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Cardio-protective Effect of Selenium and Lemon Grass (*Cymbopogon citratus*) Ethanolic Extract against Doxorubicin-induced Cardio Toxicity and DNA Fragmentation in Rats

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Abstract Doxorubicin (Dox) is a chemotherapy drug used for treatment of wide variety of cancers and the most effective antitumor agents, soon proved to be hampered by such serious problems as the development of cardiotoxicity and heart damage by producing free radicals and oxidative stress along the period of treatment. The current study aims at evaluating the cardio protective effect of *Cymbopogon citrates* known as lemon grass (LG) and the role of trace element selenium (SE), which is an essential element possessing antioxidant properties and has protective effects against toxicity of some drugs, in attenuating cardiac and heart damages induced by doxorubicin alone and in combination. Animals were divided into seven groups each of seven rats, normal control group; selenium group received (1.5mg/kg b. w., orally) for 7 days, LG group received (200mg/kg b.w. orally) for 7 days and doxorubicin -treated group injecting doxorubicin i.p. 48hrs before decapitation at a dose of (20 mg/kg b.w. / i.p). The doxorubicin - treated animals were divided into 3 groups, one (doxorubicin + SE), second group (doxorubicin + LG) and the third group (doxorubicin+ SE+ LG). Serum CK-MB, LDH, as cardiac damage markers; ALT, AST as indicator of hepatic damage and Urea, creatinine as indicator of renal damage, were measured. MDA, TAC, ROS, TP and genomic DNA, as cardiac oxidative status indices and cardiac antioxidant capacity, were also estemated. Histopathological changes in cardiac tissues were examined. Doxorubicin induced significant increase in serum lactate dehydrogenase (LDH); CK-MB; alanine aminotransferase activities, Urea, Creatinine, malondialdehyde levels, reactive oxygen species (ROS) contents. However, significant reduction in total antioxidant capacity (TAC) and total proteins (TP) were observed. Selenium and Lemon Grass pretreatment caused significant decrease in serum LDH and CK-MB levels; ALT, AST, Urea, Creatinine in serum, significant decrease in cardiac malondialdehyde, whereas, significant elevation in cardiac total antioxidant capacity (TAC) and Total protein (TP), compared to doxorubicin -treated group. Histopathological examination of cardiac heart, liver and kidney tissues confirmed with the previous biochemical results, also, genomic DNA of cardiac heart tissues confirmed with the previous biochemical results. Chronic doxorubicin administration caused cardio toxicity and DNA damage. Selenium (SE) and Lemon Grass (LG) pretreated exerted significant protection against DOX-induced cardiac damage.

Keywords Doxorubicin, *Cymbopogon citrates*, selenium, CK-MB, genomic DNA, TAC

Introduction

Doxorubicin caused cardio toxic effect in animals [1] and in patients [2]**.** It is widely accepted that oxidative stress and the production of free radicals are involved in doxorubicin both in terms of antitumor effects and cardio toxicity [3]**.** Doxorubicinol could accumulate in the heart and contribute significantly to the chronic accumulative cardio toxicity of doxorubicin therapy. Hence, different treatment schedules were used to decrease drug levels in serum and

it has also been proposed free radical scavengers and flavonoids might be effective in lessening the pathological changes observed after treatment [1,4].

Doxorubicin is an essential component of treatment of breast cancer, soft tissue sarcomas and many other cancers Kufe *et al.* [5]. Because Dox has been shown to produce free radicals, it was suggested earlier that free radical injury might be a mechanism of Dox antitumor activity. There now appears to be general agreement that oxidative stress is unlikely to be a significant contributor to the antitumor activity of Dox [6-7].

Antioxidants protect cells and tissues against free radical which caused oxidative damage and injury [8]. Selenium (SE) plays an important biological role in living organisms, mostly through its incorporation in a family of proteins; selenoproteins Safinaz *et al.* [7]. SE is an essential element possessing antioxidant properties. SE treatment has been found to display protective effect against toxicity of substances occurring in environment and food as acrylamide [9], lead [10-11] as well as against side effects of some drugs e.g.: cisplatin or neuroleptics [12-13]. Different forms of SE have been studied including inorganic selenite [14] and organic compounds [15-16] as well as selenium-enriched natural products [10-11]. Recently, the development of nanotechnology has prompted the attempts towards medical application of selenium nanoparticles [17-18].

SE has been found to affect functions of the cardiovascular system, its deficiency has been reported to induce cardiomyocyte injury [19] as well as to increase cardiotoxicity of drugs and heart dysfunctions observed in pathological conditions [20-21]. The effect of selenium supplementation in the form of sodium selenite has been studied in patients with coronary artery disease and the outcomes have been encouraging [22-23].

Cymbopogon citratus, commonly known as lemon grass (LG), belonging to the family Poaceae is used in folk medicine for analgesic, antioxidant activity, LG is also used as a blood purifier [24-25]. LG is recommended for common colds and gastric problems, infections and colitis, various pharmacological studies like anti-inflammatory and inhibition of lipoxygenase enzymes in *In-vitro* condition of LG have been reported. Though the antioxidant activity of LG was reported earlier [24-25].

LG contains several biocompounds in its leaves and essential oil extracts. Anti-oxidant, anti-inflammatory and antihypertensive evidences of LG were clearly elucidated to support initial pharmacological claims. LG was non-toxic, non-mutagenic and receives wide acceptance among alternative medicine practitioners in several developing countries [26]. LG leaves are popular among countries of South America, Asia and West Africa having been widely utilized as antiseptic, antifever, and anti-inflammatory effects. Others are febrifuge, diuretic, tranquilizer and stomachic agent [24, 26-27].

LG ethanolic extract was reported to exhibit antioxidant properties by decreasing ROS production and lipid peroxidation, as well as, increasing SOD activity and glutathione formation [28]. Recently, LG was also reported to show antioxidant property by DPPH scavenging test, the results showed that both leaves and stalk extracts possess radical scavenging ability in a dose dependent manner [26, 29]**.** In folk medicine LG of Brazil is believed to have cytoprotective, antioxidant, anti-inflammatory properties [28, 30]**.** The antihepatotoxic activity has also been reported [31-32]**.**

Therefore, this study is directed to investigate the protective effect of Selenium (SE) as an adjuvant therapy and *Cymbopogon citratus* (LG) alone and in combination against DOX-induced cardiotoxicity in rat heart from biochemical and histopathological point of view.

Material and Methods

Material

Plant extract

Plant material was identified by Applied Research Center for Medicinal Plants (ARCMP), Egypt, dried in lypholizer -50ºC until dryness, ground and the ethanolic extract was prepared by macerating the leaves of *Cymbopogon Citratus* with ethanol 70% overnight, the process was repeated three times and the extraction was filtered then evaporated to dryness under vacuum by lypholization freeze-drying. The filtrate (ethanolic extract) was kept and stored at -20◦C until used for further study [33].

Chemical agents for In-Vitro study

All chemicals, solvents and reagents used were of analytical and pure grade. *Rutin hydrate* as antioxidant standard was purchased from Sigma chemical Co., St. Lewis, USA.

Chemicals and Drugs for In-Vivo study

All chemicals were obtained from Sigma Company; Santa Lewis, USA, they obtained in analytical and purified grade TBARS, ROS, TAC were purchased from Merck India Ltd., and Doxorubicin hydrochloride (DOXO) supplied from Sigma-Aldrich Co., (St. Louis, Mo, USA).

Animals

Experiments were performed using male albino rats (120-150 g) supported from the animal house of NODCAR, Egypt. Animal were kept in a room at a constant temperature 22±1◦C with 12h light-dark cycles and had free access to diet and tap water.

Methods

In-Vitro study

Determination of Hydroxyl radical scavenging assay (OH•) of plant extract

The scavenging capacity for hydroxyl radical was determined by the method of Siddhuraju and Becker [34] using Rutin hydrate as standard antioxidant.

Total antioxidant capacity (TAC) (Phosphomolybdeum reduction potential assay) of plant extract

The determination of the antioxidative capacity is performed by the reaction of antioxidants in the samples with a defined amount of sulphoric acid, ammonium molybdate and sodium phosphate. The antioxidants in the sample eliminate a certain amount of the provided radicals. The residual was determined colorimetrically using *Rutin hydrate* as standard at wave length 695nm [35].

Ferric Reducing Antioxidant Power Method (FRAP) of plant extract

The FRAP assay is based on the reducing power of antioxidants by the reduction of the Fe^{+++} to the Fe^{++}, which form a green colored according to the method described [36-37]**.** The FRAP reagent was freshly prepared and the absorbance was measured at 700 nm after 10 min of incubation at room temperature, using *Rutin hydrate* as standard.

Determination of Nitric Oxide (NO) scavenging contents of plant extract

Nitric oxide (NO) content was measured by the determination of total nitrate and nitrite concentrations in the samples [38-39].

In-Vivo study

Experimental protocol

The rats were divided into seven groups each group of 7 rats as the following:

-Group 1 rats received saline orally every day for 7 days served as normal control.

-Group 2 rats received a sole dose of selenium (SE) every day for 7 days (1.5mg/kg b. wt., orally) [23].

-Group 3 rats received the ethanolic extract of *Cymbopogon citrates* (lemon grass) (LG) every day for 7 days (200mg/kg b. wt., orally) [25].

-Group 4 rats received Doxorubicin (20 mg/kg b. wt., i.p.) at 48hrs before decapitation served as positive control [40]

-Group 5 rats received selenium (SE) orally every day for 7 days then injected with Doxorubicin 48hrs before decapitation.

-Group 6 rats received *Cymbopogon citrates* (lemon grass) (LG) orally every day for 7 days then injected with Doxorubicin 48hrs before decapitation.

-Group 7 rats received selenium (SE) + *Cymbopogon citrates* (lemon grass) (LG) orally every day for 7 days then injected with Doxorubicin 48hrs before decapitation.

Blood samples were collected after 7 days, all animals were sacrificed and decapitated after blood collecting for determination of LDH, CK-MB, ALT, AST, Urea, Creatinine in serum. The Heart was kept at -80°C until use for measurement of TAC, MDA, ROS, TP and genomic DNA analysis on agarose 1%. A part of heart, liver and kidney tissues were fixed in 10% formaldehyde for histopathological examinations.

Determination of serum lactate dehydrogenase (LDH)

The activity of serum lactate dehydrogenase was measured at 340 nm by the method described by Lum and Gambino [41], the initial rate of the NADH oxidation is directly proportional to the catalytic LDH activity. It is determined by measuring the decrease in absorbance.

Determination of serum Creatine Kinase (CK-MB)

The activity of creatine kinase-MB (CKMB; standard Spin react kit (Ref 1001054) using immune inhibition kinetic assay) was assayed in serum according to the method of Okinaka *et al.* [42].

Determination of serum liver transaminases activity

Two transaminases namely alanine transaminase (ALT) and aspartate transaminase (AST) were determined according to the method of Young [43].

Determination of serum renal tests

Nitrogen urea (NU) and creatinine (Cr) were assayed in serum using kits provided from Biodiagnostic Co. (Giza, Egypt) according to the methods described by Fawcett and Soctt [44] and Szasz *et al.* [45], respectively.

Determination of total antioxidant capacity (TAC) assay in heart tissues

The determination of the antioxidative capacity is performed by the reaction of antioxidants in the sample with a defined amount of exogenously provide hydrogen peroxide (H_2O_2). The antioxidants in the sample eliminate a certain amount of the provided hydrogen peroxide. The residual H_2O_2 is determined colorimetrically by an enzymatic reaction which evolves the conversion of 3, 5 dichloro-2- hydroxy benzensulphonate to a colored product.

Determination of lipid peroxidation levels (MDA) in heart tissues

Lipid peroxidation was estimated by thiobarbituric acid (TBA), according to the method of Ohkawa *et al.* [46]. The determined values are expressed in terms of malondialdehyde (nmole MDA/mg protein).

Determination of reactive oxygen species (ROS) in heart tissues

The reactive oxygen species was estimated according to the method of Vrablic *et al.* [47]. The determined values are expressed as (µmole NBT/gm tissue).

Determination of total protein in tissue

Protein contents of heart were determined by the method of Lowry *et al.* [48].

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) methods for determining the total proteins

Electrophoresis is the migration of charged molecules in solution in response to an electric field. Their rate of migration depends on the strength of the field; on the net charge, size, and shape of the molecules and also on the ionic strength, viscosity and temperature of the medium in which the molecules are moving. As an analytical tool,

electrophoresis is simple, rapid and highly sensitive. It is used analytically to study the properties of a single charged species, and as a separation technique. SDS-PAGE was done according to the method of Laemmli [49].

Extraction of Genomic DNA

DNA extraction and detection of apoptosis (DNA fragmentation assay) were done according to "Salting out extraction method" of Aljanabi and Martinez [50] with some modifications of Hassab El-Nabi *et al.* [51].

Agarose gel electrophoresis for DNA fragmentation

Gel electrophoresis is a method for separation and analysis of macromolecules and their fragments, based on their size and charge. 1% agarose gel was prepared according to the reported method [52-54]**.**

Histological examination

After the experimental period animals were decapitated, heart; liver and kidney sections removed immediately, sliced and washed in saline. Tissue pieces were preserved in 10% formalin for histophathological studies. The pieces were processed and embedded in paraffin wax. Sections were taken and stained with hematoxylin and eosin and photographed [55]**.**

Statistical analysis

The obtained data were presented as the means ± S.D. One-way ANOVA was carried out and statistical comparisons among groups were performed with Duncan's test [56] using a statistical package program (SPSS, version 17.0). All p values are two-tailed, and $p<0.05$ was considered as significant for all statistical analyses in this study.

% Change = Mean of treated / Mean of control × 100

Results and Discussion

***In-Vitro* study**

Effect of Cymbopogon citrates (Lemon Grass) (LG) ethanolic extract on hydroxyl radical scavenging (OH•) as compared to Rutin hydrate (standard antioxidant).

The hydroxyl radical (OH·) in the cells can easily cross cell membranes at specific sites, react with most biomolecules and further more cause tissue damage and cell death. Thus, removing (OH·) is very important for the protection of living systems [57]**.** Table (1) shows the inhibition percentage of (OH·) radical scavenging effect of *Cymbopogon citrates* (Lemon Grass) (LG) ethanolic extract in comparison with the effect of *Rutin hydrate* at the concentrations of 50, 250, 500 and 1000 µg/ml. The inhibition percentage of ·OH radical scavenging effect of *Rutin hydrate* at the dose of 1000 µg/ml was found to be 63.1%, while the ratio at this concentration for *Cymbopogon citrates* (Lemon Grass) (LG) ethanolic extract was found to be 63.0% as similar to *Rutin hydrate* due to the active phenolic and flavonoid compounds. As indicated from these results the inhibitory effect of this extract was in concentration dependent mannar, these results were similar to the results reported [26].

Table 1: Effect of *Cymbopogon citrates* (Lemon Grass) (LG) ethanolic extract on hydroxyl radical (OH•) as compared to *Rutin hydrate* (standard antioxidant)

Treatments	**Hydroxyl radical (OH•) "I %" scavenging activities**			
	50 µg/ml	**250 µg/ml**	**500 µg/ml**	**1000 µg/ml**
Rutin hydrate (st)	47.4%	54.0%	54.5%	63.1%
Lemon grass (LG)	48.5%	49.5%	57.0%	63.0%

N. B.: (%): It was expressed as the absorbance of sample versus the absorbance of control, the Control (without antioxidant) = 100% toxicity

Effect of Cymbopogon citrates (Lemon Grass) (LG) ethanolic extract on Ferric Reducing Antioxidant power (FRAP) and Total Antioxidant Capacity (TAC) compared to Rutin hydrate (standard antioxidant).

It was reported that, the reducing capacity of a compound serves as a significant indicator of its potential antioxidant activity. To measure reductive ability, it was investigated the $Fe^{+3} \rightarrow Fe^{+2}$ transformation in the presence of *Rutin*

hydrate as standard at 700nm compared to *Cymbopogon citrates*. Higher absorbance of the reaction mixture indicated greater reducing power; the reducing power was in a concentration-dependent manner. These results suggest that *Rutin hydrate* and *Cymbopogon citrates* have a remarkable potency to donate electron to reactive free radicals, converting them into more stable non-reactive species [57]**.**

Table (2) revealed the antioxidant activity for *Cymbopogon citrates* compared to *Rutin hydrate* (100% activity). As indicated from the data the reducing power was antioxidant and phenoilc compounds-dependent as can be seen *Rutin hydrate* has higher activity by 2.18 ±0.07 µg/g dry plant (100% activity), while for *Cymbopogon citrates* by 1.28±0.04 µg/g dry plant (58.7% activity) as compared to *Rutin hydrate*. Yildirim *et al.* [58] reported that there is a direct correlation between antioxidant activities and reducing power of components. Recently, the polarity and the hydrophobicity of antioxidants, besides the above mentioned factors were found to play important roles in their activity, especially in biomembrane systems [59]

Moreover, as revealed in Table (2) the effect of Lemon Grass *(Cymbopogon citrates)* (LG) ethanolic extract on Total Antioxidant Capacity (TAC) as compared to *Rutin hydrate* (standard antioxidant) that, the inhibition percentage value of *Cymbopogon citrates* appeared to be 4.07 ±0.60 by (64.8% activity) as compared to *Rutin hydrate* 6.28±0.24 (100% activity as standard antioxidant). Our results are in agreement with Adegbegi and Oso [60].

Table 2: Effect of *Cymbopogon citrates* (Lemon Grass) (LG) ethanolic extract on Ferric Reducing Antioxidant power (FRAP) and Total Antioxidant Capacity (TAC) as compared to *Rutin hydrate* (standard antioxidant)

Treatments	***[a] FRAP assay expressed as µg/g dry plant***	***[b] TAC activity expressed as mean ± S.D***
Rutin hydrate (st)	2.18 ±0.07	6.28 ±0.24
% Change	100%	100%
Lemon grass (LG)	1.28 ±0.04	4.07 ±0.60
% Change	58.7%	64.8%

N.B.: [a,b] Values are means ± S.D. of six measurements, Data are significantly different ($p < 0.05$).
[b] Higher absorbance indicated higher reducing power.

Reduction of Nitric Oxide (NO•) contents of Cymbopogon citrates (Lemon Grass) (LG) ethanolic extract against Rutin hydrate (standard) as compared to control (100% toxicity).

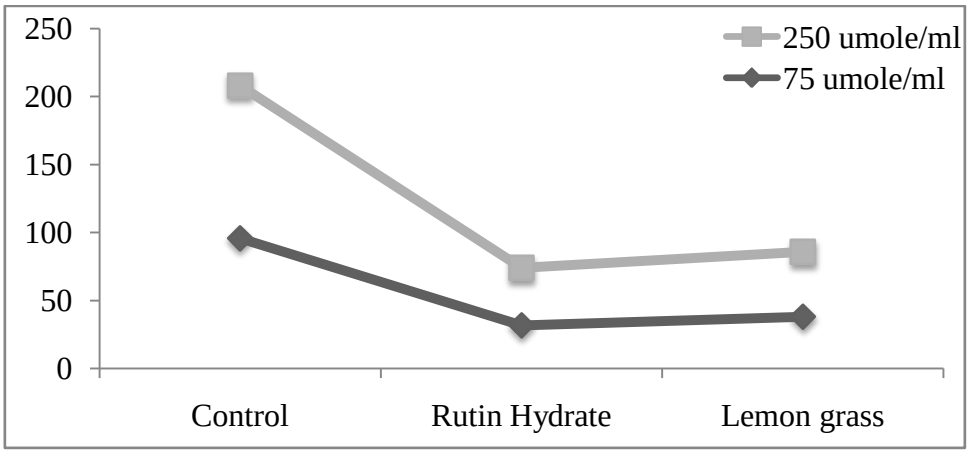


Figure 1: Nitric Oxide (NO•) content of Cymbopogon citrates (Lemon Grass) (LG) ethanolic extract against Rutin hydrate (standard) as compared to control (100% toxicity).

Figure (1) shows that *Cymbopogon Citratus* caused the significant reduction in nitrite/nitrate contents at the high concentration of 250 µmole/ml by (47.4%) against *Rutin hydrate* by (42.4%) as compared to control group (100%

toxicity) at the same concentration, which indicating that *Cymbopogon Citratus* has potential antioxidant properties similar to *Rutin hydrate* as standard antioxidant due to the active phenolic groups. *Cymbopogon Citratus* was found to have a nitric oxide scavenging activity which was confirmed with those of Saha *et al.* [61].

***In-Vivo* study:**

Doxorubicin (DOX)-induced cardiomyopathy has long been a serious side effect in treating human cancers, which limits the clinical dosage of DOX [62]**.** The mechanism of DOX-induced cardiotoxicity is attributed to the formation of ROS and subsequent changes of membrane fluidity and integrity. Oxidative stress is generally held as the mediating mechanism in the multiple biological processes leading to DOX-induced cardiotoxicity [63]. Nutritional strategies designed to augment cellular defense systems have been identified as a promising approach to combat oxidative stress associated disease conditions. In this respect, SE pretreated adjusting antioxidant enzymatic status, could offer protection in preventing free radical-induced cardiac injury. In the present study, role of trace element, selenium, in attenuating cardiac and hepatic damages induced by antitumor agent, doxorubicin was studied **[7].**

Effects of Selenium (SE); Cymbopogon citrates (Lemon Grass) (LG) extract alone and in combination against doxorubicin (DOX)-induced cardio-toxicity on weight/body weight and the ratios in rat heart.

Treatment of Doxorubicin (DOX) (20mg/kg b.w.) i.p. for acute toxicity (7 days) caused a statistically significant decrease in the body and organ weight of rats, after treatment with the plant extract, the body and heart weights were increased by different values of percentage. The effect of Selenium (SE) appeared to be highly increased in the body and organ weights than the plant extract only as indicated in Table (3). Also, *Cymbopogon citrates* (LG) plus Selenium (SE) exerted a highly percentage value more than *Cymbopogon citrates* (LG) only then Selenium (SE) only for the body and the heart weights, respectively. Antonio *et al.* [64] reported that, the effect of DOX-induced cardiotoxicity is characterized by decreased the heart weight and body weight. Chronic treatment with DOX-induced severe biochemical changes as well as oxidative damage in the heart tissues. The experimental evidence suggests the generation of free radicals in the heart tissue by chronic administration of DOX [65]**.** The generated reactive oxygen species (ROS) such as superoxide radicals and hydroxyl radicals have a potential to cause damage to various intracellular components. A deficiency of oxygen supply or glucose may damage the myocardial cells and the cell membrane becomes more permeable and ruptures, resulting in leakage of enzymes [66]**,** these results are in agreement with our findings.

Table 3: Effects of Selenium (SE); *Cymbopogon citrates* (Lemon Grass) (LG) extract alone and in combination against doxorubicin (DOX)-induced cardio-toxicity on weight/body weight and the ratios in rat heart

Treatments	**Body wt. (g)**	**heart wt (g)**	**heart wt / Body wt.**
Saline (negative control)	123.33 ± 5.00	0.522 ± 0.03	0.0042 -----
SE (1.5 mg/kg b.w)	137.78 ± 4.41	0.538 ± 0.02	0.0039
Relative change			↓7.14 %*
LG (200 mg/kg/b.w.)	126.67 ± 5.00	0.514± 0.03	0.0041
Relative change			↓ 2.38 %*
DOX (positive control) (20 mg/kg/b.w. i.p.)	**114.44 ± 7.26**	**0.500 ± 0.05**	**0.0044**
Relative change			**↑ 4.76 % ***
SR + DOX	126.67 ± 5.00	0.521 ± 0.06	0.0041
Relative change			↓ 2.38 % *
LG + DOX	145.56 ± 5.27	0.626 ± 0.14	0.0043
Relative change			↑ 2.40 % *

SE + LG + DOX	156.67 ± 8.66	0.651 ± 0.06	0.0042
Relative change			↓ 1.07 %*

Each value is the mean ±S.D. for nine rats. Significant difference from control group *P<0.05.

Effects of Selenium (SE); Cymbopogon citrates (Lemon Grass) (LG) extract alone and in combination against doxorubicin (DOX)-induced cardio-toxicity on Creatine Kinase (CK-MB) and lactate dehydrogenase (LDH) in rat serum.

Rats administered with DOX had significantly increased levels of CK-MB and LDH as compared to normal control by 521.72 ± 0.220 U/L and 1416.63 ± 0.060 U/L for CK-MB and LDH, respectively as indicated in Table (4). Pretreatment with Selenium (SE) and *Cymbopogon citrates* (LG) alone and in combination were found to inhibit the DOX-induced CK-MB and LDH release in the serum of rats. These enzymes are considered the most important markers of cardiac injury. This finding is in harmony with these stated by Viswanatha-Swamy *et al*. [67] and Safinaz *et al.* [7].

Table 4: Effects of Selenium (SE); *Cymbopogon citrates* (lemon grass) (LG) extract alone and in combination against doxorubicin-induced cardio-toxicity on Creatine Kinase (CK-MB) and lactate dehydrogenase (LDH) in rat serum

Treatments	**CK-MB (mean ± S.D.) U/L**	**LDH (mean ± S.D.) U/L**
Saline (negative control)	85.85± 0.040	1141.40 ± 0.050
Relative change	16.5%↓*	80.57%↓*
SE (1.5 mg/kg b.w)	67.69 ± 0.030	1033.46±0.010
Relative change	12.97%↓*	72.95%↓*
LG (200 mg/kg/b.w.)	97.41 ± 0.040	1273.61 ± 0.060
Relative change	18.67%↓*	89.90%↓*
DOX (positive control) (20 mg/kg/b.w. i.p.)	521.72 ± 0.220	1416.63 ± 0.060
Relative change	100%	100%
SE + DOX	194.11 ± 0.040	1225.04 ± 0.030
Relative change	37.21%↓*	84.48%↓*
LG + DOX	197.41 ±0.040	1327.58 ± 0.010
Relative change	37.84%↓*	93.71%↓*
DE + LG + DOX	140.01 ± 0.050	934.27 ± 0.020
Relative change	26.84%↓*	65.95%↓*

Significant different from positive control group at *P < 0.05

Effects of Selenium (SE); Cymbopogon citrates (Lemon Grass) (LG) extract alone and in combination against doxorubicin (DOX)-induced cardio-toxicity on liver and Kidney functions in rat serum.

Rats administered with DOX caused significantly elevation in the serum of AST, ALT, Urea (NU) and creatinine (Cr) levels to reach 37.28±0.029 U/L, 35.84±0.035U/L, 62.29±0.044 U/L and 4.31±0.019 U/L, respectively as compared to normal control as shown in Table (5). Pretreatment with Selenium (SE); *Cymbopogon citrates* (LG) alone and in combination were found to inhibit the DOX-induced release in the serum enzymes. The levels of AST, ALT, Urea (NU) and creatinine (Cr) in DOX-treated groups showed a significant increased as compared to normal control group. The increased level of these enzymes indicates myocardial injury [20]. Higher the activity, the larger is the injury size [21] these results imply that DOX when taken for long period of time could cause both liver and heart injury. Large doses of DOX over a long period of time lead to myocardial damage. However, in protective

groups, AST and ALT levels significantly decreased Urea (NU) and creatinine (Cr) as compared with DOX-treated groups. Present results suggest that treatment with SE and LG are responsible for maintenance of normal architectural integrity of cardiac muscle may inhibit myocardial damage, this is in agreement with those of Viswanatha-Swamy *et al*. [67]. Histopathological examination of liver and kidney tissues confirmed with the previous biochemical results in Figure (5) for liver tissues and (6) for kidney tissues.

Table 5: Effects of Selenium (SE); *Cymbopogon citrates* (Lemon Grass) (LG) extract alone and in combination against doxorubicin-induced cardio-toxicity on liver and Kidney functions in rat serum

Treatments	**mean ± S. D. (U/L)**		**mean ± S. D. (mg/dl)**	
	AST (GOT)	**ALT (GPT)**	**Urea (NU)**	**Creatinine (Cr)**
Saline (negative control)	27.33±0.004	27.94±0.004	46.36±0.005	1.92±0.01
Relative change	73.3%↓*	77.96%↓*	74.42%↓*	44.55%↓*
SE (1.5 mg/kg b.w)	27.62 ±0.041	29.41±0.010	52.27±0.053 8	1.83±0.023
Relative change	74.09%↓*	82.06%↓*	3.91%↓*	42.46%↓*
LG (200 mg/kg/b.w.)	28.00±0.013	29.96±0.046	47.37±0.014	1.65±0.007
Relative change	75.11%↓*	83.59%↓*	76.05%↓*	38.28%↓*
DOX (positive control) (20 mg/kg/b.w. i.p.)	37.28±0.029	35.84±0.035	62.29±0.044	4.31±0.019
Relative change	100%	100%	100%	100%
SE + DOX	32.25±0.029	33.61±0.004	51.58±0.023	1.98±0.043
Relative change	86.51%↓*	93.78%↓*	82.81%↓*	45.94%↓*
LG + DOX	28.30±0.012	33.46±0.003	49.33±0.037	2.18±0.019
Relative change	75.92%↓*	93.36%↓*	79.19%↓*	50.58%↓*
DE + LG + DOX	26.40±0.025	31.06±0.004	46.99±0.010	1.63±0.033
Relative change	70.82%↓*	88.66%↓*	75.44%↓*	37.82%↓*

Significant different from positive control group at *P < 0.05

Effects of Selenium (SE); Cymbopogon citrates (Lemon Grass) (LG) extract alone and in combination against doxorubicin (DOX)-induced cardio-toxicity on total protein (TP); total antioxidant capacity (TAC); reactive oxygen species (ROS) and malondialdehyde (MDA) in rat heart.

Results of the present study in Table (6) revealed that, 20 mg/kg b.w. total cumulative dose of DOX-induced cardiac damages, manifested biochemically by significant elevation in cardiac ROS, MDA levels and reduction in TAC and TP contents. Histopathological examination of heart section of DOX-treated groups supported these biochemical results. Selenium (SE) and Lemon Grass (LG) pretreatment, concomitant to DOX therapy, caused significant decrease in the activities of cardiac MDA, ROS levels and significant elevation in cardiac TAC and TP contents, compared to DOX-treated group values.

Our results showed that lipid peroxidation induced by DOX is significantly decreased in the pretreatment of SE, as manifested by significant reduction in the elevated level of cardiac MDA, which is consistent with previous studies **[68-69]**. Previously, it was reported that SE pretreatment can protect against free radical damages by increasing myocardial SE content and improving the expression and activity of GPx [70]. Bergendi *et al.* [69] have mentioned that drugs with antioxidant properties may supply endogenous defence system and reduce both initiation and propagation of MDA process. The decreased concentration of MDA in LG-treated rats might be due to the antioxidant and free radical scavenging activity. Pre-treating animals with LG increased the activity/ level of enzymatic/non-enzymatic antioxidants against diseased rats [25].

Various phytoconstituents present in the plant material might be responsible for the antioxidant and cardio protective activity of LG. Ochoa *et al.* [71] has reported that the polyphenol diet prevents lipid peroxidation and protects the

antioxidant enzyme from oxidative stress. The presence of flavonoid and tannin in LG was reported earlier by De Matouschek [72] since LG is a rich source of polyphenolic compounds, LG treatment attributes to the protection in myocardial infarcted rats by modulating the antioxidant enzymes. Kris-Etherton *et al.* [73] have stated that flavonoids may suppress LPO by recycling other antioxidants such as a-tocopherol. The decreased level of TBARS observed in LG-pretreated rats could be attributed to the synergistic antioxidant potential of the combination of phenols, flavonoids and tannins against free radical-mediated injury.

DOX therapy caused significant increase in MDA level. Previous studies reported similar results [74-75]. This elevation might be attributed to DOX-mediated oxidative stress. Heart tissue is rich in mitochondria, which occupy about forty percent of the total intracellular volume of cardiomyocytes [76]. DOX has high affinity for cardiolipin, a negatively charged phospholipid abundant in the mitochondrial inner membrane, leading to mitochondrial accumulation of Dox [77].

The cycling of DOX between quinone and semiquinone generates large amounts of O_2, which further give rise to a variety of ROS/RNS species [78]. ROS can damage membrane lipids and other cellular components and consequently lead to cardiomyocyte apoptosis or death [79]. Our results showed that lipid peroxidation induced by Dox is significantly decreased in the pretreatment of SE, as manifested by significant reduction in the elevated level of cardiac MDA, which is consistent with previous studies [80-81]. The concomitant overproduction of ROS is known to yield highly reactive oxygen species, which may attack and destroy important cellular biomolecules [82]. SE and LG, in the present study, caused significant decrease in the elevated cardiac ROS level shown in the DOX-treated group, which is in agreement with that reported [83]. This biochemical result is supported by the histopathological examination of heart sections of the different groups which illustrated that, in the heart sections of rats administrated DOX, congestion and kupffer cells proliferation were observed, while sections from rats administered SE, LG alone and in combination showed least heart damage.

TAC was decreased in DOX-induced cardiotoxicity in comparison with all other groups. However, in the Se only > LG only more than in SE+LG+DOX > SE+DOX > LG+DOX groups TAC was markedly increased compared to the DOX-treated groups, our results are in agreement with Vijaimohan *et al.* [84] and Wu and Huang [85], they reported that pre-administration of selenium caused a well-marked increase in TAC, Also, our results in agreement with those of Huda-Alkreathy *et al.*, [86].

Total protein content (TP) of rat heart in DOX-induced cardiotoxicity Table (6) and Figure (2) was reduced, while after treatment by SE and LG alone and in combination with DOX, the total protein was enhanced by different percentage values. The highest value of protein contents appeared to be in the mixture of SE+LG+DOX by (29.60±0.092 mg/gm tissue), then DOX+LG by (28.01±0.025 mg/gm tissue). The lowest value of total protein was produced by the mixture of SE+DOX by (24.27±0.047mg/gm tissue). It was found that the protein content was inhibited under the effect of DOX-induced cardiotoxicity; this effect was abolished by treatment of SE and other compounds, oxidative damage to DNA, RNA, proteins and cell membranes occurs when the cellular concentration of reactive oxygen species (ROS) exceeds the capacity of the cell to eliminate them [87].

Table 6: Effects of Selenium (SE); *Cymbopogon citrates* (Lemon Grass) (LG) extract alone and in combination against doxorubicin-induced cardio-toxicity on total protein (TP); total antioxidant capacity (TAC); reactive oxygen species (ROS) and malondialdehyde (MDA) in rat heart

Treatments	**Total protein mg/g tissue**	**TAC mM/L**	**ROS µmole NBT/gm tissue**	**MDA nmole MDA/mg protein**
Saline (negative control)	30.02±0.036	1.262± 0.053	82.08 ± 0.020	4.718 ± 0.028
Relative change	164.1%↑	184.5%↑*	37.7%↓*	32.17%↓*
SE (1.5 mg/kg b.w)	28.78±0.084	1.440 ± 0.011	85.16 ± 0.021	3.692 ± 0.017
Relative change	157.4%↑	210.5 %↑*	39.15%↓*	25.17%↓*

LG (200 mg/kg/b.w.)	26.44±0.022	1.400 ± 0.019	99.61±0.008	5.872 ± 0.041
Relative change	144.6%↑	288.1 %↑*	45.8%↓*	40.04%↓*
DOX (positive control) (20 mg/kg/b.w. i.p.)	18.29±0.030	0.684 ± 0.008	217.53 ± 0.019	14.667 ± 0.047
Relative change	100%	100%	100%	100%
SE + DOX	24.27±0.047	1.219 ± 0.025	97.30 ± 0.019	8.244 ± 0.014
Relative change	132.7%↑	178.2 %↑*	44.73 %↓*	56.21%↓*
LG + DOX	28.01±0.025	1.149 ±0.072	121.39 ± 0.019	8.577 ± 0.017
Relative change	153.1%↑	168.0 %↑*	55.80 %↓*	58.48%↓*
SE + LG + DOX	29.60±0.092	1.242 ± 0.037	87.30 ± 0.020	6.308 ± 0.030
Relative change	161.8%↑	181.6%↑*	40.13 %↓*	43.01%↓*

Significant different from positive control group at *P < 0.05

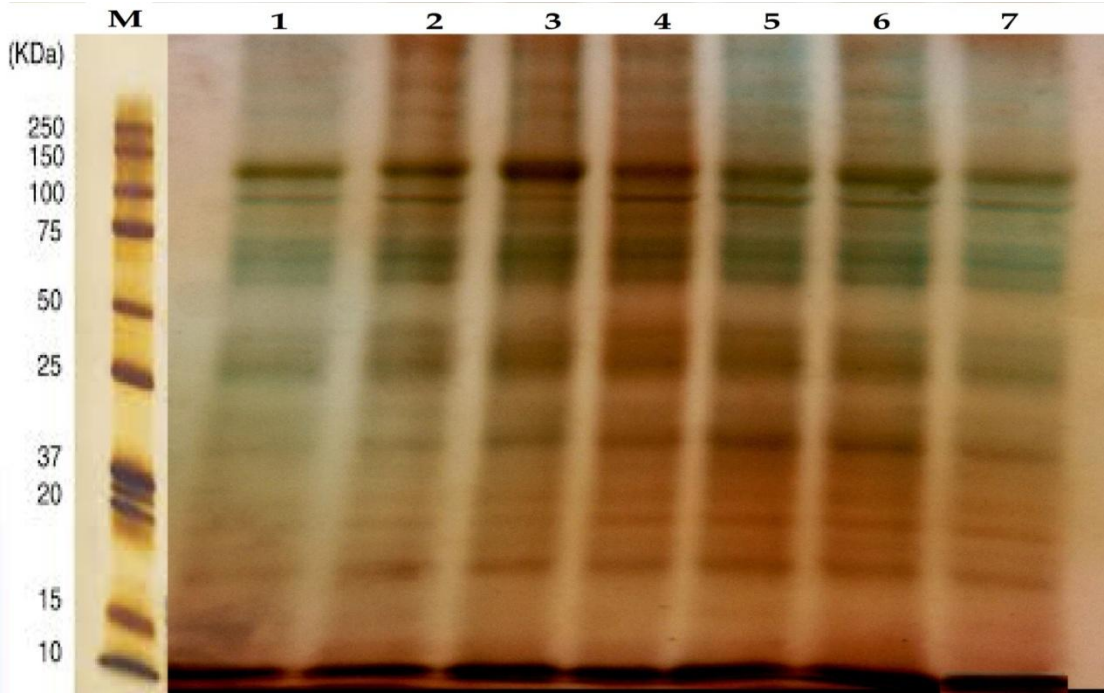


Figure 2: Silver stained SDS-PAGE, the first lane is molecular weight markers, lane 1 for normal control group, lane 2 for selenium administration group, lane 3 for lemon grass administration group, lane 4 for Doxorubicin induction group, lane 5 for group induced with doxorubicin and treated with selenium, lane 6 for group induced with doxorubicin and treated with lemon grass, finally lane 7 for group induced with doxorubicin and treated with selenium and lemon grass.

Effects of Selenium (SE); Cymbopogon citrates (Lemon Grass) (LG) extract alone and in combination against doxorubicin (DOX)-induced cardio-toxicity on genomic DNA concentration and identification on agarose in rat heart.

Figure (3) showed the identification of DNA of rat heart on agarose gel electrophoresis 1% in the treatments, the results were against DNA Ladder "200-3000bp". It was indicated from the results that DNA appeared to be damaged after treatment with DOX-induced cardiotoxicity in positive group by (20 mg/kg b.w.), while pre-administration with the SE and LG alone or in combination with DOX, this damage was improved and reduced.

All DNA bands were observed within the same molecular weight "more than 2000bp".

Our results investigated that in DOX-induced cardiotoxicity group, the DNA damage was observed, this may be due to the oxidative stress and cell membrane damage as a result to the toxic effect of free radicals of lipid peroxidation which acting on DNA, membrane proteins and lipids [88]. It was found that the antioxidant effect of some medicinal plants as LG which has a active phenolic compounds reduced the damage of DNA [60]. This is in agreement with the previous results. LG ethanolic extract administration in rat produced highly significant increase in DNA contents in either induced or non-induced toxicity groups (less damage). This enhancement in DNA concentration after treatment with the plant extracts appeared to be highly percentage in non-induced toxicity than intoxicated group. The effect of LG on DNA appeared to be dose-dependent.

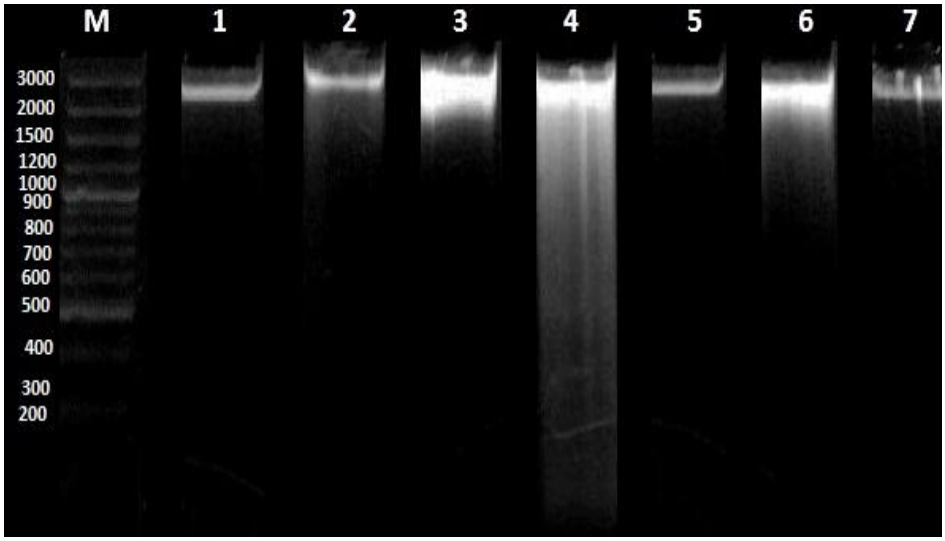
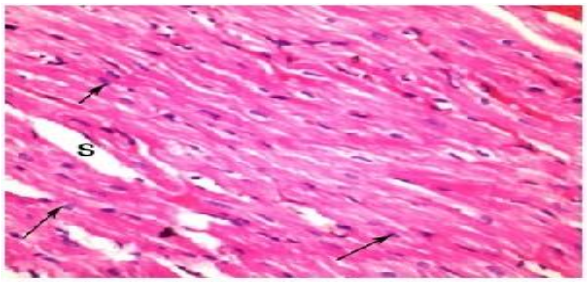


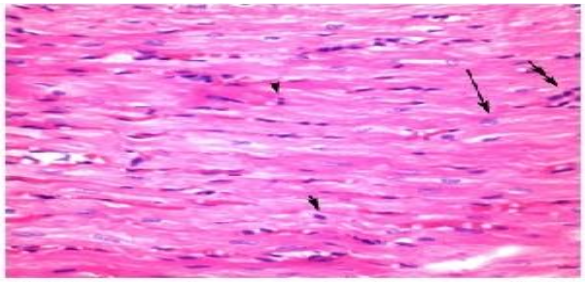
Figure 3: Genomic DNA fragmentation pattern of Heart tissues on 1% agarose gel electrophoresis. Lane M: DNA ladder, Lane 1 Saline (Untreated), Lane 2 treated with Selenium, Lane 3 Treated with lemon Grass, Lane 4 Treated with Doxorubicin (positive group), Lane 5 Treated with Selenium then Doxorubicin, Lane 6 Treated with lemon Grass then Doxorubicin, lane 7 Treated with lemon Grass+ Selenium then Doxorubicin.

Histopathological Examination of Cardiac, liver and Kidney tissues:

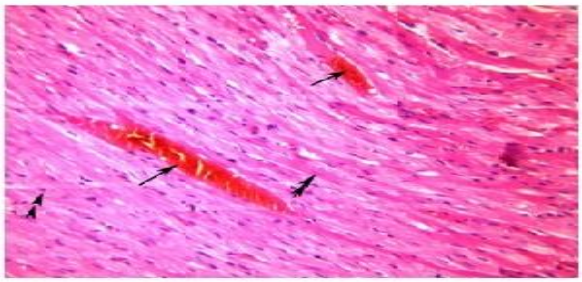
Histopathological examination of cardiac tissues in Figure (4) confirmed that, in ***G1(a)***: Microscopical examination of control group revealed, normal cardiac tissue architecture, intact cardiomyocytes with vesicular nuclei, interstitial space**. *G2(b):*** Mild to moderate pathological alterations were observed in form of sub capsular dilated blood vessels with pericardium inflammation, together with interstitial edema and acellular eosinophilic material. Focal atrophied cardiomyocytes with pyknotic nuclei. ***G3(c):*** In this group, display dilated congested cardiac vessel, cardiomyocytes with pyknotic nuclei. ***G4(d):*** In this group, display dilated congested sub capsular cardiac vessels, focal areas of pericardium edema, together with focal areas of interstitial haemorrhage, mild inflammation with focal aggregates of lipid-laden macrophage, wide areas of intact cardiomyocytes, however few cardiomyocytes display degenerative changes. ***G5(e):*** Mild to moderate pathological alterations, where cardiac vasculature were badly affected, where most of sub capsular vasculature display dilation with haemolitic blood. In their lumen, mild perivascular inflammatory infilterate, meanwhile most of cardiomyocytes display normal histologic appearance. ***G6(f):*** Histologic profile display the same picture obtained in G5, this is in addition to focal areas of cardiomyocytes display atrophy and pyknotic nuclei. ***G7(g):*** In this group, it gives the best results, where intact cardiomyocytes with vasculature could be seen, however in 50% of animals, mild vasculature injury could be seen.



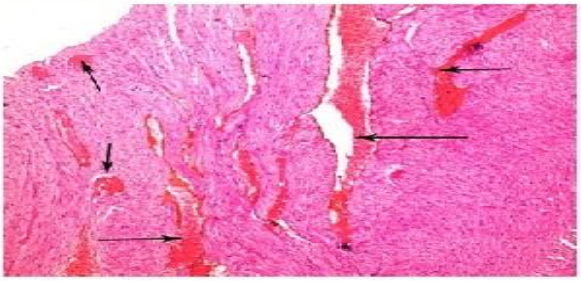
(Fig4.a) Photomicrograph of cardiac tissue of (G1) group showed intact cardiomyocytes (arrow) with vesicular nuclei, interstitial space (S). (H& E) (X:400)



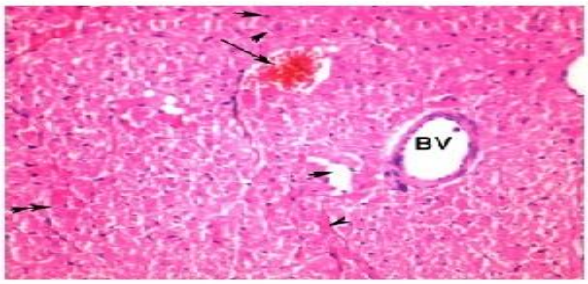
(Fig4.b) Photomicrograph of cardiac tissue of (G2) group showed cardiomyocytes with pyknotic nuclei (arrow head) and homogenous cytoplasm, intact cardiomyocytes (arrow), Lipid-Laden macrophage (double arrow). (H& E) (X:200)



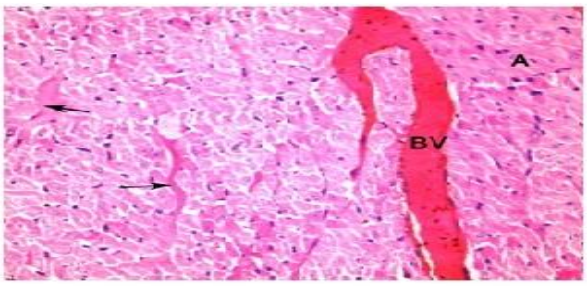
(Fig4.c) Photomicrograph of cardiac tissue of (G3) group showed congested cardiac vessel (double arrow), atrophied cardiomyocytes (arrow), separation between cardiomyocytes (S). (H& E) (X:200)



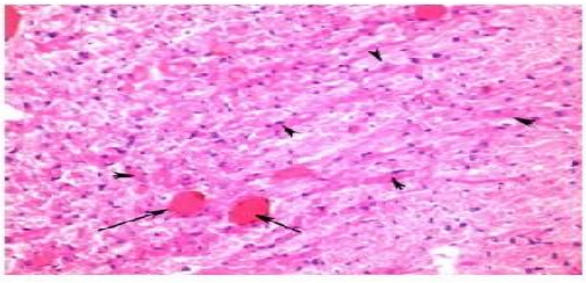
(Fig4.d) Photomicrograph of cardiac tissue of (G4) group showed dilated congested vasculature (arrow). (H& E) (X:400)



(Fig4.e) Photomicrograph of cardiac tissue of (G5) group showed dilated blood vessel (bv), extracellular haemorrhage (arrow), pyknotic nuclei (arrow head), atrophied cardiomyocytes (double arrow). (H& E)



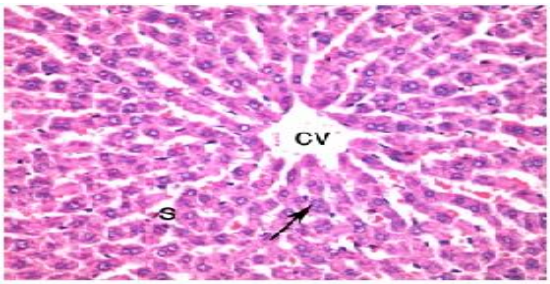
(Fig4.f) Photomicrograph of cardiac tissue of (G6) group showed atrophied cardiomyocytes (arrow) area of cardiomyocytes with pyknotic nuclei (A), dilated blood vessel (bv) with haemolytic blood. (H& E) (X:200)



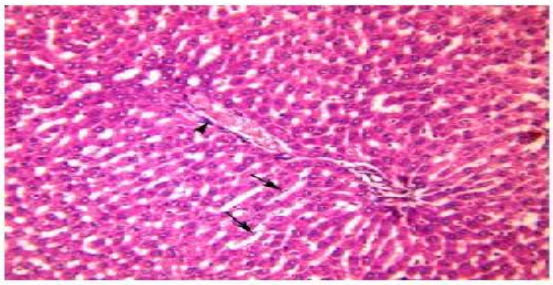
(Fig4.g) Photomicrograph of cardiac tissue of (G7) group showed congested blood vessel (arrow), atrophied cardiomyocytes (arrow head). (H& E) (X:200)

Histopathological examination of heart tissue Figure (4) showed that DOX treatment resulted in alteration of cardiac tissue structure in the form of fibrosis and apoptotic changes in cardiomyocytes. Pretreatment with SE and LG alone or in combination with DOX for 7 days ameliorated the effect of DOX administration on cardiac tissue. However,

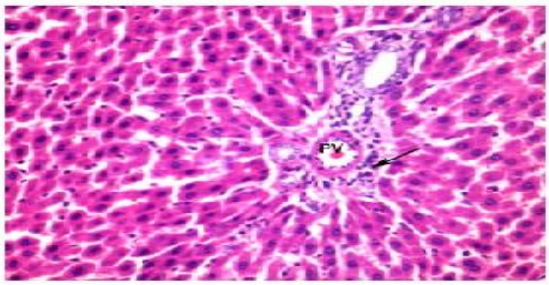
still vascular dilatation, mild congestion. Our results suggest that LG extract and SE have potentially protective against doxorubicin-induced cardiotoxicity. The biochemical data were confirmed by histopathological data which showed myocardial injury induced by DOX after treatment (Fig. 4D). It has been reported that cardiac histopathological changes were induced by DOX treatment which confirm our presented data, this is in agreement with **Safinaz *et al.* [7].**



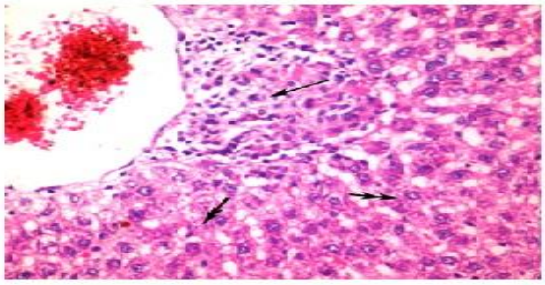
(Fig.5.a) Photomicrograph of hepatic tissue of group (G1) showed normal liver architecture, normal appearing of portal venous channels with normal sized sinusoids.



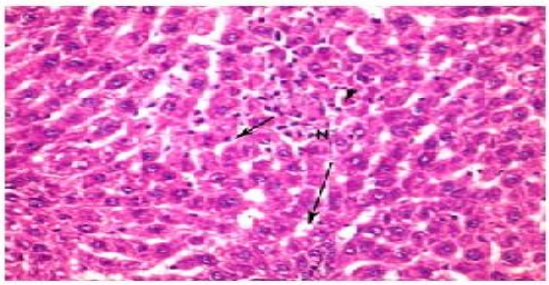
(Fig.5.b) Photomicrograph of hepatic tissue of group (G2) treated animals showing dilated sinusoids with proliferated vankupffer cells (arrow) and focal area of inflammation (arrow head). (H&E) (X=400)



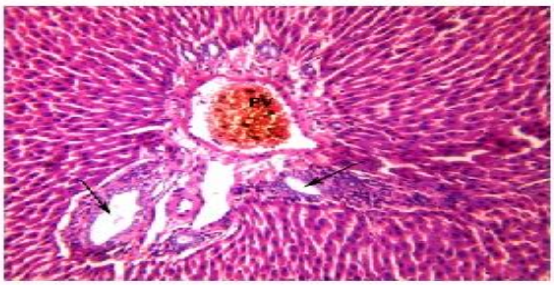
(Fig.5.c) Photomicrograph of hepatic tissue of group (G3) treated animals showing congested portal vein (PV) and perifibrotic area (arrow). (H&E) (X=400)



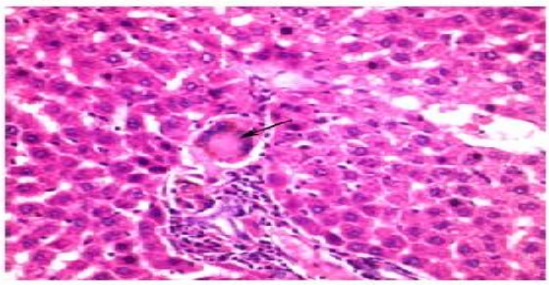
(Fig.5.d) Photomicrograph of hepatic tissue of group (G4) treated animals showing focal necrotic area (arrow) and severe vacuolated hepatocytes (double arrow). (H&E) (X=400)



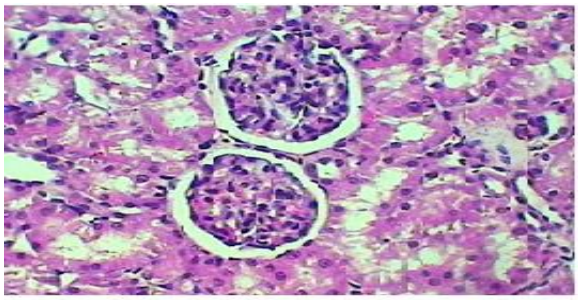
(Fig.5.e) Photomicrograph of hepatic tissue of group (G5) treated animals showing dilated sinusoids (arrow), hyaline body (arrow head) and focal necrotic area (N). (H&E) (X=400)



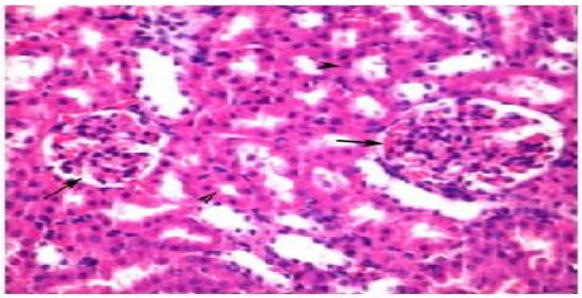
(Fig.5.f) Photomicrograph of hepatic tissue of group (G6) treated animals showing dilated congested portal vein (PV) and proliferated bile ducts (arrow). (H&E) (X=400)



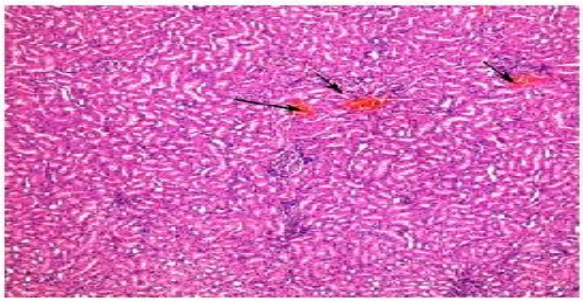
(Fig.5.g) Photomicrograph of hepatic tissue of group (G7) treated animals showing giant cell (arrow). (H&E) (X=400)



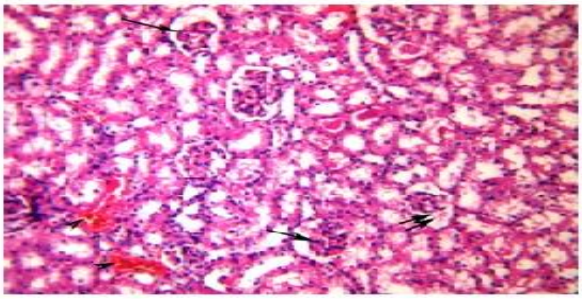
(Fig.6.a) Photomicrograph of renal tissue of (G1) group showed, normal tissue architecture, with normal glomerulus and tubules.



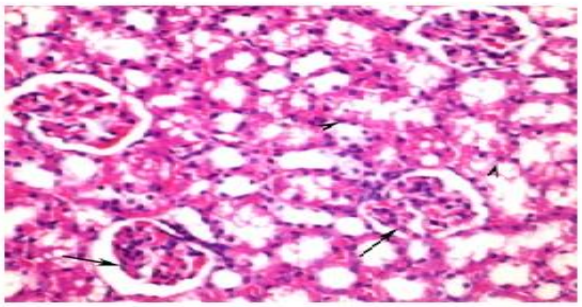
(Fig.6.b) Photomicrograph of renal tissue of (G2) group showed normal glomerular tuft (arrow), normal renal tubules (arrow head) (H& E) (X: 400)



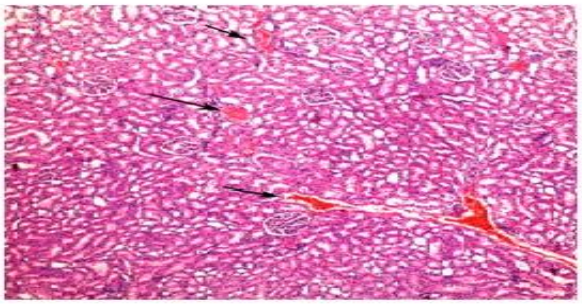
(Fig.6.c) Photomicrograph of renal tissue of (G3) group showed interstitial haemorhage (arrow). (H& E) (X: 100)



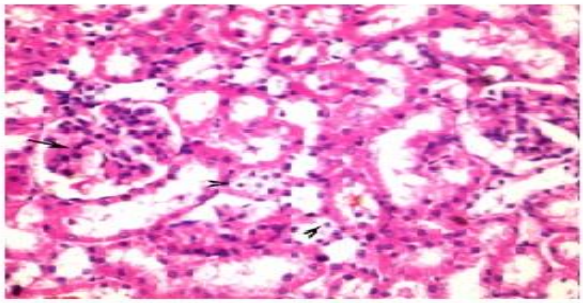
(Fig.6.d) Photomicrograph of renal tissue of (G4) group showed atrophied glomerular tuft (arrow), dilated bowman's space (double arrow) and hemorrhagic area (arrow head). (H& E) (X:200)



(Fig.6.e) Photomicrograph of renal tissue of (G5) group showed lobulated glomerular tuft (arrow), degenerative changes in epithial cells lining convoluted tubules with pyknotic nuclei (arrow head). (H& E) (X: 200)



(Fig.6.f) Photomicrograph of renal tissue of (G6) group showed interstitial haemorhage (arrow). (H& E) (X: 100)



(Fig.6.g) Photomicrograph of renal tissue of (G7) group showed hyalinized capillary in glomerular tuft (arrow), degenerative changes in epithial cells lining convoluted tubules (arrow head). (H& E) (X: 400)

Histopathological examination of liver tissues in Figure (5) confirmed that, in ***G1(a)***: revealed normal liver architecture with normal lobular pattern. The hepatocytes showed well defined central nuclei and abundant

cytoplasm, normal appearing of portal venous channels with normal sized sinusoids, bile ducts lined with normal cuboidal epithelium, also normal appearing of central vein. ***G2(b)***: The histologic profile of hepatic tissues revealed more or less normal pictures, however in 30% of animals showed portal areas with expansion and dilated congested portal vein, perifibrotic areas of proliferated bile ducts and prominent bile canaliculi. Focal areas of dilated sinusoids, with edema and inflammation were also noticed. Areas of syncetium mass of hepatocytes and focal necrotic areas were detected**.** ***G3(c):*** Insignificant pathological alterations were observed, where 1/4 of the animals showed few dilated and congested central vein, Portal area with edema and dilated sinusoids. Disturbed architecture could be seen. ***G4(d):*** Micrscopicalexamination of hepatic tissue of group (Doxorubicin) revealed that central vein displayed congestion and dilation while portal area was expanded with proliferated bile duct and edema. Inflammatory aggregates also seen, focal area of dilated sinusoids. Severe vacuolated hepatocytes were seen, this is besides many focal necrotic areas were detected. ***G5(e):*** No pathological alterations were recorded in this group, however pathological alterations were observed mainly in hepatic vasculature. ***G6(f):*** Moderate pathological alterations were recorded in this group, where few dilated congested central vein were noticed, few expanded portal areas with edema this is beside proliferated bile ducts, pericentral areas of dilated sinusoids. Hepatocytes appear intact with mild vacolation and clear nuclei together with focal necrotic areas could be detected. Giant cells, hyaline body and proliferated vankupffer cells were also observed. ***G7(g):*** Mild pathological changes were displayed in this group, where many portal areas showed restriction while others showed few expansion and inflammation, few dilated congested central vein were appeared. Hepatocytes were intact, with granulated cytoplasm and vesicular nuclei. Giant cells beside dilated sinusoids with inflammation were also observed.

Histopathological examination of kidney tissues in Figure (6) confirmed that, in ***G1(a)***: In this group revealed renal tissue architecture, preserved morphological structure along with glomerulus and tubules with no alterations. ***G2(b):*** In this group many of the renal tubules and glomerular tuft showed normal appearance. However some congested, dilated blood capillaries could be detected and many of renal vasculature revealed thickened wall with interstitial haemorrhagic area. ***G3(c):*** In this group, focal areas of mild interstitial hemorrhage, some glomerular tuft appeared with lobulation and congested dilated blood capillaries others were normal and occupied most of bowman's space with reduction in messganium cells. Mild degenerative changes in some of the cytoplasm of epithelial cells lining renal tubules were detected. ***G4(d):*** in this group treated with (Doxorubcin) showed interstitial hemorrhage, atrophied glomerular tuft with dilation of bowman's space. Moreover, there were degenerative changes in epithelial cells lining the convoluted tubules, this is beside to hyaline cast in the lumen of some of convoluted tubules were observed. ***G5(e):*** In this group revealed dilated blood vessel with thickened wall, reduction in size of glomerular tuft with dilation of bowman's space were noticed. The renal tubules display moderate atrophied cytoplasm of the epithelial cells lining them with pyknotic nuclei. ***G6(f):*** In this group showed interstitial haemorrhage. The glomerular tuft display lobulation and focally seen atherosclerotic region. This is beside to hyaline cast in the lumen of some of convoluted tubules were observed. ***G7(g):*** Mild pathological alterations were observed in this group were interstitial haemorrhage was detected. Hyalinized capillary in some glomerular tuft with mild degenerative changes in some convoluted tubules with pyknotic nuclei were still observed.

Conclusion

In conclusion, Cymbopogon citratus (LG) is recommended cardiac glycoside and the cardiac glycosides serves as defence mechanisms against cardiovascular disease and digestive problems. Thus, *Cymbopogon citratus* (LG) whole plant materials are recommended to be taken because it has many beneficial effects in human health. LG exhibited cardio protective activity and the potential was observed to be increased dose dependently. The observed cardio protective potential is mainly because of its antioxidant activity as comparable with the proven antioxidant SE. However, further studies are warranted to identify the active molecules responsible for the cardio protection.

Selenium (SE) pretreatment produced significant protection against DOX-induced cardiomyocyte damage; however, such trace element could alleviate the DOX- induced cardiac damage, as evidenced by the biochemical measurements and histopathological examinations of the cardiac tissues.

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