Study of some toxic effects of anticancer drug, doxorubicin and the use of some protective agents against doxorubicin induced toxicity
(Experiment study)

Thesis
Submitted For Partial Fulfillment of M.D Degree in Pharmacology

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دراسة لبعض التأثيرات السمية لعقار الدوكسيبروبسين وبعض المواد التي تبقى من تلك السمية (دراسة معملية)

رسالة مقدمة من الطبية / صفوه محمود سرور
مدرس مساعد قسم الأدوية والعلاج
كلية طب بنها

رسالة تؤهل للحصول على درجة الدكتوراه في الفارماكولوجي

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أستاذ ورئيس قسم الفارماكولوجي
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جامعة بنها

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<td>CPK</td>
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<td>ALT</td>
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<td>AST</td>
<td>Aspartate transaminase</td>
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<td>AIDS</td>
<td>Acquired immune deficiency syndrome</td>
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<td>AC</td>
<td>Adriamycin</td>
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<td>CHF</td>
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<td>LV</td>
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<td>LVEF</td>
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<td>MIBG</td>
<td>Metaiodobenzylguanidine</td>
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<td>SGOT</td>
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<td>Poor metabolizers</td>
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<td>Nitric oxide</td>
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<tr>
<td>cGMP</td>
<td>Cyclic granulate mono phosphate</td>
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<tr>
<td>ROS</td>
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<td>GFR</td>
<td>Glomerular filtration rate</td>
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<td>CoA</td>
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<td>CROT</td>
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"قالوا سبحانك لا علم لنا إلا ما علمتنا إنك أنت أصلح الخلق الحكيم"

صدق الله العظيم

"سورة البقرة - آية 33"
Aim of the work

Doxorubicin is one of the potent anticancer drugs which is commonly indicated in many tumors. This drug has many organ toxicities especially cardiotoxicity and this toxicity is the main limitation for its use as anticancer drug, so this study is planned to investigate the toxicity of this drug on some major organs including heart, liver and kidney.

Many researchers have expended great efforts aiming at preventing or decreasing the adverse effects of doxorubicin, so the aim of this study is to investigate the possible protective role of some chemical agents like nebivolol and some natural agents like L-carnitine against doxorubicin organ toxicity.

This will be achieved by investigating:

- **In vivo study:** The effects of doxorubicin alone, doxorubicin with nebivolol, doxorubicin with L-carnitine and doxorubicin with nebivolol & L-carnitine on:
  1- ECG and blood pressure.
  2- Cardiac enzymes as troponin I and CPK.
  3- Liver enzymes as ALT and AST.
  4- kidney function as creatinine.
  5- histopathological examination of heart, liver and kidney.

- **In-vitro study:**
  1) The effect of isoprenaline on contractility of hearts taken from another 5 groups of rats given the same medications as the groups of the in-vivo experiment.
  2) The effects of doxorubicin on isolated perfused rabbit’s heart.
Doxorubicin

Doxorubicin is a drug used in cancer chemotherapy. It is an anthracycline antibiotic, it works by intercalating DNA, with the most serious adverse effect being life-threatening cardiotoxicity. The drug is administered intravenously, as the hydrochloride salt. It is commonly used in the treatment of a wide range of cancers, including some leukemias and Hodgkin's lymphoma, as well as cancers of the bladder, breast, stomach, lung, ovaries, thyroid, soft tissue sarcoma, multiple myeloma, and others (Takimoto and Calvo, 2008). Commonly used doxorubicin-containing regimens are AC (Adriamycin, cyclophosphamide), TAC (Taxotere, AC), ABVD (Adriamycin, bleomycin, vinblastine, dacarbazine), and FAC (5-fluorouracil, adriamycin, cyclophosphamide) (Laginha, 2005).

Physical properties:

It is orange to red powder, soluble in water, normal saline, methanol and stable under normal conditions. It is light and moisture senstive (Yang et al., 2009).

Chemistry:

![Chemical structure of doxorubicin](image_url)

7-4-amino-5-hydroxy-6-methyloxan-2-oxy-6,9,11-trihydroxy-9-4-methoxy-8,10-dihydro-7H-tetracene-5 coated by Minotti et al., 2004
Pharmacokinetics:

Pharmacokinetic studies, determined in patients with various types of tumors undergoing either single or multi-agent therapy have shown that doxorubicin follows a multiphasic disposition after intravenous injection. Doxorubicin hydrochloride is not stable in gastric acid, and animal studies indicate that the drug undergoes little, if any, absorption from the gastrointestinal tract. The drug is extremely irritating to tissues and, therefore, must be administered intravenously. The initial distribution half-life of doxorubicin is approximately 5 minutes suggests rapid tissue uptake of doxorubicin, while its slow elimination from tissues is reflected by a terminal half-life of 20 to 48 hours (Lewis et al., 2006).

Binding of doxorubicin and its major metabolite, doxorubicinol to plasma proteins is about 74 to 76% and is independent of its plasma concentration of doxorubicin. Doxorubicin is excreted in the milk of the lactating patient, with peak milk concentration at 24 hours after treatment being approximately 4.4-fold greater than the corresponding plasma concentration. Doxorubicin is detectable in the milk up to 72 hours after a 15-minute intravenous infusion (Stephen et al., 1993).

Doxorubicin does not cross the blood brain barrier. Enzymatic reduction at the 7 position and cleavage of the daunosamine sugar yields aglycones which are accompanied by free radical formation, the local production of which may contribute to the cardiotoxic activity of doxorubicin. Plasma clearance of doxorubicin ranges from 324 to 809 mL/min and is predominately by metabolism and biliary excretion. Approximately 40% of the dose appears in the bile in 5 days, while only 5 to 12% of the drug and its metabolites appear in the urine during the same time period. In urine, <3% of the dose was recovered as
doxorubicinol over 7 days. Systemic clearance of doxorubicin is significantly reduced in obese women. There was a significant reduction in clearance without any change in volume of distribution in obese patients when compared with normal patients (Minotti et al., 2004).

**Mechanism of action:**

The cytotoxic effect of doxorubicin on malignant cells and its toxic effects on various organs are thought to be related to nucleotide base intercalation and cell membrane lipid binding activities of doxorubicin. Intercalation inhibits nucleotide replication and action of DNA and RNA polymerases. The interaction of doxorubicin with topoisomerase II to form DNA-cleavable complexes appears to be an important mechanism of doxorubicin cytocidal activity (Pommier et al., 2010). This inhibits the progression of the enzyme topoisomerase II, which relaxes supercoils in DNA for transcription. Doxorubicin stabilizes the topoisomerase II complex after it has broken the DNA chain for replication, preventing the DNA double helix from being resealed and thereby stopping the process of replication (Tacar et al., 2013).

Doxorubicin cellular membrane binding may affect a variety of cellular functions. Enzymatic electron reduction of doxorubicin by a variety of oxidases, reductases and dehydrogenases generates highly reactive species including the hydroxyl free radical OH•. Cells treated with doxorubicin have been shown to manifest the characteristic morphologic changes or apoptosis. Induced Apoptosis may be an integral component of the cellular mechanism of action relating to therapeutic effects, toxicities or both (Shi et al., 2011).
Clinical pharmacology of doxorubicin:

Doxorubicin has produced significant therapeutic responses in a number of solid tumours and haematologic malignancies, and is commonly used in the treatment of carcinoma of the breast, the lung, the ovary, transitional cell bladder cancer, neuroblastoma, Wilm's tumour, soft tissue sarcomas, osteosarcoma, acute lymphocytic - lymphoblastic leukaemia, acute myelogenous leukaemia, non-Hodgkin's lymphoma & Hodgkin's disease. Doxorubicin has also shown antitumour activity in some adult and paediatric malignancies as carcinoma of the thyroid, carcinoma of the endometrium, carcinoma of the head and neck, carcinoma of the stomach, primary hepatocellular carcinoma, carcinoma of the testis, carcinoma of the prostate, Ewing's sarcoma, rhabdomyosarcoma, multiple myeloma & chronic leukaemias (Yun, 2010).

Doxorubicin is a cytotoxic drug that is usually administered to cancer patients by the intravenous and, whenever appropriate, intravesical and intra-arterial route also may be used. In intravenous (IV) Administration, dosage is usually calculated on the basis of body surface area (mg/m2). The doxorubicin dose-schedule may differ depending on the therapeutic indication (e.g. solid tumours or acute leukaemias) as well as on its use within a specific regimen (e.g. as a single agent or in combination with other cytotoxics or as a part of multidisciplinary approaches which include combination with surgery and/or radiotherapy and/or hormonotherapy). Intravenous administration of doxorubicin should be performed with caution. It is recommended to administer doxorubicin IV infusion within isotonic sodium chloride or 5% glucose solution over a period of 3 to 5 minutes. This technique is intended to
minimize the risk of thrombosis or perivenous extravasation which could lead to severe cellulitis, vesication and tissue necrosis. A direct push injection is not recommended due to the risk of extravasation (Schulmeister, 2007).

Treatment of solid tumours, when doxorubicin is administered as a single agent, the recommended dose per cycle is 60-75mg/m2 every three weeks. The drug is generally given as a single dose per cycle; however, it is possible to give the drug dosage per cycle in divided administrations (e.g. day 1 and 3, or days 1 and 8) (Hughes, 1986).

In the management of acute leukaemias, bone marrow aplasia is a therapeutic achievement and intensive combination chemotherapy schedules are employed. In this situation the recommended dose of doxorubicin is 2.4mg/kg of body weight (approximately corresponding to 75-90mg/m2), to be administered divided over three consecutive days (one cycle). The time and dose of the second cycle should be determined by both the bone marrow and peripheral blood cells status. The interval between cycles should be however at least 10 days (St Marie and Camp-Sorrell, 2000).

Doxorubicin has been also used by the intra-arterial route in an attempt to produce intense local activity with reduced general toxicity. Since this technique is potentially hazardous and can lead to widespread necrosis of the perfused tissue, intra-arterial administration should only be attempted by physicians fully trained with this technique. Doxorubicin is usually administered intravenously. The solution should be injected over 3 to 5 minutes through the tubing of a freely-running infusion of physiological solution, after confirmation that the needle is correctly inserted into the vein. This technique reduces the risk of thrombosis and
perivenous extravasation of the drug that can lead to severe cellulitis and necrosis, and ensures the washing of the vein after administration. Injection in small veins and repeated injection in the same vein can lead to venous sclerosis (Hughes, 1986)

**Drug Interactions:**

Doxorubicin is extensively metabolized by the liver. Changes in hepatic function induced by concomitant therapies may affect doxorubicin metabolism, pharmacokinetics, therapeutic efficacy, and/or toxicity. Toxicities associated with doxorubicin, especially hematologic and gastrointestinal events, may be increased when doxorubicin is used in combination with other cytotoxic drugs. There is an increase in cardiotoxicity when doxorubicin is co-administered with paclitaxel due to significant decrease in doxorubicin clearance with more profound neutropenic and stomatitis episodes. When progesterone was given intravenously to patients with advanced malignancies at high doses (up to 10 g over 24 hours) concomitantly with a fixed Doxorubicin dose (60 mg/m2) via bolus injection, enhanced doxorubicin-induced neutropenia and thrombocytopenia were observed. The addition of cyclosporine to doxorubicin may result in increases in area under the curve for both doxorubicin and doxorubicinol possibly due to a decrease in clearance of parent drug and a decrease in metabolism of doxorubicinol. Adding cyclosporine to doxorubicin results in more profound and prolonged hematologic toxicity than doxorubicin alone. Coma and/or seizures have also been described (Stephen, 1993).

Necrotizing colitis manifested by typhlitis (cecal inflammation), bloody stools, and severe & sometimes fatal infections have been associated when a combination of cetarabine and doxorubicin are given.
The addition of cyclophosphamide to doxorubicin treatment does not affect exposure to doxorubicin, but may result in an increase in exposure to doxorubicinol. Doxorubicinol only has 5% of the cytotoxic activity of doxorubicin. Concurrent treatment with doxorubicin has been reported to exacerbate cyclophosphamide-induced hemorrhagic cystitis. Acute myeloid leukemia has been reported as a second malignancy after treatment with doxorubicin and cyclophosphamide (Chen et al., 2002).

**Adverse effects:**

(1) **Cardiotoxicity:**

The most dangerous side effect of doxorubicin is heart damage. When the cumulative dose of doxorubicin reaches 550 mg mg/m², the risks of developing cardiac side effects, including dilated cardiomyopathy, CHF, markedly increase. Doxorubicin cardiotoxicity is characterized by a dose-dependent decline in mitochondrial oxidative phosphorylation. Reactive oxygen species, generated by the interaction of doxorubicin with iron, can then damage the myocytes (heart cells), causing myofibrillar loss and cytoplasmic vacuolization. Morphologic damage has been quantitated by pathologists from samples obtained by myocardial biopsy. The cellular lesions are known as "myofibrillar dropout". Myofibrillar dropout consists of swelling of the sarcoplasmic reticulum and, with more advanced stages of damage, complete loss of myofibrils (Shi et al., 2011).

Anthracycline-induced cardiotoxicity may be manifested by acute or chronic event.

**Acute** doxorubicin cardiotoxicity, occurs during and within 2–3 days of its administration. The incidence of acute cardiotoxicity is
approximately 11% \cite{Swain2003}. The manifestations are usually chest pain due to myopericarditis and/or palpitations due to sinus tachycardia, paroxysmal nonsustained supraventricular tachycardia and premature atrial and ventricular beats. The electrocardiogram may reveal nonspecific ST-T changes, left axis deviation and decreased amplitude of QRS complexes. The mechanisms for these acute changes are not clear but may be due to doxorubicin-induced myocardial edema, which is reversible. Acute left-ventricular (LV) failure is a rare manifestation of acute cardiotoxicity, but it is also reversible with appropriate treatments \cite{Takemura2007}.

Chronic doxorubicin cardiotoxicity, it's incidence is much lower, with an estimated incidence about 1.7%. It is usually evident within 30 days of administration of its last dose, but it may occur even after 6–10 years after its administration. It is manifested by a reduction in LVEF (left ventricular ejection fraction) and/or signs and symptoms of congestive heart failure (CHF) such as tachycardia, dyspnea, pulmonary edema, dependent edema, cardiomegaly and hepatomegaly, oliguria, ascites, pleural effusion, and gallop rhythm. Subacute effects such as pericarditis/myocarditis have also been reported. Life-threatening CHF is the most severe form of anthracycline-induced cardiomyopathy and represents the cumulative dose-limiting toxicity of the drug \cite{Kili2007}.

Age also influences the risk of developing doxorubicin cardiomyopathy. Very young and very old individuals are more prone to develop this complication. A history of cardiovascular disease such as hypertension and reduced LV ejection fraction before therapy is also a risk factor to develop this complication. The prognosis of patients who
develop congestive heart failure is poor (50% mortality in 1 year) (Broder et al., 2008).

The histopathological changes in doxorubicin cardiomyopathy are in the form of areas of patchy myocardial interstitial fibrosis and scattered vacuolated cardiomyocytes (Adria cells). Adria cells are seen adjacent to areas of fibrosis. The areas of fibrosis are usually widespread and areas of acute myocyte damage are infrequent. There is fibroblast proliferation and histiocyte infiltration in the areas of healed myocarditis. Partial or total loss of myofibrils and myocyte vacuolar degeneration are essential features of doxorubicin cardiotoxicity. With loss of myofilaments, the remnants of Z discs are seen. There is distention of sarcoplasmic reticulum and the T-tubules. The myocyte vacuoles coalesce and form large membrane-bound spaces. The nucleus-chromatin disorganization and replacement of chromatin by pale filaments are also features of doxorubicin cardiomyopathy (Takemura and Fujiwara, 2007).

Cardiotoxicity may occur at lower doses in patients with prior mediastinal/pericardial irradiation, concomitant use of other cardiotoxic drugs, doxorubicin exposure at an early age, advanced age and pre-existing heart disease is a cofactor for increased risk of doxorubicin cardiotoxicity. In such cases, cardiac toxicity may occur at doses lower than the recommended cumulative dose of doxorubicin. Studies have suggested that concomitant administration of doxorubicin and calcium channel blockers or cardiotoxic drugs, especially those with long half-lives, e.g., trastuzumab) may increase the risk of doxorubicin cardiotoxicity (Chatterjee et al., 2010).

The total dose of doxorubicin administered to the individual patient should also take into account previous or concomitant therapy with
related compounds such as daunorubicin, idarubicin, and mitoxantrone. Although not formally tested, it is probable that the toxicity of doxorubicin and other anthracyclines or anthracenediones is additive. Cardiomyopathy and/or congestive heart failure may be encountered several months or years after discontinuation of doxorubicin therapy (Simunek et al., 2009).

The risk of acute manifestations of doxorubicin cardiotoxicity in pediatric patients may be as that in adults. Pediatric patients appear to be at particular risk for developing delayed cardiac toxicity as doxorubicin-induced cardiomyopathy impairs myocardial growth in pediatric patients and with advance in age congestive heart failure may develop in early adulthood. As many as 40% of pediatric patients may have subclinical cardiac dysfunction and 5 to 10% of pediatric patients may develop congestive heart failure on long-term follow-up. This late cardiac toxicity may be related to the dose of doxorubicin. The longer the length of follow-up, the greater the increase in the detection rate. Treatment of doxorubicin-induced congestive heart failure includes the use of digitalis, diuretics, after load reducers such as angiotensin converting enzyme (ACE) inhibitors, low salt diet, and bed rest. Such intervention may relieve symptoms and improve the functional status of the patient (Ibrahim et al., 2009).

Diagnosis of doxorubicin cardiomyopathy:

The diagnosis of doxorubicin cardiomyopathy should consist of taking appropriate history to assess the likelihood of the diagnosis. A complete examination of the cardiovascular system to detect presence of signs of overt heart failure, such as elevated jugular venous pressure and S3 gallop, is essential. An electrocardiogram should be obtained, which
usually demonstrates nonspecific ST-T wave changes and sometimes low-voltage QRS complexes. A chest X-ray is also helpful to assess cardiomegaly and signs of pulmonary venous congestion. It should be emphasized that the presence or absence of the abnormalities by these evaluations are nonspecific and nondiagnostic (Saway et al., 2011).

Echocardiography with Doppler studies is commonly used to detect early diastolic and systolic LV dysfunction. Exercise echocardiography may also be useful to assess LV contractile reserve (Tassan-Mangina et al., 2006).

Radionuclide ventriculography has been used to assess LV systolic and diastolic function. Like other types of cardiomyopathy, cardiac adrenergic denervation occurs in doxorubicin cardiomyopathy. MIBG (metiiodobenzylguanidine) nuclear imaging can be employed to assess cardiac adrenergic denervation. Impaired glucose and fatty acid metabolism has been observed in doxorubicin cardiomyopathy. Impaired myocardial glucose uptake can be assessed by positron emission tomography using fluorine-$^{18}$F-deoxyglucose (Takamura and Fujiwara, 2007).

Antimyosin antibody study with the use of $^{111}$In-labeled monoclonal antimyosin antibody is used for the diagnosis of myocarditis and has also been employed for the diagnosis of doxorubicin cardiomyopathy (Koopman et al., 1994).

Annexin V, which has high affinity for membrane-bound phosphatidylycerine, has been used to detect apoptosis induced by doxorubicin. The measurements of neurohormones and cardiac enzymes have been used for the diagnosis of doxorubicin cardiotoxicity and
presence of heart failure. Plasma levels of B-type natriuretic peptide are elevated and correlate with the severity of congestive heart failure. The troponin T or I levels may also be increased, indicating myocardial injury (Cardinale and Sandri, 2010).

The changes in neurohormones and cardiac enzymes are not diagnostic of doxorubicin cardiomyopathy and are observed in other types of cardiomyopathy. The endomyocardial biopsy may reveal characteristic diagnostic features of doxorubicin cardiomyopathy. The findings that have been suggested for the diagnosis of doxorubicin cardiomyopathy are loss of myofibrils, distention of sarcoplasmic reticulum, and vacuolization of the cytoplasm. The endomyocardial biopsy is also used to grade the severity of doxorubicin cardiotoxicity (Takemura and Fujiwara, 2007). The disadvantage of this technique is that it is invasive, and it requires considerable experience and training. Furthermore, endomyocardial biopsies are not universally used for the diagnosis of doxorubicin cardiomyopathy and its severity. Endomyocardial biopsy is recognized as the most sensitive diagnostic tool to detect anthracycline-induced cardiomyopathy; however, this invasive examination is not practically performed on a routine basis. ECG changes such as dysrhythmias, a reduction of the QRS voltage, or a prolongation beyond normal limits of the systolic time interval may be indicative of anthracycline-induced cardiomyopathy, but ECG is not a sensitive or specific method for following anthracycline-related cardiotoxicity (Cardinale and Sandri, 2010).

Pediatric patients are at increased risk for developing delayed cardiotoxicity following doxorubicin administration and therefore a follow-up cardiac evaluation is recommended periodically to monitor for this delayed cardiotoxicity. In adults, a 10% decline in LVEF to below
the lower limit of normal or an absolute LVEF of 45%, or a 20% decline in LVEF at any level is indicative of deterioration in cardiac function. In pediatric patients, deterioration in cardiac function during or after the completion of therapy with doxorubicin is indicated by a decline in LVEF of 10 percentile units or an LVEF below 55% \( (Hershman\ et\ et\ al.,\ 2008)\).

In general, if test results indicate deterioration in cardiac function associated with doxorubicin, the benefit of continued therapy should be carefully evaluated against the risk of producing irreversible cardiac damage. Acute life-threatening arrhythmias have been reported to occur during or within a few hours after doxorubicin administration \( (Chatterjee\ et\ et\ al.,2010)\).

There is no specific treatment available for the management of patients with established heart failure. Diuretics are used to relieve symptoms and signs of pulmonary and systemic venous congestion. β-Adrenergic blocking agents should be considered, as for treatment of other types of systolic heart failure \( (Malcom\ et\ et\ al.,\ 2008)\). It has been reported that metoprolol is safe and can be effective in doxorubicin-induced cardiomyopathy. Angiotensin II inhibition could also be used in doxorubicin-induced cardiomyopathy \( (Takemura\ and\ Fujiwara,\ 2007)\).

In patients with advanced heart failure and in those intolerant to angiotensin II inhibition therapy, low-dose hydralazine-isosorbide dinitrate combination treatment is often employed. In patients with malignant arrhythmias, amiodarone and implantable cardioverter & defibrillator should be considered. It should be appreciated that none of the treatments employed for ischemic or idiopathic dilated cardiomyopathy has been demonstrated to improve the prognosis of patients with doxorubicin cardiomyopathy. Cardiac transplantation has
been reported to improve long-term prognosis of the patients in whom the primary malignancy is cured following chemotherapy (Thomas et al., 2002).

In addition to staying below the cumulative doses, various preventive measures may be employed by the oncologist in order to reduce the risk of cardiotoxicity. Cardiac monitoring is recommended at 3, 6, and 9 months. There is a major effort has been done to limit the cumulative dose of doxorubicin to <450 mg (Swain et al., 2003).

The use of anthracycline analogues, alternative methods of drug delivery and continuous slow infusion instead of standard infusion protocols have also been employed to reduce the risk of dilated cardiomyopathy as it will result in a reduced plasma level and a much lower left ventricular peak concentration (Zhang et al., 2012).

A number of pharmaceutical agents have been tested, usually in experimental animal studies, to assess their potential to reduce the risk of doxorubicin cardiotoxicity. Most of the pharmacologic agents that have been tested to reduce or prevent doxorubicin cardiotoxicity have the potential to reduce oxidative stress. The mercaptopropionyl glycine (MPG), a synthetic aminothiol that exhibits antioxidant properties, has been shown to reduce doxorubicin cardiotoxicity (van Dalen et al., 2008).

Similarly, probucol, super oxide dismutase activator, and dexrazoxane with documented antioxidant properties have been reported to decrease doxorubicin cardiotoxicity (Ducroq et al., 2010).

Because the calcium channel blocker amlodipine and the β-and α-adrenergic blocking agent carvedilol have antioxidant properties they
have also been studied for their potential to reduce doxorubicin cardiotoxicity (Kalay et al., 2006).

The PDE5 inhibitor sildenafil, erythropoietin and thrombopoietin, granulocyte colony-stimulating factor, and the endothelin receptor-blocking agent bosentan have been used in experimental animal models for the protection against developing doxorubicin cardiomyopathy (Bien et al., 2007).

The potential protective role of nitric oxide and superoxide dismutase has been investigated in a transgenic mouse model. Lack of nitric oxide was associated with enhanced cardiac injury, and mitochondrial injury was attenuated by an increase in superoxide dismutase (Cole et al., 2006).

The use of liposomal preparations of doxorubicin when appropriate as the liposomal formulations of daunorubicin and doxorubicin are less toxic to cardiac tissue than the non-liposomal form because a lower proportion of drug administered in the liposome form is delivered to the heart (Lotrionte et al., 2009).

(2) Hematologic Toxicity:

As with other cytotoxic agents, doxorubicin may produce myelosuppression. Myelosuppression requires careful monitoring. Total and differential white blood cell, red blood cell, and platelet counts should be assessed before and during each cycle of therapy with doxorubicin. A dose-dependent, reversible leukopenia and/or granulocytopenia (neutropenia) are the predominant manifestations of doxorubicin hematologic toxicity and are the most common acute dose-limiting toxicities of this drug (Lyman et al., 2011).

With the recommended dose schedule, leukopenia is usually transient, occurring within 10 to 14 days after treatment with recovery usually
occurring by the 21st day. Thrombocytopenia and anemia may also occur. Clinical consequences of severe myelosuppression include fever, infections, sepsis/septicemia, septic shock, hemorrhage, tissue hypoxia, or death (Elad et al., 2010).

(3) Secondary Leukemia:

The occurrence of secondary acute meloid leukemia and myelodysplastic syndrome has been reported most commonly in patients treated with chemotherapy regimens containing anthracyclines (including doxorubicin) and DNA-damaging antineoplastic agents, in combination with radiotherapy, when patients have been heavily pretreated with cytotoxic drugs. Such cases generally have a 1–3 year latency period. Pediatric patients are also at risk of developing secondary acute meloid leukemia (Marie-Cécile et al., 2007).

(4) Hepatic Impairment:

Despite the widespread clinical usage of doxorubicin, the side effects of doxorubicin form a major concern. It has been reported that the heart is a prefer-ential target of doxorubicin-induced toxicity. However, increasing evidence shows that the antitumor agent also affects other organs including the liver, kidney and brain. Almost 40% of patients suffered liver injury after doxorubicin treatment. The main toxic effects on hepatocytes include: arrest cell cycle of hepatocytes, oxidative stress and disruption of electron transport. But the exact mechanism of hepatotoxicity of doxorubicin is not fully clarified. Due to these serious side effects, the clinical use of doxorubicin has been restricted (Deleve et al., 2013).
Since metabolism and excretion of doxorubicin occurs predominantly by the hepatobiliary route, toxicity of recommended doses of doxorubicin can be enhanced by hepatic impairment; therefore, prior to individual dosing, evaluation of hepatic function is recommended using conventional laboratory tests such as SGOT, SGPT, alkaline phosphatase, and bilirubin (Reuben et al., 2010).

To reduce the toxic effects of doxorubicin, several pharmacologic agents, such as antioxidants, hematopoietic cytokines and iron-chelating agents were used. The development of doxorubicin analogues and the production of efficacious delivery systems were also found to be effective ways. Although most of these attempts showed beneficial effects, some of these attempts failed to attenuate doxorubicin toxicity in clinical trials. Therefore, it is very necessary to search for more effective strategies against doxorubicin-induced complications while preserve or enhance its therapeutic effects (Gökçimen et al., 2007).

5) Nephrotoxicity:

Nephrotoxicity is one of the limiting factors for using doxorubicin as an anticancer chemotherapeutic. Unfortunately, the use of doxorubicin has been limited by the occurrence of dose-dependent toxicities to vital organs, as the heart, the kidney, and the liver (Carvalho et al., 2009).

The exact mechanism of doxorubicin-induced nephrotoxicity is not yet completely understood. Renal doxorubicin-induced toxicity may be part of a multiorgan damage mediated mainly through free radical formation eventually leading to membrane lipid peroxidation. Induction of apoptosis and modulation of nitric oxide are other mechanisms that may be involved in toxic adverse effects associated with doxorubicin therapy. In addition, doxorubicin may induce nephrotoxicity through its
direct renal damaging effect, as it accumulates preferentially in the kidney (*Lee and Harris*, 2011).

Doxorubicin toxic effects to other organs as the heart and the liver may modulate blood supply to the kidney and alter xenobiotic detoxification processes, respectively, thus indirectly contributing to doxorubicin-induced nephropathy. A number of antioxidant compounds have been proposed as chemopreventive therapy for doxorubicin-induced toxicity (*Granados-Principal et al.*, 2010).

(6) **Immunosuppressant Effects:**

Administration of live or live-attenuated vaccines in patients immunocompromised by chemotherapeutic agents including doxorubicin, may result in serious or fatal infections. Vaccination with a live vaccine should be avoided in patients receiving doxorubicin. Killed or inactivated vaccines may be administered; however, the response to such vaccines may be diminished (*Elad et al.*, 2010).

(7) **Teratogenecity:**

Doxorubicin can cause fetal harm when administered to a pregnant woman. Doxorubicin was teratogenic and embryotoxic. Characteristic malformations included esophageal and intestinal atresia, tracheoesophageal fistula, hypoplasia of the urinary bladder, and cardiovascular anomalies (*Autio et al.*, 2007).

(8) **Impairment of Fertility:**

Decreased fertility in female rats at the doses of 0.05 and 0.2 mg/kg/day (about 1/200 and 1/50 the recommended human dose on a body surface area basis) when administered from 14 days before mating...
through late gestation period. A single i.v. dose of doxorubicin at 0.1 mg/kg (about 1/100 the recommended human dose on a body surface area basis) was toxic to male reproductive organs, producing testicular atrophy and oligospermia in rats. Doxorubicin is mutagenic as it induced DNA damage in rabbit spermatozoa and dominant lethal mutations in mice. Therefore, doxorubicin may potentially induce chromosomal damage in human spermatozoa. Oligospermia or azoospermia were evidenced in men treated with doxorubicin, mainly in combination therapies. Men undergoing doxorubicin treatment should use effective contraceptive methods. In women, doxorubicin may cause infertility during the time of drug administration. Doxorubicin may cause amenorrhea, ovulation and menstruation may return after termination of therapy, although premature menopause can occur. Recovery of menses is related to age at treatment (Morgan et al., 2012).
Nebivolol

Nebivolol is a $\beta_1$ receptor blocker with nitric oxide-potentiating vasodilator effect used in treatment of hypertension and, also for left ventricular failure (de Boer et al., 2007).

Chemistry:

Nebivolol's molecular formula is (C$_{22}$H$_{25}$F$_2$NO$_4$•HCl) with the following structural formula:

![Nebivolol Hydrochloride](image)

Nebivolol hydrochloride (Gielen et al., 2006)

Physical properties:

Nebivolol hydrochloride is a white to almost white powder that is soluble in methanol, dimethylsulfoxide, and N-dimethylformamide, sparingly soluble in ethanol, propylene glycol, and polyethylene glycol, and very slightly soluble in hexane, dichloromethane, and methylbenzene (Gielen et al., 2006) and soluble in water (Dursun et al., 2014).

Pharmacokinetics:

Nebivolol is metabolized by a number of routes, including glucuronidation and hydroxylation by CYP2D6. The active isomer (d-nebivolol) has an effective half-life of about 12 hours in CYP2D6 extensive metabolizers (EM) (most people), and 19 hours in poor
metabolizers and exposure to d-nebivolol is substantially increased in poor metabolizers (PM). This has less importance than usual, however, because the metabolites, including the hydroxyl metabolite and glucuronides (the predominant circulating metabolites), contribute to β-blocking activity. The absolute bioavailability has not been determined. Mean peak plasma nebivolol concentrations occur approximately 1.5 to 4 hours post-dosing in EMs and PMs. Food does not alter the pharmacokinetics of nebivolol. Under fed conditions, nebivolol glucuronides are slightly reduced. Nebivolol may be administered without regard to meals (*Mangrella et. al, 1998*).

The plasma protein binding of nebivolol is approximately 98%, mostly to albumin, and is independent of nebivolol concentrations. Nebivolol is predominantly metabolized via direct glucuronidation of parent and to a lesser extent via N dealkylation and oxidation via cytochrome P450 2D6. Its stereospecific metabolites contribute to the pharmacologic activity. Elimination after a single oral administration of nebivolol, 38% of the dose was recovered in urine and 44% in feces for EMs and 67% in urine and 13% in feces for PMs. Essentially all nebivolol was excreted as multiple oxidative metabolites or their corresponding glucuronide conjugates (*Prisant, 2008*).

**Pharmacokinetics in Special Populations**

**Hepatic Disease**

Nebivolol peak plasma concentration increased 3-fold and the apparent clearance decreased by 86% in patients with moderate hepatic impairment. No formal studies have been performed in patients with severe hepatic impairment and nebivolol should be contraindicated for these patients (*Prisant, 2008*).
Renal Disease

The apparent clearance of nebivolol was unchanged following a single 5 mg dose of nebivolol in patients with mild renal impairment (ClCr 50 to 80 mL/min, n=7), and it was reduced in patients with moderate renal impairment (ClCr 30 to 50 mL/min, n=9), but clearance was reduced by 53% in patients with severe renal impairment (ClCr < 30 mL/min, n=5). No studies have been conducted in patients on dialysis (Prisant, 2008).

Mechanism of Action:

The mechanism of action of the antihypertensive response of nebivolol has not been definitively established. Possible factors that may be involved include: (1) decreased heart rate, (2) decreased myocardial contractility, (3) diminution of tonic sympathetic outflow to the periphery from cerebral vasomotor centers, (4) suppression of renin activity and (5) vasodilation and decreased peripheral vascular resistance (Brunton et al., 2006).

Pharmacological actions:

β1 Selectivity

Beta blockers help patients with cardiovascular disease by blocking β receptors, while many of the side-effects of these medications are caused by their blockade of β2 receptors, for this reason, beta blockers that selectively block β1 receptors (termed cardioselective or β1-selective beta blockers) produce fewer adverse effects (for instance, bronchoconstriction) than those drugs that non-selectively block both β1 and β2 receptors. The drug is highly cardioselective at 5 mg (Gielen et al., 2006).
However, at doses above 10 mg, nebivolol loses its cardioselectivity and blocks both β1 and β2 receptors. While the recommended starting dose of nebivolol is 5 mg, sufficient control of blood pressure may require doses up to 40 mg (De Boer et al., 2007).

**Vasodilator action**

Nebivolol is unique as a beta-blocker. Unlike carvedilol, it has a nitric oxide (NO)-potentiating, vasodilator effect (Weiss, 2006). Along with labetalol, celiprolol and carvedilol, it is one of four beta blockers to cause dilation of blood vessels in addition to effects on the heart (Bakris, 2009).

**Antihypertensive effect**

Nebivolol lowers blood pressure by reducing peripheral vascular resistance, and significantly increases stroke volume with preservation of cardiac output (Kamp et al., 2003). The net hemodynamic effect of nebivolol is the result of a balance between the depressant effects of beta-blockade and an action that maintains cardiac output (Gielen et al., 2006).

**Antioxidant effect:**

It has been proposed that nebivolol exerts endothelium-protective effects caused by its antioxidant properties. Nebivolol protected against ROS-induced endothelial damage. Furthermore, it has been shown to cause vasorelaxation via a nitric oxide/cGMP-dependent pathway in various vascular beds. In addition to its direct negative inotropic and vasodilator effects, nebivolol has been assumed to counteract oxidative stress. It has been demonstrated that nebivolol protects against hydroxyl
radical (OH)-induced injury in right ventricular rabbit cardiac trabeculae and in demembranized muscles from explanted human hearts (Agabiti and Rizzoni, 2007). In urine samples of healthy human volunteers, it was shown that nebivolol significantly decreased the levels of 8-iso-PGF$_{2\alpha}$, a marker of oxidative stress (Weiss, 2006). Oxidative stress is associated with the impairment of endothelium-dependent vasorelaxation caused by the loss of nitric oxide (·NO) bioactivity in the vessel wall (Bakris, 2009). Endothelial dysfunction, combined with enhanced levels of reactive oxygen species (ROS), is assumed to play an important role in the pathogenesis of cardiovascular diseases such as, atherosclerosis, congestive heart failure and hypertension (Baldwin and Keam, 2009).

Furthermore, it is unknown whether the observed protective effect of nebivolol during oxidative stress results from a direct scavenging action of the molecule or whether it is mediated by a different mechanism. If indeed nebivolol is a scavenger of reactive oxygen species, this could imply that the molecule itself is modulated by this reaction. Such a molecular modification could possibly lead to a change in pharmacological activity (Gielen et al., 2006).

**Indications:**

Nebivolol is indicated for the treatment of hypertension, to lower blood pressure. Nebivolol may be used alone or in combination with other antihypertensive agents. Lowering blood pressure reduces the risk of fatal and nonfatal cardiovascular events, primarily strokes and myocardial infarctions (Moen & Wagstaff, 2006)
Dosage and administration:

Hypertension

The dose of nebivolol must be individualized to the needs of the patient. For most patients, the recommended starting dose is 5 mg once daily, with or without food, as monotherapy or in combination with other agents. For patients requiring further reduction in blood pressure, the dose can be increased at 2-week intervals up to 40 mg. A more frequent dosing regimen is unlikely to be beneficial (Chobanian et al., 2013).

Renal Impairment

In patients with severe renal impairment (ClCr less than 30 mL/min) the recommended initial dose is 2.5 mg once daily; titrate up slowly if needed. Nebivolol has not been studied in patients receiving dialysis (Prisant, 2008).

Hepatic Impairment

In patients with moderate hepatic impairment, the recommended initial dose is 2.5 mg once daily. Nebivolol has not been studied in patients with severe hepatic impairment and therefore it is not recommended in that population (Agabiti and Rizzoni, 2007).

Drug interaction:

When nebivolol is co-administered with CYP2D6 inhibitors as quinidine, propafenone, fluoxetine, paroxetine the dose of nebivolol may need to be reduced (Moen and Wagstaff, 2006).
Patients receiving catecholamine-depleting drugs such as reserpine or guanethidine should be closely monitored because the added β-blocking action of nebivolol may produce excessive reduction of sympathetic activity. In patients who are receiving nebivolol and clonidine, discontinue nebivolol for several days before the gradual tapering of clonidine (Wojciechowski and Papademetriou, 2008).

Both digitalis glycosides and β-blockers slow atrioventricular conduction and decrease heart rate. Concomitant use of nebivolol can increase the risk of bradycardia (Brunton et al., 2006).

Nebivolol can exacerbate the myocardial depressants effects of calcium channel blockers or inhibitors of AV conduction, such as certain calcium antagonists (particularly of the phenylalkylamine [verapamil] and benzothiazepine [diltiazem] classes), or antiarrhythmic agents, such as disopyramide (Olga, 2009).

**Precautions:**

Sudden discontinuation of nebivolol therapy in patients with coronary artery disease may cause severe exacerbation of angina, myocardial infarction and ventricular arrhythmias have been reported in patients with coronary artery disease following the abrupt discontinuation of therapy with β-blockers. Myocardial infarction and ventricular arrhythmias may occur with or without preceding exacerbation of the angina pectoris. Taper nebivolol over 1 to 2 weeks when possible. If the angina worsens or acute coronary insufficiency develops, re-start nebivolol promptly, at least temporarily (Bakris, 2009). Patients with bronchospastic diseases should not receive β-blockers (Tafreshi and Weinacker, 1999).
β-blockers may mask some of the manifestations of hypoglycemia, particularly tachycardia. Nonselective β-blockers may potentiate insulin-induced hypoglycemia and delay recovery of serum glucose levels. It is not known whether nebivolol has these effects (Poirier et al., 2001).

β-blockers may mask clinical signs of hyperthyroidism, such as tachycardia. Abrupt withdrawal of β-blockers may be followed by an exacerbation of the symptoms of hyperthyroidism or may precipitate a thyroid storm (Pessina, 2001).

β-blockers can precipitate or aggravate symptoms of arterial insufficiency in patients with peripheral vascular disease (Bakris, 2009).

Because of significant negative inotropic and chronotropic effects in patients treated with β-blockers and calcium channel blockers of the verapamil and diltiazem type, monitor the ECG and blood pressure in patients treated concomitantly with these agents (Olga, 2009).

Renal clearance of nebivolol is decreased in patients with severe renal impairment. Nebivolol has not been studied in patients receiving dialysis (Prisant, 2008).

Metabolism of nebivolol is decreased in patients with moderate hepatic impairment. (Agabiti and Rizzoni, 2007).

While taking β-blockers, patients with a history of severe anaphylactic reactions to a variety of allergens may be more reactive to repeated accidental, diagnostic, or therapeutic challenge. Such patients may be unresponsive to the usual doses of epinephrine used to treat allergic reactions (Pessina, 2001). In patients with known or suspected
pheochromocytoma, initiate an $\alpha$-blocker prior to the use of any $\beta$ blocker (Punzi et al., 2010).

Adverse effects:

Nebivolol is associated with a number of serious risks. Nebivolol is contraindicated in patients with severe bradycardia, heart block greater than first degree, cardiogenic shock, decompensated cardiac failure, sick sinus syndrome (unless a permanent pacemaker is in place), severe hepatic impairment and in patients who are hypersensitive to any component of the product (Gielen et al., 2006).

Nebivolol therapy is also associated with warnings regarding abrupt cessation of therapy, cardiac failure, angina and acute myocardial infarction, bronchospastic diseases, anesthesia and major surgery, diabetes and hypoglycemia, thyrotoxicosis, peripheral vascular disease, non-dihydropyridine calcium channel blockers use, as well as precautions regarding use with CYP2D6 inhibitors, impaired renal and hepatic function, and anaphylactic reactions. Finally, Nebivolol is associated with other risks for example, a number of treatment-emergent adverse events with an incidence greater than or equal to 1 percent in nebivolol -treated patients and at a higher frequency than placebo-treated patients were identified in clinical studies, including headache, fatigue, and dizziness (De Boer et al., 2007).

In clinical trials and worldwide post marketing experience, there were reports of nebivolol overdose. The most common signs and symptoms associated with nebivolol overdosage are bradycardia and hypotension. Other important adverse reactions reported with nebivolol overdose include cardiac failure, dizziness, hypoglycemia, fatigue and vomiting.
Other adverse reactions associated with β-blocker overdose include bronchospasm and heart block. Because of extensive drug binding to plasma proteins, hemodialysis is not expected to enhance nebivolol clearance (Germino, 2009).

**Contraindications:**

Nebivolol is contraindicated in severe bradycardia, heart block greater than first degree, patients with cardiogenic shock, decompensated cardiac failure, sick sinus syndrome (unless a permanent pacemaker is in place), patients with severe hepatic impairment and patients who are hypersensitive to nebivolol (Germino, 2009).
L-Carnitine

L-Carnitine is a naturally occurring compound that facilitates the transport of fatty acids into mitochondria for beta-oxidation. Exogenous L-carnitine is used clinically for the treatment of carnitine deficiency disorders. It is synthesized in the liver and kidneys and stored in the skeletal muscles, heart, brain, and sperm. (Volek, 2008).

Carnitine has been proposed as a treatment for many conditions because it acts as an antioxidant. Antioxidants fight harmful particles in the body known as free radicals, which damage cells and DNA. Antioxidants can neutralize free radicals and may reduce or help prevent some of the damage they cause (Othman et al., 2010).

Chemistry:

Carnitine is a quaternary ammonium compound biosynthesized from the amino acids lysine and methionine. In living cells, it is required for the transport of fatty acids from the cytosol into the mitochondria during the breakdown of lipids for the generation of metabolic energy. It is widely available as a nutritional supplement. Carnitine exists in two stereoisomers: Its biologically active form is L-carnitine, whereas its enantiomer, D-carnitine, is biologically inactive (Steiber et al., 2004).

Pharmacokinetics of L- Carnitine:

In humans, the endogenous carnitine pool, which comprises free L-carnitine and a range of short-, medium- and long-chain esters, is maintained by absorption of L-carnitine from dietary sources,
biosynthesis within the body and extensive renal tubular reabsorption from glomerular filtrate. In addition, carrier-mediated transport ensures high tissue-to-plasma concentration ratios in tissues that depend critically on fatty acid oxidation. The absorption of L-carnitine after oral administration occurs partly via carrier-mediated transport and partly by passive diffusion (Charle, 2006).

L-Carnitine and its short-chain esters do not bind to plasma proteins and, although blood cells contain L-carnitine, the rate of distribution between erythrocytes and plasma is extremely slow in whole blood. After intravenous administration, the initial distribution volume of L-carnitine is typically about 0.2-0.3 L/kg, which corresponds to extracellular fluid volume (Evans and Fornasini, 2003).

L-Carnitine is eliminated from the body mainly via urinary excretion. Under baseline conditions, the renal clearance of L-carnitine (1-3 mL/min) is substantially less than glomerular filtration rate (GFR), indicating extensive (98-99%) tubular reabsorption. The threshold concentration for tubular reabsorption (above which the fractional reabsorption begins to decline) is about 40-60 micromol/L, which is similar to the endogenous plasma L-carnitine level. Therefore, the renal clearance of L-carnitine increases after exogenous administration, approaching GFR after high intravenous doses. Patients with primary carnitine deficiency display alterations in the renal handling of L-carnitine and/or the transport of the compound into muscle tissue. Similarly, many forms of secondary carnitine deficiency, including some drug-induced disorders, arise from impaired renal tubular reabsorption. Patients with end-stage renal disease undergoing dialysis can develop a secondary carnitine deficiency due to the unrestricted loss of L-carnitine.
through the dialyser, and L-carnitine has been used for treatment of some patients during long-term haemodialysis (Charle, 2006).

Pharmacodynamics of L- Carnitine:

- Role in fatty acid metabolism:

  Carnitine transports long-chain acyl groups from fatty acids into the mitochondrial matrix, so they can be broken down through β-oxidation to acetyl CoA to obtain usable energy via the citric acid cycle. In some organisms, such as fungi, the acetate is used in the glyoxylate cycle for gluconeogenesis and formation of carbohydrates. Fatty acids must be activated before binding to the carnitine molecule to form 'acylcarnitine'. The free fatty acid in the cytosol is attached with a thioester bond to coenzyme A (CoA). This reaction is catalyzed by the enzyme fatty acyl-CoA synthetase and driven to completion by inorganic pyrophosphatase (Pekala et al., 2011).

  The acyl group on CoA can now be transferred to carnitine and the resulting acylcarnitine transported into the mitochondrial matrix. This occurs via a series of similar steps: 1- Acyl CoA is transferred to the hydroxyl group of carnitine by carnitine acyltransferase I (palmitoyltransferase) located on the outer mitochondrial membrane. 2- Acylcarnitine is shuttled inside by a carnitine-acylcarnitine translocase. 3- Acylcarnitine is converted to acyl CoA by carnitine acyltransferase II (palmitoyltransferase) located on the inner mitochondrial membrane. The liberated carnitine returns to the cytosol (Marcovina et al., 2013).

  Human genetic disorders, such as primary carnitine deficiency, carnitine palmitoyltransferase I deficiency, carnitine palmitoyltransferase
II deficiency and carnitine-acylcarnitine translocase deficiency, affect different steps of this process (Olpin, 2005).

Carnitine acyltransferase I and peroxisomal carnitine octanoyl transferase (CROT) undergo allosteric inhibition as a result of malonyl-CoA, an intermediate in fatty acid biosynthesis, to prevent futile cycling between β-oxidation and fatty acid synthesis (Volek et al., 2008).

- Role in doxorubicin cardiotoxicity:

Carnitine has a protective effect against doxorubicin cardiotoxicity as showed in the study done by Sayed-Ahmed et al., 2000a that studied doxorubicin cardiotoxicity in absence and presence of propionyl L-carnitine (PLC) on palmitoyl-CoA and palmitoyl-carnitine oxidation and on the activity of carnitine palmitoyltransferase (CPT 1). The results showed that doxorubicin induced concentration-dependent inhibition of substrates oxidation and inhibition of CPT I and that PLC completely reversed this inhibition to the control values. They concluded that doxorubicin induced its cardiotoxicity by inhibition of CPT I and beta-oxidation of long-chain fatty acids with the consequent depletion of ATP in cardiac tissues, and that PLC can be used as a protective agent against doxorubicin-induced cardiotoxicity.
In doxorubicin cardiomyopathic rat model, Sayed-Ahmed et al. (2001) have initiated another study to confirm their in vitro findings and investigated the protective effects of PLC against doxorubicin induced cardiotoxicity. Chronic administration of doxorubicin (3 mg/kg, intraperitonial) significantly increased creatine kinase-MB, lactate dehydrogenase and malondialdehyde and decreased reduced glutathione. Treatment with PLC induced complete reversal of these effects without decreasing the antitumour activity of doxorubicin.

In another study, Sayed-Ahmed et al., 2000b investigated the effects of doxorubicin on mRNA expression of heart fatty acid binding protein (H-FABP) using northern blot analysis. Results showed that chronic administration of doxorubicin caused dose-dependent and cumulative
inhibition of H-FABP gene expression and daily administration of L-carnitine protected against this effect in cardiac tissues.

Figure (2): Inhibition of H-FABP mRNA expression in the heart as a novel mechanism for DOX-induced cardiotoxicity and for L-carnitine mediated protection against this effect. FFA: free fatty acid; CT: carnitine/acyl-carnitine translocase; FA-CoA: fatty acyl-CoA; CAT: carnitine acetyl transferase; FA-Carnitine: fatty acyl-carnitine; H-FABP: heart-type fatty acid binding protein; CPT I: outer carnitine palmitoyl transferase; CPT II: inner carnitine palmitoyl transferase; OCTN2: organic cation/carnitine transporter; (−) and (+) indicate inhibition and stimulation, respectively. (Sayed-Ahmed et al. (2000b).

- Effect on Atherosclerosis

There may be a link between dietary consumption of carnitine and atherosclerosis, but there is also evidence that it lowers the risk of mortality and arrhythmias after an acute myocardial infarction. When certain species of intestinal bacteria were exposed to carnitine from food, they produced a waste product, trimethylamine, which is transformed in
the liver to trimethylamine N-oxide (TMAO). TMAO may be associated with atherosclerosis. The presence of large amounts of TMAO-producing bacteria was a consequence of a long-term diet rich in meat. However, when the authors compared the risk of cardiovascular events to the levels of carnitine and TMAO, they found that the risk was higher in those with higher TMAO levels, independent of the carnitine levels. Vegetarian and vegans who ate a single meal of meat had much lower levels of TMAO in their blood stream than did regular meat-eaters, as vegetarian and vegans had lower levels of the intestinal bacteria that converts carnitine into TMAO (Koeth and Robert, 2013).

- **Effects on bone mass:**

  In the course of human aging, carnitine concentration in cells diminishes, affecting fatty acid metabolism in various tissues. Particularly adversely affected are bones, which require continuous reconstructive and metabolic functions of osteoblasts for maintenance of bone mass (Pekala et al., 2011)

- **Antioxidant effects:**

  The carnitines exert antioxidant action, thereby providing a protective effect against lipid peroxidation of phospholipid membranes and against oxidative stress induced at the myocardial and endothelial cell level (Othman et al., 2010).

Possible health effects

Carnitine has been proposed as a supplement to treat a variety of health conditions including myocardial infraction or angina (Dinicolantonio et
al., 2013), heart failure, angina and diabetic neuropathy, improving exercise performance (Pekala et al., 2011).

**Uses:**

Heart Conditions

- Angina: carnitine can be used along with conventional treatment for stable angina. Several clinical trials show that L-carnitine and propionyl-L-carnitine can help reduce symptoms of angina and improve the ability of people with angina to exercise without chest pain (Witte and Clark, 2006).
- A few studies have found that carnitine may help when used with conventional medicines after a heart attack, but not all studies agree. Some small studies suggest that people who take L-carnitine supplements soon after a heart attack may be less likely to have another heart attack, die of heart disease, have chest pain and abnormal heart rhythms, or develop heart failure. However, other studies have shown no benefit. Treatment with oral carnitine may also improve muscle weakness (Xue, 2007).
- Heart failure: A few small studies have suggested that carnitine (usually propionyl-L-carnitine) can help reduce symptoms of heart failure and improve exercise capacity in people with heart failure (Witte & Clark, 2006).

Peripheral Vascular Disease

Decreased blood flow to the legs from atherosclerosis or hardening of the arteries where plaque builds up in the arteries often causes an aching or cramping pain in the legs while walking or exercising. This pain is called intermittent claudication, and the reduced blood flow to the legs is
called peripheral vascular disease (PVD). Several studies show that carnitine can help reduce symptoms and increase the distance that people with intermittent claudication can walk. Most studies have used propionyl-L-carnitine (Carrero and Grimble, 2006).

Diabetic Neuropathy

Diabetic neuropathy happens when high blood sugar levels affect nerves in the body, especially the arms, legs, and feet, causing pain and numbness. Some small preliminary studies suggest acetyl-L-carnitine may help reduce pain and increase feeling in affected nerves. It is also possible that carnitine can help nerves regenerate (Head, 2006).

Exercise Performance

Carnitine is often taken to improve exercise performance (Hiatt, 2001).

Weight Loss

Although L-carnitine has been marketed as a weight loss supplement, there is no scientific evidence to show that it works. Some studies do show that oral carnitine reduces fat mass, increases muscle mass, and reduces fatigue, which may contribute to weight loss in some people (Villani, 2000).

Alzheimer's disease and memory impairment

The evidence is mixed as to whether carnitine is useful in treating Alzheimer's disease. Several early studies showed that acetyl-L-carnitine, might help slow down the progression of Alzheimer's disease, relieve depression related to senility and other forms of dementia, and improve
memory in the elderly. But larger and better-designed studies found it didn’t help at all (Pettegrew, 2000).

Kidney Disease and Dialysis

Because the kidneys make carnitine, kidney disease could lead to low levels of carnitine in the body (Lynch, 2008).

Male Infertility

Low sperm counts have been linked to low carnitine levels in men. Several studies suggest that L-carnitine supplements may increase sperm count and mobility (Sinclair, 2000).

Erectile Dysfunction

Preliminary studies suggest propionyl-L-carnitine may help improve male sexual function. One study found that carnitine improved the effectiveness of sildenafil in men with diabetes who had not previously responded to sildenafil. In another study, a combination of propionyl-L-carnitine and acetyl-L-carnitine improved the effectiveness of sildenafil in men who had erectile dysfunction after prostate surgery (Cavallini et al., 2005).

Peyronie's Disease

Peyronie's disease is characterized by a curvature of the penis that leads to pain during an erection. One promising study compared acetyl-L-carnitine to the medication tamoxifen in 48 men with this condition. Acetyl-L-carnitine worked better than tamoxifen at reducing pain during sex and reducing the curve of the penis. Acetyl-L-carnitine also had fewer side effects than tamoxifen (Biagiotti, and Cavallini, 2001).
Hyperthyroidism

Some research suggests that L-carnitine may help prevent or reduce symptoms of an overactive thyroid, such as insomnia, nervousness, heart palpitations, and tremors. In fact, in one study, a small group of people with hyperthyroidism saw these symptoms improve, and their body temperature become normal, when taking carnitine. But a larger, better-designed clinical trial is needed to see if carnitine really works. In addition, researchers think carnitine may work by blocking the action of thyroid hormone, which could be dangerous for people with low thyroid levels (Benvenga et al., 2001).

Dietary Sources

The highest concentrations of carnitine are found in red meat and dairy products. Carnitine can be found at significantly lower levels in many other foods including nuts and seeds (e.g. sunflower, sesame, beans, peas, lentils, peanuts), vegetables (asparagus, beet greens, broccoli, brussels sprouts, collard greens, garlic, mustard greens, okra, parsley, kale), fruits ( bananas), cereals (buckwheat, corn, millet, oatmeal, rice bran, rye, whole wheat, wheat bran, wheat germ) and other foods (bee pollen, brewer's yeast, carob). In general, 20 to 200 mg is ingested per day by that on usual diet, whereas those on a strict vegetarian or vegan diet may ingest as little as 1 mg/day. No advantage appears to exist in giving an oral dose greater than 2 g at one time, since absorption studies indicate saturation at this dose (Carrero and Grimble, 2006).

Available Forms

Carnitine is available as a supplement in a variety of forms.
- L-carnitine: the most widely available and least expensive
- Acetyl-L-carnitine: Often used in studies for Alzheimer's disease and other brain disorders
- Propionyl-L-carnitine: Often used in studies for heart disease and peripheral vascular disease

Avoid D-carnitine supplements. They interfere with the natural form of L-carnitine and may produce unwanted side effects. In some cases, L-carnitine may be taken by prescription or given intravenously by a health care provider. Recommended doses of L-carnitine vary depending on the health condition being treated. The usual dose is between 1 - 3 g per day (Steiber et al., 2004).

Precautions:

Side effects are generally mild. High doses (5 or more grams per day) may cause diarrhea. Other rare side effects include increased appetite, body odor, and rash.

People with the following conditions should talk to their health care provider before taking carnitine: Peripheral vascular disease, high blood pressure, liver disease from alcoholism (cirrhosis), kidney disease, history of seizures, diabetes (Steiber et al., 2004).

Possible Interactions

In a laboratory study, L-carnitine supplements protected muscle tissue against toxic side effects from Azalatine, a medication used to treat HIV and AIDS. Treatment with L-carnitine may protect heart cells against the toxic side effects of doxorubicin, a chemotherapy medication used to treat cancer, without making the medication any less effective. In
case of isotretinoin which is a strong medication used for severe acne, can cause liver problems, as measured by a blood test, as well as high cholesterol and muscle pain and weakness. These symptoms are like those seen with carnitine deficiency. Carnitine may stop thyroid hormone from getting into cells, and theoretically may make thyroid hormone replacement less effective. The anti-seizure medication valproic acid may lower blood levels of carnitine. Taking L-carnitine supplements may prevent any deficiency and may also reduce the side effects of valproic acid. However, taking carnitine may increase the risk of seizures in people with a history of seizures (Othman et al., 2010).
Materials and Method

Materials:  
A- Drugs and Chemicals:

- **CaCl$_2$H$_2$O**(crystals): El-Nasr Pharmaceutical Cehmical Company was dissolved in distilled water.
- **Creatinine acid reagent** (solution): Sanbio Laboratory Texas, U.S.A.
- **Creatinine base reagent** (solution): Sanbio Laboratory Texas, U.S.A.
- **Creatinine standered** (solution): Sanbio Laboratory Texas, U.S.A.
- **cTnl Enzyme Conjugate Reagent** (solution): GenWay Biotech. for protein and antibody solutions SanDiego
- **Doxorubicin HCL** (solution): EIMC United Pharmaceuticals. A.R.E.
- **Formaline** (neutral 10% formaline): El-Gomhoria Pharmaceutial Chemical Co. A.R.E
- **Glucose** (crystals): El-Nasr Pharmaceutical Cehmical Company was dissolved in distilled water.
- **Hematoxylin and eosin**: E. Mark, Darmastadt. U.S.A.
- **Isoprenaline HCL** (powder): Sigma,U.S.A was dissolved in distilled water.
- **KCl** (crystals): El-Nasr Pharmaceutical Cehmical Company was dissolved in distilled water.
- **L-alanine** solution GenWay Biotech. for protein and antibody solutions SanDiego
- **L-aspartate** solution GenWay Biotech. for protein and antibody solutions SanDiego
- **Lactate dehydrogenase** solution GenWay Biotech. for protein and antibody solutions SanDiego
- **L-Carnitine** (powder. Eva Pharm. A.R.E) was dissolved in distilled water.
- **Malate dehydrogenase** solution GenWay Biotech. for protein and antibody solutions SanDiego
- **NaCl** (crystals): El-Nasr Pharmaceutical Cehmical Company was dissolved in distilled water.
- **NaH₂PO₄** (crystals): El-Nasr Pharmaceutical Cehmical Company was dissolved in distilled water.
- **Nebivolol HCL** (powder): Sigma, U.S.A was dissolved in distilled water.
- **Phosphate buffer** (solution): GenWay Biotech. for protein and antibody solutions SanDiego
- **Reduced nicotine adenine dinucleotide phosphate** (solution): GenWay Biotech. for protein and antibody solutions SanDiego
- **5-Stop solution** (diluted hydrochloric acid) (solution): GenWay Biotech. for protein and antibody solutions SanDiego
- **Tetramethyl-benzidine** (TMB) Reagent (solution): GenWay Biotech. for protein and antibody solutions SanDiego

**B- Animals:**

- **Rats:** Adult male albino rats [from Helwan Farm], weighting 120-200 gm were used.
- **Rabbits:** of local strains ranging from 1-1.5 kg of both sexes were used for experiments on isolated heart
Ethical consideration of experimental animals:
Experimental rats will be under complete healthy conditions all over the experiment in the form of:
- Clean environment.
- Good ventilation
- Good nutrition in the form of free access to water and diet containing cereals and bread.
- Number of rats in each cage is six
- The experimental rats will be under care of professional technicians and qualified researchers.

C- Apparatus:

1- Harvard tension transducer and universal oscillography FT03 for ECG recording and measurement of blood pressure *(Harvard, UK)*.

3- Modified langendorff's apparatus *(Harvard, UK)*.

4- Spectrophotometer for biochemical assessment.

D- Physiological solutions:

Ringer's solution (for isolated heart):

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Mg/l</th>
</tr>
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<tbody>
<tr>
<td>NaCl</td>
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<tr>
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</tr>
<tr>
<td>NaH₂PO₄</td>
<td>0.1</td>
</tr>
<tr>
<td>CaCl₂H₂O</td>
<td>0.2</td>
</tr>
<tr>
<td>Glucose</td>
<td>10</td>
</tr>
</tbody>
</table>
Methods:

(A) In vivo experiment:
Rats were randomly divided into 5 groups (6 rats for each).

Animal groups:
1- Group (I): control group:
The control group will receive saline intraperitoneal in comparative volume to the tested groups.

2- Group (II): Doxorubicin administered group (Dox):
This group will receive doxorubicin in a dose of 3 mg / kg / day intraperitoneally every other day for 12 days (Amani et al., 2011).

3- Group (III): Doxorubicin + Nebivolol treated group (Dox + Neb):
This group will receive doxorubicin in a dose of 3 mg / kg / day intraperitoneally every other day for 12 days and nebivolol in a dose of 1mg / kg / day orally daily for 12 days (Amani et al., 2011).

4- Group (IV): Doxorubicin + L-Carnitine treated group (Dox + L-car):
This group will receive doxorubicin in a dose of 3 mg / kg / day intraperitoneally every other day for 12 days (Amani et al., 2011) and L-carnitine in a dose of 200 mg / kg body weight intraperitonially daily for 12 days (Nathalie A. et al., 1999).

5- Group (V): Doxorubicin + Nebivolol & L-Carnitine group (Dox + Neb + L-car):
This group will receive doxorubicin in a dose of 3 mg / kg / day intraperitoneally every other day for 12 days, nebivolol in a dose of 1mg / kg / day orally and L-Carnitine in a dose of 200 mg / kg body weight intraperitonially daily for 12 days.
Procedures:

(a) **Cardiac investigations:**

(1) **ECG:**

Adult male albino rats, weighing about 150-200gm of each were used. The animals were anaesthetized with urethane in a dose of 1.5-1.75 gm/kg body weight. Half of the dose was injected intraperitoneally, to induce rapid onset and the other half subcutaneously, to insure long maintenance of the anaesthetic effect.

After complete anaesthesia, the rats were led on their back. ECG records were done using needle electrodes. The four limb electrodes were fixed to the animal’s four limbs and records were done using the standard lead II at rate of 25m/min. The use of lead II was more informative (in rats) than other leads (*Chan et al., 1987*).

(2) **Blood pressure measurement:**

The blood pressure of rats was determined following the method of *Cangiano et al. (1978)*. The method can be outlined as follows:

The carotid artery was freed from its surrounding fascia for a distance about 1.5 cm and then two loose ligatures were placed, one at either end and around the freed artery. The artery was ligated at the end far from the heart and the end near to the heart was temporarily occluded with small bulldog clip. Now, half way across the artery was cut and the arterial cannula filled with glucose and heparin solution (20,000 I.U liter of 5% glucose solution) was inserted towards the heart. The cannula was connected to the previously calibrated blood pressure transducer P.T and the pressure was recorded as tracing by oscillograph 400 M.D. 4C. palemr bioscience, Washington. A strain gauge copuler FC. 137 were used.
(3) **Biochemical assessment.**

Blood samples were collected from the retrobulbar sinus of rat's eye by using heparinized capillary tubes (*Schermer, 1967*). Blood samples were delivered in clean, dry test tubes and allowed to clot at room temperature. The serum was separated by centrifugation at 2000 rounds/minute for 10 minutes.

**a-Serum Tropinin I.**

Serum Tropinin I activity was determined according to Bodor method (*Bodor, 1994*).

**Sample:**

Serum sample from rats used in this study.

**Reagents:**

1. Antibody coated wells
2. Reference standered set
3- cTnl enzyme conjugate reagent
4- Tetramethyl-benzidine (TMB)Reagent
5- Stop solution (diluted hydrochloric acid)

**Procedure:**

1. Secure the desired number of coated wells in the holder
2. Dispense 100µ of standers, specimens, and controls into appropriate wells.
3. Gently mix for 10 minutes
4. incubate at room temperature
5. Strike the wells sharply into absorbent paper to remove all residual water droplets
6. Dispense 100µ of TMB Reagent into each well
7. Read absorbance at 450nm with microtiter well reader within 15 minutes

**Calculations:**

Calculate the mean absorbance value for each set of reference standards, controls and samples. Construct a standard curve. Using the mean absorbance value for each sample we determine the corresponding concentration of troponin I.

**b- Serum Creatine phosphokinase:**

Creatine phosphokinase activity was determined according to Bergemeyer methods (*Bergemeyer, 1974*).

**Principle:**

Creatine phosphokinase in the serum catalyzes the removal of phosphate moiety from p-nitrophenylphosphate. The rate of phosphate release was measured calorimetrically. It is used as a measure for the activity of the enzyme.

**Sample:**

Serum sample from rats used in this study were used.

**Reagents:**

1. Diethanolamine buffer solution.
2. Substrate solution contained p-nitrophenylphosphate.

**Procedure:**

1. The working solution was prepared by mixing 3 ml of substrate solution to 10 ml of buffer solution. The solution was kept in opaque plastic bottle. It is kept at 8°C for maximal of 3 days before use.
2. 2-0.01ml of serum was pipetted into clean, dry plastic cuvette of 1 ml volume.
3. 0.5 ml of working reagent solution was added to each cuvette then carefully mixed and incubated for 1 minute at 37°C.
4. The absorbance of the solution was read at 405nm using spectrophotometer. The instrument was calibrated using air as blank.
5. The absorbance was read again after 2 and 3 minutes.

(4) **Histopathological examination of the heart at end of the study:**

Under ether anesthesia, sacrifice of all animals was done at the was opened and the heart was excised as a whole, preserved in formaline and referred to histopathological examination.

(b) **Hepatic investigations:**

(1) **Biochemical assessment:**

- **Serum alanine transaminase**

  Serum alanine transaminase activity was determined according to Reitman and Frankel methods *(Reitman and Frankel, 1957)*

**Principle:**

Alanine transaminase content of the serum was used to catalyze the transfer of amino group from L-alanine to α-oxoglutarate leading to formation of L-glutamate and pyruvate.

Lactic dehydrogenase in reagent was used to catalyze the reduction of pyrovate by NADH to L-lactate with formation of oxidized NAD.

The change in rate of absorbance as a result of the latter reaction was determined at 340nm.

**Reagents:**

1. Buffer substrate solution contains.
Materials and Method

- Phosphate buffer 80nmol/L at pH 7.4.
- L-alanine 0.8mol/L.

2. Enzyme/coenzyme/α-oxoglutarate solution
Lactic dehydrogenase more than 2U/ml.
Reduced nicotine adenine dinucleotide phosphate (NADH) 0.18mmol/L.

Procedure:
1. 0.2ml of enzyme/coenzyme/α-oxoglutarate solution was pippted to clean dry plastic cuvette of 1 ml volume.
2. 0.2 ml of serum sample was added to cuvette. The cuvette was mixed carefully and transferred to 1 cm plastic cuvette.
3. The absorbance of the sample was read by spectrophotometer at 340 nm. The instrument was calibrated using air as blank. The absorbance was read again after 2 and 3 minutes.

- Serum aspartate transaminase:
Serum aspartate transaminase activity was determined according to Reitman & Frankel methods (Reitman and Frankel, 1957).

Principle
Aspartate transaminase content of the serum was used to catalyze the transfer of amine group from L-alaine to α-oxoglutarate leading to formation of L-glutamate and oxaloacetate.

Malate dehydrogenase in reagent was used to catalyze the reduction of oxaloacetate by NADH to L-lactate with the formation of oxidized NAD.

The change in rate of absorbance as a result of the latter reaction was determined at 340nm.
Sample:
Serum sample from rats used in this study.

Reagents:
1. Buffer substrate solution contains:
   - Phosphate buffer 80mmol/l, pH7.4.
   - L-aspartate 200mmol/l.

2. Enzyme/coenzyme/α-oxoglutarate solution contains:
   - Malate dehydrogenase more than 0.6U/ml.
   - Lactate dehydrogenase more than 1.2 U/ml.
   - α oxoglutarate 12 mmol/l.
   - Reduced nicotine adenine dinucleotide phosphate (NADH) 0.18mmol/L.

Procedure:
1. Working solution was prepared by mixing buffer substrate solution with enzyme/coenzyme/α-oxoglutarate solution in 1:1/volume ratio. 2-0.2 ml of the serum sample was put in clean dry plastic cuvette of 1 ml volume.
2. 0.2 ml of the serum sample was put in clean dry plastic cuvette of 1 ml volume.
3. 1 ml of enzyme/coenzyme/α-oxoglutarate solution was added. The tubes were carefully mixed and incubated at 37°C for 1 minute.
4. The solution was transferred to 1 ml plastic cuvette.
5. The absorbance of the sample was read by spectrophotometer at 240nm. The instrument was adjusted to zero using air as blank.
6. The absorbance was read again after 2 and 3 minutes.
(2) **Histopathological examination of the liver at the end of the study.**

Under ether anesthesia, sacrification of all animals was done at the end of the experiments by a sharp blow on the head followed by decapitation. Liver specimens were taken fixed in 40% formalin and referred to histopatholgical examination *(Drury and Wallington, 1967).*

(c) **Renal investigations:**

(1) **Serum creatinine level measurement**

Serum creatinine activity was determined according to Jaffe methods *(Jaffe, 1886).*

**Sample:**

Serum sample from rats used in this study.

**Reagents:**

- Creatinine acid reagent 3.6 mol/l.
- Creatinine base reagent 240 mol/l.
- Creatinine standered 5-mg /dl.

**Procedure:**

- Set spectrophotometer cuvette temperature to 37°C
- Put 1 ml of working reagent into test tubes and prewarm at37°C for 3 minutes
- Transfer 0.050 ml of standred to test tube , mix and immediately place in cuvette tube
- After exactly 20 seconds read and record absorbance (A₁)
- At exactly 60 seconds after reading (A₁) read and record (A₂)
- Calculate change in absorbance Δ A by subtracting (A₁ - A₂ )

**Calculation:**
Serum creatinine (mg /dl) = Δ Au (unknown) X 5 .

Δ As (standered)

(2) Histopathological examination of the kidney at the end of the study.

Under ether anesthesia, sacrification of all animals was done at the end of the experiments by a sharp blow on the head followed by decapitation. The abdomen will be opened and the kidney will be excised as a whole, preserved in formaline and referred to histopathological examination.

(b) In vitro experiment:

Langendorff’s technique for isolated perfused heart was used. The animal was sacrificed by cutting the throat. The chest was opened, the heart quickly excised, and transported to a dish containing oxygenated Ringer’s solution. The heart was thoroughly squeezed to expel any blood from the chambers of the heart. The tissue was cleaned and fixed through the aorta to the cannula of the Langendorff’s apparatus. The temperature was kept constant by means of an electric automatic thermostat, a thermometer was inserted through one side of the cannula to ensure a constant temperature of the heart at 37°C throughout the experiment. A hook was attached to the ventricular wall of the heart and was attached by a thread to a side way lever which recorded the contractions of the ventricle on slowly moving drum. Drugs were added to the cannula. The experiment was repeated at least 6 times.

(A) The effect of isoprenaline on contractility of hearts taken from another 5 groups of rats given the same medications as the groups of the in-vivo experiment.

(B) The effect of doxorubicin on contractility of isolated normal mammalian heart
**Statistical Analysis:**

All data were expressed as Mean ± SEM. Difference between the groups were compared by Student’s T-test with P-value < 0.05 selected as the level of statistical significance (Hill, 1971).

**Statistical methods**

The processing of the data required the calculation of the following parameters:

1. - Arithmetic mean is used as a measure of the central tendency

\[
\bar{X} = \frac{\sum X}{n}
\]

\( \bar{X} \) = Arithmetic mean

\( \sum X \) = Sum of values recorded.

n= number of observations.

2. - Standard error of the mean (S.E.) is used as measure of precision and statistical reliability of the mean:

\[
S.E = \sqrt{\frac{\sum d^2}{n(n-1)}
\]

\( d^2 \) = Spread of differences between different values and the mean (X and \( \bar{X} \)).

3. - Standard deviation (S.D.) is used as a measure of dispersion

\[
S.D = \sqrt{\frac{\sum d^2}{n-1}}
\]

4. - Student's "t": test of significance of the difference between two mean values (m₁ and m₂).
The statistically significance of data was analyzed by using paired student's t-test for intra group comparison and unpaired student's t-test for inter group comparison.

* All data are expressed as mean ± S.E.M.

* A "p" of less than 0.05 was regarded as significant.

The statistical analysis was carried out using the computer program SPSS (Statistical program for social science).
Results

I. In vivo experiments:

(A) Cardiac results:
1- Effect of treatment with nebivolol & L-Carnitine on doxorubicin induced changes in T wave in rats:

Administration of doxorubicin in a dose of 3 mg / kg / day intraperitoneally every other day for 12 days resulted in significant elevation (P< 0.01) in T wave voltage from 0.3 ± 0.17 m volt in control group to 0.6 ± 0.07 m volt in Dox group (Table 1 & Fig 3 ).

In Dox +Neb group giving nebivolol in a dose of 1mg / kg / day orally for 12 days reduced T wave voltage significantly (P < 0.05) to 0.45 ± 0.06 m volt compared to Dox group ( Table 1 & Fig 3 ).

In Dox+L-car group giving L- carnitine in a dose of 200 mg / kg body weight intraperitonially for 12 days reduced T wave voltage significantly (P<0.05) to0.46± 0.02 m volt compared to Dox group( Table 1& Fig 3 ).

In Dox+Neb+L-car group giving both nebivolol in a dose of 1mg / kg / day for 12 days & L-Carnitine in a dose of 200 mg / kg body weight intraperitonal for 12 days reduced T wave voltage significantly (P < 0.05) to 0.4 ± 0.04 m volt compared to Dox group (Table 1 & Fig 3 ).

Comparing the result of Dox+Neb +L- car group with that of Dox+Neb group, adding L-carnitine to nebivolol was not significantly (P > 0.05) more effective than nebivolol alone in reducing T wave voltage ( Table 1 & Fig 3 ).

Comparing the result of Dox+Neb+L- car group with that of Dox +L-car group, adding nebivolol to L- carnitine was not significantly (P > 0.05) more effective than nebivolol alone in reducing T wave voltage ( Table 1 & Fig 3 ).
Table (1): Showing changes of T wave voltage in various groups:

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>T wave voltage (m volt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td></td>
<td>0.3 ± 0.07</td>
</tr>
<tr>
<td>Dox</td>
<td></td>
<td>0.6 ± 0.15 &lt; 0.01</td>
</tr>
<tr>
<td>Dox + Neb</td>
<td></td>
<td>0.45 ± 0.09 &lt; 0.05</td>
</tr>
<tr>
<td>Dox + L-car</td>
<td></td>
<td>0.46 ± 0.08 &lt; 0.05</td>
</tr>
<tr>
<td>Dox + Neb + L-car</td>
<td></td>
<td>0.4 ± 0.04 &lt; 0.05</td>
</tr>
</tbody>
</table>

- Data represented as Mean ± SEM (n = 6)
- Significant level at P < 0.05

P: compared with control group.
P1: compared with Dox group.
P2: compared with Dox + Neb group.
P3: compared with Dox + L-car group

Figure (3): Histogram showing changes of T wave voltage in various groups.

* Significant compared with control group

** Significant compared with Dox group
2- Effect of treatment with nebivolol & L-Carnitine on doxorubicin induced changes in heart rate:

Administration of doxorubicin in a dose of 3 mg / kg / day intraperitoneally every other day for 12 days resulted in significant rise (P< 0.01) in heart rate from 230 ± 17.7 beat /minute in control group to 313.3± 19.1 beat /minute in Dox group (Table 2 & Fig. 4 ).

In Dox +Neb group giving nebivolol in a dose of 1mg / kg / day orally in for 12 days resulted in insignificant change in the heart rate (P > 0.05) to 303± 34.8 beat /minute compared to Dox group ( Table 2 & Fig 4 ).

In Dox+L-car group giving L- carnitine in a dose of 200 mg / kg body weight intraperitoneally for 12 days resulted in insignificant change in the heart rate (P > 0.05) to 310 ± 13.6 beat /minute compared to Dox group ( Table 2 & Fig 4 ).

In Dox+Neb+L-car group giving both nebivolol in a dose of 1mg / kg / day for 12 days & L-Carnitine in a dose of 200 mg / kg body weight intraperitoneally for 12 days resulted in insignificant change in the heart rate (P > 0.05) to 312± 18.9 beat /minute compared to Dox group ( Table 2 & Fig 4 ).

Comparing the result of Dox+Neb +L- car group with that of Dox+Neb group, adding L-carnitine to nebivolol was not significantly (P > 0.05) more effective than nebivolol alone in changing heart rate g the result of Dox+Neb+L- car group with that of Dox +L-car group, adding nebivolol to L- carnitine was not significantly (P > 0.05) more effective than nebivolol alone in changing heart rate ( Table 2 & Fig 4 ).
Results

Table (2): Showing changes of heart rate in various groups:

<table>
<thead>
<tr>
<th>Group</th>
<th>Heart rate (beat/second)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>230 ±17.7</td>
</tr>
<tr>
<td>Dox P</td>
<td>313.4 ± 19.1</td>
</tr>
<tr>
<td>Dox + Neb P&lt;sub&gt;1&lt;/sub&gt;</td>
<td>303.3 ± 34.8</td>
</tr>
<tr>
<td>Dox + L- car P&lt;sub&gt;1&lt;/sub&gt;</td>
<td>310 ± 13.6</td>
</tr>
<tr>
<td>Dox + Neb + L- car P&lt;sub&gt;1&lt;/sub&gt;</td>
<td>312 ± 18.9</td>
</tr>
</tbody>
</table>

*Data represented as Mean ± SEM (n = 6)  - Significant level at P < 0.05
P: compared with control group.
P<sub>1</sub>: compared with Dox group.
P<sub>2</sub>: compared with Dox + Neb group.
P<sub>3</sub>: compared with Dox + L- car group

Figure (4): Histogram showing changes of heart rate in various groups.

* Significant compared with control group
3- Effect of treatment with nebivolol & L-Carnitine on doxorubicin induced changes in QRS voltage in rats:

Administration of doxorubicin in a dose of 3 mg / kg / day intraperitoneally every other day for 12 days resulted in insignificant change (P > 0.05) in QRS voltage respectively from 8.3 ± 0.11 m volt in control group to 7.9 ± 0.13 m volt in Dox group (Table 3 & Fig.5 ).

In Dox+Neb group giving nebivolol in a dose of 1mg / kg / day orally for 12 days resulted in insignificant change (P > 0.05) in QRS voltage respectively from 8.1 ± 0.03 m volt to 7.9 ± 0.13 m in Dox group ( Table 3 & Fig 5 ).

In Dox+L-car group giving L- carnitine in a dose of 200 mg / kg body weight intraperitonially for 12 days resulted in insignificant change (P > 0.05) in QRS voltage respectively from 8.2 ± 0.15 m volt to 7.9 ± 0.13 m volt in Dox group ( Table 3 & Fig 5 ).

In Dox+Neb+L-car group giving both nebivolol in a dose of 1mg / kg / day for 12 days & L-Carnitine in a dose of 200 mg / kg body weight intraperitontially for 12 days resulted in insignificant change (P > 0.05) in QRS voltage respectively from 8 ± 0.07 m volt to 7.9 ± 0.13 m volt in Dox group ( Table 3 & Fig 5 ).

Comparing the result of Dox+Neb +L- car group with that of Dox+Neb group, adding L-carnitine to nebivolol was not significantly (P > 0.05) more effective than nebivolol alone in changing QRS voltage ( Table 3 & Fig 5 ).

Comparing the result of Dox+Neb+L- car group with that of Dox +L-car group, adding nebivilol to L- carnitine was not significantly (P > 0.05) more effective than nebivilol alone in changing QRS voltage ( Table 3 & Fig 5 ).
Table (3): Showing changes of QRS voltage in various groups:

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>QRS voltage (m volt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td></td>
<td>8.3 ± 0.13</td>
</tr>
<tr>
<td>Dox</td>
<td></td>
<td>7.9 ± 0.11</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Dox + Neb</td>
<td></td>
<td>8.1 ± 0.13</td>
</tr>
<tr>
<td>P1</td>
<td></td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Dox +L-car</td>
<td></td>
<td>8.2 ± 0.15</td>
</tr>
<tr>
<td>P1</td>
<td></td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Dox +Neb+L-car</td>
<td></td>
<td>8 ± 0.07</td>
</tr>
<tr>
<td>P1</td>
<td></td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>P2</td>
<td></td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>P3</td>
<td></td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

*Data represented as Mean ± SEM (n = 6)  - Significant level at P < 0.05

P: compared with control group.
P1: compared with Dox group.
P2: compared with Dox + Neb group.
P3: compared with Dox + L-car group

Figure (5): Histogram showing changes of QRS voltage in various groups.
4- Effect of treatment with nebivolol & L-Carnitine on doxorubicin induced changes in systolic blood pressure & mean blood pressure:

Administration of doxorubicin in a dose of 3 mg / kg / day intraperitoneally every other day for 12 days resulted in significant drop (P< 0.001) in systolic blood & mean blood pressure respectively from 121 ± 4.8 mmHg and 97.7 ± 2.6 mmHg in control group to 88.3± 4 mmHg and 76.3 ± 2.8 mmHg in Dox group (Table 4 & Fig. 6,7).

In Dox+Neb group giving nebivolol in a dose of 1mg / kg / day orally for 12 days resulted in significant elevation in systolic blood pressure & mean blood pressure (P < 0.05) to 99.8 ± 0.17 and 92 ± 1 mmHg respectively compared to Dox group (Table 4 & Fig. 6,7).

In Dox +L-car group giving L- carnitine in a dose of 200 mg / kg body weight intraperitonially for 12 days resulted in significant elevation in systolic blood pressure & mean blood pressure (P < 0.001) to 123.3 ± 4.9 and 94.5±4.3 mmHg respectively compared to Dox group (Table 4 & Fig. 6,7).

In Dox+Neb+L-car group giving both nebivolol in a dose of 1mg / kg / day orally for 12 days & L-Carnitine in a dose of 200 mg / kg body weight intraperitonially for 12 days resulted in significant elevation in systolic & mean blood pressure (P < 0.01) to 120 ± 6.3 & 96 ± 6.7 mmHg respectively compared to Dox group (Table 4 & Fig. 6,7).

Comparing the result of Dox +Neb+L-car group with that of Dox+Neb group, adding L-carnitine to nebivolol was significantly (P >0.05) more effective than nebivolol alone in elevating systolic & mean blood pressure(P< 0.05) (Table 4 & Fig. 6,7).

Comparing the result of Dox+Neb+L-car group with that of Dox+L-car group, adding nebivolol to L- carnitine was not significantly (P > 0.05) more effective than nebivolol alone in elevating systolic blood pressure & mean blood pressure (Table 4 & Fig. 6,7).
Table (4): Showing changes of systolic blood pressure & mean blood pressure in various groups:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Systolic blood pressure (mmHg)</th>
<th>Mean blood pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>121.7 ± 4.8</td>
<td>97.7 ± 2.6</td>
</tr>
<tr>
<td>Dox</td>
<td>88.3 ± 4</td>
<td>76.3 ± 2.8</td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Dox +Neb</td>
<td>99.8 ± 0.17</td>
<td>92 ± 1</td>
</tr>
<tr>
<td>P&lt;sub&gt;1&lt;/sub&gt;</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Dox +L- car</td>
<td>123.3 ± 4.9</td>
<td>94.5 ± 4.3</td>
</tr>
<tr>
<td>P&lt;sub&gt;1&lt;/sub&gt;</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Dox+Neb+L- car</td>
<td>120 ± 6.3</td>
<td>96 ± 6.7</td>
</tr>
<tr>
<td>P&lt;sub&gt;1&lt;/sub&gt;</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>P&lt;sub&gt;2&lt;/sub&gt;</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>P&lt;sub&gt;3&lt;/sub&gt;</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

*Data represented as Mean ± SEM (n = 6) - Significant level at P < 0.05

**P**: compared with control group.

**P<sub>1</sub>**: compared with Dox group.

**P<sub>2</sub>**: compared with Dox+ Neb group.

**P<sub>3</sub>**: compared with Dox+L- car group
**Results**

![Graph showing systolic blood pressure](image1)

**Animal groups**

Figure (6): Histogram showing Systolic blood pressure in various groups.

* Significant compared with control group
** Significant compared with Dox group

![Graph showing mean arterial blood pressure](image2)

**Animal groups**

Figure (7): Histogram showing mean arterial blood pressure in various groups.

* Significant compared with control group
** Significant compared with Dox group
Fig. (8): Blood pressure and ECG measurement of control group.
(A typical trace of 6 separate experiments)

Fig. (9): Blood pressure and ECG measurement of Dox group.
(A typical trace of 6 separate experiments)

Fig. (10): Blood pressure and ECG measurement of Dox+ Neb group.
(A typical trace of 6 separate experiments)
Fig. (11): Blood pressure and ECG measurement of Dox+ L-Car group. (A typical trace of 6 separate experiments)

Fig. (12): Blood pressure and ECG measurement of Dox + Neb + L-car group. (A typical trace of 6 separate experiments)
5- Effect of treatment with nebivolol & L-Carnitine on doxorubicin induced changes in cardiac enzymes in rats:

Administration of doxorubicine in a dose of 3 mg / kg / day intraperitoneally every other day for 12 days resulted in significant rise (P< 0.001) in cardiac enzymes creatine phosphokinase and troponin I from 317 ± 86 u / l and 0.26 ±0.17 ng / ml respectively in control group to 1187.6 ±63 u / l and 1.2±0.23 ng / ml in Dox group (Table & Fig.  ).

In Dox+Neb group giving nebivolol in a dose of 1mg / kg / day orally for 12 days reduced the cardiac enzymes creatine phosphokinase and troponin I significantly (P < 0.01) to 869 ± 28.7 u / l and 0.75±0.03 ng / ml respectively compared to Dox group ( Table 5 & Fig. 13,14 ).

In Dox +L-car group giving L- carnitine in a dose of 200 mg / kg body weight intraperitonially for 12 days reduced the cardiac enzymes creatine phosphokinase and troponin I significantly (P < 0.01) to 851 ± 46.2  u / l and 0.7 ± 0.054 ng / ml respectively compared to Dox group ( Table  5 & Fig. 13,14 ).

In Dox +Neb+ L-car group giving both nebivolol in a dose of 1mg / kg / day for 12 days & L-carnitine in a dose of 200 mg / kg body weight intraperitonially for 12 days reduced the cardiac enzymes significantly (P< 0.01) to 755 ± 43.9 u / l & 0.63 ± 0.036 ng / ml respectively compared to Dox group ( Table  5 & Fig. 13,14 ).

Comparing the result of Dox+Neb+L-car group with that of Dox+Neb group, adding L-carnitine to nebivolol was not significantly (P > 0.05) more effective than nebivolol alone in reducing the cardiac enzymes ( Table 5 & Fig. 13,14 ).

Comparing the result of Dox+Neb+ L- car group with that of Dox + L-car group, adding nebivolol to L- carnitine was not significantly (P > 0.05) more effective than nebivolol alone in reducing the cardiac enzymes ( Table 5 & Fig. 13,14 ).
Table (5): Showing changes of CPK & troponin I level in various groups:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>CPK (u/l)</th>
<th>Troponin I (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>317 ± 34</td>
<td>0.26 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>Dox</td>
<td>1187.6 ± 45</td>
<td>1.2 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Dox + Neb</td>
<td>869 ± 28.7</td>
<td>0.75 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>P₁</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>Dox + L-car</td>
<td>851.2 ± 26.2</td>
<td>0.7 ± 0.054</td>
</tr>
<tr>
<td></td>
<td>P₁</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>Dox + Neb + L-car</td>
<td>755 ± 33.2</td>
<td>0.63 ± 0.036</td>
</tr>
<tr>
<td></td>
<td>P₁</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>P₂</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>P₃</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

- Data represented as Mean ± SEM (n = 6) - Significant level at P < 0.05

P : compared with control group.
P₁ : compared with Dox group.
P₂ : compared with Dox + Neb group.
P₃ : compared with Dox + L-car group.

Figure (13): Histogram showing changes of CPK level in various groups.

* Significant compared with control group
** Significant compared with Dox group
Results

![Graph showing troponin I levels in various groups]

Figure (14): Histogram showing changes of troponin I level in various groups.

* Significant compared with control group
** Significant compared with Dox group

5- Histopathological evaluation of cut sections of the heart:

In the Dox group, there was cardiac myocyte degenerative changes in the form of cell swelling, loss of cytoplasmic striations, with mild interstitial fibrosis (Fig. 16,17).

The doxorubicin induced myocardial damage was improved in nebivolol and l-carnitine and nebivolol & l-carnitine treated rats in comparison to the Dox group, as the myocyte degeneration as well as the interstitial fibrosis were decreased (Fig. 18,19,20).
**Fig. (15):** Cut section of normal myocardial tissues (H & E × 100).

**Figure (16):** Cut section of myocardium of Dox group showing, (A) eosynophylic cytoplasm, inflammatory infiltration(B) intestinal edema congested capillaries between irregular wavy-directed cardiomiocytes. (H x & E x 100).
Figure (17): Cut section of myocardium & percardium of Dox group showing, (A) eosynophytic cytoplasm, inflammatory infiltration (B) interstitial edema congested capillaries. (H x & E x 100).

Figure (18): Cut section of myocardium of Dox+ Neb group showing (A) eosynophytic cytoplasm, inflammatory infiltration (B) interstitial edema congested capillaries (H x & E x 100).
Results

Figure (19) : Cut section of myocardium of Dox +L-car group showing (A) eosynophylic cytoplasma, inflammatory infiltration (B) interstitial edema, congested capillaries (H x & E x 100).

Figure (20) : Cut section of myocardium of Dox+Neb +L-carnitine group showing interstitial edema (H x & E x 100).
(B) Hepatic results:
1- Effect of treatment with nebivolol & L-Carnitine on doxorubicin induced changes in liver enzymes ALT & AST level in rats:

Administration of doxorubicin in a dose of 3 mg / kg / day intraperitoneally every other day for 12 days resulted in significant rise (P< 0.001) in liver enzymes ALT & AST level from 34 ± 5.77 u / l and 47 ± .95 u / l respectively in control group to 89.2 ± 3.08 u / l and 95 ± 2.46 u / l in doxorubicin administered group ( Table 6 & Fig. 21,22 ).

In Dox + Neb group giving nebivolol in a dose of 1mg / kg / day orally for 12 days reduced the cardiac liver enzymes ALT& AST significantly (P < 0.01) to 45.7 ± 2.08 u / l and 55 ± 2.39 u / l respectively compared to Dox group ( Table 6 & Fig. 21,22 ).

In Dox + L-Car group giving L- carnitine in a dose of 200 mg / kg body weight intraperitonially for 12 days reduced the liver enzymes ALT& AST significantly (P < 0.01) to 48 ± 2.65 u / l and 60 ± 2.86 u / l respectively compared to Dox group ( Table 6 & Fig. 21,22 ).

In Dox +Neb + L_Car group giving both nebivolol in a dose of 1mg / kg / day orally for 12 days & L-Carnitine in a dose of 200 mg / kg body weight intraperitonially for 12 days reduced the cardiac enzymes significantly (P< 0.01) to 44 ± 1.78 u / l & 50 ± 2.86 u / l respectively compared to Dox group ( Table 6 & Fig. 21,22 ).

Comparing the result of Dox +Neb + L_Car group with that of Dox + L-car group, adding nebivolol to L- carnitine was not significantly (P > 0.05) more effective than nebivolol alone in reducing the liver enzymes ( Table 6 & Fig. 21,22 ).

Comparing the result of Dox +Neb + L_Car group with that of Dox + Neb group, adding L-carnitine to nebivolol was not significantly (P > 0.05) more effective than nebivolol alone in reducing the liver enzymes ( Table 6 & Fig. 21,22 ).
Table 6: Showing changes of ALT & AST level in various groups:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>ALT (u/l)</th>
<th>AST (u/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>34 ± 5.77</td>
<td>47 ± 0.95</td>
</tr>
<tr>
<td></td>
<td>Dox</td>
<td>89.2 ± 3.08</td>
<td>95 ± 2.46</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>Dox + Neb</td>
<td>45.7 ± 2.08</td>
<td>55 ± 2.39</td>
</tr>
<tr>
<td></td>
<td>P&lt;sub&gt;1&lt;/sub&gt;</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>Dox + L- car</td>
<td>48 ± 2.65</td>
<td>60 ± 2.86</td>
</tr>
<tr>
<td></td>
<td>P&lt;sub&gt;2&lt;/sub&gt;</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>Dox+ Neb + L- car</td>
<td>44 ± 1.78</td>
<td>50 ± 2.86</td>
</tr>
<tr>
<td></td>
<td>P&lt;sub&gt;1&lt;/sub&gt;</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>P&lt;sub&gt;2&lt;/sub&gt;</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>P&lt;sub&gt;3&lt;/sub&gt;</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

*Data represented as Mean ± SEM (n = 6) - Significant level at P < 0.05

P: compared with control group.
P<sub>1</sub>: compared with Dox group.
P<sub>2</sub>: compared with Dox+Neb group.
P<sub>3</sub>: compared with Dox+ L- car group.
Results

Figure (21): Histogram showing changes of ALT level in various groups.

* Significant compared with control group
** Significant compared with Dox group

Figure (22): Histogram showing changes of AST level in various groups.

* Significant compared with control group
** Significant compared with Dox group
2- Histopathological evaluation:

The liver of the Dox group showed marked hydropic changes, some necro-inflammatory foci and portal tract inflammation (fig. 24).

The doxorubicin induced liver damage was improved in nebivolol and l-carnitine and nebivolol & l-carnitine treated rats in comparison to the Dox group, as hydropic changes, necro-inflammatory foci and portal tract inflammation were decreased (fig. 25, 26, 27).

Figure (23): A photomicrograph of a cut section in the liver of a control rat showing normal liver architecture composed of hexagonal or pentagonal lobules with central veins and peripheral hepatic triads or tetrads embedded in connective tissue. Hepatocytes are arranged in trabecules running radiantly from the central vein and are separated by sinusoids containing Kupffer cells. (H x & E x 400).
Figure (24): A photomicrograph of a cut section in the liver of Dox group showing preserved liver architecture, (A) hepatocytes show marked hydropic changes, (B) some necro-inflammamatory foci & (C) portal tract inflammation (H & E x 400)

Figure (25): A photomicrograph of a cut section in the liver in Dox+Neb group, showing preserved liver architecture, hepatocytes show (A) hydropic changes, no necro-inflammamatory foci (B) congested central vein (H & E x 400)
Results

Figure (26): A photomicrograph of a cut section in the liver in Dox + L-car group, showing preserved liver architecture, (A) hepatocytes show hydropic change (B) inflammatory foci (C) congested central vein (H & E x 400).

Figure (27): A photomicrograph of a cut section in the liver in Dox+ Neb+L-car group showing preserved liver architecture, (A) hepatocytes show moderate hydropic changes, (B) few inflammatory foci and no portal tract inflammation. (H & E x 400).
(C) -Renal results:

1- Effect of treatment with nebivolol & L-Carnitine on doxorubicin induced changes in creatinine level in rats:

Administration of doxorubicin in a dose of 3 mg / kg / day intraperitoneally every other day for 12 days resulted in significant rise (P< 0.001) in creatinine level from 0.65 ± 0.05 mg / dl in control group to0.95 ± 0.02 mg / dl in Dox group (Table 7 & Fig 28).

In Dox +Neb group giving nebivolol in a dose of 1mg / kg / day orally for 12 days reduced the creatinine level significantly (P < 0.01) to 0.72 ± 0.04 mg / dl compared to Dox group (Table 7 & Fig 28).

In Dox +L- Car group giving L- carnitine in a dose of 200 mg / kg body weight intraperitoneally for 12 days reduced the creatinine level significantly (P < 0.01) to 0.74 ±0.07 mg / dl compared to Dox group (Table 7 & Fig 28).

In Dox +Neb + L- car group giving both nebivolol in a dose of 1mg / kg / day orally for 12 days & L-Carnitine in a dose of 200 mg / kg body weight intraperitoneally for 12 days reduced the creatinine level significantly (P< 0.001) to 0.69 ±0.02 mg / dl respectively compared to Dox group (Table 7 & Fig 28).

Comparing the result of Dox+Neb+L- car group with that of Dox+ L-car group, adding nebulolol to L- carnitine was not significantly (P > 0.05) more effective than nebulolol alone in reducing the creatinine level (Table 7 & Fig 28).

Comparing the result of Dox+Neb + L- car group with that of Dox +Dox+Neb group, adding L-carnitine to nebulolol was not significantly (P > 0.05) more effective than nebulolol alone in reducing the creatinine level (Table 7 & Fig 28).
Table (7): Showing changes of creatinine level in various groups:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>0.65± 0.05</td>
</tr>
<tr>
<td></td>
<td>Dox</td>
<td>0.95 ± 0.02</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Dox + Neb</td>
<td>0.72 ± 0.04</td>
</tr>
<tr>
<td>P₁</td>
<td></td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>Dox + L- car</td>
<td>0.74 ± 0.07</td>
</tr>
<tr>
<td>P₁</td>
<td></td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>Dox+ Neb + L- car</td>
<td>0.69 ±0.02</td>
</tr>
<tr>
<td>P₁</td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>P₂</td>
<td></td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>P₃</td>
<td></td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

- Data represented as Mean ± SEM (n = 6)  
- Significant level at P < 0.05

P : compared with control group.  
P₁ : compared with Dox group.  
P₂ : compared with Dox+Neb group.  
P₃ : compared with Dox+L- car group
**Results**

<table>
<thead>
<tr>
<th>Creatinine level (mg/dl)</th>
<th>Control</th>
<th>Dox</th>
<th>Dox+Neb</th>
<th>Dox+L-car</th>
<th>Dox+Neb+L-car</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.65</td>
<td></td>
<td></td>
<td>0.95*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.72**</td>
<td></td>
<td></td>
<td>0.74**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.69**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant compared with control group
** Significant compared with Dox group

**Figure (28):** Histogram showing creatinine level in various groups.

2- **Histopathological evaluation:**

The kidney of the Dox group shows glomerulopathy characterized by mesangial proliferation, tubular atrophy & dilatation and congestion of interstitial capillaries (Fig. 30).

The doxorubicin induced kidney damage was improved in nebivolol and l-carnitine and nebivolol & l-carnitine treated rats in comparison to the Dox group, as the mesangial proliferation, tubular atrophy & dilatation and congestion of interstitial capillaries was decreased (Fig. 31, 32, 33).
Fig. (29): Cut section of normal kidney tissue (H & E × 100)

Fig. (30): Cut section of kidney tissue of Dox group showing glomerulopathy characterized by (A) mesangial proliferation, (B) tubular atrophy & (C) dilatation and congestion of interstitial capillaries (H & E × 100).
Fig. (31): Cut section of kidney tissue of Dox+Neb group showing (A) mesangial proliferation and (B) some congestion of interstitial capillaries (H & E × 100).

Fig. (32): Cut section of kidney tissue of Dox+L-car group showing (A) mesangial proliferation (B) congestion of interstitial capillaries (H & E × 100)
Fig. (33): Cut section of kidney of Dox+Neb+L-car group showing (A) some mesangeal proliferation (H & E × 100)
II. In vitro experiments:

(A) The effect of isoprenaline on contractility of hearts taken from rats treated as the groups of the in-vivo experiment:

- Effect of isoprenaline on isolated perfused rat’s hearts of control group:

It was noticed that isoprenaline in a gradually increasing doses (2, 4 and 8 μg/bath) produced an increase in the force of spontaneous contraction of isolated perfused rat’s heart in dose related manner.

This increase of the force of spontaneous contractions of the isolated perfused rabbit’s heart was significant (P < 0.05) with the dose of 2μg/bath with percentage of increase 63.6 % compared to pre-isoprenaline contraction. Also it was significant (P < 0.05) with the dose of 4μg/bath compared to preisoprenaline contraction and with percentage of increase 85.2 %. Meanwhile it was significant (P < 0.01) with the dose of 8μg/bath compared to preisoprenaline contraction and with percentage of increase 110.5 % (Table 8 & Fig 34, 35).

Table (8): Effect of isoprenaline on the amplitude of spontaneous rhythmic contraction of isolated perfused rat heart in control group.

<table>
<thead>
<tr>
<th>Dose of isoprenaline μg/ml bath</th>
<th>Amplitude of contraction before isoprenaline (cm)</th>
<th>Amplitude of contraction after isoprenaline (cm)</th>
<th>Percentage changes (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3.24 ± 0.6</td>
<td>5.3 ± 0.2</td>
<td>↑63.6 %</td>
<td>&lt; 0.05*</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>6 ± 0.3</td>
<td>↑85.2 %</td>
<td>&lt; 0.05*</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>6.8 ± 0.26</td>
<td>↑110.5 %</td>
<td>&lt; 0.01*</td>
</tr>
</tbody>
</table>

Data represented as mean ± SEM of six experiments.

* Significant level at (P < 0.05) compared to pre isoprenaline value.
Figure (34): Histogram showing the effect of isoprenaline on isolated rat's heart of control group.

* Significant (P < 0.05) compared to pre isoprenaline value.

Fig. (35): A record demonstrating the effect of gradually increasing concentrations of isoprenaline on the isolated perfused rat's heart contractions in control group (A typical trace of 6 separate experiments)
• Effect of isoprenaline on isolated perfused rat’s hearts of Dox group:

It was noticed that isoprenaline in increasing doses (2 and 4μg/bath) produced a non significant increase in the force of spontaneous contraction of isolated perfused rat’s heart ($P > 0.05$).

This increase of the force of spontaneous contractions of the isolated perfused rat’s heart was significant ($P < 0.05$) with the dose of 8g/bath with percentage of increase 59.1 % compared to preisoprenaline contraction (Table 9 & Fig 36, 37).

Table (9): Effect of isoprenaline on the amplitude of spontaneous rhythmic contraction of isolated perfused rat heart in Dox group.

<table>
<thead>
<tr>
<th>Dose of isoprenaline (µg/ml bath)</th>
<th>Amplitude of contraction before isoprenaline (cm)</th>
<th>Amplitude of contraction after isoprenaline (cm)</th>
<th>Percentage changes (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.15 ± 0.27</td>
<td>1.2 ± 0.28</td>
<td>↑ 4.3 %</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>4</td>
<td>1.43 ± 0.34</td>
<td>1.83 ± 0.48</td>
<td>↑ 59.1 %</td>
<td>&lt; 0.05*</td>
</tr>
</tbody>
</table>

Data represented as mean ± SEM of six experiments.

* Significant level at ($P < 0.05$) compared to pre isoprenaline value.
Results

Figure (36): Histogram showing the effect of isoprenaline on isolated rat’s heart of Dox group.

* Significant (P < 0.05) compared to pre isoprenaline value.

Fig. (37): A record demonstrating the effect of gradually increasing concentrations of isoprenaline on the isolated perfused rat's heart contractions in Dox group (A typical trace of 6 separate experiments)
- **Effect of isoprenaline on isolated perfused rat’s hearts of Dox + Neb group:**

  It was noticed that isoprenaline in increasing doses (4 and 8 μg/bath) produced an increase in the force of spontaneous contraction of isolated perfused rat’s heart in dose related manner.

  This increase of the force of spontaneous contractions of the isolated perfused rat’s heart was not significant (P > 0.05) with the dose of 2μg/bath with percentage of increase 27.3 %. Meanwhile it was significant (P < 0.05) with the dose of 4μg/bath with percentage of increase 51.5 % and it was significant (P < 0.01) with the dose of 8μg/bath and with percentage of increase 118.2 % compared to preisoprenaline contraction (Table 10 & Fig 38, 39).

**Table (10): Effect of isoprenaline on the amplitude of spontaneous rhythmic contraction of isolated perfused rat heart in Dox+ Neb group.**

<table>
<thead>
<tr>
<th>Dose of isoprenaline (μg/ml bath)</th>
<th>Amplitude of contraction before isoprenaline (cm)</th>
<th>Amplitude of contraction after isoprenaline (cm)</th>
<th>Percentage changes (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.65 ± 0.22</td>
<td>2.1 ± 0.17</td>
<td>↑ 27.3 %</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>2.5 ± 0.27</td>
<td>↑ 51.5 %</td>
<td>&lt; 0.05 *</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>3.6 ± 0.41</td>
<td>↑ 118.2 %</td>
<td>&lt; 0.01 *</td>
</tr>
</tbody>
</table>

Data represented as mean ± SEM of six experiments.

* Significant level compared to pre isoprenaline value.
Figure (38): Histogram showing the effect of isoprenaline on isolated rat’s heart of Dox +Neb group.
* Significant compared to pre isoprenaline value.

Fig. (39): A record demonstrating the effect of gradually increasing concentrations of isoprenaline on the isolated perfused rat's heart contractions in Dox+Neb group (A typical trace of 6 separate experiments)
• Effect of isoprenaline on isolated perfused rabbit’s hearts of Dox+L- car group:

It was noticed that isoprenaline in increasing doses (4 and 8 μg/bath) produced an increase in the force of spontaneous contraction of isolated perfused rabbit’s heart in dose related manner.

This increase of the force of spontaneous contractions of the isolated perfused rabbit’s heart was not significant (P > 0.05) with the dose of 2μg/bath with percentage of increase 25.5 %. Meanwhile it was significant (P < 0.05) with the dose of 4μg/bath percentage of increase 36.2 % and it was significant (P < 0.05) with the dose of 8μg/bath and with percentage of increase 58.7 % compared to preisoprenaline contraction (Table 11 & Fig 40, 41).

Table (11): Effect of isoprenaline on the amplitude of spontaneous rhythmic contraction of isolated perfused rat heart in Dox+L- car group.

<table>
<thead>
<tr>
<th>Dose of isoprenaline (μg/ml bath)</th>
<th>Amplitude of contraction before isoprenaline (cm)</th>
<th>Amplitude of contraction after isoprenaline (cm)</th>
<th>Percentage changes (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2.35 ± 0.33</td>
<td>2.9 ± 0.38</td>
<td>↑ 25.5 %</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>4</td>
<td>3.2 ± 0.41</td>
<td>3.6 ± 0.38</td>
<td>↑ 58.7 %</td>
<td>&lt; 0.05 *</td>
</tr>
<tr>
<td>8</td>
<td>3.6 ± 0.38</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data represented as mean ± SEM of six experiments.

* Significant level at (P < 0.05) compared to pre isoprenaline value.
Results

Figure (40): Histogram showing the effect of isoprenaline on isolated rat’s heart of Dox+ L-car group

* Significant (P < 0.05) compared to pre isoprenaline value.

Fig. (41): A record demonstrating the effect of gradually increasing concentrations of isoprenaline on the isolated perfused rat's heart contractions in Dox+L-car group (A typical trace of 6 separate experiments)
• **Effect of isoprenaline on isolated perfused rat’s hearts of Dox + Neb + L- car group:**

It was noticed that isoprenaline in increasing doses (2, 4 and 8 μg/bath) produced an increase in the force of spontaneous contraction of isolated perfused rat’s heart in dose related manner.

This increase of the force of spontaneous contractions of the isolated perfused rat’s heart was significant (P < 0.01) with the dose of 2μg/bath with percentage of increase 48.6 % , also it was significant (P < 0.01) with the dose of 4μg/bath and with percentage of increase 79.4 % and it was significant (P < 0.001) with the dose of 8μg/bath and with percentage of increase 106.2 % compared to preisoprenaline contraction ( Table 12 & Fig 42, 43 )

**Table (12): Effect of isoprenaline on the amplitude of spontaneous rhythmic contraction of isolated perfused rat heart in Dox + Neb + L- car group:**

<table>
<thead>
<tr>
<th>Dose of isoprenaline (μg/ml bath)</th>
<th>Amplitude of contraction before isoprenaline (cm)</th>
<th>Amplitude of contraction after isoprenaline (cm)</th>
<th>Percentage changes (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2.4 ± 0.31</td>
<td>3.6 ± 0.46</td>
<td>↑ 48.6 %</td>
<td>&lt; 0.01 *</td>
</tr>
<tr>
<td>4</td>
<td>2.4 ± 0.31</td>
<td>4.37 ± 0.47</td>
<td>↑ 79.4 %</td>
<td>&lt; 0.01 *</td>
</tr>
<tr>
<td>8</td>
<td>2.4 ± 0.31</td>
<td>5.1 ± 0.41</td>
<td>↑ 106.2 %</td>
<td>&lt; 0.001 *</td>
</tr>
</tbody>
</table>

Data represented as mean ± SEM of six experiments.

* Significant level at (P < 0.05) compared to pre isoprenaline value.
Results

Figure (42): Histogram showing the effect of isoprenaline on isolated rat’s heart of Dox + Neb +L-car group.

* Significant (P < 0.01) compared to pre isoprenaline value.

Fig. (43): A record demonstrating the effect of gradually increasing concentrations of isoprenaline on the isolated perfused rat's heart contractions in Dox+Neb+L-car group (A typical trace of 6 separate experiments)
### Table (13): Percent change of contraction of isolated perfused rat heart in different groups after isoprenaline different doses 2,4,8 µg:

<table>
<thead>
<tr>
<th>Dose of isoprenaline µg/ml bath</th>
<th>Control group</th>
<th>Doxorubicin group</th>
<th>Nebivolol group</th>
<th>L-carnitine group</th>
<th>Nebivolol &amp; L-carnitine group</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>↑ 63.6 %</td>
<td>↑ 4.3 %</td>
<td>↑ 27.3 %</td>
<td>↑ 25.5 %</td>
<td>↑ 48.6 %</td>
</tr>
<tr>
<td>4</td>
<td>↑ 85.2 %</td>
<td>↑ 24.2 %</td>
<td>↑ 51.5 %</td>
<td>↑ 36.2 %</td>
<td>↑ 79.4 %</td>
</tr>
<tr>
<td>8</td>
<td>↑ 110.5 %</td>
<td>↑ 59.1 %</td>
<td>↑ 118.2 %</td>
<td>↑ 58.7 %</td>
<td>↑ 106.2 %</td>
</tr>
</tbody>
</table>

- In the control group the percent change of contraction of isolated perfused rat heart ↑ 63.6 %, ↑ 85.2 %, ↑ 110.5 % after isoprenaline different doses 2,4,8 µg respectively but in the Dox group the percent change was markedly decreased to ↑ 4.3 %, ↑ 24.2 %, ↑ 59.1 % and in the Dox+Neb group the percent change was markedly improved compared to Dox group to be ↑ 27.3 %, ↑ 51.5 %, ↑ 118.2 % respectively, in the L-carnitine group the percent change was also markedly improved compared to Dox group to be ↑ 25.5 %, ↑ 36.2 %, ↑ 58.7 % respectively and finally in the Dox + Neb +L-car group the percent change showed the best improvement compared to Dox group to be ↑ 48.6 %, ↑ 79.4 %, ↑ 106.2 % respectively.
(B) The effect of Doxorubicin on contractility of isolated rabbit's heart:

It was noticed that doxorubicin in a gradually increasing doses (1/2, 1, 2 and 4 μg / bath) produced a decrease in the force of spontaneous contraction of isolated perfused rabbit's heart in dose related manner (fig. 44)

Also it was noticed that doxorubicin decrease the positive inotropic effect of isoprenaline in a dose of 2 μg / bath on isolated perfused rabbit’s heart (fig. 44)

Fig. (44) : A record demonstrating the effect of gradually increasing concentrations of doxorubicine on the isolated perfused rabbit's heart (A typical trace of 6 experiments)
Fig. (45) : A record demonstrating the effect of gradually increasing concentrations of doxorubicin on isoprenaline effect on the isolated perfused rabbit's heart (A typical trace of 6 experiments)
Discussion

Doxorubicin is one of the potent anticancer drugs. It is commonly used in the treatment of a wide range of cancers, including leukemia and Hodgkin's lymphoma, as well as cancer bladder, breast, stomach, lung, ovaries, thyroid, soft tissue sarcoma, multiple myeloma (Takimoto and Calvo, 2008). This drug has many organ toxicities especially cardiotoxicity and this toxicity is the main limitation for its use as anticancer drug (Tangpong et al., 2011).

It causes disruption in basal body metabolism and shows toxic effect especially in heart, liver and kidney tissues. It is known that doxorubicin is considered the most toxic anthracyclines which may cause death (Sayed et al., 2010b).

Many researchers have expanded great efforts aiming at preventing or decreasing the adverse effects of doxorubicin. In this study, the possible cardioprotective, hepatoprotective and nephroprotective effect of some chemical agents like nebivolol and some natural agents like L-carnitine was studied in a model of cardiotoxicity, hepatotoxicity, nephrotoxicity induced by doxorubicin. Also the effect of isoprenaline on isolated rat heart of the treated rat groups and effect of doxorubicin on isolated rabbit heart were studied.

Animals used in the in-vivo study were rats. The choice of rats was because they are cheap, easy to deal with and to take samples, as well as it is easy to take sufficient amount of blood easily through puncture of the retro bulbar sinus by capillary tube.

Parameters studied in this work were ECG, blood pressure measurement, cardiac enzymes troponin I and serum creatine phosphokinase, as a markers for cardiac cell injury and liver enzymes ALT, AST as they can be used as a markers of hepatic cell injury and
creatinine level as markers of renal cell function also histopathological evaluation of heart, liver and kidney was done.

In the present study acute toxicity was induced in experimental animals by administration of doxorubicin in a dose of 3 mg / kg / day intraperitoneally every other day for 12 days (Amani et al., 2011). The potential organ protective effect of nebivolol was tested by concomitant oral administration of nebivolol in a dose of 1mg / kg / day orally daily for 12 days (Amani et al., 2011) and the potential organ protective effect of L-carnitine was tested by concomitant administration of L-carnitine in a dose of 200 mg / kg body weight intraperitonially daily for 12 days (Nathalie et al., 1999).

The present study showed that doxorubicin administration caused ECG changes as increased T wave voltage in Dox group compared to control group, concerning heart rate and QRS voltage there was insignificant change in Dox group compared to control group also there was significant decrease in blood pressure indicating deterioration of cardiac function, There was also histopathological changes including cardiac myocyte degenerative changes in the form of cell swelling, loss of cytoplasmic striations, with mild interstitial fibrosis accompanied by elevation of cardiac enzymes troponin I and CPK which indicate structural affection of the heart by doxorubicin toxicity.

The result of present study showed that treatment of rats of Dox+Neb group with nebivolol for 12 days produced a significant reduction in T wave voltage, significant elevation in blood pressure and significant reduction in cardiac enzymes troponin I & CPK in comparison to Dox group & treatment of rats of Dox+L-Car group with L-carnitine produced also a significant reduction in T wave voltage, significant elevation in blood pressure and significant reduction in cardiac enzymes troponin I & CPK in comparison to Dox group. When rats of Dox+Neb+
L-Car group given both nebivolol and L-carnitine, there was also significant reduction in T wave voltage, significant elevation in blood pressure and significant reduction in cardiac enzymes troponin I & CPK in comparison to Dox group but without significant difference when compared with groups Dox+Neb and Dox+L-Car group.

In the Dox group there was cardiac myocyte degenerative changes in the form of cell swelling, loss of cytoplasmic striations, with mild interstitial fibrosis. The doxorubicin induced myocardial damage was improved in groups treated with nebivolol, L-carnitine and in group treated with nebivolol & L-carnitine in comparison to the Dox group, as the present work showed marked decrease of the area of hydropic degeneration and area of inflammation and decrease in inflammatory infiltrate in cardiac sections of nebivolol and in L-Carnitine treated group.

As regards effect of isoprenaline on isolated perfused rat heart of the treated rats groups, isoprenaline produced concentration-dependent increase in force of contraction in control group. The positive inotropic effect induced by isoprenaline was significantly reduced (P<0.05) in hearts from the Dox group compared to control rats (p<0.05). Both Dox+Neb and Dox+L-car groups showed significant improvement in power of cardiac contractility in response to increasing doses of isoprenaline compared to doxorubicin group (p<0.05). There were insignificant difference between the Dox+Neb and Dox+L-car groups in the percent change of contraction when compared to each other.

Calcium homeostasis disturbances related to the appearance of oxidative stress could explain why the response to isoprenaline was attenuated in the doxorubicin group. The dysfunction of sarcoplasmic reticulum could induce changes in intracellular calcium concentrations (Hideo et al., 1991) leading to impairment of cardiac contractility and/or
relaxation. Moreover, it has been reported that anthracycline treatment could induce reduction of β-adrenergic receptor density on myocardial membrane (Nagami et al., 1997).

The present work runs in consistence with Ibrahim et al., 2009 that demonstrated occurrence of marked cardiotoxicity and nephrotoxicity with doxorubicin intake as they found increased cardiac enzymes troponin I, CPK and increased heart rate. Ibrahim et al., 2009 also found decreased QRS voltage which isn't in agreement with our result as we found non significant change in QRS voltage in Dox group compared to control this may be due to difference between the two studies in doses and duration.

These results are also in agreement with Nathalie et al., 1999, Atiar et al., 2007, Shi , et al ., 2011, Amani et al., 2011 & Imbaby et al., 2014 that demonstrated the toxic effect of doxorubicin in rat models that found increased heart rate, decreased blood pressure, histopathological changes and elevation of cardiac enzymes troponin I and CPK and this is also in agreement with Jessica et al., 2011 who studied doxorubicin toxicity in humans.

This is also in agreement with Paolo et al., 2004 who confirmed that doxorubicin induces oxidative stress, mitochondrial dysfunction, and histopathological lesions in the cardiac tissue in doxorubicin induced mitochondrial mediated cardiomyopathy.

Doxorubicin causes inhibition of nucleic acid and protein synthesis, release of vasoactive amines, altered adrenergic function and decreased expression of cardiac-specific genes are other proposed mechanisms. It is possible that more than one mechanism is operative (Monti et al., 1995)

There is also down regulation of α-actin, myosin light and heavy chains, troponin-I, and desmin proteins by doxorubicin has been suggested as a potential mechanism of its cardiotoxicity. Decreased expression of the contractile proteins is associated with myofibrillar loss
and reduced myocardial contractile function. Down regulation of sarcoplasmic reticular ATPase may cause abnormal myocardial diastolic function (Aries et al., 2004).

It has been suggested that doxorubicin can inactivate extracellular signal-regulated kinase (ERK) (Lou et al., 2005). There is evidence that doxorubicin induces apoptosis of cardiomyocytes. Formation of hydrogen peroxide and superoxide has been implicated in doxorubicin-induced cardiomyocyte toxicity (Zhang et al., 2012).

Among the proposed mechanisms of cardiac injury, doxorubicin-induced generation of reactive oxygen species (ROS) that can cause the following:

Doxorubicin causes myofilament apoptosis causing myocyte cell death from the doxorubicin-induced generation of ROS, which, in turn, activate a multiplicity of signaling pathways that determine cell fate. A key pathway involves activation of the tumor suppressor protein p53 (Sano et al., 2007) p53-dependent apoptosis involves the transcriptional activation or inhibition of certain target gene pathways such as mitogen-activated protein kinases (MAPK).

Doxorubicin causes suppression of myofilament protein synthesis through depletion of cardiac progenitor cells (CPCs) is also postulated to contribute to doxorubicin-induced cardiac injury, De Angelis et al., 2010 reported that doxorubicin exposure significantly reduced the population of CPCs, raising the possibility that CPC death may represent a primary event responsible for impaired myocyte turnover and the onset of ventricular dysfunction. Interestingly, delivery of CPCs into doxorubicin-induced failing hearts caused regeneration of cardiomyocytes, leading to improved LV performance and overall survival.
Ultrastructural changes to myocytes by ROS which cause alterations in calcium homeostasis leading to systolic (contractile) and diastolic dysfunction (Kemi et al., 2008). Doxorubicin stimulates calcium release and inhibits sarcoplasmic reticulum calcium reuptake, resulting in cytosolic calcium overload (Saeki et al., 2002). This calcium overload may contribute to impaired contractile function by (1) promoting release of the proapoptotic factor cytochrome c (Logue et al., 2005) and/or (2) activating the cysteine protease calpain. Calpains initiate turnover of both regulatory and structural myofibrillar proteins through cleavage and release of large polypeptide fragments (Gustafsson and Gottlieb, 2003).

Doxorubicin causes alterations in cardiac energy metabolism; the heart requires ATP to sustain contraction and relaxation. As such, deficiencies in cellular homeostasis are important factors in the development of cardiomyopathy (Ingwall and Weiss, 2004). Doxorubicin reduces cardiac energy reserves by lowering ATP and phosphocreatine levels as well as the phosphocreatine/ATP ratio. In the normal heart, AMP-activated protein kinase (AMPK) plays a crucial role in protecting cardiac cells from perturbations in energy homeostasis via activation of catabolic pathways to generate ATP (Neumann et al., 2003). Doxorubicin reduces the level of both AMPK protein and its basic activation state, which leads to decreased phosphorylation of anti-acetyl-CoA carboxylase (ACC), an AMPK downstream target. Lack of ACC inhibition results in impairment of fatty acid oxidation; thus, interventions that increase AMPK expression and/or normalize myocyte metabolism may be an effective strategy to offset cardiotoxicity. The mechanisms underlying inhibition of AMPK are not clear; alterations in gene expression and upstream signaling require further investigation. Other important factors activated in response to metabolic perturbations include hypoxia-
inducible factor-1 and peroxisome proliferator-activated receptor gamma coactivator 1-α (Tokarska-Schlattner et al., 2005).

Doxorubicin increased oxidative stress appears to induce toxic damage to the mitochondria of cardiomyocytes. Several mitochondrial enzymes such as NADH dehydrogenase, cytochrome P-450 reductase and xanthine oxidase are involved in generating oxygen free radicals (reactive oxygen species) (Takemura and Fujiwara, 2007). Doxorubicin also increases superoxide formation by increasing endothelial nitric oxide synthase which promotes intracellular hydrogen peroxide formation (Zhang et al., 2012).

These results are in agreement with the results of other experimental studies demonstrating the cardioprotective and the antioxidant effects of nebivolol as the study done by Amani et al., 2011 & Imbaby et al., 2014, they demonstrated that oxidative damage plays an important role in the pathogenesis of doxorubicin cardiotoxicity, as they found significant elevation in the level of MDA in doxorubicin cardiotoxicity which is decreased significantly in nebivolol group.

Nebivolol was used to protect against different drug toxicity, it was tried in rat model of anthracycline induced cardiotoxicity and nephrotoxicity (Amani et al., 2011) and was used with curcumin against doxorubicin-induced cardiac toxicity in rats (Imbaby et al., 2014) and it was found that the oral administration of curcumin and/or nebivolol attenuated doxorubicin cardiotoxicity as manifested by increasing survival rate, improvement in body weight, heart index, and ECG parameters, increase in ventricular isoprenaline responses, and improvement in cardiac enzymes.

A study by Filomena de Nigrisa et al., 2008 reported a beneficial effect of BBs on anthracycline treated hearts. In particular, the use of
nebivolol or carvedilol with anthracyclines have reduced the release of glutathione (GSSG) and reduced glutathione (GSH). Since nebivolol predominantly affects nitric oxide (NO) pathway, the common protective pathway of nebivolol could be the beta blocker properties coupled to antioxidant and NO release. Previous studies showed that patients treated with β1-selective antagonists without intrinsic sympathomimetic activity (ISA) had more adrenoceptors than control subjects and that the adenylate cyclase activation by the β-adrenoceptor agonist isoprenaline is also enhanced in this situation (Trochu et al., 1999).

The intrinsic sympathomimetic activity is therefore an important criterion for the therapeutical usefulness of BBs in heart failure patients.

The cardiac effects of BBs are varying in human cardiac tissue. The cardiodepressant effects of beta-blocker may not correlate with its β1-selectivity but result from the combined effects of β1-selectivity, intrinsic sympathomimetic properties and inverse agonist (Klara et al., 2001).

The prominent cardioprotective effects of the third-generation beta-blocker nebivolol against anthracycline-induced cardiotoxicity, using the model of an isolated perfused rat heart, were examined by De Nigris et al., 2008 who used Sprague-Dawley rats were treated with doxorubicin in combination with nebivolol or carvedilol. The cotreatment of beta-blockers and anthracyclines had reduced the release of GSSG and GSH.

A more significant reduction was shown with nebivolol than with carvedilol. Also, significant reductions of CPK and troponin I activities were observed when the hearts were treated with nebivolol. At the same time, glutathione peroxidase (GSHPx), Manganese superoxide dismutase (MnSOD), and nitrite/nitrate release were increased after the co-treatment. Three cardiac parameters have been used to evaluate the cardiac toxicity of both anthracyclines and beta-blocker-anthracycline combinations. The left ventricular pressure developed under a constant
perfusion pressure (LVDP), then the variation rates of this parameter were observed during contractility (LV/dt)max and relaxation LV(dP/dt)min. Nebivolol in combination with anthracyclines had exerted the most significant protection of heart tissue.

It has been demonstrated that nebivolol affected vasodilatation through NO production, stimulated NO release, enhanced NO bioavailability and prevented NO deactivation (Maffei et al., 2007).

A study by Angelo et al., 2006 demonstrated that nebivolol stimulates NO production in the heart. This action of nebivolol is exerted via a signaling pathway starting from the activation of β3-adrenergic receptors and leading to overexpression of inducible NO synthase.

Nebivolol, a last-generation beta blocker, which is a selective β1-adrenergic receptor antagonist, it leads to (1) an increase in the renal NO excretion (2) a significant increase in the renal plasma flow and glomerular filtration rate (GFR),(3) suppression of the rennin angiotensin aldosterone system and inhibition of angiotensin II (Greven and Gabriels, 2000) and reduces endothelin-1,and (iv) has an antioxidant effect (Mason et al., 2006).

Patrianakos et al., 2005 compared the efficacy of nebivolol versus carvedilol on left ventricular (LV) function and exercise capacity in patients with non ischemic dilated cardiomyopathy (NIDC). Both nebivolol and carvedilol appear relatively safe, with beneficial effects on LV systolic and diastolic function as well as exercise capacity in patients with non ischemic dilated cardiomyopathy (NIDC) after 12 months' treatment. However, carvedilol exhibits more favorable effects on LV function than does nebivolol.

The effects of L-carnitine and propionyl-L-carnitine (PLC) on doxorubicin induced cardiomyopathy have extensively been investigated.
Several experimental and clinical studies have reported that doxorubicin induces its cardiomyopathy by inhibition of beta-oxidation of long-chain fatty acids with the consequent depletion of cardiac ATP and that L-carnitine supplementation protects the myocardium against this toxicity (Sayed-Ahmed et al., 2000a and Waldner et al., 2006).

In early experiments, Alberts et al., 1978 investigated the protective effects of carnitine against acute (high-dose) and chronic (intermittent, low dose) doxorubicin toxicity in rat and mice. They found that carnitine was able to decrease both acute and chronic doxorubicin linked lethality in normal rats without decreasing its antineoplastic activity or raising its bone marrow toxicity.

Paterna et al., 1984 investigated whether L-carnitine could prevent doxorubicin induced cardiomyopathies in rabbits by promoting free fatty acid utilization for energy production and neutralizing long-chain free fatty acid toxicity occurring after ischemia. The results showed that L-carnitine reduced the frequency of cardiomyopathies and increased survival rate. Histopathological examination of myocardial tissues under light and electron microscopes revealed a marked decrease in mitochondrial lesions. Also, the possible protective effects of L-carnitine against doxorubicin induced cardiotoxicity were studied by Neri et al., 1986.

Rat heart slices were incubated with doxorubicin (14 mg/ml), L-carnitine (600 mg/ml), and doxorubicin plus L-carnitine for 60 min. They measured cellular oxygen uptake, ATP, intracellular Ca$^{2+}$ concentration. L-carnitine significantly reduced doxorubicin induced cardiac metabolic damage. The oxygen uptake inhibition decreased from 38% to 7%, the decrease in ATP concentration was reduced from 78% to 27% and the
inhibition of protein synthesis from 11% to 6%. Therefore, according to Neri et al., 1986, L-carnitine appears to be a useful drug in the prevention of doxorubicin induced cardiotoxicity. The protective effects of L-carnitine against doxorubicin induced cardiotoxicity in rats were also studied by McFalls et al. (1986). Cardiomyopathy was induced by weekly intravenous injection of doxorubicin (2 mg/kg) over a period of 6–7 weeks.

Using doxorubicin cardiomyopathic rat model, Shug (1987) designed an experiment to find an explanation for the protective effect of L-carnitine against doxorubicin induced cardiotoxicity. After 6 weeks of intravenous administration of doxorubicin, rats treated without carnitine had a lower ejection fraction and left ventricular pressure than control or rats treated with doxorubicin plus L-carnitine. Total carnitine in plasma was significantly higher in rats treated with doxorubicin than control and doxorubicin plus carnitine. They suggested that the increase in carnitine ester in plasma of doxorubicin treated rats was due to doxorubicin induced inhibition of long-chain fatty acid oxidation. Myocardial carnitine concentration was not altered by doxorubicin, but plasma carnitine concentration was increased resembling those of cardiomyopathies in adults.

In isolated rat heart myocytes and mitochondria, Sayed-Ahmed et al., 2000a studied the effects of different concentrations of doxorubicin in absence and presence of propionl- L-carnitine (PLC) on palmitoyl-CoA and palmitoyl-carnitine oxidation and on the activity of carnitine palmitoyltransferase (CPT 1). The results showed that doxorubicin induced concentration-dependent inhibition of substrates oxidation and inhibition of CPT I and that PLC completely reversed this inhibition to the control values. They concluded that doxorubicin induced its
cardiotoxicity by inhibition of CPT I and beta-oxidation of long-chain fatty acids with the consequent depletion of ATP in cardiac tissues, and that PLC can be used as a protective agent against doxorubicin -induced cardiotoxicity.

In DOX cardiomyopathic rat model, Sayed-Ahmed et al., 2001 have initiated another study to confirm their in vitro findings and investigated the protective effects of PLC against DOX-induced cardiotoxicity. Chronic administration of DOX (3 mg/kg, I.P) significantly increased creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH) and malondialdehyde and decreased reduced glutathione. Treatment with PLC induced complete reversal of these effects without decreasing the antitumour activity of DOX. In another study, Sayed-Ahmed et al., 2000b investigated the effects of DOX on mRNA expression of heart fatty acid binding protein (H-FABP) using Northern blot analysis. Results showed that chronic administration of DOX caused dose -dependent and cumulative inhibition of H-FABP gene expression and that daily administration of L-carnitine protected against this effect in cardiac tissues. Authors concluded that DOX induces its cardiotoxicity by inhibition of gene expression of H-FABP.

More recently, Sayed-Ahmed et al., 2010b reported that chronic DOX therapy decreased the expression of H-FABP and OCTN2 mRNA expression and increased the expression of apoptotic genes in cardiac tissues that should be viewed as a mechanism during development of DOX-induced cardiomyopathy.

Waldner et al., 2006 examined the effects of L-carnitine with a view to reducing cardiotoxicity of DOX-containing chemotherapy in 20 patients with Non-Hodgkin lymphoma. Patients were scheduled to
receive 3 g L-carnitine before each chemotherapy cycle, followed by 1 g L-carnitine/day during the following 21 days. Carnitine-treated patients showed a rise in plasma carnitine which led to an increase of relative mRNA levels from CPT 1 and OCTN2. They concluded that biochemical and molecular analyses indicated a stimulation of oxidative metabolism in white blood cells through carnitine uptake.

These results are in agreement with Nathalie et al., 1999 and Maccari and Ramacci, 1981 that demonstrated the protective role of L-carnitine in doxorubicin cardiotoxicity as evident by improvement in ECG changes in T wave voltage and the improvement in cardiac enzymes troponin I & CPK in L-carnitine treated group.

Zeidan et al., 2002 evaluated the heart and liver responses after doxorubicin toxic aggression, with and without exogenous L-carnitine protection. Results showed that doxorubicin induced long term cardiac subcellular pathology included loss, disruption and disassembly of myofibrils, and mitochondrial swelling and condensation. On the other hand, the doxorubicin induced subcellular hepatic alterations consisted of polymorphic mitochondria, cytoplasmic vacuolization and accumulation of lipid droplets. However, these alterations were of less severity in carnitine treated group in both heart and liver, suggesting that carnitine could be used as a possible protective agent against doxorubicin induced toxicity.

As regard liver function data presented in this study demonstrated that doxorubicin caused significant elevation in serum liver enzymes including ALT & AST compared to control group. These elevations of ALT and AST are due to hepatocellular damage and leakage of these enzymes and appearance in serum.
Administration of nebivolol in combination with doxorubicin in Dox+Neb group caused significant reduction in liver enzymes when compared to Dox group. These results suggested that nebivolol might have protective effect against doxorubicin induced liver damage.

This is supported in our work by the improvement occurring in the histopathological picture of liver cut section of nebivolol treated group which showed less hydropic changes, less portal tract inflammation and less congestion in portal vein.

Damage, at the cellular level by oxidants as doxorubicin, is attenuated by antioxidant enzyme such as superoxide dismutase (SOD), glutathione peroxidase (GSHPx), catalase (CAT) and glutathione reductase (GR) (Koc et al., 2005). Superoxide dismutase is one of the major enzymatic antioxidant mechanisms against superoxide radical, prevents liver toxicity induced by doxorubicin (Yagmurca et al., 2007). Catalase and GSHPx catalyze dismutation of the superoxide anion (O$_2^-$) into hydrogen peroxide (H$_2$O$_2$) which then converting H$_2$O$_2$ to water thus providing protection against reactive oxygen species (Sayed-Ahmed et al., 2010a). The reduction in activity of these enzymes may be caused by the increase in free radical production during doxorubicin metabolism (Brookins Danz et al., 2009 and Fisher-Wellman et al., 2009).

Kolodziejczyt et al., 2011 study showed decrease in the gene expression levels of GSHPx, CAT and GR in liver tissue with the cumulative dose of doxorubicin with decrease in their activity in the serum. These data showed that doxorubicin not only increase the free radical formation but also decrease its ability to detoxify reactive oxygen species. The formation of superoxide radicals together with nitric oxide (NO) might form peroxynitrite induced by doxorubicin causes tissue damage. This was in agreement with Injac et al., 2009. In contrast to this
study, Kalender et al. 2005 found that administration of doxorubicin (5 mg/week for 6 weeks) increased the levels of SOD and the activity of both GSHPx and catalase enzymes.

Administration of nebivolol in combination with doxorubicin in Dox + Neb group cause significant reduction in liver enzymes when compared to Dox group, the action of nebivolol as antioxidant might play this protective role as discussed above in it’s role in doxorubicin cardiomyopathy.

Also administration of L-carnitine in combination with doxorubicin cause significant reduction in liver enzymes when compared to doxorubicin group. These results suggested that L-carnitine may also have protective effect against doxorubicin induced liver damage. This protective effect may be due to stabilization of hepatocyte membranes by L-carnitine with the consequent decrease in the leakage of liver enzymes. L-carnitine have been found to offer protection against doxorubicin induced liver damage (Yapar et al., 2007).

Data presented by Kolodziejczyt et al., 2011 demonstrate that doxorubicin increased serum liver enzymes including ALT, ALP and total bilirubin. These elevations of ALT and ALP are due to hepatocellular damage. These results were previously reported in doxorubicin induced hepatotoxicity model done by Andreadou et al., 2007 and Cosan et al., 2008. This study established that doxorubicin significantly decrease the total carnitine in liver tissues. This could be a secondary effect following inhibition of endogenous carnitine biosynthesis and/or decreased carnitine transport in doxorubicin induced hepatocytes damage. This hypothesis is consistent with data presented by Laub et al., 1986 which showed that biosynthesis of carnitine is
decreased in pediatric patients receiving valproic acid. The level of carnitine in hepatocytes is controlled by the specific carnitine transporter (OCTN-2) and endogenous synthesis (Hwu et al., 2007 and Fujita et al., 2000). Decreased expression of OCTN-2 has been reported in the acute hepatitis (Chang et al 2005). Also, OCTN-2 located on hepatocyte membranes might be destroyed when exposed to ROS induced by doxorubicin.

L-carnitine have been found to protect against doxorubicin induced liver damage (Yapar et al., 2007). It was used to prevent the toxic effect of cisplatin-induced nephrotoxicity by normalized kidney function, where oxidative stress and lipid peroxidation play a major role in this toxicity. In addition, L-carnitine attenuated the reduced GSH levels, (El-Awady et al., 2011) in which there were no toxic effects of it on approved doses. Sayed-Ahmed et al., 2010 reported that L-carnitine administration in combination with doxorubicin significantly increase antioxidant enzyme and decrease the lipid peroxidation. The increase in GSH level lead to increase in the activity of GSHPx in which the former acts as a cofactor for later. These results are consistent with previous studies reported that L-carnitine had similar non-enzymatic free-radical scavenging and anti-lipid peroxidation activities (Kolodziejczyk et al., 2011).

As regard kidney function data presented in this study demonstrated that doxorubicin caused significant elevation in creatinine level in Dox group compared to the control group. Administration of nebivolol or L-carnitine in combination with doxorubicin caused significant reduction in serum creatinine level in Dox+Neb group and Dox+L-car group compared to Dox group. These results suggested that nebivolol and L-carnitine may have protective effect against doxorubicin induced kidney damage.
Also, the cut sections of kidney of the Dox group showed glomerulopathy evident by increase in serum creatinine associated with mesangial proliferation, swelling and vacuolation of epithelial cells, moderate tubular atrophy and dilatation and congestion of interstitial capillaries.

The doxorubicin induced kidney damage was improved in Dox+Neb group and Dox+ L-car groups in comparison to the Dox group, as the mesangial proliferation, tubular atrophy & dilatation and congestion of interstitial capillaries was markedly decreased. These results are consistent with previous studies reported by other investigators Lebrecht et al., 2004 that studied doxorubicin induced cardiotoxicity and nephrotoxicity in normal rats. Coadministration of nebivolol with doxorubicin was able to ameliorate up to almost reverse doxorubicin induced myocardial injury, glomerular filtration disturbance and renal tubular damage. According to the reported results for doxorubicin induced toxicity, almost all organs can be attacked and damaged via the formation of free oxygen radicals and some of the organ specific pathways. Several mechanisms have been proposed to account for doxorubicin cardiotoxicity, e.g., free radical stress, calcium overloading, and mitochondrial dysfunction but the exact mechanism of doxorubicin induced nephrotoxicity is not yet known. However, it has been suggested by many investigators that cellular damage induced by doxorubicin is mediated by the formation of an iron anthracycline free radical, which in turn causes severe damage to the kidney cell plasma membrane (Washio et al.,1994)

Moreover, nephrotoxicity and oxidative stress induced by doxorubicin in rats were investigated by Boonsanit et al., 2006 who found that L-carnitine can prevent renal impairment functionally, biochemically as evident by the improvement in serum creatinine and histopathologically
as evident by decreased mesangeal proliferation, swelling and vacuolation of epithelial cells, moderate tubular atrophy and dilatation and congestion of interstitial capillaries with a corresponding reduction of oxidative stress.

Our results are in agreement with previous studies reported that L-carnitine had similar non-enzymatic free radical scavenging and anti-lipid peroxidation activities (Kolodziejczyt et al., 2011) and that it could be used to prevent the toxic effect of cisplatin-induced nephrotoxicity by normalizing kidney function, where oxidative stress and lipid peroxidation play a major role in this toxicity.
Summary and Conclusion

Doxorubicin is a widely used anthracycline antibiotic in cancer chemotherapy, the most serious adverse effect being life-threatening heart damage. It is commonly used in the treatment of a wide range of cancers, including some leukemias and Hodgkin's lymphoma, as well as cancers of the bladder, breast, stomach, lung, ovaries, thyroid, soft tissue sarcoma, multiple myeloma, and others. Commonly used doxorubicin-containing regimens are AC (Adriamycin, cyclophosphamide), TAC (Taxotere, AC), ABVD (Adriamycin, bleomycin, vinblastine, dacarbazine), and FAC (5-fluorouracil, adriamycin, cyclophosphamide).

Nebivolol is a $\beta_1$ receptor blocker with nitric oxide-potentiating vasodilator effect used in treatment of hypertension and, also for left ventricular failure.

L-Carnitine is a natural substance that helps the body turn fat into energy. Our body makes it in the liver and kidneys and stores it in the skeletal muscles, heart, brain, and sperm. Usually, our body can make all the carnitine it needs. Some people, however, may not have enough carnitine because their bodies cannot make enough or can’t transport it into tissues so it can be used.

The present study was carried out to find out and compare the protective effects of nebivolol and L-carnitine against doxorubicin cardiotoxicity, hepatotoxicity and nephrotoxicity, also the in vitro studies were done to investigate the effect of doxorubicin on contractility of isolated normal mammalian heart and the effect of increasing doses of isoprenaline on contractility of hearts taken from another 5 groups of rats given the same medications as the groups of the in-vivo experiment.
Rats were randomly divided into 5 groups (6 rats for each group). The control group received saline intraperitoneal in comparative volume to the tested groups, Dox group received doxorubicin intraperitoneally every other day for 12 days, Dox + Neb group received doxorubicin intraperitoneally every other day for 12 days and nebivolol orally daily for 12 days, Dox +L-car group received doxorubicin intraperitoneally every other day for 12 days and L-Carnitine intraperitoneally daily for 12 days and Dox + Neb +L-car group received doxorubicin intraperitoneally every other day for 12 days nebivolol orally and L-Carnitine intraperitoneally daily for 12 days.

At the end of the study period, ECG, blood pressure, troponin I, CPK, creatinine, ALT, AST were measured and histopathological examination of heart, liver and kidney was done.

It was found that doxorubicin administration caused significant increase in T wave voltage, significant reduction in blood pressure and significant elevation in troponin I, CPK, ALT, AST, creatinine serum levels and prominent histopathological changes compared to control group. This study revealed that nebivolol and L-carnitine produced a significant reduction in T wave voltage, significant elevation in blood pressure and significant reduction in troponin I, CPK, ALT, AST, creatinine serum levels and improved markedly histopathological changes compared to Dox group.

Comparing the result of Dox+Neb +L-car group with that of Dox+Neb group and Dox + L-car group, adding L-carnitine to nebivolol was not significantly more effective than nebivolol or L-carnitine alone in reducing T wave voltage.

Comparing the result of Dox+Neb+L-car group with that of Dox+Neb group, adding L-carnitine to nebivolol was significantly more effective than nebivolol alone in elevating systolic & mean blood pressure.
pressure and comparing the result of Dox+Neb+L-car group with that of Dox + L-car group, adding nebulolol to L-carnitine was not significantly more effective than nebulolol alone in elevating systolic blood pressure & mean blood pressure also was found that comparing the result of Dox+Neb+L-car group with that of Dox+Neb group and Dox + L-car group adding L-carnitine to nebulolol was not significantly more effective than nebulolol or L-carnitine alone in reducing the cardiac enzymes.

Comparing the result of Dox+Neb+L-Car group with that of Dox+Neb group and Dox + L-car group, adding nebulolol to L-carnitine was not significantly more effective than nebulolol alone in reducing the liver enzymes. Comparing the result of Dox+Neb+L-car group with that of Dox+Neb group and Dox + L-car group, adding L-carnitine to nebulolol was not significantly more effective than nebulolol alone in reducing the creatinine level.

In-vitro study, It was noticed that isoprenaline in increasing doses (2, 4 and 8 μg/bath) produced an increase in the force of spontaneous contraction of isolated perfused rat’s heart in dose related manner.

In Dox group it was noticed that isoprenaline in increasing doses (2, 4 μg/bath) produced an non significant increase in the force of spontaneous contraction of isolated perfused rabbit’s heart. This increase of the force of spontaneous contractions of the isolated perfused rabbit’s heart was significant with the dose of 8μg/bath, with percentage of increase 59.1 % compared to preisoprenaline contraction.

In Dox + Neb group it was noticed that isoprenaline in increasing doses (4 and 8 μg/bath) produced an increase in the force of spontaneous contraction of isolated perfused rat’s heart in dose related manner.

This increase of the force of spontaneous contractions of the isolated perfused rat’s heart was not significant with the dose of 2μg/bath.
In Dox + L-car group it was noticed that isoprenaline in increasing doses (4 and 8 μg/bath) produced an increase in the force of spontaneous contraction of isolated perfused rabbit’s heart in dose related manner.

This increase of the force of spontaneous contractions of the isolated perfused rabbit’s heart was not significant (P > 0.05) with the dose of 2μg/bath.

In Dox + Neb +L-car group it was noticed that isoprenaline in increasing doses (2, 4 and 8 μg/bath) produced an increase in the force of spontaneous contraction of isolated perfused rat’s heart in dose related manner.

It was noticed that doxorubicin in a gradually increasing doses (1/2, 1, 2 and 4 μg/bath) produced a decrease in the force of spontaneous contraction of isolated perfused rabbit’s heart in dose related manner.

Also it was noticed that doxorubicin decrease the positive inotropic effect of isoprenaline in a dose of 2 μg / bath on isolated perfused rabbit’s heart.

In conclusion, doxorubicin can induce cardiotoxicity, hepatotoxicity and nephrotoxicity which can be ameliorated by using nebivolol and L-carnitine which are effective and may be recommended for protection against doxorubicin cardiotoxicity, hepatotoxicity and nephrotoxicity.
On the lights of the results of this study we recommended the following:

1- Doxorubicin has severe toxicity on three major organs heart, liver, kidney and it is better to avoid its use and less toxic anthracycline are better used.

2- β-blockers nebivolol may have a role in treatment of doxorubicin toxicity to reduce progression of this toxicity.

3- L-carnitine as a natural agent with less adverse effects can be used with doxorubicin administration to limit the development of doxorubicin induced toxicity without affecting its anticancer activity.

4- Further studies may be needed to show the effect of free radical scavengers as vit C and E on doxorubicin toxicity.
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الملخص العربي

يعتبر عقار الدوكسيبروبسين واحد من أهم العقارات المستخدمة لعلاج مرض السرطان و لهذا العقار عدة أثار جانبية أهمها تأثيره على الأعضاء الأساسية كالقلب والكبد والكلى.

هذه الأثار الجانبية هي التي تحد من استخدامه في علاج السرطان لذا فإن هناك العديد من الجهود المبذولة لمحاولة حل تلك المشكلة وتقليل سمية هذا العقار حتى يتاح استخدامه على نطاق واسع.

يعتبر النبيبيفيلول من الأدوية المستخدمة في علاج ارتفاع ضغط الدم عن طريق غلق مستقبلات بيتا في القلب وزيادته اكسيد النيتروجين ذو التأثير الموسع للاوعي الدموية وقد وجد أن له تأثير مضاد للأكسدة مما قد يتيح استخدامه في الوقاية من سمية بعض الأدوية التي تضر الجسم عن طريق زيادته الأكسدة في الجسم ومن ضمن هذه الأدوية عقار الدوكسيبروبسين.

أيضاً فإن ال كرنيتين من المواد الطبيعية التي تساعد على حرق الدهون في الجسم لاستخراج الطاقة وهو يتم تصنيعه في جسم الإنسان في الكبد و الكلى ويتم تخزينه في العضلات و القلب و المخ والحيوانات المنوية. لهذا الدواء أيضا تأثير مضاد للأكسدة مما قد يتيح استخدامه في الوقاية من سمية بعض الأدوية.

وقد أجري هذا البحث لدراسة التأثير الوقائي المحتمل النبيبيفيلول وال كرنيتين على سمية القلب و الكبد والكلى المحدثة تجريبياً بواسطة عقار الدوكسيبروبسين لحيوانات التجارب من خلال دراسة رسم القلب وضغط الدم و انزيمات القلب التربوزين أي و الكرياتين فوسفو كيناز و انزيمات الكبد وقياس الكرياتينين بالنسبة للكلي مع دراسة هستولوجيا الأعضاء الثلاثة الصلبة القلب و الكبد و الكلى.
أيضًا فإن هذه الدراسة قد اجريت لبحث تأثيرِ الدوكرسيربوسين وكذلك تأثير الدوكرسيربوسين مع الأيزوبرينالين على الانقباض التلقائي لعضلة القلب المعزول من الأرنب وكذلك تأثير جرعات مختلفة من الأيزوبرينالين على الانقباض التلقائي لعضلة القلب المعزول من الفنر في مجموعات الدراسة.

بالنسبة للدراسة التي أجريت داخل جسم الحيوان فإن فنران التجرب قد قسمت إلى خمسة مجموعات متساوية كل مجموعة مكونه من سته فنران كالتالي:

المجموعة الأولى (لم تعالج بأي عقار)، المجموعة الثانية (المعالجة بجرعات من الدوكرسيربوسين بجرعة 3 مجم/ كجم حقنة واحدة في الغشاء البرتونى)، المجموعة الثالثة (المعالجة بجرعات من الدوكرسيربوسين بجرعة 3 مجم/ كجم حقنة واحدة في الغشاء البرتونى بالإضافة إلى النيبيفيلول بجرعة 1 مجم/ كجم يوميا عن طريق الفم)، والمجموعة الرابعة (المعالجة بجرعات من الدوكرسيربوسين بجرعة 3 مجم/ كجم حقنة واحدة في الغشاء البرتونى بالإضافة إلى النيبيفيلول بجرعة 200 مجم/ كجم حقنة واحدة في الغشاء البرتونى) والمجموعة الخامسة (المعالجة بجرعات من الدوكرسيربوسين بجرعة 3 مجم/ كجم حقنة واحدة في الغشاء البرتونى بالإضافة إلى النيبيفيلول بجرعة 1 مجم/ كجم يوميا عن طريق الفم وال كرنيتين بجرعة 200 مجم/ كجم حقنة واحدة في الغشاء البرتونى) كل هذه مجموعات أخذت العلاج لمدة 12 يوم.

وقد وجد أن عقار النيبيفيلول وال كرنيتين لهما تأثير ذو دلالة إحصائية على خفض انزيمات القلب التروبينئي و الكرياتين فوسفوكينيز و انزيمات الكبد ونسب الكرياتين بالنسبة للكلئ،
أظهرت الدراسة أيضاً أن كل من عقار النبيفيفلول و ال كرنيتين كان لهما دور في تقليل حدوث التغيرات الخلوية المصاحبة داخل القلب و الكبد و الكلى.

أيضاً فإن هذه الدراسة وجدت أن الدوكسيروبسين قلل الانقباض التلقائي لعضله القلب المعزول من الأرنب وكذلك قلل من تأثير الأيزوبرينالين على الانقباض التلقائي لعضله القلب المعزول من الأرنب وكذلك قلل من تأثير جرعات مختلفه من الايزوبرينالين على الانقباض التلقائي لعضله القلب المعزول من الفئران في مجموعات أخذت نفس جرعات الأدوية مثل الفئران في مجموعات الدراسة. هذا التأثير تحسن في المجموعات المعالجة بالنبيفيفلول و ال كرنيتين تحسن ذو دلاله إحصائية.

ويستنتج من هذه الدراسة أن عقار النبيفيفلول و ال كرنيتين ذو تأثير فعال يمكن استخدامه في الوقاية من سمية القلب و الكبد و الكلى المحدثة بواسطة الدوكسيروبسين لما لهما من تأثير على خفض انزيمات القلب تروبين اى و الكرياتين فوسفو كيناسي و انزيمات الكبد ونسبه الكرياتينين بالنسبة للكلى.