



Tocotrienol Mitigating Adverse Effect of Doxorubicin on Pancreas Tissue in Male Rats

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ABSTRACT

Doxorubicin (DAX) as chemotherapy agent, it is known to induced acute pancreatitis in a patient with breast cancer, Tocotrienol (T3) has multiple biological properties such as antioxidant and anti-inflammatory agent. The role of T3 in pancreas protection during chemotherapy still not investigated. Aim: study has undertaken to investigate the role of T3 in the preservation of DAX-damaged pancreas. Method: thirty male rats were divided into five treatment groups, namely, normal saline, olive oil only, T3 only, DAX and DAX with T3. The treatment was given for 30 days. After killing, pancreas tissues were eviscerated and examined histologically. Results: pancreas tissues were significant reduction in size in the DAX group compared with the normal group (DAX versus normal, mean area \pm SD; 3.12 ± 0.24 vs. 5.61 ± 0.28 cm²; $p < 0.005$), whilst synchronized administration of T3 with DAX leads to conservation of pancreas tissues (DAX+ T3 vs. DAX, mean area \pm SD; 3.12 ± 0.24 vs. 4.35 ± 0.18 cm²; $p < 0.005$). DAX showed abnormal atrophy in islet of Langerhans with highly degeneration in beta cells, interstitial edema, increased vascularity and inflammatory cell infiltration. These changes were reversed by concurrent T3 administration with DAX. To our knowledge, this is the first case of protection acute pancreatitis as deleterious effects of DAX administration by T3 supplementation.

Keywords: Tocotrienol, pancreas tissues, antioxidant, DAX.

Introduction

Chemotherapy drug doxorubicin, which has been used for treating cancer for over 30 years. Although doxorubicin remains one of the most effective anticancer agents. Doxorubicin causes life-threatening toxicity to most major organs, primarily life-threatening toxicity (Tacar et al., 2013). Consequently, it is well known that pancreatitis is frequently secondary to biliary tract disease and alcohol abuse, it can also be caused by drugs, trauma and viral infections, or even be

associated with metabolic and connective tissue disorders (Sakorafas & Tsiotou, 2000). Knowledge of the true incidence of drug-induced acute pancreatitis is dependent on the clinician's ability to exclude other possible causes, and by promptly reporting the occurrence. Several studies have been reported about the drug-induced acute pancreatitis, the estimated overall incidence ranges from between 0.1 and 2% of pancreatitis cases (Tacar et al., 2013; Dionne et al., 1993; Sakorafas & Tsiotou, 2000).

In particular, drug-induced acute pancreatitis is of mild severity and usually resolves without significant complications (Tonsi et al., 2009). Many authors have reported that categorize the risk of drugs causing acute pancreatitis. A previous classification system described by Mallory and Kern (1980), categorized drugs associated with acute pancreatitis. Trivedi et al. proposed a newer classification system for commonly used medications associated with drug-induced pancreatitis according to severity of influence (Trivedi & Pitchumoni, 2005).

The vitamin E family consists of eight isomers known as alpha-, beta-, gamma-, and delta-tocopherols and alpha-, beta-, gamma-, and delta-Tocotrienol. Numerous studies focused on the health benefits of these isomers have been performed since the discovery of vitamin E in 1922. Recent discoveries on the potential therapeutic applications of tocotrienols have revolutionized vitamin E research. Nevertheless, despite the abundance of literature, only 1% of vitamin E research has been conducted on tocotrienols. Many new advances suggest that the use of tocotrienols for health improvement or therapeutic purposes is promising (Wong & Radhakrishnan, 2012) Tocotrienols possess powerful neuroprotective, antioxidant, anti-cancer and cholesterol lowering properties that often differ from the properties of tocopherols.

Experimental research examining the antioxidant, free radical scavenging effects of tocotrienols revealed that the distribution in the fatty layers of the cell membrane therefore, T3s considered as cardio-protective (Chou et al., 2009), anticancer (Park et al., 2005), neuro-protective (Kuhad & Chopra, 2009) and cancer prevention activities (Wada et al., 2005). The effect of T3 as a chemo protective agent to maintenance certain affected tissue due to chemotherapy remains to be elucidated. The aim of this study is to further characterize morphologic changes in pancreatic tissue following with DAX and the effect of T3 on the said islet Langerhans changes when administered in combination with DAX.

Methods

This is a prospective, randomized, controlled, interventional animal study using rats as the subject. This study was reviewed and approved by local authorities in the Laboratory Animal Care that was in accordance with institutional guidelines Thirty adult male Sprague–Dawely rats weighing between (150–250g) and an age between 12-16 weeks were used in the experiments.

Experimental groups and procedures

The rats were randomly divided into five experimental groups. The first group was given 0.15 ml normal saline intraperitoneally (i.p.; normal control), the second group given olive oil only (0.1 ml, oral gavage), the third group treated with T3 only (given 60 mg/kg body weight T3, oral

gavage) 10, the fourth group with 15mg/kg body weight DAX injected i.p.4 and the fifth group was administered the same dose of DAX, in addition to 60 mg/kg body weight T3 by oral needle gavages 10. All treatments were given for 30 days. After the administration of the last dose the animals, all the animals were euthanized under anesthesia in a chamber containing diethyl ether. Pancreatic tissue was eviscerated and fixed in 10% formalin, subsequently embedded in paraffin, serially sectioned at 3 mm thickness and stained with hematoxylin and eosin. They were viewed under the light microscope.

Statistical analysis

All data were subjected to statistical analysis by one way analysis of variance using Statistical Package for the SPSS version 19. Significant differences were analyzed by Duncan's triplicates range test. Results were presented as mean \pm standard deviation; statistical significance was taken significant if the difference is at p value < 0.05 .

Results

Pancreatic tissues size

The pancreatic tissues in DAX-treated group was significantly smaller than normal control (DAX vs. normal). Meanwhile co-administration of T3 with DAX leads to significant preservation of pancreatic tissues, with the size being comparable with the normal group (Figure 1).

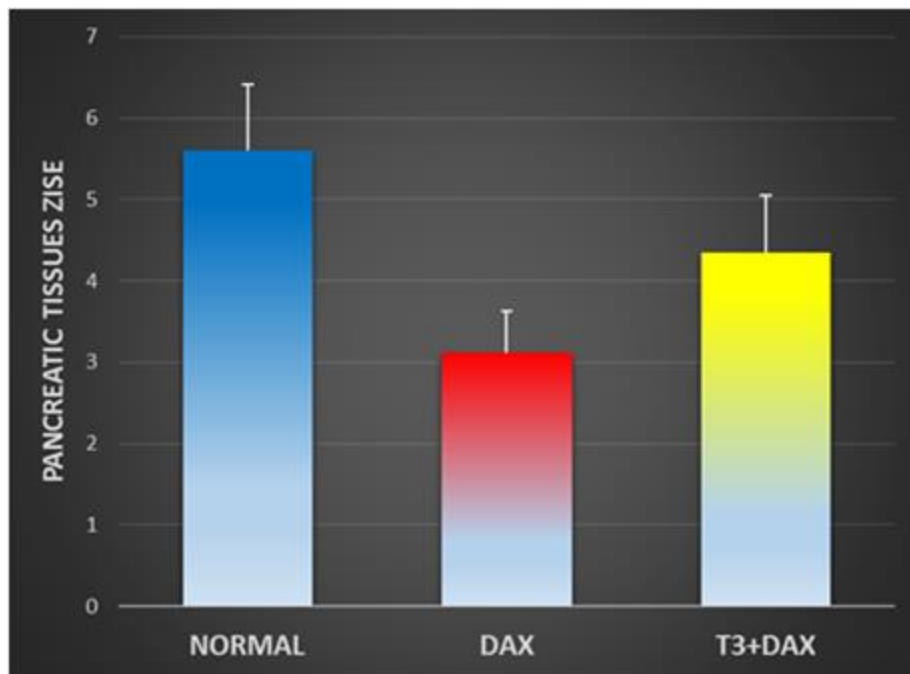


Figure 1. Pancreatic tissues size

In a control group, the histological findings of pancreatic tissues showing normal architecture structure also the cellular integrity was intact (Figure 2). The islets of Langerhans covered by thin capsule of connective tissue and appeared regular in shape as like a round clusters of cells embedded in the exocrine tissue.

In rats treated with DAX (Figure 3), the findings were degenerative and necrotic changes, and shrinkage in the islets of Langerhans. The islets were relatively small, atrophied, and showed a reduction in the number of polygonal islet cells. The nucleus of necrotic cells indicated marginal hyperchromatic. There was mostly hydropic degeneration and degranulation in the cytoplasm of the degenerative and necrotic cells, while some of the cells with a pyknotic nucleus had a dark eosinophilic cytoplasm. However, it was evident improvement with T3 supplementation group that showed in Figure 4. Since there was protection to the majority of the Langerhans islets' cell which appeared regular in shape and not so much similar to the normal tissue and these effects were not as dramatic as in the all other groups.

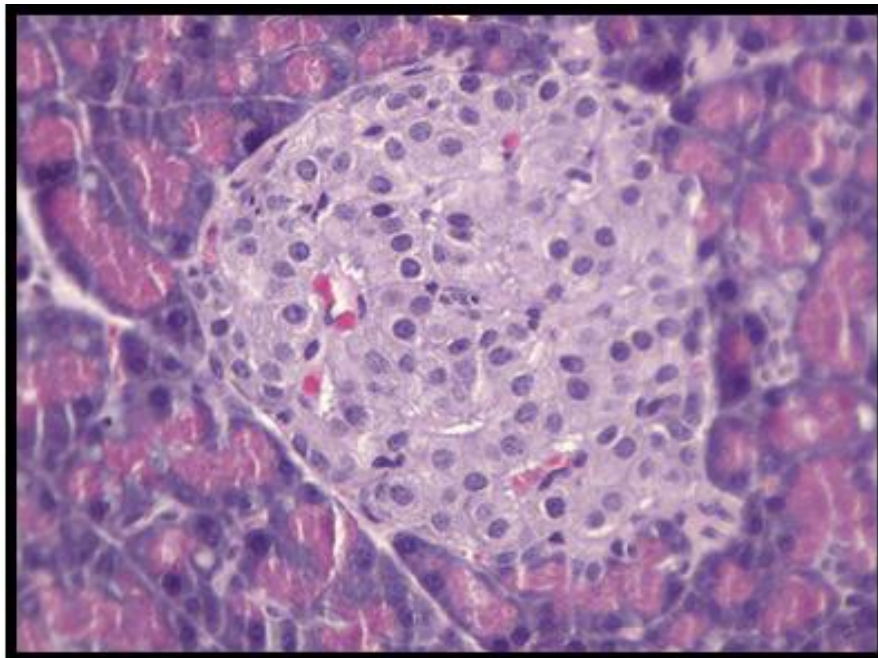


Figure 2. Islet of Langerhans of normal group, showing normal cells and normal architecture of Islet of Langerhans (H&E Staining _ 40 magnification)

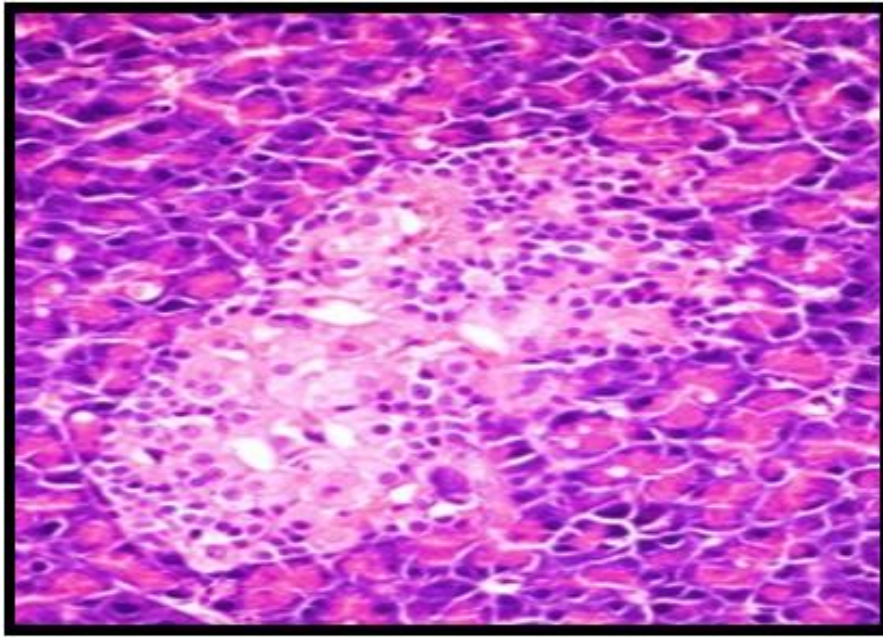


Figure 3. DAX –treated Rats. Showing reduction in number of cells, necrotic change Islets showed disrupted cytoplasm in certain areas displaying small vacuoles (H&E Staining-40 magnification)

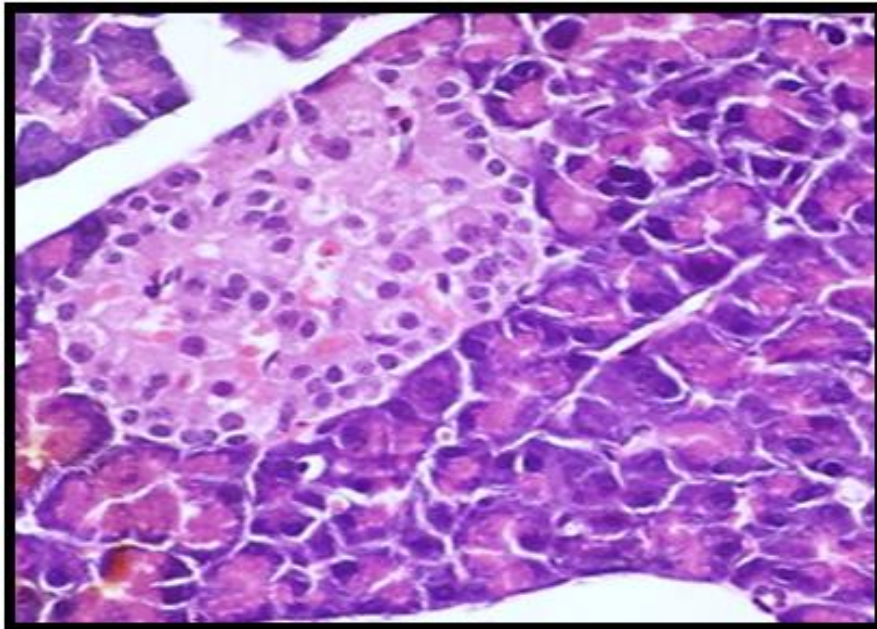


Figure 4. Islets of Langerhans of group treated with combine DAX+T3, showing normal shape. The islets of Langerhan appeared regular nuclei in shape and consisted of polygonal cells similarity with normal control (H&E Staining-40 magnification)

Discussion

Doxorubicin is known as chemotherapy-induced acute pancreatitis which is one of the potential complications 1 and 8. Nevertheless, despite the abundance of literature, only 1% of vitamin E research has been conducted on tocotrienols. Many new advances suggest that the use of T3 for health improvement or therapeutic purposes is promising due to anti-oxidative ability. This scenario highlights the importance of chemotherapy administration safety.

The results of this study has shown the restoration of pancreas tissue size in combination DAX and T3 group that might be concerned to the activity of T3 as potent anti-oxidation, Therefore antioxidant treatment has great promise in alleviating some of the detrimental effects of oxidative stress (Finkel, 2005). Whereas DAX administration resulted in reduction pancreatic tissue size that may attribute to atrophy of islet Langerhans tissue was due to a cytotoxic effect of DAX. It was corresponding to recent study which referred that DAX causes life-threatening toxicity in most major tissues in whole body (Tacar et al., 2013). In same context, these results consist with previous study had administrated that Effect of hypoxia on rat pancreas induced disintegration in morphological features (Dionne et al., 1993).

In this current study, we have investigated that the DAX- treated rats indicated that cells clusters of islet of Langerhans were irregular in shape. As well as, decreased in cells size and some cells had undergone necrosis. Histopathological study also showed degeneration of pancreatic islet cells. Our present findings were consistent with many results from previous studies which were found the same histological changes (Standl et al., 2003; Mythili et al., 2004; Attia, 2009). The pathophysiology behind drug-induced pancreatic injury was disclosure by different studies (Salvador et al., 2014; Badalov et al., 2007). Potential mechanisms underlying such pancreatic injury might be related to drug hepatotoxicity which can be secondary to intrinsic toxicity of the drugs affecting the tissue. In the vast majority of the cases, production of toxic intermediate metabolites (Badalov et al., 2007).

Based on our data of rats supplementation T3 during chemotherapy treatment, we found the lowering for degenerative and necrotic changes in the islets cells compared with DAX treatment group. Furthermore, results had demonstrated that DAX- treated rats with T3 was almost regenerative effect on islets of Langerhans cells and a picture like normal was appeared and increased in size of islets. In agreement with our results, the morphology of pancreatic islets of Langerhans showed similar size as appeared in normal control and the size of pancreatic cell was reduced.

In this study was consistent with previous studies which showed that may be a sign of regeneration. Signs of regeneration of islets cells have been reported following consumption of plant extracts (Ali et al., 2005; Jelodar. et al., 2005; Patel et al., 2012). Several authors have proposed that a regenerative effect of plants on pancreatic tissue with staining along with ordinary H&E staining has been used to clarify the effect of applied plants on pancreatic cells (Noor et al., 2008; Item et al., 2010). It is well-established that physiological role of T3 in a free radical scavenger in the body.

The presence of unsaturated isoprenoid side chain in T3 results in its increased fluidity and mobility between the membranes (Atkinson et al., 2008). Thus, that was leading to an increase in the efficiency of T3 interaction with lipid radicals compared with tocopherols (Serbinova et al., 1991). In addition, T3 is incorporated and transferred in between the cell membrane at a rapid rate (Yoshida et al., 2003) due to shorter side chain of its chromosols (Salvador et al., 2014). The protective effect of T3 on oxidative stress has also been reported in carbon tetrachloride-induced liver damage as well as it reduces the serum level of hepatic enzymes, as a marker of hepatic cell injury due to the oxidative damage caused by carbon tetrachloride (Yachi et al., 2010).

Conclusions

These protective effects might be due to antioxidant and anti-inflammatory properties of T3 that prevent the damage to pancreatic cells. In conclusion, this study evidently indicated that DAX treatment markedly damaged pancreatic tissue that might result in impairment tissue function and that treatment with T3 might prevent this toxicity in rats. Acute pancreatitis after chemotherapy exposure is highly suggestive to decrease role these chemotherapeutic agents as adverse effects.

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