International Journal of Scientific Research in Science and Technology
Available online at : www.ijsrst.com
Print ISSN: 2395-6011 | Online ISSN: 2395-602X
doi : https://doi.org/10.32628/IJSRST52310693

Hepatoprotective effect of D-Limonene Against Adriamycin Induced Hepatotoxicity in Experimental Rats

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ARTICLEINFO

Article History:

Accepted: 15 Dec 2023

Published: 05 Jan 2024

Publication Issue :

Volume 11, Issue 1

January-February-2024

Page Number :

08-13

ABSTRACT

The presence study is to evaluate hepatoprotective function of *d*-limonene against adraimycin induced hepatotoxicity. Monoterpenes plays an essential role to fight against various diseases. Among the various monoterpenes an efficient *d*-Limonene plays a fundamental role to fight against hepatotoxicity caused by cancer chemotheraphy treatment. Male albino Wistar rats were administrated with ADR (15mg/Kg body weight) in six equal injections and the protection efficacy of *d*-limonene (100mg/Kg body weight) was examined with reference to tissue Aspartate aminotransferase (AST) level and the pathological studies was examined by microscopic study. Rats treated with ADR results in elevated level of liver AST marker enzymes, whereas the level of AST was controlled when administrated with *d*-Limonene. However Histopathological proof added more protective role of rats treated with d-limonene against hepatotoxicity. ADR administration of 15mg/Kg body weight of rats increase the level of hepatotoxicity by increasing the marker enzyme activity and show severe morphological changes. The final outcome from our result suggests that *d*-limonene (100mg/Kg body weight) a vibrant monoterpene act as latent hepatoprotective negotiator by attenuating ADR induced hepatotoxicity.

Keywords: Adriamycin, *d*-limonene, AST, Histopathology, Hepatotoxicity

I. INTRODUCTION

Since 1960's doxorubicin or Adriamycin (ADR) an anthracycline anti-neoplastic drug widely used in treatment of major route of cancer, including Osteosarcoma (Singal P.K et al., 2000), Soft tissue Sarcomas (Schneider M.B, Matsuzaki H et al., 2001), Breast cancer (Quiles JL et al., 2002), gastric, liver (Tangpong J, cole M, Sultana R et al., 2007), esophageal carcinomas (Shri N, Mohammed A. Xiaogu D 2004), Hodgkin's lymphomas and various other cancers. However, rate limiting effect of ADR on treating cancer was hampered by various side effects on chemotherapy strategies like toxicity in central nervous system (Tangpong J, cole M, Sultana R et al., 2007), nephrotoxicity (Shri N, Mohammed A. Xiaogu D et al., 2004), hepatotoxicity and most awful condition leads to cardiomyopathy and congestive

heart failure (Weiss R, 1992). Various reports suggest cardiac damage and hepatotoxicity caused by ADR prion to be higher compare to other side effects (Kufe DW et al ., 2003). ADR mediated hepatic damage is probably by the formation of free radicals causes oxidative stress and damage. However the exact mechanism for ADR causing hepatotoxicity remains to be unclear but it is believed free radical formation (P Kufe DW et al., 2003). One of the best monocyclic monoterpene *d*-limonene is highly present in all essential citrus fruits and citrus oil with lemon aroma. Recent studies reveals administration of *d*-limonene shows low toxicity after repeated dose upto one year. Administration of *d*-limonene gradually inhibits lipid peroxidation and prevents free radical damage (Pandima Devi K et al., 2004; Clegg R.J et al., 1980). It also reported *d*-limonene have various import at biological properties like anti-oxidant potential (Clegg R.J et al., 1982; Yang X et al., 2010), anti-inflammatory (Bakkali F et al., 2008) and chemo preventive properties against numerous types of cancer (Miller J et al., 2008). The purpose of the study was intended to test the hypothesis whether a nutritional strategy like chronic administration of *d*-limonene could prevent ADR induced hepatotoxicity and hamper the free radicals production in albino rats.

II. MATERIALS AND METHODS

Chemicals: *d*-limonene and Adriamycin were purchased from Sigma-Aldrich (St Louis, MO, USA), and all other chemicals used for biochemical estimation were used analytical grade.

Animals: Adult male albino Wistar rats of (140 - 160g) were used for the study. Rats were kept in polypropylene cages and maintain under standard temperature of (25±2^{O}C) in large air conditioned room, fed with standard chow. The animals had free access to water. All the experiments were designed and conducted according to the ethical norms approved by animal ethics committee guidelines University of madras with approved number.

Experimental design

The experimental animals were divided into four groups, each group comprising of six animals.

Group I: Normal control rats fed with standard diet and drinking water for 6 weeks

Group II: Adriamycin (15mg/kg body weight) was dissolve in normal saline and given six equal injections via, intra peritoneal.

Group III: Rats treated with *d*-limonene alone (100mg/kg body weight) was dissolved in corn oil and administrated intra gastric gavage for period of four weeks.

Group IV: Rats pre-treated with *d*-limonene (100mg/kg Body weight) was dissolved in corn oil and administrated intra gastric gavage for period of four weeks and two weeks prior to adriamycin administration.

Preparation of tissue sample: The portion of liver was rinsed completely in phosphate buffer saline of 0.05M at PH 7.4 by using tissue homogenizer (10%) whole homogenate. Obtained homogenate was centrifuged by using semi centrifuge at 8000 rpm for the period of 30 mins. Separated tissue supernatant was finally used for the analysis of enzyme markers.

Morphometric studies: After sacrifice, the portion of liver tissue were removed surgically and rinsed completely by using normal physiological cold saline. The portion of liver tissue were fixed in 10% of buffered formalin and washed completely in 70% alcohols and embedded in paraffin wax. Cut the paraffin sections at 5µm thickness and stained with H&E for morphological assessment under light microscope.

Statistical Analysis: Results were analysed by mean ±S.D values significance between the groups was statistically performed by one way ANOVA followed by Tukey's multiple comparisons. Statistical comparison was followed by using graphpad prism software. P value is significantly less than 0.05.

III. RESULTS

Effect of *d*-limonene on liver marker

Fig. 3: The tissue liver marker AST of control and experimental group of rats. The animals treated with ADR showed significantly increased levels of AST when compared to control animals ($p<0.05$). Animals treated with *d*-limonene failed to induce the level of enzymes in significant ranges when compared to ADR treatment animals comparatively. Drug control animals did not show any changes and remain same as control group.

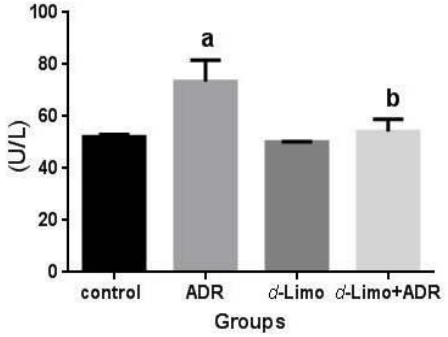

Fig. 3 : Level of AST tissue marker in the liver of control and experimental group of rats.

Effect of *d*-limonene on macroscopic finding

Fig. 4: (ADR induced) liver toxicity as a sign of enlarged and decolourisation of liver after two weeks. (ADR+*d*-limonene) treatment group of animals show greatly reduce in shape and colorization of liver when compared to ADR induced group. Whereas, no abundant changes were observe in group of control and *d*-limonene alone treated animals

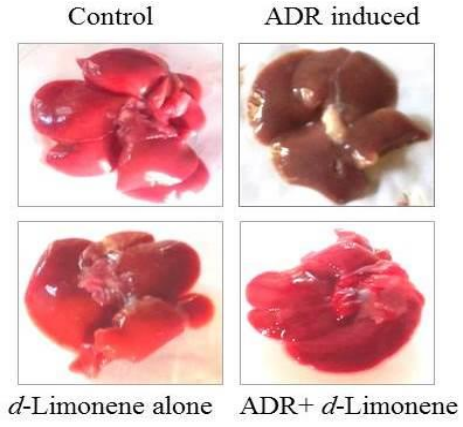

Fig. 4 : Represents the macroscopic appearance of liver on control and experimental group of rats

Effect of *d*-Limonene on histopathological studies

The histology of the heart tissue sections, stained with hematoxylin and eosin (H&E) was assessed under a light microscope. Control hepatic tissue (Fig. 5A) show increase number of polygonal cells and well-shaped rounded nuclei with eosinophilic cytoplasm. The hepatic lobes appeared lobe formed of hepatocytes with normal architecture. In contrast, ADR treated animals (Fig. 5B) showed massive pathological disarrangement, focused mainly on degeneration of hepatic cords with congestion and kupffer cells proliferation leads to destroyed cells and appear to be damaged architecture, compared to control animals. In case of rats treated with *d*-Limonene (Fig. 5D) shows less degenerated nuclei with normal hepatocytes with complete central and portal vein. In addition, most damages were followed by increased number of necrotic and apoptotic hepatocytes with dilation in blood sinusoids.

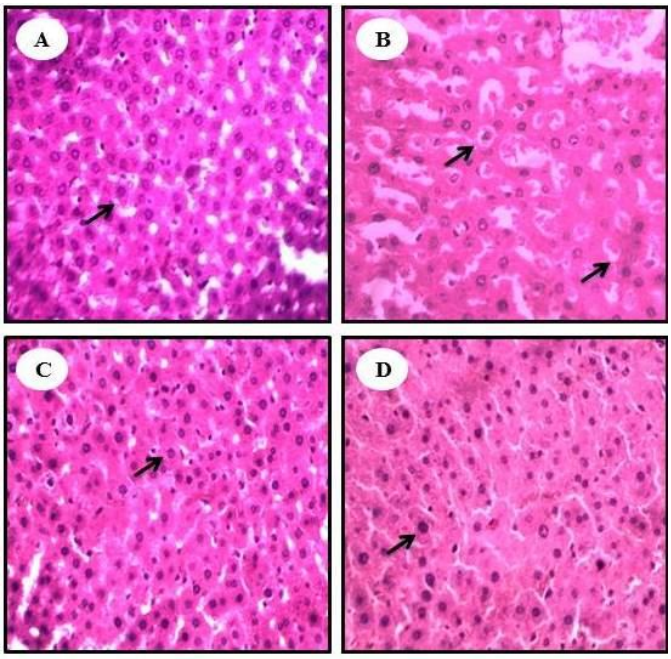

Fig. 5 : Photomicrographs (40x) obtained from 5. A. Control group, 5. B. ADR group, 5. C. *d*-Limonene alone group and 5. D. ADR+*d*-Limonene group.

IV. DISCUSSION

ADR a wide used chemotherapeutic drugs used for treating different haematological and solid tumors.

However hepatotoxicity is highly limited by dose dependent toxicity in cancer treatment condition (Horan PG et al., 2006). The exact mechanism for ADR meditate toxicity in various organs was mediated by formation of free radical leads to oxidative stress (Chularojmontri L et al., 2005). Oxygen derived free radicals causes severe lethal damage to plasma membrane and disturbs assembly of cytoskeleton (Uchiyama M et al., 1978). Oxidative stress redox mediating free radical production (Vasquezvivar J et al., 1997), mitochondrial DNA damage (Lebrecht D et al., 2005). Calcium and iron homeostatis (Solem LH et al., 1994; Kotamraju S et al., 2002). ROS and RNS are generated by various endogenous systems by different physical and pathological conditions. Due to various oxidative stresses the level of free radical shoot up to abnormal condition leads to various hepatological diseases. To regulate the over whelm of free radicals, anti-oxidant is necessary to regulate the physiological functions. Hence external source of anti-oxidant can pallitate the therapeutic strategies by controlling oxidative damage (Lobo V et al., 2011). Liver is the major target site for overall metabolism and Aspartate transaminase is one of the best marker enzymes for liver damage. ADR administrated gradually decrease the activity of Aspartate aminotransaminase level and increase the level of lipid peroxidation is highly noticeable that demonstrate the changes in cellular damages. Elevated serum Aspartate amino transaminase influence ADR toxicity, which moderately increase the outflow of these enzymes from injured liver cell. However treatment with *d*-limonene (100mg/Kg body weight) gradually decreases the negative potential of ADR treated animals thus provide extensive defence against hepatic injury. More over the histopathological finding revealed that animals treated with (100mg/kg) of *d*-limonene has protected hepatic tissues from necrosis induced by ADR. So it has finally revealed that the animals pre-treated with *d*-limonene protect hepatic damage against ADR induced hepatic toxicity in experimental group of animals.

V. CONCLUSION

Finally, the present study reveal that *d*-limonene plays an important role in defending against ADR induced hepatic damage or hepatotoxicity by ameliorating free radicals and iron chelating properties by scavenging lipid alkoxyl and peroxyl radical. On the basic of these finding it suggest the subsequent level of *d*-limonene prior to ADR will slowly reduce the impairment to live in cancer chemotherapy. Further clinical studies are abundantly requisite to find the beneficial and therapeutic functions of *d*-limonene in cancer treatment patients.

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Cite this article as :

Indumathi Selvanathan, "Hepatoprotective effect of D-Limonene Against Adriamycin Induced Hepatotoxicity in Experimental Rats", International Journal of Scientific Research in Science and Technology (IJSRST), Online ISSN : 2395-602X, Print ISSN : 2395-6011, Volume 11 Issue 1, pp. 08-13, January-February 2024. Available at doi :
https://doi.org/10.32628/IJSRST52310693
Journal URL : https://ijsrst.com/IJSRST52310693