *Klebsiella pneumoniae, Shigella dysenteriae,* Methicillin Resistant *Staphylococcus aureus, Streptococcus pneumoniae* and *Candida albicans.* Amoxicillin (Amx) at a concentration of 10 µg was used as control drug. *A. vasica* was used in the following concentrations: 1 mg/ml, 1.5 mg/ml and 2 mg/ml. A strong anti-microbial activity of *A. vasica* was evidenced. (Table 1; Figure 6) There was a dose-dependent increase in the zone of inhibition which emphasizes the anti-bacterial activity of the extract.

For evaluating antimicrobial activity of *Adhatoda vasica* zebrafish infection model was used. Methicillin Resistant *Staphylococcus aureus* was used for infecting the animals. There was a decline in the colony forming units cultured from the tissue (site of infection) of treated animals with respect to time as well as with respect to dose (Figure 7). 20 mg/Kg showed a strong anti-bacterial activity compared to other doses. The infection was visible with a white patch beneath the skin at the site of intramuscular injection (Figure 8). There was a dose-dependent decrease in gene expression levels of virulence genes such as *spA* and *mecA*. (Figure 9)

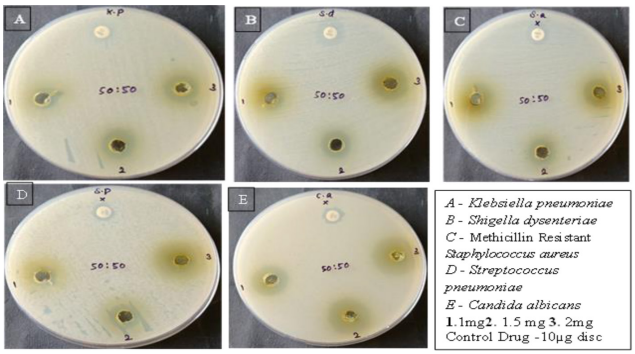

Figure 6: *In vitro* antimicrobial activity.

MRSA shows strong inhibition by *Adhatoda vasica* leaves hydroalcoholic extracts and followed by *Candida albicans.*

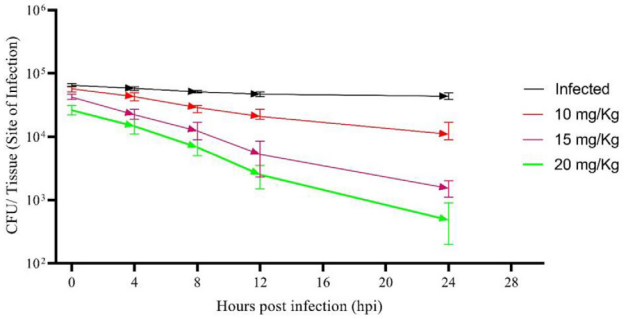

Figure 7: *In vivo* antimicrobial activity.

Colony forming units was significantly decreasing when treated animals with respect to time as well as with respect to dose wise.

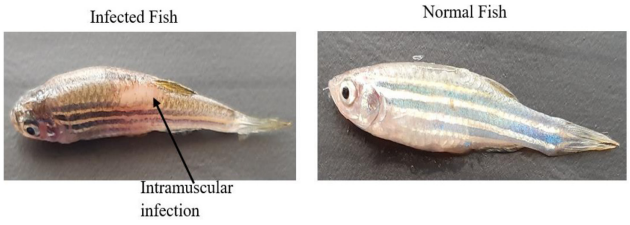

Figure 8: Intramuscular infections.

(0.5 OD at 600 nm. 5 µL bacterial suspension injection by intra muscular and infected fish has damaged skin with white patches)

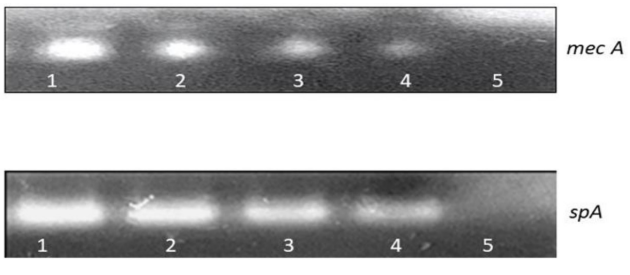

Figure 9: Bacterial gene.

(mec A, spA) expression analysis after treatment with various concentrations of *A. vasica* extract

DISCUSSION

In spite of being effective against a diverse array of cancers, doxorubicin's clinical utility is compromised for its wide range of adverse side-effects. Cardiomyopathy is one of the life-threatening side effects of doxorubicin. Doxorubicin has been demonstrated to mimic various types of cardiomyopathies.[18] In the current study, we attempted to neutralize the side-effect of doxorubicin-mediated cardiomyopathy using hydroalcoholic extract of *Adhatoda vasica*.[19] *Adhatoda vasica* extensively being utilized in folk medicines. We used zebrafish as model organism to study the cardio protective effect of *Adhatoda vasica* against doxorubicin-induced cardiomyopathy.

Catalase is an anti-oxidant enzyme whose expression is essential for the clearance of oxidative stress in a variety of tissues including the heart. It is effective in decomposing hydrogen peroxide into water and oxygen. Catalase has been shown to attenuate cardiac reperfusion injury effectively.[20] Therefore, we evaluated the activity of catalase in doxorubicin and hydroalcoholic extract of *Adhatoda vasica*. Treated animals. Surprisingly, catalase activity increased with increasing concentrations of the extract. Kang *et al*., have demonstrated that cardiac-specific overexpression of catalase in mice offered protection against doxorubicin-induced oxidative stress and cardiotoxicity.[21,22] In addition to controlling oxidative stress, cardiac specific overexpression of catalase also restores contractile dysfunction.[23] From our experiments it is evident that hydroalcoholic extract of *Adhatoda vasica* protects the heart by promoting catalase activity.

GSH is the key anti-oxidant in almost all organisms. It is mainly involved in scavenging free radicals such as ROS, RNS etc. GSH also is involved in regulating metal homeostasis, detoxifying endogenously produced toxicants.[24] In addition, GSH also acts as cofactor of multiples of enzymes. Abrogation of glutathione activity elevates oxido-reductive stress and subsequently contributes to cardiomyopathy in rodent model.[25] It is noteworthy that glutathione-related substances are pivotal in maintaining contractile function during hypoxic conditions.[26] Doxorubicin treatment impaired the activity of GSH. (Figure 2) However, hydroalcoholic extract of *Adhatoda vasica* restored the activity of GSH. Higher concentration of the extract strongly induced the activity of GSH to a level greater than the control. Therefore, it is reasonable to speculate that the extract might enhance the cardiac activity by not only controlling the oxidative stress but also by regulating other activities such as metal homeostasis.

Superoxide radicals are scavenged effectively by a group of enzymes collectively known as superoxide dismutase. Mn-SOD and Cu/Zn-SOD are responsible for the neutralization of superoxide radicals. Chouchani *et al*., have derived the possible mechanism of ischemia-reperfusion injury emphasizing the role of superoxide molecules.[27] Doxorubicin elevated the concentration of superoxide radicals in the hearts of treated animals. Treatment with hydroalcoholic extract of *Adhatoda vasica* significantly reduced the amount of superoxide radicals. Mitochondria are the sites of action of Mn-SOD which helps the cell survival in doxorubicin treated cardiomyocytes.[28] Since the superoxide concentration drastically reduced in higher concentration of *Adhatoda vasica* it is evident that mitochondrial free radical scavenging by Mn-SOD is increased drastically.

Lipid peroxidation has been well established in doxorubicin treated heart.[29] Lipid molecules have been found to be oxidatively degraded by doxorubicin. Wide range of adverse effects originate from lipid peroxidation. For example, impaired glucose transport has been associated with lipid peroxidation induced by doxorubicin.[30] Neurotoxicity associated with doxorubicin treatment can also be associated with lipid peroxidation.[31] Consistent with earlier observations, doxorubicin treatment increased the lipid peroxidation greater than two-fold. Supplementation of 20 µg/ml of hydroalcoholic extract of *Adhatoda vasica* significantly restored the peroxidation levels to normal. Earlier reports also suggests that *Adhatoda vasica* effectively controls the lipid peroxidation.[32] In our experiment also the plant extract efficiently controlled the lipid peroxidation suggestive of the restoration of cardiac function.

Histopathological section of cardiac tissue also revealed the protective effect of hydroalcoholic extract of *Adhatoda vasica* in restoring the microarchitecture of the cardiac tissue. Doxorubicin induced a number of derangements in the organization of the cardiac tissue. Alongside the disrupted microarchitecture of the heart infiltration of neutrophils was also evident from the cardiac tissue sections (Figure 9 panel B). Neutrophil infiltration into the cardiac tissue happens as early as 24 hr in mouse model which marks the inflammatory status of the tissue.[33] On the other hand, treatment with hydroalcoholic extract of *Adhatoda vasica* restored the microarchitecture of the cardiac tissue to normal. Supplementation with the plant extract could effectively reduce the inflammation of the cardiac tissue as evidenced by the reduced number of neutrophils in the cardiac tissues. Overall, it is evident from the histological sections that the supplementation with the

plant extract effectively ameliorated the adverse effects of doxorubicin.

Antibacterial activity of the plant extract has been evaluated both *in vitro* and *in vivo*. The extract shows a very strong antimicrobial activity against variety of bacteria and yeast. Our results are in agreement with earlier findings.[34] Therefore, the antimicrobial activity can be attributed to pyrroloquinazoline alkaloids as suggested by Singh and Sharma (2013). Among the screened organisms, Methicillin Resistant *Staphylococcus aureus* showed the highest sensitivity to the extract and *Candida albicans* showed a relatively lesser sensitivity compared to MRSA but higher than the other tested organisms. These results are indicative of the potential effect of *A. vasica* against a variety of microorganisms. In the current experiment we choose MRSA to test the *in vivo* anti-bacterial activity of *A. vasica*, since MRSA has been known to cause in prosthetic valve endocarditis.[35] Moreover, MRSA has poses a great threat during cardiac surgery.[31] *A. vasica* has been shown to possess a number of bioactive compounds which include vasicine, vasicinone, deoxyvasicine, vasicol, adhatodinine, and vasicinol. The antibacterial activity of the extract could be due to the combined synergistic effect of the compounds rather than the effect of a single compound.

Gene expression analysis was performed to analyse the expression of genes which offer antibiotic resistance and virulence to the bacteria. Our results revealed that the expression levels of *SpA* and *MecA* genes downregulated by the plant extract in a dose-dependent manner. *SpA* gene limits the immune system of the host by holding the IgG antibodies in inverted position so that the antibody-mediated phagocytosis is prevented.[36,37] Therefore, it is apparent that the antibodies are ineffective through prevention of phagocytosis of the pathogen. When treated with *A. vasica* the virulence of the pathogen is compromised by rendering the bacteria devoid of *SpA*. (Figure 8)

Mec A is another gene which encodes a protein known as penicillin-binding protein family PBP2a, a transpeptidase, which has a lower affinity for beta lactam antibiotics and therefore allows the building of cell wall. *mecA* Gene Is Widely Disseminated in Staphylococcus aureus Population; Factors Contributing to the Evolution of mecA-Mediated β-lactam Resistance in Staphylococci: Update and New Insights from Whole Genome Sequencing (WGS) However, our results show that there was a dose dependent reduction in the expression of *Mec A* upon treatment with the plant extract of *A. vasica* indicating that the plant extract suppresses the antibiotic resistance gene to make the pathogen sensitive to antibiotics.

CONCLUSION

In conclusion, *Adhatoda vasica* is an effective in promoting anti-oxidant effects. The plant extract restores the activities of catalase, superoxide dismutase and GSH which were impaired by doxorubicin. Subsequently, the extract prevents the lipid peroxidation in doxorubicin treated cardiac tissues. *A. vasica* has a strong anti-microbial activity against MRSA, which causes infections during cardiac surgical procedures and during cardiac implantations. Furthermore, the tissue sections also indicate the improvement of cardiac tissue architecture disrupted by the doxorubicin and reduces the inflammation induced by the anti-neoplastic drug.

ACKNOWLEDGEMENT

The authors would like to thank the Deanship of Scientific Research, Jazan University. This review is part of the Research Groups funding programme (Vector-Borne Diseases Research Group, Grant no. RG-2-1) of Jazan University. Authors like to thank the funding programme Vector-Borne Diseases Research Group, Grant no. RG-2-1.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

MRSA: methicillin-resistant Staphylococcus aureus; **ROS:** Reactive oxygen species; **GSH:** Reduced Glutathione; **DTNB:** 5,5'-dithio-bis-(2-nitrobenzoic acid); **NBT:** Nitro blue tetrazolium; **NADH:** nicotinamide adenine dinucleotide (NAD) + hydrogen (H); **PMS:** phenazine methosulfate; **TBARS:** Thiobarbituric acid reactive substances; **SDS:** Sodium Dodecyl Sulphate; **TBA:** thiobarbituric acid; RNS: Reactive nitrogen species; **Mn-SOD:** Manganese superoxide dismutase; **Cu/Zn-SOD:** Copper zinc superoxide dismutase; **IgG:** Immunoglobulin G.

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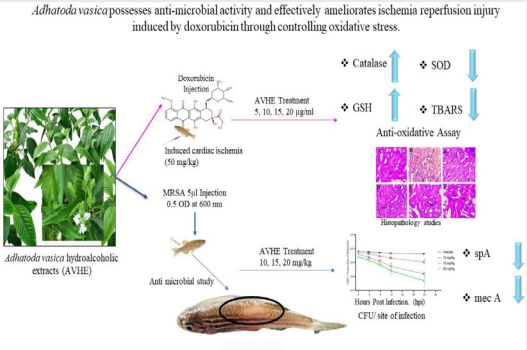
PICTORIAL ABSTRACT



SUMMARY

- Doxorubicin-induced cardiotoxicity limits the clinical utility of the drug. One of the major causes of cardiotoxicity is oxidative stress.
- *Adhatoda vasica* is widely used in Chinese and East Asian folk medicines which has been shown to possess strong anti-oxidant activity.
- Supplementation with *Adhatoda vasica* extract to doxorubicin reduces the oxidative stress induced by doxorubicin through improvement of catalase, superoxide dismutase and GSH activities and subsequently reduces lipid peroxidation.
- Doxorubicin mediated inflammatory response in cardiac tissue is neutralized by supplementation with *Adhatoda vasica* plant extract and improves the cardiac tissue architecture.
- *Adhatoda vasica* plant extract has been shown to possess anti-microbial activity against a variety of microorganisms including yeast. Most importantly, *A. vasica* plant extract has potent anti-bacterial effect against MRSA through suppression of *SpA* and *MecA* genes.

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Cite this article: Hershan AA, Abdulhaq AA. *Adhatoda vasica* Possesses Anti-microbial Activity and Effectively Ameliorates Ischemia Reperfusion Injury Induced by Doxorubicin through Controlling Oxidative Stress. Indian J of Pharmaceutical Education and Research. 2022;56(4):1172-80.