

COMPARATIVE STUDY ON THE EFFECTS OF LYCOPENE AND SAFFRON ON DOXORUBICIN-INDUCED CARDIOTOXICITY IN ADULT MALE ALBINO RATS: A HISTOLOGICAL AND BIOCHEMICAL ASSESSMENT

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ABSTRACT

Background: Doxorubicin (DOX), is one of the most widely-used chemotherapeutic drugs. Despite its broad therapeutic efficacy, clinical studies have reported that, the major limiting factor of DOX chemotherapy is its significant cardiotoxic effects, which often results in irreversible degenerative cardiomyopathy and heart failure. Lycopene and saffron are powerful antioxidants against free radicals and oxidative attacks. **Aim of the work:** the aim of this study was to investigate and compare the protective effects of tomato Lycopene (LYC) and saffron against DOX induced cardiotoxicity in adult male rats. **Material and methods:** the study was conducted on 48 adult male albino rats, divided into six groups; group I served as (Control group), group II (Lycopene treated group), group III (Saffron treated group), group IV (Doxorubicin treated group), groupV (Doxorubicin and Lycopene treated group), groupVI (Doxorubicin and saffron treated group). At the end of experiment, all rats were sacrificed and their blood and heart tissues were subjected to biochemical analysis, while the remaining heart tissues were stained for histopathological study. **Results:** Doxorubicin co-administration with either tomato LYC or saffron showed significant improvements of all studied parameters with the superiority of LYC as regards cardiac enzyme parameters and cardiac troponin. **Recommendations:** Human trials should be carried out to establish the potential protective effects of lycopene and saffron. Future experiments are required to evaluate the possible protective molecular mechanisms of lycopene and saffron against DOX-induced cardiotoxicity

Keywords: Doxorubicin, Lycopene, Saffron, cardiotoxicity.

INTRODUCTION:

Doxorubicin (Dox), an anthracycline chemotherapeutic drug, is used in treatment of many solid tumors as well as malignant hematologic diseases. Despite its broad therapeutic efficacy, clinical studies showed that the major limiting factor of DOX chemotherapy is its cardiotoxic effects, which often leads to irreversible degenerative cardiomyopathy with heart failure (Tokarska-Schlattner et al., 2006; Octavia et al., 2012). Even

at lower doses, some patients develop heart disease many years after treatment (Lipshultz et al., 2013).

Several potential mechanisms of DOX-induced cardiotoxicity were suggested, and after comprehensive basic and clinical investigation, the free radical hypothesis was acknowledged (Corna et al., 2004; Shi et al., 2011; Ichikawa et al 2014).

Considering that the heart tissues is vulnerable to free radicals due to less development of antioxidant defense

mechanisms (**Doroshov et al., 1980**), cellular injury can be attributed to DOX-induced oxidative stress. As free radicals play a basic role in DOX-induced cardiotoxicity, antioxidants could protect the heart tissues against DOX-toxicity (**Razmaraii et al., 2016 C**).

Among several studies attempt to decrease cardiotoxic effect of this valuable drug, scavenging of ROS by natural antioxidants is considered as favorable cardioprotective against DOX-induced cardiotoxicity both in vitro and in vivo (**Mohamad et al., 2009; Li et al., 2010**).

Lycopene (ψ , ψ -Carotene) (LYC) is a natural carotenoid, which can be found in ripped tomato fruit, watermelon as well as pink grapefruit. Several studies proved its properties in decreasing many chronic or inflammatory diseases (**Bramley, 2000**).

Lycopene has good antioxidant properties include scavenging singlet oxygen and free radical (**Johnson, 2002**). Recently, it has been used as a food additive as well as a natural antioxidant. Additionally, LYC exhibited potent neuroprotective, anti-inflammatory, anti-proliferative, maintenance of normal cell metabolism, cognition enhancing properties and regulating blood lipid metabolism (**Kumar et al 2009; Maiani, et al., 2009; Ried and Fakler, 2011; Sachdeva and Chopra, 2015 ;Datta et al., 2016**).

Crocus sativus L. known as saffron belongs to the Iridaceae family and has a dried red stigma, which is used in folk medicine for many purposes including anti-spasmodic, eupeptic, gingival, sedative, anti-catarrhal, carminative, diaphoretic, expectorant, stimulant,

stomachic, aphrodisiac and emmenagogue effects (**Shen and Qian, 2006**).

Saffron contains many pharmacologically active compounds as crocin, picrocrocin and safranal. Crocin, a water-soluble carotenoid has anticarcinogen (**Fernández, 2006**), radical scavenging (**Assimopoulou et al., 2005**), anti-hyper lipidemic (**Soeda et al., 2007**) and memory improving actions (**Hosseinzadeh and Ziaei, 2006**).

Papandreou et al., (2006) stated that, Saffron antioxidant effect was compared with other vegetables rich in carotenoids antioxidant (carrots and tomatoes), and that, the extract of *C. sativus* stigmas had stronger antioxidant effect than carrots and tomatoes.

Therefore, with this background on Doxorubicin, lycopene, and saffron, we aimed to investigate and compare the possible beneficial effects of lycopene, and saffron against Doxorubicin-induced cardiotoxicity in adult male albino rats.

MATERIALS & METHODS:

Ethical Consideration of Study

Experimental procedures as well as the use of laboratory animals were approved by the Animal Research Committee in Zagazig University. Painless procedures were conducted. Animal housing and handling were ethically considered.

Animals

In this study 48 male Sprague-Dawley mature albino rats, 200–220 g weight each, were used. Animals were fed ad libitum and housed in pairs in steel cages, having a temperature-controlled environment ($22 \pm 2^\circ\text{C}$) with 12 h light/dark cycles.

Chemicals

Doxorubicin HCl (Adriblastina®) (50mg) was obtained from Pharmacia S.P.A. Inc., Milan, Italy.

Lycopene: Tomatoes (*Solanum lycopersicum* L., Solanaceae) were purchased from local commercial sources. The botanical authentication and extraction were done at the Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University according to **Luo and Wu (2011)**.

The obtained LYC extract weighed 4 grams. This amount was prepared in 80 mL of corn oil (50 mg/ml) and preserved in a refrigerator at 4 °C in a dark container to avoid oxidation of LYC.

Saffron manufactured by Sigma-aldrich company, Louis st., USA, and purchased from Sigma-Egypt.

Experimental Protocol

Animals were randomly divided into 6 groups.

Group I (control group): contains 18 rats and is further divided into 3 equal subgroups as follows:

- Ia → negative control - received regular diet and water.
- Ib → saline treated group - received 1ml saline (0.9%) once daily.
- Ic → corn oil treated group - received 0.5 mL corn oil daily (used for preparation of oral suspension of LYC) by oral gavage.

- Group II (**Lycopene treated group**) contains 6 rats; each received LYC in a dose of 200 mg/kg/day for 4 weeks by oral gavage (**Stacewicz-Sapuntzakis and Bowen, 2005**).

- Group III (**Saffron treated group**) contains 6 rats; each received saffron in a dose of 20mg/kg once a daily for 4 weeks dissolved in 1ml saline (0.9%) (**Goyal et al., 2010**).

-Group IV (**Doxorubicin treated**

group) contains 6 rats; each received DOX in a dose of 2.5mg/kg dissolved in normal saline by intra muscular route in the form of 6 injections over a period of 2 weeks to end up in a total cumulative dose of 15mg/kg. (**Koti et al., 2009**).

-Group V (**Doxorubicin and Lycopene treated group**) contains 6 rats; each received lycopene for 4 weeks starting 2 weeks before DOX administration and then for two additional weeks concomitantly with DOX administration at the same dose levels in the aforementioned groups.

-Group VI (**Doxorubicin and saffron treated group**) contains 6 rats; each received saffron for 4 weeks starting 2 weeks before DOX administration and then for two additional weeks concomitantly with DOX administration at the same dose levels in the aforementioned groups.

Rats' body weight was recorded daily. At the end of experiment, blood samples were obtained then all animals were scarified under light ether anesthesia, serum was collected and stored at -20 °C until measurement. Hearts were removed and weighed then stained for histopathological studies.

Method used for measurement of blood pressure and heart rate:

Principle:

Non-invasive Blood Pressure measurement was done according to **NIBP250 User's Guide (2013)**. During occlusion cuff inflation, tail blood experiences 3 distinct phases.

1. **Phase I** occurs once the cuff has been inflated to its maximum pressure. At this point, the pressure is so great that it occludes the artery and no blood passes. It is then followed by the release of this pressure. This phase is represented by the flat-line before the

systolic point.

2. **Phase II** occurs once the pressure drops below the animal's systolic blood pressure. It is at this time that blood can force its way through the partially occluded artery and generate a signal for the sensor cuff. This phase is represented on the graph by the ascending waveform between the systolic and diastolic points.

3. **Phase III** takes place when the pressure is not enough to restrict blood flow at all, and the waveform from the signal cuff resumes its regular form and amplitude. The high-amplitude portions of the waveform at the beginning and end represent the animal's heart rate (**Bunag et al., 1982**).

Biochemical parameters:

Venous blood samples were obtained from animals by means of capillary glass tubes from the retro-orbital plexus under light ether anesthesia (**Semler, 1992**).

The collected blood was used to measure the following parameters:

- **Oxidative marker:**

1-MDA (nmol/ ml) that was assayed colorimetrically according to **Ohkawa and colleagues (1979)**.

2- GSH (ng/ml) that was assayed colorimetrically according to **Beutler and colleagues (1963)**.

3-Catalase activity (U/ml) that was assayed colorimetrically according to **Aebi, (1983)**.

- **Cardiac Troponin I:** was measured according to **Collinson and fellow researchers (2001)**.

- **Assesment of serum level of cardiac marker enzymes:**

Serum level of lactate dehydrogenase (LDH) and creatinine-MB isoenzyme (CK-MB) enzymes were determined according to guidelines of some locally available

commercial kits using previously described principles of **Buhl and Jackson, (1978)** and **Wu and Bowers, (1982)**.

- **Tissue parameters:**

Hearts were immediately dissected out and grossly inspected to assess any gross abnormalities then washed with cold normal saline and used for histopathological study.

Light microscope examination:

They were fixed in 10% formalin solution. After which, tissues were embedded in paraffin blocks and processed into 5 u. thickness sections. They were stained by Hematoxylin and Eosin (**Horobin and Bancroft, 1998**) and examined using light microscope.

Statistical analysis

Data were analyzed by Statistical Package of Social Science (SPSS), software version 22.0 (**SPSS Inc., 2013**).

RESULTL

Group I (**G1**) → control.

- Group Ia (**G1a**) → negative control.

- Group Ib (**G1b**) → saline treated group.

- Group Ic (**G1c**) → corn oil treated group.

Group II (**GII**) → Lycopene-treated group.

Group III (**GIII**) → Saffron-treated group.

Group IV (**GIV**) → Doxorubicin-treated group.

GroupV (**GV**) → Doxorubicin and Lycopene treated group.

GroupVI (**GVI**) → Doxorubicin and saffron treated group.

Biochemical findings among G1a, G1b, G1c, GII and GIII were not statistically significant as regards all studied parameters (**Table 1, 2, 3**). So,

G1a was used as the control group for comparison with other treated groups.

Findings confirmed that body and heart weights decreased after DOX treatment but increased after lycopene and saffron treatment. (Table 4, 7).

Doxorubicin treated group showed increased cardiac troponin I, LDH and CPK. Furthermore, disturbance of oxidative marker in heart tissue was reversed by supplementation of lycopene or saffron with superiority of lycopene (Table 5, 8).

A significant decrease in the heart rate was detected in the DOX-treated group. Also, Dox caused a decrease in

the systolic, diastolic, mean BP which was reversed by lycopene or saffron with superiority of lycopene (Table 6, 9).

Histopathological result:

Left ventricles of GI showed normal myocardial architecture (Fig.1). While histopathological examination of GIV revealed myocardial cell damage, necrosis, and massive interstitial hemorrhage (Figs.2).

Upon supplementation of either lycopene or saffron there was partial reversal of these pathological changes (Fig.3, 4).

Table (1): Statistical comparison among negative control (G1a), saline (G1b), corn oil (G1c), lycopene (GII) and saffron (GIII) treated groups as body and cardiac weights using ANOVA test.

	G1a	G1b	G1c	GII	GIII	F	P
Body wt	199.32± 1.02	198.11± 1.02	197.98± 1.01	199.02± 1.22	198.91± 1.21	1.725	>0.05
Heart wt	0.90±0. 0201	0.91± 0.021	0.90± 0.010	0.92± 0.011	0.91± 0.022	1.388	>0.05

NB All values are expressed as Mean± SD N: Number of rats in each group=6 rats
SD: standard deviation P: < 0.05 significant

Table (2): Statistical comparison among negative control (G1a), saline (G1b), corn oil (G1c), lycopene (GII) and saffron (GIII) treated groups as regard mean values of LDH, CBK, cardiac troponin I, oxidative stress markers using ANOVA test.

	G1a	G1b	G1c	GII	GIII	F	P
LDH	369.12 ± 4.91	367.99± 4.95	368.55± 4.85	365.95± 3.99	366.45± 3.45	0.556	>0.05
CBK(MB)	337.98 ± 3.89	336± 3.45	335.99± 4.12	336.55± 4.44	335.99± 3.85	0.283	>0.05
Cardiac treponin I	50.33± 4. 35	49.35± 4.33	51.12± 3.44	49.55± 4.05	48.99± 3.09	0.290	>0.05
MDA (nmol/ ml)	159.55 ± 50.75	160.05± 50.63	175.71 ± 55.23	166.98± 54.95	165.55± 53.55	0.091	>0.05
GSH (ng/ml)	0.90 ± 0.28	0.89 ± 0.27	0.85 ± 0.25	0.87 ± 0.27	0.86 ± 0.28	0.035	>0.05
catalase activity (U/ml):	5.19 ± 0.85	5.38 ± 0.86	4.98 ± 0.74	5.00 ± 0.64	5.15 ± 0.75	0.265	>0.05

Values are expressed as Mean± SD SD: standard deviation P: < 0.05 significant

Table (3): Statistical comparison among negative control (GIa), saline (GIb), corn oil (GIc), lycopene (GII) and saffron (GIII) treated groups as regards SBP, DBP, MBP and HR using ANOVA test.

	GIa	GIb	GIc	GII	GIII	F	P
SBP	136.6± 5.399	135± 6.498	135.4± 6.398	132.4± 6.132	135± 6.733	0.361	>0.05
DBP	114.2± 6.663	113.1± 5.971	115.2± 6.143	114.3± 6.144	113.2± 5.972	0.118	>0.05
MBP	106.3± 3.020	106.4± 3.022	105.3± 5.021	107.2± 5.888	106.9± 4.023	0.168	>0.05
HR	166.9± 10.493	167.8± 9.493	166.1± 12.069	116.8± 12.055	166.5± 11.069	0.019	>0.05

Values are expressed as Mean± SD SD: standard deviation P: < 0.05 significant
 SBP: systolic blood pressure DBP: diastolic blood pressure MBP: mean blood pressure HR: heart rate

Table (4): Statistical comparison among negative control (GIa), Doxorubicin (GIV) treated, Doxorubicin and lycopene (GV) treated and Doxorubicin and saffron (GVI) treated groups as regards body weight and cardiac weight using ANOVA test

	GIa	GIV	GV	GVI	F	P
Body wt	199.32± 1.02	168.02±1.03	189.30± 1.04	180.05± 1.01	1,015.725	<0.0001*
Heart wt	0.90±0.0201	0.65± 0.0220	0.84± 0.0121	0.77± 0.0120	234.891	<0.0001*

Values are expressed as Mean± SD SD: standard deviation P: < 0.05 significant

Table (5): Statistical comparison among negative control (GIa), Doxorubicin (GIV) treated, Doxorubicin and lycopene (GV) treated and Doxorubicin and saffron (GVI) treated groups as regards mean values of LDH, CBK, Cardiac troponin I, oxidative stress markers using ANOVA test

	GIa	GIV	GV	GVI	F	P
LDH	369.12 ± 4.91	425.45± 4.54	380.55± 2.99	398.85± 3.98	208.519	<0.0001*
CBK	337.98 ± 3.89	398.85± 3.55	345.54± 2.45	358.22± 2.98	414.173	<0.0001*
Cardiac treponin I	50.33±4 .35	89.16± 17.28	60.15± 18.33	66.85± 17.18	6.876	(0.002) <0.05*
MDA (nmol/ml)	159.55 + 50.75	315.66 + 95.45	181.35 55.65	211.88+ 67.55	5.926	(0.005) <0.05*
GSH (ng/ml)	0.90 + 0.28	0.32+ 0.10	0.85+0.12	0.74+ 0.23	10.712	<0.0001***
catalase activity (U/ml)	5.19 + 0.85	2.44 + 0.72	3.99 + 0.75	3.40 + 0.55	15.080	<0.0001***

Values are expressed as Mean± SD SD: standard deviation
 P:< 0.05 significant P: <0.0001 highly significant

Table (6): Statistical comparison among negative control (GIa), Doxorubicin (GIV) treated, Doxorubicin and lycopene (GV) treated and Doxorubicin and saffron (GVI) treated groups as regards mean values of SBP, DBP, MBP and HR using ANOVA test

	GIa	GIV	GV	GVI	F	P
SBP	136.6± 5.399	100± 5.448	125.4± 3.212	115.4± 4.338	65.862	>0.0001
DBP	114.2± 6.663	85.1± 4.981	104.3± 4.122	95.2± 6.221	29.773	>0.0001
MBP	106.3± 3.020	80.4± 3.125	99.2± 3.285	90.3±4 .021	65.781	>0.0001
HR	166.9± 10.493	120.8± 10.495	155.8± 9.155	140.1± 11.069	22.554	>0.0001

Values are expressed as Mean± SD SD: standard deviation P: < 0.05 significant
P: <0.0001 highly significant SBP: systolic blood pressure DBP: diastolic blood
pressure MBP: mean blood pressure HR: heart rate

Table (7): Least significant difference test (LSD) for comparison of the changes of the mean values of body weight and cardiac weight in-between groups.

Group Parameter	Control	Doxorubicin treated group	Doxorubicin and Lycopene treated group	Doxorubicin and Saffron treated group
Body weight: a=versus control b=versus Doxorubicin treated group c=versus Doxorubicin and Lycopene treated group	0.94 a		0.94 a	0.0083** a
	0.24 b			0.94 b
	0.0008** c			
Heart weight: a=versus control b=versus Doxorubicin treated group c=versus Doxorubicin and Lycopene treated group	0.82 a		<0.0001** a	<0.0001** a
	<0.0001** b			0.119 b
	<0.0001** c			

Number of sacrificed rats for each group was 6 rats.

*: significant (p<0.05) **: highly significant (p<0.01). NS: non-significant.

Table (8): Least significant difference test (LSD) for comparison of the changes of the mean values of LDH and CBK in-between groups.

Group Parameter	Control	Doxorubicin treated group	Doxorubicin and Lycopene treated group	Doxorubicin and Saffron treated group
LDH: a=versus control b=versus Doxorubicin treated group c=versus Doxorubicin and Lycopene treated group	0.67 a		<0.0001** a	<0.0001** a
	<0.0001** b			0.079 b
	<0.0001** c			
CBK: a=versus control b=versus Doxorubicin treated group c=versus Doxorubicin and Lycopene treated group	0.93 a		<0.0001** a	<0.0001** a
	0.42 b			0.911 b
	<0.0001** c			

Number of sacrificed rats for each group was 6 rats.

*: significant (p<0.05)

**: highly significant (p<0.01).

NS: non-significant.

Table (9): Least significant difference test (LSD) for comparison of the changes of the mean values of cardiac troponin I and oxidative stress markers in-between groups.

Group Parameter	Control	Doxorubicin treated group	Doxorubicin and Lycopene treated group	Doxorubicin and Saffron treated group
Cardiac troponin I a=versus control b=versus Doxorubicin treated group c=versus Doxorubicin and Lycopene treated group	0.0016** a		0.69 a	0.277 a
	0.019 * b			0.089 b
	0.87 c			
MDA (nmol/ ml) a=versus control b=versus Doxorubicin treated group c=versus Doxorubicin and Lycopene treated group	0.0047 ** a		0.95 a	0.57 a
	0.016* b			0.077 b
	0.87 c			
GSH ((ng/ml) a=versus control b=versus Doxorubicin treated group c=versus Doxorubicin and Lycopene treated group	0.0003** a		0.97 a	0.51 a
	0.0008** b			0.007** b
	0.77 c			
catalase activity (U/ml) a=versus control b=versus Doxorubicin treated group c=versus Doxorubicin and Lycopene treated group	<0.0001** a		0.044* a	0.002** a
	** b			0.134 b
	0.51 c			

Number of sacrificed rats for each group was 6 rats.

*: significant (p<0.05)

**: highly significant (p<0.01).

NS: non-significant.

Table (10): Least significant difference test (LSD) for comparison of the changes of the mean values of SBP, DBP, MBP and HR in-between groups.

Group	Control	Doxorubicin treated group	Doxorubicin and Lycopene treated group	Doxorubicin and Saffron treated group
Parameter SBP a=versus control b=versus Doxorubicin treated group c=versus Doxorubicin and Lycopene treated group	<0.0001** a	<0.0001** a	<0.0001** a	<0.0001** a
	<0.0001** b			<0.0001** b
	0.0002** c			
DBP a=versus control b=versus Doxorubicin treated group c=versus Doxorubicin and Lycopene treated group	<0.0001** a	<0.0001** a	<0.0001** a	0.0018** a
	0.0015** b			<0.0001** b
	0.0045** c			
MBP a=versus control b=versus Doxorubicin treated group c=versus Doxorubicin and Lycopene treated group	<0.0001** a	<0.0001** a	<0.0001** a	0.0002** a
	<0.0001** b			<0.0001** b
	<0.0001** c			
HR a=versus control b=versus Doxorubicin treated group c=versus Doxorubicin and Lycopene treated group	<0.0001** a	<0.0001** a	<0.0001** a	0.09 a
	0.001** b			<0.0001** b
	0.0086** c			

Number of sacrificed rats for each group was 6 rats.

*: significant (p<0.05)

** : highly significant (p<0.01).

NS: non-

significant.

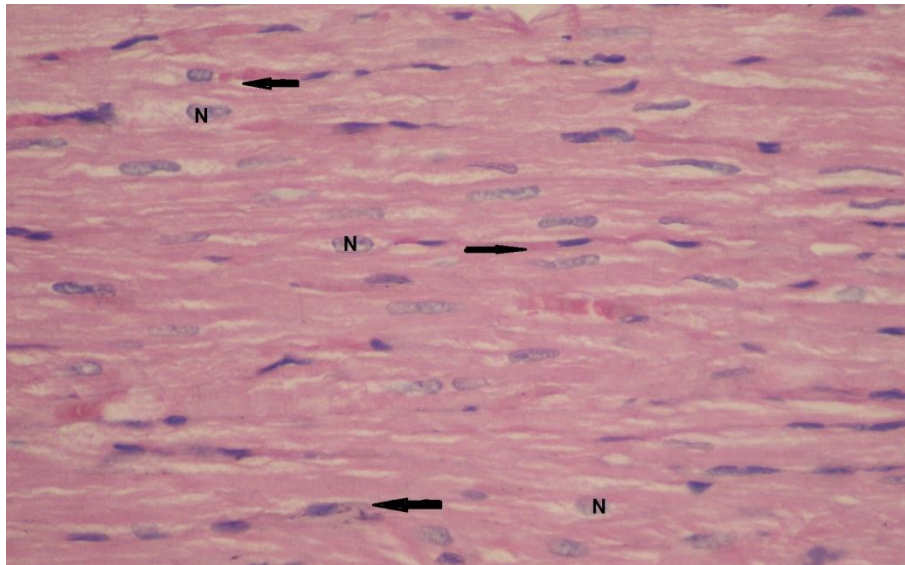


Figure (1): A photomicrograph of a section from heart of the control group showing muscle fibers in different directions that contains acidophilic sarcoplasm (arrow), central pale oval nuclei (N) and delicate connective tissue separating cardiac myocytes. (H & E X 400).

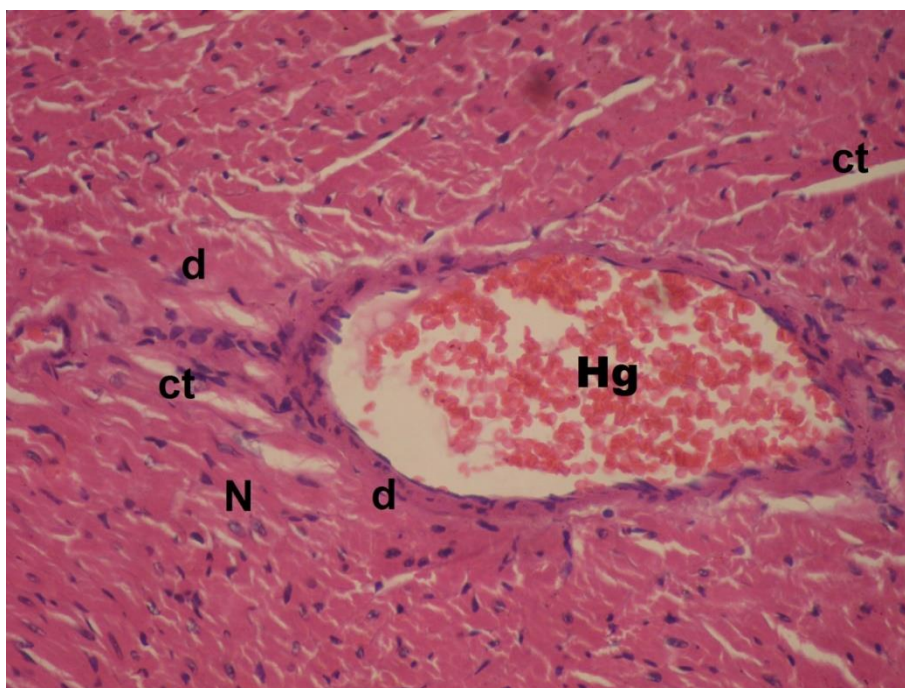


Figure (2): A photomicrograph of a section from the heart of a doxorubicin treated rat showing areas of hemorrhage (Hg). Nuclei were either lost or darkly stained (N). Muscle fibers were either separated by wide connective tissue spaces (ct) or appeared degenerated and pale (d). (Hx & E X 400)

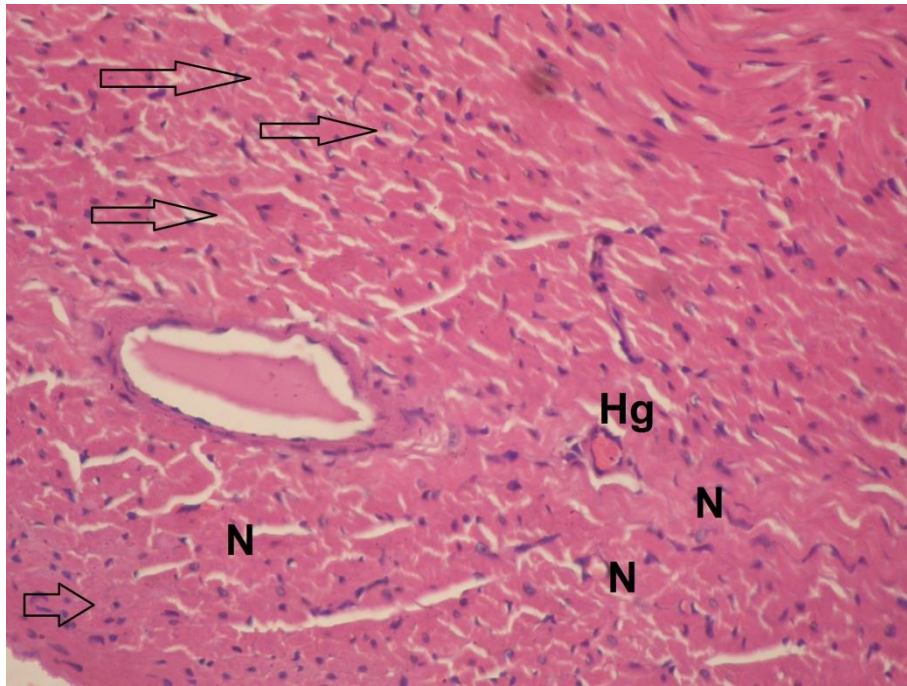


Figure (3): A photomicrograph of a section from heart of a doxorubicin and saffron treated rat showing that some of muscle fibers are normal and contained acidophilic sarcoplasm and central pale oval nuclei (N). Many muscle fibers showing necrosis and their nuclei were lost or darkly stained (arrow) with hemorrhage of heart interstitium (Hg) (H & E X 400).

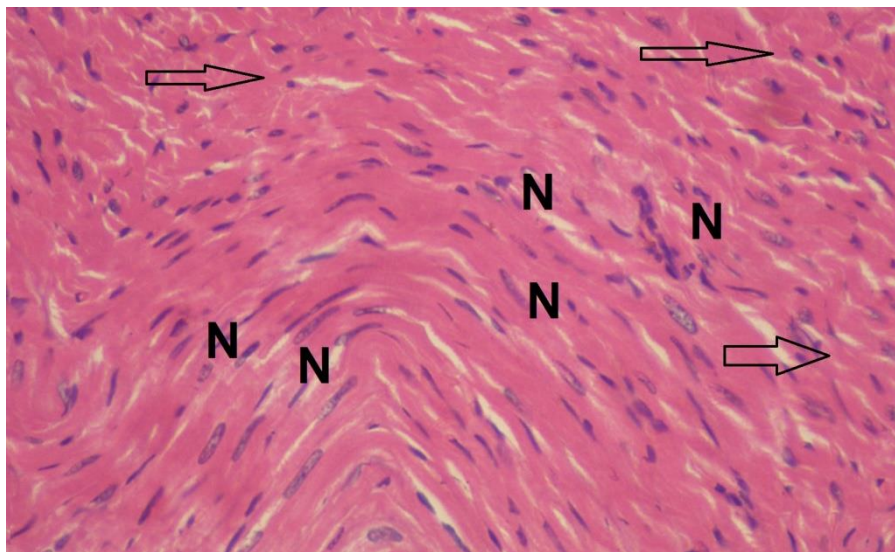


Figure (4): A photomicrograph of a section from heart of a doxorubicin and lycopene treated rat showing that most of muscle fibers are normal and contained acidophilic sarcoplasm and central pale oval nuclei (N). Some muscle fibers showed necrosis and their nuclei were lost or darkly stained (arrow) (H & E X 400).

DISCUSSION

Doxorubicin (DOX) is one of the commonest used antineoplastic drug (Binaschi et al, 2001). Despite its beneficial effects in treating cancer, the clinical use of DOX has a serious limitation due to its cardiotoxic effect (Jones et al., 2006; Simunek et al., 2009). This cardiotoxicity is a common cause of morbidity and premature mortality (Scott et al., 2011). So, strategies to decrease its cardiotoxic side effects are necessary (Dolinsky et al., 2013). Adjuvant therapies that protect the heart tissues without interference with tumor treatment are needed. Such drugs could be beneficial in preventing cardiomyopathy as well as permitting the use of more effective dosages of DOX (Liu et al., 2014).

While research has suggested that the DOX-induced cardiotoxicity is multifactorial (Yi et al., 2006; Scott et al., 2011), many studies support the involvement oxidative stress as a contributing factor (Simunek et al., 2009). So, we aimed to evaluate and compare the possible beneficial effects of lycopene, and saffron as antioxidants against Doxorubicin- induced cardiotoxicity in adult male albino rats.

The present findings indicated that the combined treatment with either lycopene or saffron improved the cardiotoxic effects of doxorubicin in rats. These findings included a decrease of LDH and CBK-MB in serum, the preservation of heart histopathological morphology, a decrease in oxidative stress markers in cardiac tissue as well as an improvement in blood pressure.

Our findings have confirmed the previous reports that, DOX treatment leads to decreased both body and heart weights in animals (Li et al., 2009; Razmaraii et al., 2016c). We found

that, lycopene and saffron treatment increased body and heart weights, as compared to DOX treated group.

Doxorubicin (DOX)- induced heart toxicity is characterized by free radicals' generation in the heart (Gao et al., 2015; El-Sayed et al. 2016). The present study proved that the activity of SOD and CAT were significantly decreased in the DOX group and the co-treatment with either lycopene or saffron increased SOD and CAT activity. MDA is a marker for lipid peroxidation (Torun et al., 2009). In the present study, blood levels of MDA were markedly increased in the DOX group, and this was significantly reversed in antioxidants treated groups.

The protective effects of saffron and its antioxidant effect have been shown against many chemical-induced cardiovascular toxicity as Diazinon (Razavi et al., 2013), and isoproterenol (Goyal et al., 2010). In line with our study, Chahine et al (2016) mentioned that, SAF has antioxidant therapeutic effect against DOX cardiotoxicity.

Yilmaz et al. (2006) mentioned the protective role of lycopene in DOX-induced cardio and nephro toxicities using both biochemical and histopathological assessments and explained the role of lycopene in inhibiting lipid peroxidation and improve antioxidant parameters in both heart and kidneys.

This study showed significant deterioration of cardiac function in the DOX-treated rat group compared to the control group. A number of parameters were investigated to assess cardiac damage after DOX treatment. Heart rate has been considered as an independent risk factor in cardiovascular disease (Cooney et al., 2010). This study showed a significant

decrease in the heart rate in the DOX-treated group. Also, DOX caused a decrease in the systolic, diastolic, mean BP. These results were in line with previous results of (Ozdoğan et al., 2011; Momin et al., 2012; Ammar et al., 2015). This hemodynamic disturbance caused by DOX could be attributed to disruption of the myofibrils (Weinberg and Singal, 1987; Momin et al., 2012).

When saffron was admitted as a co-treatment it was able to prevent the development of DOX induced hemodynamic disturbance which was consistent with Sachdeva et al. (2012) who reported that saffron treatment for 4 weeks improved systolic, diastolic, and mean arterial pressure in rat myocardium exposed to large doses of isoproterenol. Also, another study was stated that, Crocin (20 mg/kg, IP, for 28 days) improved toxic effects of diazinon by decreasing lipid peroxidation and restoring disturbed contractile and relaxant responses in rat aorta. Also, concurrent administration of crocin and diazinon could restore the effects of subchronic diazinon administration on both systolic blood pressure and heart rate in rats (Razavi et al., 2013; 2014).

The improvement in cardiac function may come from the anti-arrhythmic effect of saffron and the preventive loss of contractile proteins (Troponin C and α -actinin). Also, saffron scavenges ROS in the affected myocardium leading to less damage in cells and preservation of contractile proteins (Nader et al., 2016).

Regarding cardiac function in combined DOX-lycopene treated group, there was significant increase in the systolic, diastolic, mean BP, heart rate in combined DOX & lycopene

treated group when compared to DOX treated group. This protective effect of lycopene on DOX-induced hemodynamic instability came in agreement with Aman et al., (2012), who found that supplementation of LYP significantly attenuated the hemodynamic changes occur in myocardium exposed to toxic doses of isoproterenol.

The serum LDH and CPK-MB enzyme activities are considered as important measures in early and late phases of cardiac injury. These enzymes are released from the heart into the blood during myocardial damage due to deficiency of oxygen or glucose, the cell membrane become permeable or may rupture and results in the leakage of it in the serum (Arya et al., 2006). This study stated that, serum LDH and CPK-MB were increased after Dox administration in rats. These results are in good agreement with findings of (Koti et al., 2009; Swamy, et al., 2012).

Both CPK-MB and LDH are not specific for myocardial injury individually; however, evaluation of these two enzymes together may be an indicator of myocardial injury (Swamy, et al., 2012).

Regarding serum LDH and CPK-MB enzyme in antioxidant treated groups, there was highly significant difference in combined DOX and LYC when compared to DOX treated group, while there was significant difference in combined DOX and SAF in comparison to DOX only treated group. The potential protective effects of LYC and SAF against cardiac enzymes changes in the present study were consistent with previous work of Lin et al., (2016) and Mehdizadeh et al. (2013) respectively.

Cardiac troponin is a complex of three regulatory proteins (troponin C, I, and T), attached to the actin filament in skeletal or cardiac muscle tissue, and is important for muscle contraction. Its levels are used as a diagnostic marker for different heart disorders including DOX-induced cardiotoxicity (**Mahajan and Jarolim, 2011**). Increased level of cardiac troponin in the DOX-treated rats of this study was in line with several studies (**Thompson K et al., 2010; Lipshultz et al., 2012; Desai et al., 2013**).

Cardiac troponin I levels were highly significantly decreased in the DOX-lycopene treated rats when compared to DOX-treated rat group, and significantly decreased in DOX-saffron when compared to DOX-treated rat group.

Previous reports have documented that saffron consumption could prevent cardiac toxicity and decreased serum levels of cardiac troponin I in rats with isoproterenol induced myocardial toxicity (**Joukar et al., 2010**), and that lycopene, given in a dose of 4mg/kg, orally once daily for 21 days, could improve cardiac lesion parameters, including cardiac troponin in isoproterenol-induced cardiotoxicity (**Mohamadin et al., 2012**).

In the present study, rats of the control group did not show any histopathological changes and heart showed normal appearance (**fig.1**).

On the contrary, various histopathological changes such as interstitial edema, focal myocardial fibrosis, perinuclear vacuolation and myocardial necrosis were observed following DOX treatment (**fig.2**). These results were reported in different experimental animal studies (**Yilmaz et al., 2006; Dogan et al., 2010;**

Razmaraii et al., 2016 a, c).

Treatment with either saffron or lycopene demonstrated less disruption of the myofibrils and less vacuolization of the cytoplasm (**fig.3, 4**).

In line with the present study, **Karimi et al. (2005)** who stated that doxorubicin induced histopathological changes in cardiac tissues which prevented by lycopene treatment.

Also, Yilmaz et al., (2006) found similar results.

According to **Joukar et al. (2010)** saffron administration decrease histopathological cardiac lesions in rats exposed to isoproterenol induced myocardial toxicity.

Razmaraii et al., (2016 B) showed that saffron treatment significantly improved DOX-induced structural changes in the myocardium.

Many studies try to identify the mechanism of DOX induced cardiotoxicity (**Singal et al 1997; Minotti et al., 2004; Razmaraii et al., 2016 c**), but no single explanation was able to clarify the cause of it (**Tokarska-Schlattner et al., 2006**).

The most important cause of DOX related cardiotoxicity was attributed to oxidative stress. So, antioxidants can be protective against this by free radical scavenging ability (**Horenstein et al., 2000; Ferreira AL et al., 2008**).

Currently, dexrazoxane, is the only antioxidant drug approved by the US Food and Drug Administration for clinical use with Doxo to decrease its cardiotoxicity. Despite that, its administration is limited due to the controversial results linking its use with the risk of secondary malignancies (**Tebbi et al., 2007**). So, searching for other beneficial antioxidants is mandatory.

Many studies have been proved that

lycopene has powerful antioxidant activity both in vitro and in vivo 100 times more efficiently than vitamin E and 125 times more than glutathione (Stahl and Sies, 2007; Mein et al., 2008).

Saffron high antioxidant capacity was investigated by many studies (Ochiai et al., 2004; Magesh et al. 2006; Kanakis et al. 2007; Soeda et al. 2007; Tavakkol-Afshari et al. 2008; Asdaq and Inamdar 2010; Bathaie and Mousavi 2010; Karimi et al. 2010; Makhoulf et al. 2011). Several mechanisms explain the protective effects of saffron against cardiotoxicity induced by many chemical, including modulation of cardiac hemodynamic, histopathological and ultrastructural impairments (Goyal et al., 2010), improvement in cardiac markers such as CK-MB (Razavi et al., 2013), and (Mehdizadeh et al., 2013), and alleviation of lipid peroxidation (Razavi et al., 2013).

CONCLUSION

Both SAF and LYC have beneficial effects against doxorubicin induced-cardiotoxicity due to their potent antioxidant effects, with the advantage to LYC over SAF. The advantage of LYC over SAF in the current study may be due its observed cardioprotective effects.

RECOMMENDATION:

As the findings in the current study have not yet been verified by clinical investigations, so, human trials should be carried out, to establish the potential protective effects of lycopene and saffron in human intoxications. Future experiments are required to evaluate the possible protective molecular

mechanisms of lycopene and saffron against DOX-induced cardiotoxicity. Further animal investigations to examine the possible beneficial effects of combined treatment of lycopene and saffron against DOX-induced cardiotoxicity, are necessary.

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دراسة مقارنة عن آثار الليكوبين والزعفران على التسمم القلبي المسبب بالدوكسوروبيسين في ذكور الجرزان البيضاء البالغة: تقييم نسيجي وبيوكيميائي

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الملخص العربي

المقدمة: دوكسوروبيسين هو أحد أدوية العلاج الكيميائي المستخدمة على نطاق واسع. على الرغم من فعاليته العلاجية واسعة، وقد ذكرت الدراسات السريرية أن العامل الرئيسي الذى يحد من استخدامه كعلاج كيميائي هو آثاره القلبية لأنه غالباً يتسبب فى اعتلال عضلة القلب. ويعتبر الليكوبين والزعفران من مضادات الأكسدة القوية. **هدف البحث:** تم إجراء هذا البحث لدراسة ومقارنة الآثار الواقية من الطماطم الليكوبين والزعفران ضد اعتلال عضلة القلب الناتج من استخدام الدوكسوروبيسين فى ذكور الجرزان البيضاء. **خطة البحث:** أجريت الدراسة على 48 من من الذكور الجرزان البيضاء مقسمة إلى ست مجموعات كالتالى: (المجموعة الضابطة السالبة)، المجموعة الثانية (المجموعة المعاملة بالليكوبين)، المجموعة الثالثة (المجموعة المعالجة بالزعفران)، المجموعة الرابعة (المجموعة المعالجة دوكسوروبيسين)، المجموعة الخامسة (المجموعة المعالجة بالدوكسوروبيسين و الليكوبين)، المجموعة السادسة (المجموعة المعالجة بالدوكسوروبيسين و الزعفران). فى نهاية التجربة، تم الحصول على عينات دم وانسجة من كل فئران للدراسة النسيجية والبيوكيميائية. **النتائج:** أظهرت النتائج ان استخدام الليكوبين والزعفران ادى الى تحسن فى اعتلال عضلة القلب الناجم عن استخدام الدوكسوروبيسين. وقد كان هذا التحسن اكثر مع اليكوبين. **التوصيات:** وينبغي إجراء التجارب البشرية، لتحديد الدور الوقائى المحتمل لكل من الليكوبين والزعفران فى التسمم. و عمل تجارب تكميلية لدراسة الالية العمل لكل منهما فى الوقاية من العتلال القلبي المسبب من الديكسوروبيسين .