“Be the change that you wish to see in the world.”
— Mahatma Gandhi

SUMMARY AND CONCLUSION
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Cardiomyopathy belongs to cardiovascular disease and it represents one of the largest groups of disease in world wide. More than 100 million of people are attributed due to cardiomyopathy. The reason for the death are age, race, sex, familial history plays a vital role for reason behind death due to cardiomyopathy. On other hand, other major causes for cardiomyopathy was due to administration of anti-cancer drug due to prolonged period which may intend to produce toxicities in vital organ like heart and damages cardiomyocytes and leads to cardiac damages. Insufficient knowledge among people of molecular and cellular mechanism of their pathophysiology incompletely causes cardiac damage. Therefore to prevent continuous death due to chemotherapy, certain clinical trial should be improved to prevent the cardiomyopathy by cellular and molecular levels.

Heart is a primary organ to get affect by various anti-cancer drugs. Despite our understanding was fully focused on the production of reactive oxygen species and accumulation of oxidant products which are important reason for cardiomyopathy by various chemotherapeutic drugs. Due to accumulation of oxidant products it damages the mitochondria in heart cell apart from which even DNA, lipids and protein also damaged. Reactive oxygen species may damage extracellular matrix and also causes inflammatory responses. Moreover it may also damage mitochondria and finally mediates apoptotic cell death and also triggers MAPK signaling cascade. To prevent critical damages, the present work was done to explore the valuable factor which prevents adriamycin inducing cardiomyopathy.
*d*-Limonene is one of the naturally founding mono-terpenoid mainly isolated from citrus fruits and possess high anti-oxidant potential activity, free radical scavenging potential, anti-inflammatory and anti-apoptotic properties. Effectiveness of *d*-Limonene alters ADR inducing cardiomyopathy was controlled by administrating (100mg/kg body weight for 4 weeks). Whereas, various molecular changes, histological changes and biochemical observation are summarized as follows.

- There were significant changes in heart; body weight and gross appearance in heart of ADR administration rats. Pre-co-treated with *d*-Limonene effectively alter and prevents these changes completely.

- Serum and tissue parameters like CK-MB, LDH, CPK, Troponin-T, C-reactive protein, SGOT, SGPT in ADR mediating rats shows abnormalities and are elevated in significant ranges. On *d*-Limonene treated activities of the biochemical parameter were significantly reduced shows that *d*-Limonene strongly stabilize the cell membrane integrity and prevent tissue membrane intoxication.

- Blood pressure, heart rate was significantly increased in ADR administrated rats significant reduction was measured by rats pre-co-treated with *d*-Limonene.

- Electrocardiogram pattern in ADR mediating rats shows high pathological elevation in Q-T and S-T intervals, where as *d*-Limonene administrated pre-co-treated rats shows nearly valuable ranges to normal was highly noted.
• Oxidative stress in ADR administrated rat’s shows sufficient production of Reactive oxygen species (ROS) which was completely prevented in rats pre-co-treated with d-Limonene and prevent production of ROS in cardiac cell. Anti-oxidant property of d-Limonene was highly confirmed by attenuating ROS generation in H9c2 cardiomyocytes in invitro study.

• Cardiac damages also accompanied by production of lipid peroxidase and it was excessively prevented by pre-co-treated with d-Limonene and maintain myocardial membrane injury.

• ADR reduces the activity of enzymic and non-enzymic anti-oxidant and it was enhanced by pre-co-treated with d-Limonene which maintains anti-oxidant defense system and act as defense barrier to maintain cardiac integrity.

• Studies on histopathological findings shows microcytolysis and damages in cardiac muscles cells by ADR mediating cardiomyopathy which was further decreased by high potential activity of d-Limonene in pre-co-treated.

• Disruption of collagen by special stains like massion trichrome, decreasing glococonjugates by PAS, increasing mast cell count by toluidine blue stains, increasing iron (Fe$^{2+}$) by Perl’s Prussion stains and increase in production of calcium by Von kossa stain in ADR administration was significantly reduced by potential activity of d-Limonene and prevent cardiotoxicity bearing cardiomyopathy.
- Scanning electron microscopic images shows the level of collagen fiber disruption in ADR rats was completely altered by pre-co-treated with d-Limonene shows it maintain the muscular stability and maintain cardiac architecture by maintaining systolic and diastolic integrity.

- Elevated lipid profile was significantly reduced by pre-co-treated with d-Limonene shows it reduced alteration in lipid profile and prevents progression of cardiomyopathy.

- Studies on mitochondria shows abnormalities in TCA cycle enzymes and ETC complex enzymes in ADR induced rats, once treated with d-Limonene significantly reduces the enzymatic abnormalities in mitochondria and prevents kinetic modulations.

- Abnormalities in membrane bounded ATPases in ADR induced cardiomyopathy was partially altered by rats treated with d-Limonene and prevents ion channels homeostasis.

- Mitochondrial studies show ultrastructural changes in ADR mediating mitochondrial swelling was significantly prevented by administration of d-Limonene.

- The rats pre-co-treated with d-Limonene prevents the depletion of mitochondrial ATP ratio which was highly affected by ADR mediating cardiomyopathy.

- Increased expression of MMP-2 and MMP-9 in ADR inducing cardiac extracellular matrix changes and decreasing expression of TIMP-2 were
measured. *d*-Limonene treated rats decreases matrix metalloproteinase and increases TIMP-2 levels, thereby maintaining membrane integrity and preventing dialation.

- Significant elevations in level of mitochondrial calcium were observed in ADR induced rats. Treated with *d*-Limonene were reduced significantly and maintain calcium ions in subcellular levels.

- ADR induced free radicals production was enhanced by iron (Fe\textsuperscript{2+}) through redox cycling. Pre-co-treated with *d*-Limonene minimizes the iron formation prevents production of oxidants and safeguard cardiac damages.

- Inflammatory markers like COX-2 was highly expressed in ADR induced cardiac dysfunction further, pre-co-treated rats with *d*-Limonene decreases significantly and inhibit inflammatory response.

- Activation of cytokines like TNF-α, IL-1β and IL-6 were highly expressed in ADR inducing cardiac inflammatory activation. *d*-Limonene pre-co-treatment shows decrease in activation of cytokines and proves the anti-inflammatory response in damaged cardiac cells.

- DNA damage was noted by extensive DNA fragmentation in ADR mediating rats. *d*-Limonene which consequently reduces the predominant damages and prevent cell death.

- Apoptosis major execution for ADR were studied by increased expression of cytochrome-c, caspase-3, caspase-9, cleaved PARP, P53, Bax in downregulation, where *d*-Limonene up-regulating Bcl-2 (anti-apoptotic
marker) and inhibit apoptosis in treated rats. AO/EB, DAPI staining was also demonstrated anti-apoptotic effect of \(d\)-Limonene in H9C2 cardiomyocytes.

- Major consequences for ADR inducing cardiomyopathy was further denoted by MAPK signaling pathway. Increase in expression of P-JNK, P-P38 in ADR was noted. Pre-co-treated with \(d\)-Limonene finally inhibit via, MAPK signaling pathway indicating cardiopreventive role of \(d\)-Limonene against ADR inducing myocardial myopathy.

**CONCLUSION**

In summary, the present study demonstrated that administration of \(d\)-Limonene performed to be excellent anti-oxidant potential mediating by ADR inducing cardiomyopathy. Drawback for ADR mediating cardiomyopathy was due to over production of reactive oxygen species which damages normal cells. \(d\)-Limonene minimizes production of ROS in cardiac cells and act as potent cardioprotective agent by increasing anti-oxidant, anti-inflammatory and cardiac protectivity. Further, combination of anti-oxidant enhancing drugs will stabilize the various cardiac side effect in current anti-neoplastic drugs, exact mechanism for cardiopreventive action of \(d\)-Limonene have to be emphasizes in cardiovascular diseases.

This study shows that \(d\)-Limonene elicit a typical cardiopreventive role on ADR related cardiomyopathy in MAPK signaling pathway. Hence, protective effect mainly inhibit oxidative stress by anti-oxidant defense, thus prevent mitochondrial dependent apoptotic pathways. Finally \(d\)-Limonene can be used as
Cardiopreventive role of \( d \)-Limonene against ADR induced cardiomyopathy

Schematic figuration