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**Research Article**

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# *Effect of Roselle (Hibiscus sabdariffa) against Adriamycin Induced-cardiotoxicity in Male Rats*

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**ABSTRACT**

*Adriamycin (ADR) is an antitumor antibiotic drug has been shown to be highly effective in the treatment of cancer and a variety of malignant. However, the successful use of ADR has been hampered by its cardiotoxicity, which mediated through oxidative stress. Hibiscus Sabdariffa (HS) has shown to possess powerful antioxidant properties. This study aimed to investigate the impact of HS extract (HSE) against ADR-induced cardiotoxicity in male rats. The study was conducted on four groups; control, ADR (10 mg/kg), and HSE+ADR groups 500 and 750 (mg/kg), rats received HSE for 1 week before ADR injection. After 3 days from ADR injection, rats were subject to electrocardiography (ECG) analysis, and plasma were separation for determination of lactate dehydrogenase (LDH) and creatine kinase (CK-MB). Heart tissues were subjected for determination of malondialdehyde (MDA) level and glutathione peroxidase (GPx) activity, as well as examined microscopically. Results revealed that, treatment of rats with ADR significant increased heart rate, ST height, QT interval and T amplitude, as well as increased LDH and CK-MB, while it decreased QRS interval. Cardiac MDA level was significant increase with significant decrease in GPx activity as compared with control rats. Pretreatment of ADR-treated rats with HSE resulted in significant improvement in ECG readings and tested heart enzymes, as well as normalize cardiac contents of MDA and GPx as compared with ADR group. Histopathological examination of heart sections revealed that, ADR injection caused many myocardial injury, while HSE pretreatment showed either mild or slight congestion of myocardial blood vessels at low dose (500 mg/kg) or no histopathological change at high dose (750 mg/kg). These results suggested that, HSE is effective in ameliorated ADR-induced cardiotoxicity in male rats, probably through its active compounds that have antioxidant properties.*

**Keywords**: *Adriamycin, Cardiotoxicity, Hibiscus Sabdariffa, Antioxidant, Rats.*

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## INTRODUCTION

Cardiotoxicity has a rising relevance as a consequence of the global improvement in cancer management, where adverse effects of treatments have significant consequences on patient outcome [1][2] [3]. New targeted therapies have widened the cardiotoxic spectrum of antineoplastic drugs. Most patients remain asymptomatic during and after drug infusion, however electrocardiographic abnormalities such as QT interval prolongation and nonspecific ventricular repolarization changes can acutely appear in approximately 11 % of patients [4]. Adriamycin (ADR) or Doxorubicin is an antitumor antibiotic of the anthracycline group. It has been shown to be highly effective anti- neoplastic drugs, in the treatment of several adult and pediatric cancers. The successful use of ADR has been hampered by toxicities such as hematopoietic suppression, nausea, vomiting, extravasation and alopecia, yet the most feared side-effect is cardiotoxicity, the probability of developing cardiomyopathy is largely dose-dependent, even at low doses [5][6].

*Hibiscus sabdariffa* (HS) calyces, *Malvaceace* family, are a famous plant cultivated and used widely. It is an annual herbaceous shrub used in traditional medicine [7]. *Hibiscus sabdariffa* comprises of many bioactive flavonoids compounds as anthocyanins, quercetin, cyanidin, kaempferol, hydrocitric acid, saponins, tannins, hemidesmine, hemidesmol, hemidesterol, stearoptin, pregnane glycosides, *β*-sitosterol, indicusin, coumarin, volatile oils and triterpines [8] [9] [10]. *Hibiscus sabdariffa* has been used traditionally for the treatment of blood disorders [8]. It had hypoglycemic [11], antioxidant, antithrombotic [12], anti-inflammatory [13], antiulcerogenic [9], antidiabetic [14], hepatoprotective [15], cardioprotective [16], neuroprotective [17], renoprotective [18], hypocholesterolemic and antihypertensive properties [19]. It is Also known to has diuretic properties [20-21], and effective on treating pyrexia and liver damage [22]. Numerous studies in *vitro* experiments have evaluated the effects of HS extracts against various cancer cell lines through its antioxidant activity [23] [24] [25]. Therefore, the present study aims to evaluate the effect of HSE as protective natural agent against ADR -induced cardiotoxicity in male rats.

## MATERIALS AND METHODS

### Plant material

*Hibiscus Sabdriffa* extract, in Tablet form, was obtained from SWANSON Health Products Company. Each capsule contained (400 mg) of full spectrum HS flower, with purity (99%), there is up to a milligram of microcrystalline cellulose.

### Drug, kits and chemicals

Arimaycin (Doxorubcin hydrochloride), red color powder, was obtained from King Abdulaziz University hospital. Lactate Dehydrogenase (LDH) (product number ab102526), glutathione peroxidase (product number ab102530), and lipid peroxidation (MDA) (product number ab118970) Elisa kits were purchased from Abcam plc Global Medical Supplies, Creatine kinase-MB (CK-MB) Elisa kit (product number EC2.7.3.2) was purchased from Cayman Chemical Company USA. All chemicals were with the highest laboratory purity are purchased from Sigma Chemical Co., (St. Louis, MO, USA).

### Animals

Male Wister albino rats (n= 24) (180-200 g) were obtained from the Animal experimental unit of King Fahd Center for Medical Research, King Abdulaziz University. All animals were allowed to one week acclimatize in animal housing standard laboratory conditions before being used for this study. They fed standard nutritionally balanced diet according to Reeves [26], and drinking water *ad libitum.*

### Pretreatment with Hibiscus Sabdriffa

*Hibiscus Sabdriffa* extract was prepared by dissolving HS powder in distilled water at the room temperature. Two oral doses of HS (500 and 750 mg/ kg) were administrated by gavage to rats, the dose 500 mg/kg was chosen according to Kunworarath *et al*. [27].

### Experimental design

After adaptation rats were classified into two main groups. **Group (I)** Control (n=6) rats received daily an oral dose of dist. water 1 ml, and intraperitioneal (i.p) injected with phosphate- buffered saline (PBF). **Group (II)** (n=18) classified into three sub-groups; **control positive group (ADR)** rats received the same daily oral dose of dist. water 1 ml, and i.p injected with a single dose of ADR (10 mg/kg b.w). **Treated groups with HSE**; rats injected with a single i.p dose of ADR and treated daily with an oral dose of HSE at two doses levels (500 and 750 mg/kg b.w) dissolved in 1 ml of dist. water. Treated groups with HSE were orally received HSE for 7 days before and daily after ADR injection.

**Electrocardiography (ECG) assessment**

**Cardiac contractility recording**

Invasive real-time recording of cardiac contractility was carried out according to Radovits *et al*. [28] with slight modifications. After urethane anesthesia, animals were placed on controlled heating pads and body temperature, measured *via* a rectal probe, they was maintained at 37°C. A microtip pressure transducer (SPR-320; Millar Instruments, Houston, TX, USA) was inserted into the right carotid artery and advanced into the left ventricle under pressure control. After stabilization for 5 min, the signals were continuously recorded at a sampling rate of 1000 $s^{-1}$. The microtip catheter was connected to a Power Lab Data Interface Module connected to a PC running Lab Chart professional software (version 7.3; ADI Instruments, Bella Vista, Australia) containing a blood pressure module, which detects and calculates the slopes of the systolic pressure increment (dP/dt) and the diastolic pressure decrement (−dP/dt), the systolic and diastolic duration, and the contractility index [29].

**Blood collection and serum separation**

At the end of the experimental 3 days after ADR injection. Blood samples were withdrawn by heparinized capillary tube from the retro orbital plexu of each rat under anesthesia with diethyl ether, then centrifuged at 3000 rpm for 15 min. to separate serum, which stored at -20° C for the biochemical analysis [30].Heart specimens collected and kept either frozen or in buffered 10% formalin solution.

**Biochemical analysis**

**Determination of cardiac functions enzymes**

Serum lactate dehydrogenase (LDH) was assessed according to Dichtl *et al*. [31]. In this assay LDH reduces NAD to NADH, which then interacts with a specific probe to generate color at 450 nm. Serum creatine kinase-MB (CK-MB) was assessed according to Guzy [32]. The CK-MB enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm.

**Determination of oxidative stress in heart tissues**

Glutathione peroxidase (GPx) activity was assessed according to Rotruck *et al*. [33]. GPx reduces cumene hydroperoxide (CuOOH) while oxidizing GSH to GSSG. The generated GSSG is reduced to GSH with consumption of NADPH by GR. The decrease of NADPH measured at 340 nm is propotional to GPx activity. Thiobarbituric acid reactive substances (TBARS) to assay malondialdehyde (MDA) according to Ohkawa *et al*. [34]. The MDA-TBA adducts formed by the reaction of MDA and TBA under light temperature and acidic conditions is measured calorimetrically at 540 nm.

**Histological examination**

Heart from each group was removed, washed immediately with saline and then fixed in 10% buffered formalin. Then embedded in paraffin, sections cut at 3-5 μm and stained with hematoxylin and eosin [35]. These sections were then examined under a light microscope for histological changes.

**Statistical analysis**

Statistical analyses of data were carried out using SPSS version 22. Data were expressed as the mean ± SEM. Comparison between groups were done by one-way analysis of variance (ANOVA), followed by L.S.D [36].

## RESULTS

**Electrocardiography (ECG)**

The present study result showed a normal pattern of ECG measurement in control rats, on the other hand ADR (10 mg/kg) treated rats showed prominent T wave. Moreover, treatment of rats with ADR significantly increased heart

rate, ST height, QT interval and T amplitude while significantly decreased QRS interval compared to the control rats value. Moreover, pretreatment of ADR treated rats with HSE (500 mg/kg) significantly increased heart rate, ST height and QT interval but returned both T amplitude and QRS interval to the control rats values. Pretreatment of ADR treated rats with HSE (750 mg/kg) significantly increased heart rate and QT interval. On the other hand, pretreatment of ADR treated rats with HSE (750 mg/kg) returned ST height, T amplitude and QRS interval to the control rats values (Figure 1 and Table 1).

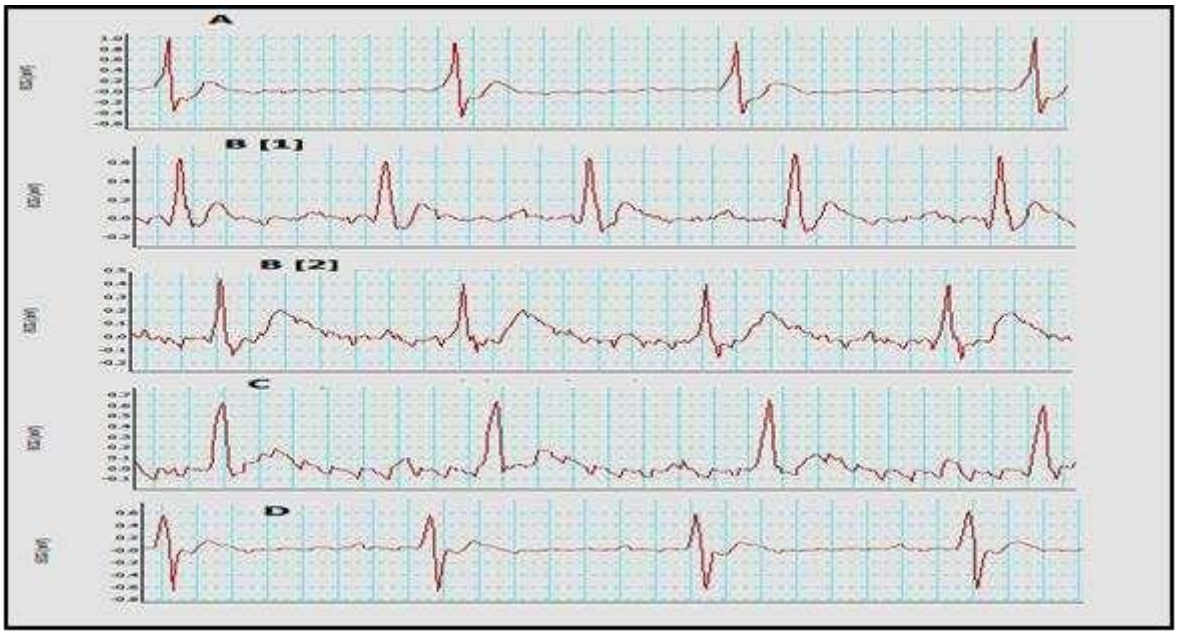



**Figure (1): Effect of HSE 500 and 750 (mg/kg) pretreatment on ADR-induced alterations in ECG pattern.** A: Control group, B [1 & 2]: ADR group, C: ADR + HSE 500 (mg/kg) group, D: ADR + HSE 750 (mg/kg) group. ECG tracing of control treated rats showed a normal heart rate, ST height, QT interval, T amplitude and QRS interval. ADR-treated group shows tachycardia, ST segment elevation, prolonged QT interval, increase T amplitude and narrowing of the QRS complex. HSE at both doses decreased heart rate, ST height and QRS complex.

**Table (1):** Effect of HSE on the electrocardiographic (ECG) parameters measured in ADR-induced cardiotoxicity in rats

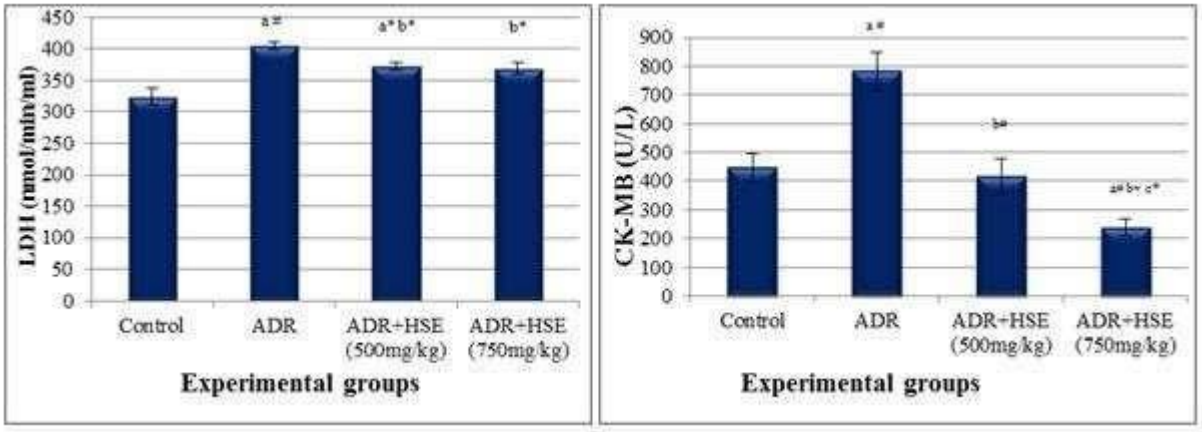
| ECG Parameters | Control | ADR | ADR + HSE (500 mg/kg) | ADR + HSE (750 mg/kg) |
|---|---|---|---|---|
| **Heart Rate** (BPM) | 324 ± 14.0 | 405 ± 6.0 $^{a+}$ | 372 ± 6.0 $^{a*\ b\#}$ | 369 ± 3.0 $^{a\#\ b+}$ |
| **ST Height** (mV) | 0.05 ± 0.01 | 0.17 ± 0.01$^{a+}$ | 0.09 ± 0.001 $^{a\#\ b+}$ | 0.04 ± 0.01 $^{b+\ c\#}$ |
| **QT Interval** (s) | 0.07 ± 0.01 | 0.09 ± 0.004$^{a*}$ | 0.13 ± 0.02 $^{a*}$ | 0.11 ± 0.01$^{a*}$ |
| **T Amplitude** (mV) | 0.14 ± 0.05 | 0.30 ± 0.05$^{a*}$ | 0.15 ± 0.05 $^{b*}$ | 0.17 ± 0.02 $^{b*}$ |
| **QRS Interval** (s) | 0.020 ± 0.002 | 0.014 ± 0.001$^{a\#}$ | 0.019 ± 0.002$^{b*}$ | 0.019 ± 0.001$^{b\#}$ |

**ECG**: Electrocardiographic **ADR:** Adriamycin **HSE:** *Hibiscus sabdariffa* L. extract
Data are presented as mean ± SEM (n=6). [a] Significant versus control group. [b] Significant versus ADR group. [c] Significant between ADR + HSE (500 mg/kg) and ADR+ HSE (750 mg/kg) groups. ($^{*}p \leq 0.05$, $^{\#}p \leq 0.01$ and $^{+}p \leq 0.001$).

**Cardiac functions enzymes**

Injection with ADR significantly increased both serum LDH and CK-MB concentrations compared with control rats serum concentrations. Pretreatment of ADR injected rats with HSE 500 and 750 ( mg/kg) significantly decreased both serum LDH and CK-MB concentrations compared to ADR rats. Moreover, treated rats with HSE (500 mg/kg) returned CK-MB serum concentration to the control value while significantly increased serum LDH concentration compared to the control rats serum concentration. In addition, treated rats with HSE (750 mg/kg) returned serum

LDH to the control rats serum concentration and decreased serum CK-MB concentration. Treated rats with HSE (750 mg/kg) significantly decreased serum CK-MB concentration compared to HSE (500 mg/kg) treated rats serum concentration . On the other hand, there is no significant different between HSE (750 mg/kg) and HSE (500 mg/kg) in regard to LDH serum concentration Figure (2).



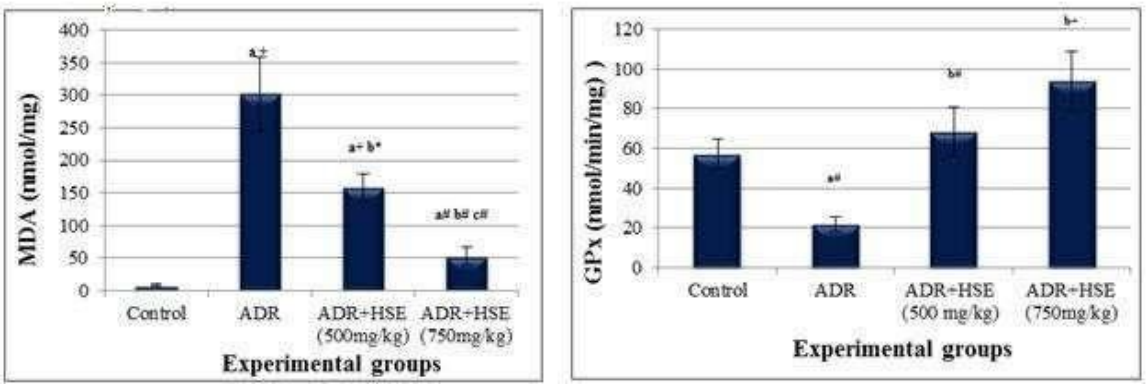

**ADR:** Adriamycin **HSE:** *Hibiscus sabdariffa* L. extract

Data are presented as mean ± SEM (n=6). [a] Significant versus control group. [b] Significant versus ADR group. [c] Significant between ADR + HSE (500 mg/kg) and ADR+ HSE (750 mg/kg) groups. ($^{*}p \leq 0.05$, $^{\#}p \leq 0.01$ and $^{+}p \leq 0.001$).

**Figure (2):** Effect of HSE on serum lactate dehydrogenase (LDH) and creatine kinase-MB (CK-MB) enzymes activity measured in ADR-induced cardiotoxicity in rats

**Oxidative stress in cardiac tissues**

Treatment of rats with ADR significantly increased both myocardial muscle MDA content and GPx enzyme activity compared to control rats values while treated rats with HSE (500 mg/kg) significantly decreased both myocardial muscle MDA content and GPx enzyme activity. In addition, treated rats with HSE (750 mg/kg) significantly decreased both myocardial muscle MDA content and GPx enzyme activity compared to ADR-treated rats. Treated rats with HSE 500 and 750 (mg/kg) significantly increased myocardial muscle MDA content compared to the control rats value. On the other hand, treated rats with HSE (500 and 750 mg/kg) returned myocardial muscle GPx enzyme activity to the control rats value. Finally, pretreatment of ADR treated rats with HSE (750 mg/kg) significantly decreased myocardial muscle MDA content compared to HSE (500 mg/kg) treated rats. On the other hand, there is no significant different between HSE (750 mg/kg) and HSE (500 mg/kg) in regards to myocardial muscle GPx enzyme activity Figure(3).



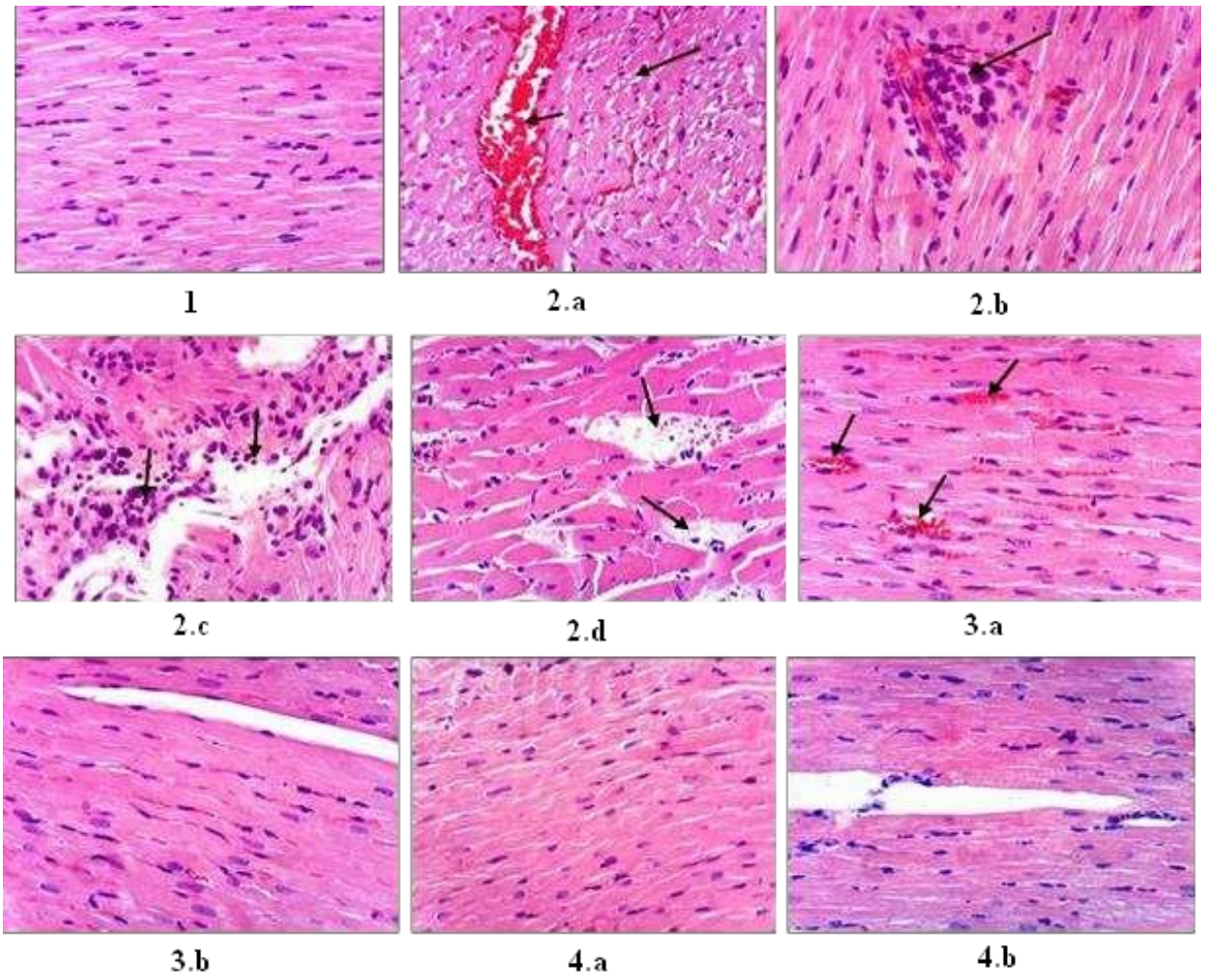

**ADR:** Adriamycin **HSE:** *Hibiscus sabdariffa* L. extract

Data are presented as mean ± SEM (n=6). [a] Significant versus control group. [b] Significant versus ADR group. [c] Significant between ADR + HSE (500 mg/kg) and ADR+ HSE (750 mg/kg) groups. ($^{*}p \leq 0.05$, $^{\#}p \leq 0.01$ and $^{+}p \leq 0.001$).

**Figure (3):** Effect of HSE on the myocardial tissues lipid peroxidation measured as MDA and GPx activity measured in ADR-induced cardiotoxicity in rats

**Histological examination**

Sections from control rats, showing normal histological structure of cardiac myocytes Figure (4.1). Sections from rats treated with ADR (10 mg/kg) showed dilatation and congestion of myocardial blood vessel, hyalinosis of myocardial blood vessel wall, necrosis of sporadic myocytes, focal mononuclear cells infiltrating the cardiac myocytes and intermuscular oedema Figures (4(2 a-d)). Sections from ADR rats pretreated with HSE (500 mg/kg) showed either congestion of myocardial blood vessels or no histopathological changes Figures (4(3 a-b)). Sections from ADR rats pretreated with HSE (750 mg/kg) showed either no histopathological changes, expect some sections showed mild congestion of myocardial blood vessels Figure (4 (4.a-b)).




**Figures (4) Effect of HSE on the cardiac histopathological changes occurred in ADR-induced cardiotoxicity in rats.** Control rats showing normal histological structure of cardiac myocytes (1). Rats injected with ADR showing dilatation and congestion of myocardial blood vessel and necrosis of sporadic myocytes (2.a), focal mononuclear cells infiltrating the cardiac myocytes (2.d), focal myocarditis (2.c) and inter muscular edema (2.d). ADR rats pretreated with HSE (500 mg/kg) showed congestion of myocardial blood vessels (3.a), expect some sections showed apparently no histopathological changes (3.b). ADR rats pretreated with HSE (750 mg/kg) showed no histopathological changes (4.a), expect some sections showed mild congestion of myocardial blood vessels (4.b). (H&E x 400)

## DISCUSSION

Adriamycin (ADR) is an effective anthracycline chemotherapeutic agent clinically used for treatment of several types of haematological malignancy and solid tumour [37][38]. However, the risk of cardiac, renal, pulmonary, testicular, and hematological toxicities largely limits its effective and widespread use in clinical oncology [39]. Adriamycin-induced cardiotoxicity causes severe changes in the blood pressure, heart rate and heart weight, as well as loss of myocardium contractility [40][41]. It is apparent that oxidative stress augmentation is a key aspect in development of myocardial infarction and cardiovascular toxicities [42]. The heart is more susceptible to ROS mediated cardiotoxicity because of its high energy requirement and high mitochondrial density. Furthermore, the heart also lacks the antioxidant enzymes needed to detoxify superoxide anions and hydrogen peroxide; thus, the generated free radicals accumulate and cause severe lipid peroxidation, leading to extensive destruction of the cardiac cellular mitochondrial membranes, endoplasmic reticulum, and nucleic acid [43][44].

*Hibiscus sabdariffa* (HS) is a tropical annual herbal shrub which has been evaluated for treatment of various diseases like tumors, inflammation, heart diseases, diabetes, hyperlipidemia, and diarrhea [45][46]. Its flowers contains many active constituents, mainly, cyanidin, quercetin, calcium oxalate, thiamine, riboflavin, niacin and ascorbic acid [7] [47]. The most important bioactive constituent of HSE is anthocyanin, that have been found to counteract the oxidative cellular damage during many diseases and toxicity conditions [48]. By understanding the free radical mechanism of anthracycline-induced cardiotoxicity, the hypotheses of this study is to investigate the possible protective role of HS extract (HSE) on ADR-induced cardiac damage in male albino rats.

**Effect of HSE on ADR-induced on Electrocardiography (ECG) changes**

The results of the present experiment showed that, treatment of rats with ADR (10 mg/kg) after 72 hrs significantly increased heart rate, ST height, QT interval and T amplitude compared to the control rats value. In addition, treatment of rats with ADR significantly decreased QRS interval compared to control rats. In agreement with our results, Consistent with this study results, the study done El-Sayed *et al*. [49] and Xin *et al*. [50] reported that treatment of rats with ADR (15 mg/kg) for 72 h resulted in several alterations in the ECG pattern of rats mainly significant increase in heart rate, elevation of ST segment, prolongation of QT interval, and increase in T wave amplitude.

The ECG pattern themselves are sensitive and sophisticated measures which could be readily used to explain both short and long-term dose-dependent effects of cardiotoxicants [51]. The severity of ECG changes have been reported to parallel the effects of ADR cardiotoxicity in patients [52]. The significant increase in heart rate noticed in rats after ADR injection may be a baroreflex mechanism in response either to decreased conduction (reflected by prolongation of action potential duration) or to acute cardiomyopathy produced by the high single dose of ADR, which is mainly characterized by decreasing myocardium contractility. This decrease in contractility was previously illustrated in *vitro* by Tokarska-Schlattner *et al*. [53] and in *vivo* by Dragojevic-Simic *et al*.[54].

Adriamycin induced $Ca^{2+}$ overload *via* a free radical accumulate mechanism, thus considered a myocardial mechanism for delayed after depolarizations and ADR-induced arrhythmogenic activity[55]. It also increased T wave amplitude, which is one of the earliest ECG changes following coronary artery occlusion and ischemia [56]. In addition, ST segment elevation caused by ADR injection reflect myocardial ischemia which may result from ADR- induced ROS production mainly superoxide anion and hydrogen peroxide [49] [53]. Also, the consecutive cellular membrane damage due to oxidative stress might be characterized by ST segment elevation [57].

Prolonged QT interval are related to cardiac vagal dysfunction, representing cardiac toxic potential as increased risk of ventricular arrhythmia, cardiac dysfunction, and sudden cardiac collapse [58]. Furthermore, prolongation of QT interval and increase in T wave amplitude could be attributed to the ability of ADR to block the delayed rectifier $K^+$ current, resulting in delayed ventricular repolarization and prolonged ventricular action potential duration [59] [60]. An altered membrane function due to ADR-induced lipid peroxidation is responsible for the ECG changes that follow ADR treatment. Membrane stabilization could affect the propagation phase of lipid peroxidation, in that the mobility of lipid peroxyl radicals would be prevented, and thus their freedom to interact with adjacent membrane polyunsaturated fatty acids would be restricted [61].

In contrary to this study results, Hazari *et al*. [51] has studied the same heart disease model, where they gave male WKY and SH rats 3 different ADR doses consists of 1.5, 2.5 and 5 (mg/kg/week) for 3 weeks and sacrificed animals after 24 h from the final injection. It found that 5 mg/kg of ADR causes decrease in heart rate and eventually heart failure, as well as ST depression and T-wave inversion in SH rats. The difference occurred between Hazari et al. [51] results and our results may be due to the different rats strains and the different ADR dosage regimen (concentration and intervals).

This study results showed that, pretreatment of ADR-treated rats with HSE 500 and 750 (mg/kg) significantly decreased heart rate, ST height and T amplitude, with non-significant increase in QT interval and a significant increase in QRS interval compared to ADR-treated rats. In addition, pretreatment of ADR-treated rats with HSE (500 mg/kg) significantly increased heart rate, ST height and QT interval compared to control rats while it returned both T amplitude and QRS interval to the control rats values. Pretreatment of ADR with HSE (750 mg/kg) significantly increased heart rate and QT interval compared to control rats while it returned ST height, T amplitude and QRS interval to the control rats values. Pretreatment of ADR treated rats with HSE (750 mg/ kg) significantly decreased ST height compared to HSE (500 mg/kg) treated rats value.

To the best of our knowledge, this study is the first research which examine the effect of HSE on the ECG component during ADR-induced cardiotoxicity, the effect of HSE is probably related to the presence of its major phytochemical compounds. In agreement with this study results, Ashour *et al*. [62] showed a potential protective effect of bilberry extract which is rich in anthocyanine, flavonoids and phenolic acids against ADR-induced cardiotoxicity in rats. They reported that, pretreatment of ADR (15 mg/kg)-injected rats with alcoholic extract of billberry (100 mg/kg) for 10 consecutive days resulted in ST segment depression and prolongation QT intervals.

**Effect of HSE on ADR-induced changes heart function enzymes**

This study results revealed that, treatment of rats with ADR (10 mg/kg) significantly increased both serum LDH and CK-MB concentrations compared to the control rats. In agree with our findings, Goyal *et al*. [63] reported that injection of rats with ADR resulted in increase in serum CK-MB and LDH. Furthermore, the results of El-Sherbiny *et al*. [64] revealed that, injection of Sprague Dawley rats with ADR (3.5 mg/kg) twice weekly for 3 weeks significantly increased serum CK-MB and LDH. Also, coincide with this study results, El-Sayed *et al*. [49]. On the other hand, Oliveira *et al*. [65] results showed that injection of Wister rats with ADR (5 mg/kg) weekly for four weeks resulted in normal serum biochemical profile for CK-MB and LDH which may be explained by the low dose of ADR administered over long period of time used in this experiment.

Adriamycin may cause deficiency of oxygen supply or glucose supply to the myocardial cell membrane leading to damage and/or rupture of the cell membrane so that the enzymes leaks out. These enzymes are also called as specific biomarkers which can be estimated to check the damage [66]. In addition, increasing LDH and CK-MB effect could be a secondary event following ADR-induced lipid peroxidation of cardiac membranes with the consequent increase in the leakage of LDH and CK-MB [67][68]. Furthermore, CK-MB has higher specificity for the myocardium because it's concentration in myocytes is arguably much greater. It is considered also an important marker of early and late cardiac cell injury during ADR therapy which may be due to ADR-induced inhibition of nucleic acid and protein synthesis [69][70].

Our study results showed a cardioprotective effect of HSE against ADR-induced cardiotoxicity. As, pretreatment of ADR-treated rats with HSE (500 mg/kg and 750 mg/kg) significantly decreased both serum LDH and CK-MB concentrations compared to ADR-treated rats. Surprisingly, pretreatment of ADR with HSE (750 mg/kg) returned serum LDH to the control rats serum concentration while significantly decreased serum CK-MB concentration compared to the control rats serum concentration. In agreement of this study results, Obouayeba *et al*. [71] showed a cardioprotective effect of HSE against ADR-induced cardiotoxicity. They reported that, pretreatment of ADR (15 mg/kg)-injected wistar rats for 24 h with alcoholic extract of HS (100 mg/kg and 200 mg/kg) for 1 week resulted in a significant decrease in both serum LDH and CK-MB compared to ADR treated rats. Liu *et al*. [40] explored the effect of blueberry anthocyanins-enriched extracts for seven days on cyclophosphamide (100 mg/kg)-induced cardiac injury. The results showed that, daily administration of 20 and 80 mg/kg blueberry anthocyanins-enriched extracts to cyclophosphamide-treated rats resulted in an obvious reversal of cyclophosphamide-induced increase in CK-MB and LDH to the normal value. According to Obouayeba *et al*. [71], the cardioprotective property of the HSE is probably related to the presence of its major phytochemical compounds including flavonoids (gossypetin, hibiscetin, quercetin and sabdaretin) and anthocyanins (delphinidin 3-O-sambubioside and cyanidin 3-O-

sambubioside). As the works of different authors have demonstrated the cardioprotective property of phenolic compounds in particular anthocyanins and flavonoids [72] [73][74].

**Effect of HSE on ADR-induced changes in heart oxidative stress**

The present study results showed that, treatment of rats with ADR (10 mg/kg) significantly increased both myocardial muscle MDA content and GPx enzyme activity compared to control rats values. In agreement with our findings, Goyal *et al*. [63] recently, reported that, injection of ADR resulted in increased MDA content in the myocardium. Furthermore, many previous studies, reported that, treatment of rats with ADR significantly increased MDA content in the myocardium [66][75] [76]. In agreement with our findings, previous studies, reported that treatment of rats with ADR significantly decreased GPx activity in the myocardium [75][76].

Adriamycin induced myocardiopathy is mainly believed to involve the oxidative stress and production of ROS that promotes lipid perioxidation. The principle is that ADR is bound with ferric iron to induce the generation of free radicals [77] [78]. In addition, ADR is converted to semiquinone by mitochondrial, lysosomal and cytosolic enzymes. Semiquinone is a charged moiety that readily donates an electron to an oxygen molecule, resulting in generation of an oxygen free radical or superoxide ion/hydroxyl radicals. Due to the presence of less developed antioxidant defense mechanisms of heart, they are particularly vulnerable to damage by anthracycline-induced ROS [53][79]. This damage caused by ADR may be associated with reactive oxygen species that promotes lipid perioxidation [77]. In addition, Chen *et al*. [80] explained the decreased in GPx enzyme activity in ADR-treated animals by its excessive use to overcome ADR-induced ROS production.

Pretreatment of ADR treated rats with HSE (500 mg/kg and 750 mg/kg) significantly decreased both myocardial muscle MDA content and GPx enzyme activity compared to ADR-treated rats. Pretreatment of ADR treated rats with HSE (500 mg/kg and 750 mg/kg) returned myocardial muscle GPx enzyme activity to the control rats value. In the same line, the study of Liu *et al*. [40] who showed that, blueberry anthocyanins-enriched extracts attenuated cyclophosphamide-induced increased MDA levels, which suggested that blueberry anthocyanins-enriched extracts have antioxidant properties.

Oxidative stress reflects an imbalance between the systemic manifestation of ROS and the ability of biological systems to readily detoxify the ROS or repair the resulting damage, which is usually evaluated by monitoring ROS- induced lipid peroxide (MDA) and antioxidative systems such as SOD and GPx expression [81][82]. The protective effect of HSE against ADR-induced increase in MDA and decrease in GPx could be attributed to HSE high content of several antioxidants including anthocyanine, flavonoids and phenolic acids [83][84]. These compounds were reported to inhibit ADR-induced oxidative stress in rats heart [62].

In addition, these compounds were reported to inhibit oxidative processes in other tissues [85]. They have been shown to effectively scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide [86], improve the ferric- reducing antioxidant power in plasma [87] and suppress the DNA damage induced by ADR [88]. Surprisingly, HS whole extract was found to be a better scavengers of superoxide anions radicals, hydroxyl radical and hydrogen peroxide as compared to quercetin and α-tocopherol [84].

**Effect of Effect of HSE on ADR-induced heart histopathological changes**

The present study results showed that injection of rats with ADR (10 mg/kg) resulted in many histopathological defects in myocardial muscle and heart blood vessels. Three days after ADR the heart sections showed dilatation and congestion of myocardial blood vessel, hyalinosis of myocardial blood vessel wall, necrosis of sporadic myocytes, focal mononuclear cells infiltrating the cardiac myocytes, intermuscular oedema and focal myocarditis. In agreement with this study results, Goyal *et al*. [63] showed that injection of a single dose of ADR (67.75 mg/kg) for 48 h induced severe histological lesions in the heart muscle mainly, cytoplasmic vacuolization and myofibrillar loss. Similarly, Momin *et al*. [66] reported that the heart sections obtained after injection of albino rats with ADR (15 mg/kg) in six divided doses for 2 weeks showed abundant areas of necrosis, aggregation of acute inflammatory cells and damaged vascular spaces. Furthermore, Abdel-Sattar *et al*. [89] showed that injection of a single dose of ADR (15 mg/kg) for 3 days induced severe histological lesions in the heart muscle mainly, cytoplasmic vascular degeneration, interstitial edema and fibrotic bands.

The present study result showed that pretreatment of ADR-treated rats pretreated with HSE (500 and 750 mg/kg) showed either mild congestion of myocardial blood vessels or no histopathological changes. In agreement with this study results, Liu *et al*. [40] showed that blueberry anthocyanins-enriched extract attenuates cyclophosphamide-induced left ventricular leukocyte infiltration as well as cardiomyocyte apoptosis. Furthermore, Ashour *et al*. [62] showed a potential protective effect of bilberry anthocyanins-enriched extract against ADR-induced cardiac muscle histopathological changes. As administration of bilberry extract with ADR resulted in mild congestion and some interstitial edema of the myocardium.

## CONCLUDION

**In conclusion,** consumption of HS attenuated the cardiotoxicity induced by ADR. The results revealed that, the most effective dose is the high dose (750 mg/kg) which has therapeutic effects as antioxidants activity. Therefore, it is recommended that dietary supplements with HSE as natural treatment for cancer patient after ADR treatment. Further studies should be conducted for longer period and with different doses .

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