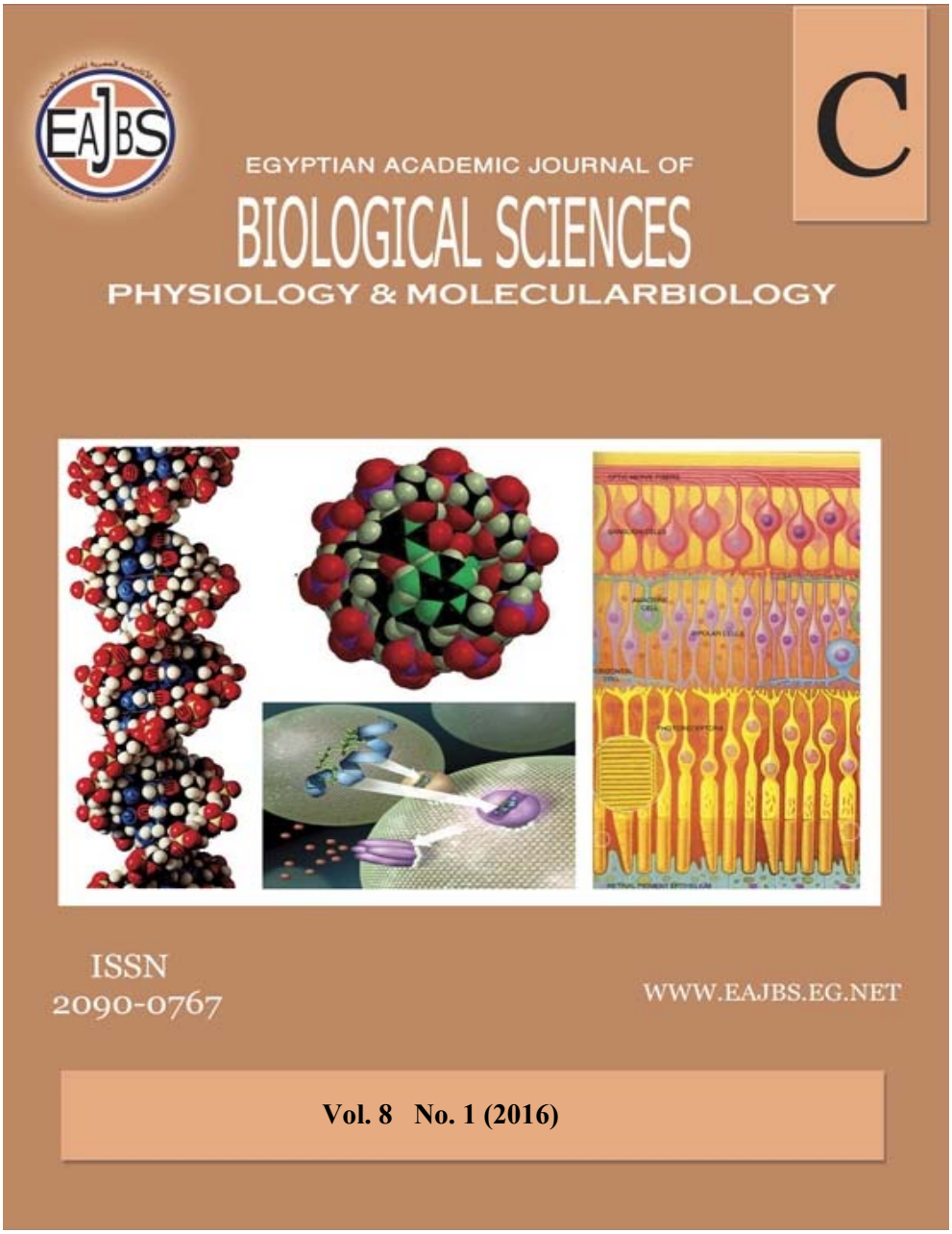

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Comparative Physiological Studies on the Effect of Nano-magnetic Particles (iron oxid) and Graviola Leaves Extract on Adriamycin Induced Cardiotoxicity in Male Albino Rats

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ABSTRACT

Adriamycin (ADR) or Doxorubicin (DOX), is an effective antineoplastic agent commonly used for the treatment of various cancers. ADR is an anthracycline chemotherapeutic containing a quinone group that is known to produce reactive oxygen species (ROS) in heart. . Because of its cardiotoxicity side effects in various tissues, which lead to cardiomyopathy and congestive heart failure, the clinical application is limited. The present study was designed to evaluate the cardioprotective effect of nano-magnetic particles (NFe3O4) and Graviola *(Annona muricata)* leaves extract and combination of them against ADR -induced cardiomyopathy in rats which are divided into 5 groups including one control and four experimental (10 rats per group). They received saline (normal), ADR alone (15 mg/kg body weight, i.p.) (control), ADR followed by NFe3O4 (15 mg/kg body weight, i.p.), ADR followed by Graviola (200mg/kg body weight, oral), and ADR followed by NFe3O4 and Graviola. Animals were sacrificed 28 days after treatment and evaluations were made by measuring cardic enzymes (CK and LDH) in serum. Also the activities of antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) as well as the level of malnodialdhyde (MDA) as a marked of lipid peroxidation . In the ADR-exposed rats, CK, LDH, and MDA significantly increased, while GSH, SOD and CAT enzymes decreased when compared to normal animals. In the Group NFe3O4, graviola leaves extract, and (NFe3O4 +graviola leaves extract alone), CK, LDH and MDA levels significantly decreased, while GSH, SOD and CAT enzymes levels significantly increased when compared to ADR treated animals. The results showed that there is a possibility that the ethanolic extract of graviola and NFe3O4 and combination of them ameliorate the toxicity induced by ADR in rats.

INTRODUCTION

Adriamycin (ADR) is an effective antineoplastic drug commonly used to treat different types of cancer such as ovarian, thyroid, gastric, breast, non-Hodgkin's and Hodgkin's lymphoma, multiple myeloma, and sarcomas (*Cortés-Funes and Coronado, 2007*). Studies around its cardiotoxicity which cause cardiomyopathy and congestive heart failure side effect (*Swain et al., 2003 and Carvalho et al., 2009*).

This side effect is mainly due to ADR–mediated free radical formation (*DeAtley et al., 1998*). Topoisomerase II can be activated by ADR that caused breaks in DNA strands and forming quinine type of free radicals.

It produces cardiotoxicity as a unique adverse effect (*Tripathy, 2010). Olson (1990*) declared that Tissues with less developed antioxidant defense mechanism such as the heart are highly susceptible to injury by anthracycline induced oxygen radicals. Many investigators have described the role of reactive oxygen species including hydroxyl radical in ADR–induced cardiotoxicity (*Sarvazyan et al., 1995*).

The level of ADR-induced oxidative stress is up to 10 times greater in the heart than in the other tissues (liver, kidney, spleen) (*Doroshow* and *Davies, 1986 and Mukherjee et al., 2003).*

Some nanoparticles, such as nickel, cobalt and iron are known as magnetic nanoparticles because of magnetic properties and stability *(Lu et al., 2007*). On the other hand, Due to unique size and physical properties of nanoparticle (NP) materials, they have many uses and advantages (*Faraji, et al., 2010*). Among these metallic nanoparticles is iron oxide (IO) which have received special attention because of their variety of scientific and technological applications such as hyperthermic cancer treatments, cell sorting and targeted drug delivery (*Gupta et al, 2005 and Lida et al., 2007*).

It appears that nano materials hold excessive potential to pass some of the barriers to efficient targets of cells and molecules in many diseases (*Said et al., 2012*). Still further studies are needed to find the mechanism of the nano material defensive effects.

Graviola (*Annona muricata* L.) is a genus of tropical fruit trees belonging to the family Annonaceae, of which there are approximately 119 species. It is known as soursop in English-speaking countries and is referred to by numerous common names (*Blench et al., 2007*). It is more known as soursop, guanabana, nangka blanda, prickly custard apple or durian belanda.

Studies done on the leaves of graviola have been resulted in the separation of eight cytotoxic Annonaceae acetogenins (*kim et al., 1998*).

Padmaa et al., (2009) showed that various biological properties are owned by Acetogenins (Ace) including the cytotoxic effect against the neoplastic cells which suggests their potential use as the antitumor agents. Acetogenins possess the capacity to reduce the mouse colon crypts that is induced by azoxymethane (Azo) and was found that 50% reduction in the amount of crypts in the animals treated with acetogenin when compared with the level determined in mice treated with Azo (*Padmaa Paarakh et al., 2009).*

The leaves of graviola are also hepatoprotective against carbon tetrachloride and acetaminophen-induced liver damage and in streptozotocin-treated diabetic rats *(Adewole and Ojewole, 2008*). In addition, graviola leaves extracts have antioxidant (*Baskar et al., 2007*) and molluscicidal properties (*Luna et al., 2006).*

The acetogenins in graviola leaves and seeds used as anticancer medication are selective, which means that normal cells are not killed. The cytotoxic effect of acetogenins from graviola leaves and seeds has been studied in vitro on many cancer cell lines, such as human hepatoma, lung carcinoma, human breast solid tumor, prostate adenocarcinoma, pancreatic carcinoma, colon adenocarcinoma, human lymphoma and multi-drug resistant human breast adenocarcinoma (*Gholse et al., 2012).*

According to world health organization (WHO), greater than 80% of the total world's population depends on the traditional medicines in order to satisfy their primary health care needs. Laboratory research suggests that graviola derived substances may have potential for various future applications since they have shown antinociceptive

and anticancer effects in laboratory experiments *(De Sousa et al.,* 2010). Therefore, the present study was designed to study the effect of NFe3O4 and graviola leaves extract on antioxidant enzymes and cardic enzymes after exposure to ADR in male albino rats.

MATERIALS AND METHODS

Animals:

50 adult male albino rats at age (2-3 month) and weight about (180-200 g) were obtained from the animal house of the Egyptian Organization for Biological Products and Vaccines (VACSERA, Helwan, Cairo, Egypt). They were kept under standard conditions of temperature (23±2oC), and 12h light/dark period, and fed with a standard pellet diet and water ad libitum. In this study, the experimental animals were divided at random into 5 groups of 10 animals of each group.

Drugs and chemicals:

Adriamycin (Doxorubicin hydr-ochloride)

ADR is an anthracycline antibiotic represents a class of anticancer agents composed of an amino sugar (daunosamine) linked by an O-glycosidic bond to an aglycone (doxorubicinol) was obtained from Ebewe Pharma co. Austria.

Graviola (Annona muricata)

Commonly called sour-sop or graviola, is a small erect evergreen tropical fruit tree plant belonging to the family Annonaceae, growing 5-6 meters in height. Graviola's leaves has been reported to contain several groups of substances collectively called annonaceous acetogenins. Graviola were obtained from farme (Deshna, Qena, Egypt) in January 2014. They were taxonomically identified by the (Botany Department, Faculty of Science, South Valley University).

Ethanolic extract of graviola leaves:

Graviola fresh leaves were air-dried at room temperature .The air-dried leaves of the plant were milled into fine powder in a waring commercial blender 150 g of the powdered leaves were extracted with 500 ml ethanol for three days (with occasional shaking .(The extract was concentrated in a rotary evaporator at a reduced pressure to yield crude ethanolic extract ،the crude extract thus obtained was refrigerated at 40^{o}C (Gavamukulya *et al*., 2015) and subsequently used in this study 200 mg of this extract/kg body weight was dissolved in distilled water and administered to the animals

Magnetic Iron Oxide nanoparticles:

Magnetic iron oxide nanoparticles was used in this experiment as an antioxidant and synthesized by co-precipitation method in an electronics and nano-devices lab, Physics department, South Valley University, Qena, Egypt. For biological and biomedical applications, magnetic iron oxide nanoparticles are the primary choice because of their biocompatibility and chemical stability (Matheson *et al*., 1994 and Sudhanshu *et al*., 2012).

Preparation of iron oxide nano particles (NFe3O4) suspension:

NFe3O4 particles were suspended in deionized water, the solution from above was affected by ultrasonic continuously for 60 min, then cooled rapidly to below 10ºC, the solution was centrifuged for 20 min and 104r/min, an amount of black magnetite was aggregated at the bottom of the centrifuge tubes, the supernatant liquid collected from tubes was passed through 0.22 micron filter and then the sample was obtained, In order to avoid the aggregation of the particles fresh suspension was prepared before every use (Xia Zefeng *et al*. , 2005).

Experimental Design

The first group (normal): rats were intraperitoneal (i.p.) received saline solution for 36 days.

The second group (control): rats were intraperitoneal (i.p.) received ADR alone (5mg/kg/day) for 3 consecutive

days and then received saline solution for the remaining of 36 days.

The third group: rats were intraperitoneal (i.p.) received ADR (5mg/kg /day) for 3 consecutive days and after one day, rats were intrapertioneal (i.p.) received NFe3O4 (5mg/kg/day) for 3 consecutive days, and then received saline solution for the remaining of 36 days.

The fourth group: rats were intraperitoneal (i.p.) received ADR (5mg/kg/day) for 3 consecutive days, one day later, rats were received graviola orally(200 mg/kg/day) for 28 consecutive days and then received saline solution for the remaining of 36 days.

The fifth group: rats were intraperitoneal (i.p.) received ADR (5 mg/kg/day) for 3 consecutive days and after one day ,rats received NFe3O4 (i.p.) (5mg/kg/day) for 3 consecutive days and one day later, rats received graviola (200 mg/kg/day) orally for 28 consecutive days.

The end of experiment, the animals was sacrificed by decapitation. Blood samples of all animals prepared from retro orbital eye vein. Samples were collected in clean tubes at room temperature to clot then after an hour; serum was separated by centrifugation for 30 minutes at 3000 rpm. The serum were collected in labeled eppendorf tubes and stored at -20 °C until used for biochemical analysis. (0.3gm) of Heart were washed with ice-cold buffer saline, blotted with a piece of filter paper and homogenized using a Branson sonifier (250, VWR Scientific) and stored at -80°C until used for determination of GSH, CAT, SOD, and MDA content.

Biochemical analysis:

Lactate dehydrogenase activity was estimated in serum by commercially available LDH kit (Biosystems S. A. co. Egypt) according to the colorimetric method of (Tietz ,1994 and Friedman and young, 1997). Creatine kinase activity was estimated in serum by commercially available CK assay kit (BioAssay Systems, USA) according to the method of (Bishop *et al*., 1971).

The antioxidant enzymes (GSH, SOD, CAT and MDA) were brought from bio-diagnostic co. Giza.Egypt.

GSH was determined by colorimetric method described by (Beutler *et al*., 1963), SOD was determined by colorimetric method described by (Nishikimi *et al*., 1972), CAT was determined by colorimetric method described by (Aebi, 1984) and Determination of MDA was carried out according to the method of (Ohkawa *et al*., 1979).

Statistical analysis:

The variability degree of results was expressed as Means S.D. The data were statistically analyzed by one-way ANOVA analysis of variance (prism computer program, year) and the least significant difference (L.S.D) was used to test the difference between treatments. Results were consider statistically significant when $P < (0.01)$.

RESULTS

A) Serum cardiac enzymatic parameters

1): Effects of NFe3O4, graviola leaves extract and (NFe3O4 + graviola leaves extract alone) on ADR-induced alterations in serum creatine kinase (CK) and lactate dehydrogenase (LDH) activities in rats.

In ADR treated animals a highly significant increase in the level of cardiac markers i.e CK and LDH at ($p<0.01$) levels were observed when compared with normal animals. The CK level in NFe3O4, graviola and (NFe3O4 + graviola leaves extract alone) treated animals showed a highly significant decrease at ($p<0.01$) as compared to ADR treated animals, but it was still more than normal animals. Whereas LDH level in NFe3O4, graviola and (NFe3O4 + graviola leaves extract alone) treated animals showed a highly

significant decrease at ($p<0.01$) as compared to ADR treated animals, but it was still more than normal animals. (Table 1, Figs.1& 2).

Table.1: Effects of NFe3O4, graviola leaves extract and (NFe3O4 + graviola leaves extract alone) on ADR-induced alterations in serum creatine kinase (CK) and lactate dehydrogenase (LDH) activities in rats.

Parameters Group	C.K (U/L) Mean ± S.D.	LDH (U/L) Mean ± S.D.
Normal rats	257.7± 34.72	360.3± 39.14
Control rats(ADR)	637.7± 29.85 ++a	681.3 ± 29.80++a
ADR+NFe3O4	436.0 ±19.19++a--b	397.3 ± 42.31 +a-b
ADR+ graviola	496.3 ± 41.3++a--b	457.3± 25.24++a-b
ADR +NFe3O4+graviola	344.7± 22.40++a--b	396.6 ± 16.25+a-b

The result is presented the mean± S.D. of 10 rats.
++ Highly significant increase at ($p<0.01$).
– – Highly significant decrease at ($p<0.01$).
+ Significant increase at ($p<0.05$).
– Significant decrease at ($p<0.05$).
a → significantly different from normal rats.
b → significantly different from control group.

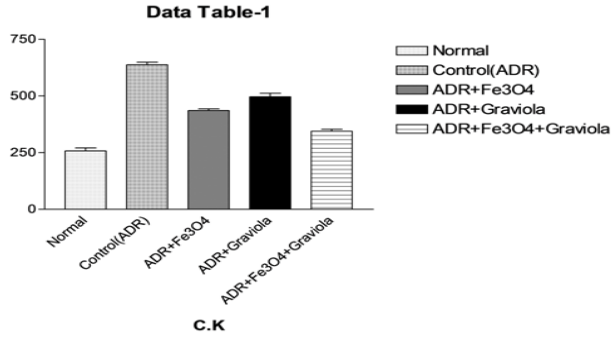

Fig. 1: Effects of NFe3O4, graviola leaves extract and (NFe3O4 + graviola leaves extract alone) on ADR-induced alterations in serum creatine kinase (CK) activities in rats.

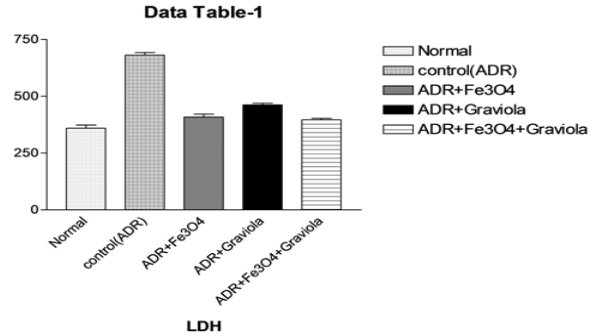

Fig. 2: Effects of NFe3O4, graviola leaves extract and (NFe3O4 + graviola leaves extract alone) on ADR-induced alterations in serum lactate dehydrogenase (LDH) activities in rats.

B) Heart homogenate biochemical analysis.

1) Effects of NFe3O4, graviola leaves extract and (NFe3O4+graviola leaves extract alone) on ADR-induced alterations in oxidative stress biomarkers (CAT, SOD, MDA and GSH activities) in cardiac tissues of rats:

In this study ،The ADR treated animals showed a highly significant decrease in the level of CAT as

compared to normal animals. NFe3O4, graviola leaves extract and(NFe3O4 + graviola leaves extract alone) treated animals showed a highly significant ($p<0.01$) increase in CAT level compared with ADR treated animals, but when compared with the normal rats the results indicated that CAT activity were improved but not approached to the normal level (Table 2, Fig 3).

ADR treated animals showed a highly significant decrease in the level of SOD as compared to normal animals. NFe3O4 and (NFe3O4 + graviola leaves extract alone) treated animals showed a highly significant ($p<0.01$) increase in SOD level compared to that of ADR treated animals and graviola leaves extract showed a significant ($p<0.05$) increase in SOD level compared with ADR treated animals, but when compared with the normal rats the results indicated that the SOD activity still lower than normal rats (Table 2, Figs 4).

ADR treated animals showed a highly significant increase in the levels of MDA as compared with normal animals. NFe3O4, graviola leaves extract and (NFe3O4 + graviola leaves extract alone) treated animals showed a highly significant ($p<0.01$) decrease in MDA levels compared to ADR treated animals, While the concentrations of MDA still higher than the normal level(Table 2, Fig 5).

Results showed that ADR treated animals caused a highly significantly decreased in the level of GSH as compared with normal animals. However, NFe3O4, graviola leaves extract and (NFe3O4 + graviola leaves extract alone) treated animals showed a highly significant ($p<0.01$) increase in GSH level compared with that of ADR treated aninals while GSH activity still lesser than the normal level (Table 2, Fig 6).

Table. 2: Effect of NFe3O4, graviola and NFe3O4 + graviola leaves extract alone on CAT, SOD, MDA and GSH activities in ADR- induced Cardiotoxicity in cardic tissues of rats.

Parameters / Group	CAT (U / g.tissue) (Mean ± S.D.)	SOD (U / g.tissue) (Mean ± S.D.)	MDA (µmol/gm tissue) (Mean ± S.D.)	GSH (µmol/gm tissue) (Mean ± S.D.)
Normal rats	1.46± 0.22	592.7± 7.04	15.12± 0.27	3.78± 0.12
Control rats(ADR only)	1.26 ±0.03 $^{--a}$	504.1± 0.07 $^{--a}$	18.52 ±0.24 $^{++a}$	1.20± 0.07 $^{--a}$
ADR+NFe3O4	1.34± 0.026 $^{--a++b}$	542.3± 5.39 $^{--a++b}$	16.19 ±0.35 $^{++a--b}$	3.05 ±0.11 $^{--a++b}$
ADR+ graviola	1.32± 0.02 $^{--a++b}$	526.4± 4.30 $^{--a+b}$	16.30±0.26 $^{++a--b}$	2.80±0 .10 $^{--a++b}$
ADR +NFe3O4+graviola	1.40 ±0.02 $^{--a++b}$	566.4± 3.76 $^{--a++b}$	15.72±0.18 $^{++a--b}$	3.24± 0.06 $^{--a++b}$

The result is presented the mean ± S.D. of 10 rats.
++ Highly significant increase at ($p<0.01$) from normal rats.
– – Highly significant decrease at ($p<0.01$) from control rats.
a → significantly different from normal rats.
b → significantly different from control rats.

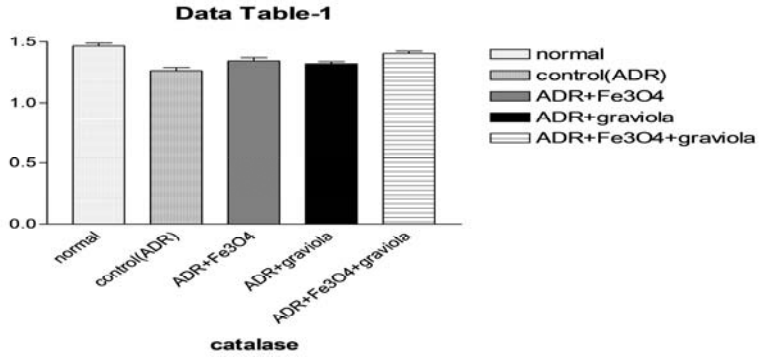

Fig. 3: Effects of NFe3O4 graviola leaves extract and (NFe3O4+graviola leaves extract alone) on ADR-induced alterations in CAT activity in cardiac tissues of rats.

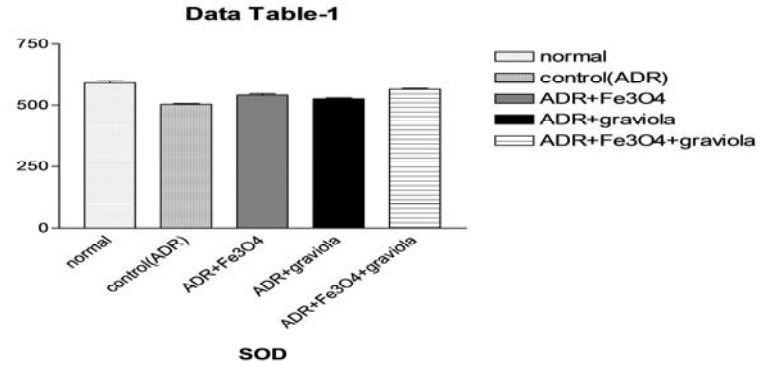

Fig. 4: Effects of NFe3O4 ،graviola leaves extract and (NFe3O4+graviola leaves extract alone) on ADR-induced alterations in SOD activity in cardiac tissues of rats.

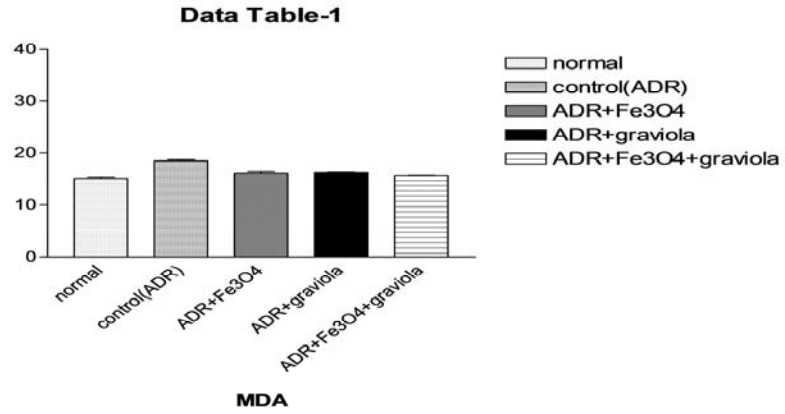

Fig. 5: Effects of NFe3O4, graviola leaves extract and (NFe3O4+graviola leaves extract alone) on ADR-induced alterations in MDA activity in cardiac tissues of rats.

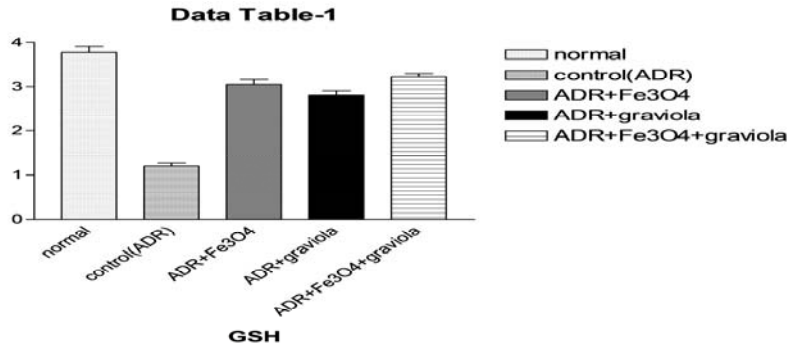

Fig. 6: Effects of NFe3O4 ،graviola leaves extract and (NFe3O4+graviola leaves extract alone) on ADR-induced alterations in GSH activity in cardiac tissues of rats.

DISCUSSION

Adriamycin (ADR) is an anthracycline antibiotic having a very potent antitumor action, which is widely used for the treatment of various cancers. The clinical use of ADR is limited. It is a dose dependence cardiotoxicity which may lead to severe and irreversible form of cardiomyopathy with congestive heart failure and high mortality is one of the factors that limit its use (*Alkreathy et al., 2010*). The mechanisms of cardiac toxicity are not fully understood and are thought to include heightened oxidative stress status leading to apoptosis of endothelial cells and cardiomyocytes (*Mukhopadhyay et al., 2009*).

One of the known mechanisms of antitumor activity of anthracyclines, like ADR, is the generation of free radicals. ADR undergoes a redox-cycling reaction during which superoxide and hydrogen

peroxide (ROS) are produced. Subsequently, iron ions can catalyze the generation of hydrogen radicals from hydrogen peroxide by Fenton-type reaction, the formation of which can break mitochondria, lipids, proteins, DNA and other structures in tumor cells and finally lead to apoptosis or necrosis (*Ravi et al., 2004*).

In present study, increased lipid peroxidation and reduction in superoxide dismutase activity in response to ADR administration all together support an oxidative mechanism of ADR-toxicity. Many investigators have described the role of reactive oxygen species including hydroxyl radical in ADR induced cardiotoxicity (*Dorr, 1996*). A relationship between ADR induced cardiotoxicity and oxidative stress has been confirmed in many experimental models.

Due to highly oxidative metabolism and relatively poor antioxidant defenses that occur in heart that make the cardiac cells more susceptible to free radical damage. Furthermore, ADR also has high affinity for the phospholipid component of mitochondrial membrane in cardiac myocytes, leading to accumulation of ADR in cardiac tissue *(Neha et al., 2014*).

Data of the present study have indicated that, treatment with ADR led to severe cardiomyopathy as indicated from the increase in serum activities of cardiac enzymes such as lactate dehydrogenase (LDH) and creatine kinase (CK). These enzymes are present in sufficiently high content in myocardial tissue so that the death of a relatively small amount of tissue results in a substantial increase in measured enzyme activity in serum. *Parker et al.,* (2001) suggest that mitochondria are the target organelle of doxorubicin-induced free radical toxicity in myocytes .

An important factor, which can mediate the damaging action of ADR in myocardial tissues, especially in mitochondria, is high affinity binding of ADR to cardiolipin, an anionic phospholipid in the inner mitochondrial membrane (*Parker et al., 2001*) leading to dissociation of cardiolipin-associated peripheral proteins from the inner mitochondrial membrane, like cytochrome c and mitochondrial creatine kinase resulting in initiation of programmed cell death (*Tokarska-Schlattner et al., 2005*).

Similar observations were obtained by *(Swamy et al., 2011 and Octavia et al., 2012).* Generation of free radical extensively damage the myocardium result in increased membrane permeability leads to leakage of LDH, CK. *Ragavendran et al., (2012)* reported that ADR treatment increases the morbidity and mortality of cancer patients due to the heart failure (*Ragavendran et al., 2012)* .

In agreement with the present finding, many authors found also significant increase in LDH and CK at dose (20 mg/kg) of ADR (*Ihab T et al., 2009)* and at dose (10 mg/kg) (*Neha et al., 2014*). In the present study, it was observed the increasing level of MDA and decreasing level of GSH, SOD and CAT in heart tissue in ADR treated animals. *Ihab et al., (2009)* found out lipid peroxidation increasing and superoxide dismutase activity reduction in response to ADR administration, all together support an oxidative mechanism of ADR-toxicity. Also, *Su et al., (2009*) reported that doxorubicin administration to rats significantly increased lipid peroxide expressed as TBARS, and decreased both glutathione peroxidase and superoxide dismutase activities in cardiac tissues.

In the present study, ADR significantly decreased the level of tissue GSH in accordance with the previous studies (*Neha et al., 2014*). Decrease in the levels of GSH represents its increased utilization by myocardial cells due to

oxidative stress.

In present study, a significant decrease in levels of SOD and CAT enzymes in ADR treated animals was observed.

A decrease in the activity of SOD can result in the decreased removal of superoxide ion, which can be harmful to the organs. The observed elevated CAT levels in ADR treated animals support the above hypothesis that this increase is possibly required to overcome excessive oxidative stress (*Li et al., 2000*).

The aim of the present study is to evaluate the effects of ADR on the antioxidant defense system and the cardioprotection afforded by NFe3O4, Graviola and (NFe3O4 + Graviola alone).

The results of the present work showed that group of rats injected with NFe3O4 showed a significant decrement of LDH and CK. Other present results are consistent with other studies stated that NFe3O4 were found to inhibit the ADR-induced CK and LDH release in serum. In accordance with the present study Mihaela Radu (2015) found a significant decrease of lactate dehydrogenase (LDH) in CD-1 mice lungs injected with a single dose of iron oxide nanoparticles coated with phospholipid-based polymeric micelles (IONPs-PM) at dose of 5 and 15 mg/kg B.W. The results of this study also showed an in increase in the levels of SOD, CAT and GSH and a decrease in MDA.

Minotti et al., (2004) indicated that the tumor cell one-electron redox cycling of ADR and iron occurs. One electron addition to the quinone moiety in ring C of ADR is known to result in formation of a semiquinone that quickly regenerates its parent quinone by reducing oxygen to ROS like superoxide anion (O_2 −) and hydrogen peroxide (H_2O_2). This futile cycle is supported by a number of NAD (P) H-oxidoreductases. The reaction is accompanied by the release of iron (II) from ferritin *(Minotti et al., 2004*). ADR releases Fe (II) through direct interactions of O_2 with the ferritin core or through electron (e−) tunneling from its semiquinone to the iron core, a mechanism likely shared by physiologic cellular reductants. The presence of ions Fe_{2+} increases the number of free radicals in the tumor. Fenton reaction is highly probable: iron catalyzed hydrogen peroxide conversion to more powerful and destructive hydroxyl free radical, which may cause molecular damage and cell death. ADR and iron generate reactive oxygen and nitrogen species-induced apoptosis and necrosis and the surface of the magnetic nanoparticle offers a suitable substrate for the catalysis to occur (*Orel et al., 2015*).

In addition to improvement of serum cardiac enzymes like LDH and CK, NFe3O4 also ameliorated the altered oxidative stress biomarkers. NFe3O4 markedly increased the reduced glutathione (GSH) levels and augmented the superoxide dismutase (SOD) activity and catalase (CAT) in heart tissues that was attenuated by doxorubicin treatment. On the other hand, Iron oxide nanoparticles decreased the elevated lipid peroxide (TBARS) levels in ADR-treated rats.

The results of the present work showed that group of rats administered Graviol showed a significant decrease of LDH and CK.

Phytochemical analysis helps detect the chemical constituents of plants extract in search of bioactive agents as basis for drug synthesis. The presence of saponins, condensed tannins and glycosides as the major constituents and trace amounts of flavonoids contribute immensely to the bioactivity of graviola and also to its usage in treating various diseases. These have included antioxidant activity (*Adewole and Ojewole, 2009*), hepatoprotective effect and antibacterial agent (Jeya Sheela *et al*., 2012).

Gavamukulya Y et al., (2014)

declare that the previous study, reported that ethanolic leaves extracts of graviola showed anticancer and antioxidant activities, so the antioxidant activities lead to decrease in LDH and CK.

Antioxidants are substances that either directly or indirectly protects cells against adverse effects of xenobiotics, drugs, carcinogens and toxic radical reactions *(Halliwell, 1995).* Its antioxidants work in several ways by reducing the energy of the free radicals; stop the free radical from forming in the first place; or interrupt an oxidizing chain reaction to minimize the damage of free radicals.

The results of the present work showed that group of rats administered Graviola showed a significant decrease of MDA. The protective activity supported by increased myocardial antioxidant enzyme activity and decrease extent of lipid peroxidation. Treatment with graviola has significantly restored the GSH levels, this effect could be attributed either to increased biogenesis of GSH or the reduction in oxidative stress levels leading to decreased generation of toxic free-radical species.

The antioxidant enzymes such as SOD and CAT constitute the major supportive team of defense against free radicals.

In present study, a significant decrease in levels of SOD and CAT enzymes in ADR treated group was observed. Graviola *leaves* extract treatment significantly reversed the changes in antioxidant levels induced by ADR. A decrease in the activity of SOD can result in the decreased removal of superoxide ion, which can be harmful to the organs. Moreover, the enhanced SOD activity in graviola *leaves* extract treated group might be involved in the scavenging of O2 - generated from ADR.

Graviola leaves extract efficiently counteracted the ADR induced cardiac tissue damage by significantly decreasing the MDA levels and increasing the GSH, SOD and CAT activities. Lipid peroxidation is known to cause cellular damage and is primarily responsible for ROS induced organ damage *(Halliwell, 1989*). Present study shows that ADR has considerably increased the MDA levels, which was significantly prevented by *graviola extract* treatment.

Wohaib and Godin (1987) showed when used graviola leaves extract caused significant decreases in the MDA levels of the diabetic rats. A significant elevation of hepatic activities of CAT, SOD and GSH level were also observed in the graviola leaves extract-treated diabetic rats.

The results of the present work showed that group of rats administered NFe3O4 + Graviola showed a significant decrease of LDH , CK and MDA levels and increase of levels of GSH , SOD and CAT .

CONCLUSION

The present study indicated that the toxicity of ADR on rat hearts is mediated through oxidative stress mechanisms and treatment with NFe3O4 and graviola leaves extract and combination of them reversed most of these negative effects induced by ADR as evidenced biochemically.

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ARABIC SUMMERY

دراسات فسيولوجية مقارنة على تأثير جسيمات النانو المغناطيسية لأكسيد الحديد و مستخلص أوراق نبات القشطة على السمية المستحدثة بواسطة الأدرمايسين على القلب فى ذكور الفئران البيضاء.

عبدالرحيم على الشاطر١- رانا عبدالستار على٢- هدى ياسين جداوى٣
١-٢-٣ قسم علم الحيوان- كلية العلوم- جامعة جنوب الوادى

يعتبر الأدرمايسين (الدوكسوريبسين) مضاد حيوي من فئة الانثراسيكلين ، هو يمثل نوع من مضادات السرطان الاكثر فاعليه يستخدم عادة لعلاج أنواع مختلفة من السرطان مثل سرطان المبيض والغدة الدرقية و المعدة ، وسرطان الثدي ، غير هودجكين و سرطان الغدد الليمفاوية ، المايلوما المتعددة ، و الأورام اللحمية . وبالرغم من ذلك فان تطبيقاته السريريه تكون محدودة نتيجة آثاره الجانبية الضارة ، و أهمها هو تسمم القلب والذى يؤدى إلى اعتلال عضلة القلب و قصور القلب الاحتقاني .

ولقد أشارت الدراسات الحديثه التى أجريت على عقارالادمايسين بان لهذا العقار أجهاداً تاكسدياً ناتج من إنتاج الشوارد الحره او من عدم كفاية العديد من أنظمة الدفاع المضادة للأكسدة والتى لها دورا مهما فى تسمم القلب بفعل الادرمايسين .

تهدف هذه الدراسه إلى دراسه التأثير العلاجى لجسيمات النانو المغناطيسية لأكسيد الحديد ومستخلص اوراق نبات القشطة على الأضرار الناجمه عن الأثار الجانبيه من إستخدام الادرمايسين فى علاج الأورام السرطانيه.

وقد تم فى هذا البحث إستخدام الفئران البيضاء التى تزن الواحده منها (١٨٠-٢٠٠) جرام وقسمت الفئران إلى ٥ مجموعات وتشمل المجموعه ١٠ فئران.

المجموعه الأولى: المجموعه الضابطه والتى اعطيت محلول ملحى من كلوريد الصوديوم ٠.٩% فقط لمدة ٣٦ يوم .

المجموعه الثانيه: مجموعه تم حقنها داخل الغشاء البريتونى بثلاث جرعات متتالية من عقار الادرمايسين بتركيز ٥مجم /كجم من وزن الجسم ثم اعطيها محلول ملحى من كلوريد الصوديوم ٠.٩% بقيه ٣٦ يوم .

المجموعه الثالثه: المجموعه المعالجه وتم حقنها داخل الغشاء البريتونى بثلاث جرعات متتالية من عقار الادرمايسين بتركيز ٥ مجم/كجم من وزن الجسم ثم عولجت الحيوانات بعد يوم واحد من حقنها بجرعه الادرمايسين باستخدام جسيمات النانو المغناطيسية لاكسيد الحديد (٥ مجم /كجم من وزن الجسم) عن طريق حقنها داخل الغشاء البريتونى لمده ٣ ايام متتالية ثم اعطيها محلول ملحى من كلوريد الصوديوم ٠.٩% بقيه ٣٦ يوم .

المجموعه الرابعه: هذه المجموعه تم حقنها داخل الغشاء البريتونى بثلاث جرعات متتالية من عقار الادرمايسين بتركيز٥ مجم/كجم من وزن الجسم ثم عولجت الحيوانات بعد يوم واحد من حقنها بجرعه الادرمايسين بإستخدام مستخلص اوراق نبات القشطة (٢٠٠ مجم /كجم من وزن الجسم) عن طريق الفم يوميا لمده ٢٨ يوم ثم اعطيها محلول ملحى من كلوريد الصوديوم ٠.٩% بقيه ٣٦ يوم .

المجموعه الخامسه: هذه المجموعه تم حقنها داخل الغشاء البريتونى بثلاث جرعات متتالية من عقار الادرمايسين بتركيز٥ مجم/كجم من وزن الجسم ثم عولجت الحيوانات بعد يوم واحد من حقنها بجرعه الادرمايسين بجسيمات النانو المغناطيسية لاكسيد الحديد (٥ مجم /كجم من وزن الجسم) لمدة ثلاث ايام متتالية ثم عولجت بعد يوم واحد بمستخلص اوراق نبات القشطة (٢٠٠ مجم /كجم من وزن الجسم) عن طريق الفم يوميا لمده ٢٨ يوم.

وبعد الإنتهاء من حقن الادرمايسين والعلاج تم ذبح الحيوانات طبقا لخطه البحث، وتم تجميع الدم فى انابيب نظيفة لاجراء التحليلات البيوكيميائية و بعد ذلك تم فصل الدم والحصول على السيرم لقياس كل من إنزيمات القلب (لاكتيك ديهيدروجينيزLDH ، كراتين كينيزCK) .

تم وزن جزء معلوم من قلب الحيوانات وحفظه فى درجه حراره -٢٠ لحين طحنه لعمل القياسات الفسيولوجيه لمضادات الأكسده فى أنسجه القلب (MDA، SOD , CAT,GSH).

التحاليل الكيموحيويه فى السيرم أظهرت المجموعة التى حقنت بالادرمايسين زياده ذات دلاله إحصائيه فى مستويات إنزيمات القلب (اللاكتيك ديهيدروجينيز(LDH) والكراتين كينيز(CK) .

اظهرت النتائج أن المعالجه بواسطه جسيمات النانو المغناطيسية لاكسيد الحديد أو مستخلص اوراق نبات القشطة أو كليهما معاً أسفرت عن إنخفاضاً ملحوظاً فى نشاط إنزيمات القلب وذلك عند مقارنه النتائج بالمجموعه الضابطه.

عند قياس الإنزيمات المضاده للأكسده فى نسيج القلب أظهرت النتائج فى تلك القياسات أن الأكسده المستحدثه بواسطه الادرمايسين عن زياده ملحوظه فى كميه المالون ثنائى ألدهيدى (MDA) وإنخفاض نشاط كلا من الجلوتاثيون (GSH) وإنزيم الكتاليز (CAT) وإنزيم السوبر أكسيد ديسميوتيز(SOD) بالمقارنه بالمجموعه الضابطه.

اما الحيوانات المعالجه بجسيمات النانو المغناطيسية لاكسيد الحديد أو مستخلص اوراق نبات القشطة أو كليهما معاً فلقد اظهرت تحسناً كبيراً تمثل فى إنخفاض ملحوظ لكميه للمالون ثنائى الالدهيدى وزياده فى كميه الجلوتاثيون و نشاط إنزيم الكتاليز والسوبر أكسيد ديسميوتيز.

ومن النتائج السابقه نستنتج أن الحيوانات التى تم حقنها بعقار الادرمايسين ثم عولجت بجسيمات النانو المغناطيسية لاكسيد الحديد أو مستخلص اوراق نبات القشطة أو كليهما معاً اظهرت تحسناً ملحوظاً فى فى وظائف القلب كما اوضحت النتائج ايضاً تحسنا ملحوظاً فى نشاط الإنزيمات المضاده للأكسده فى نسيج القلب .