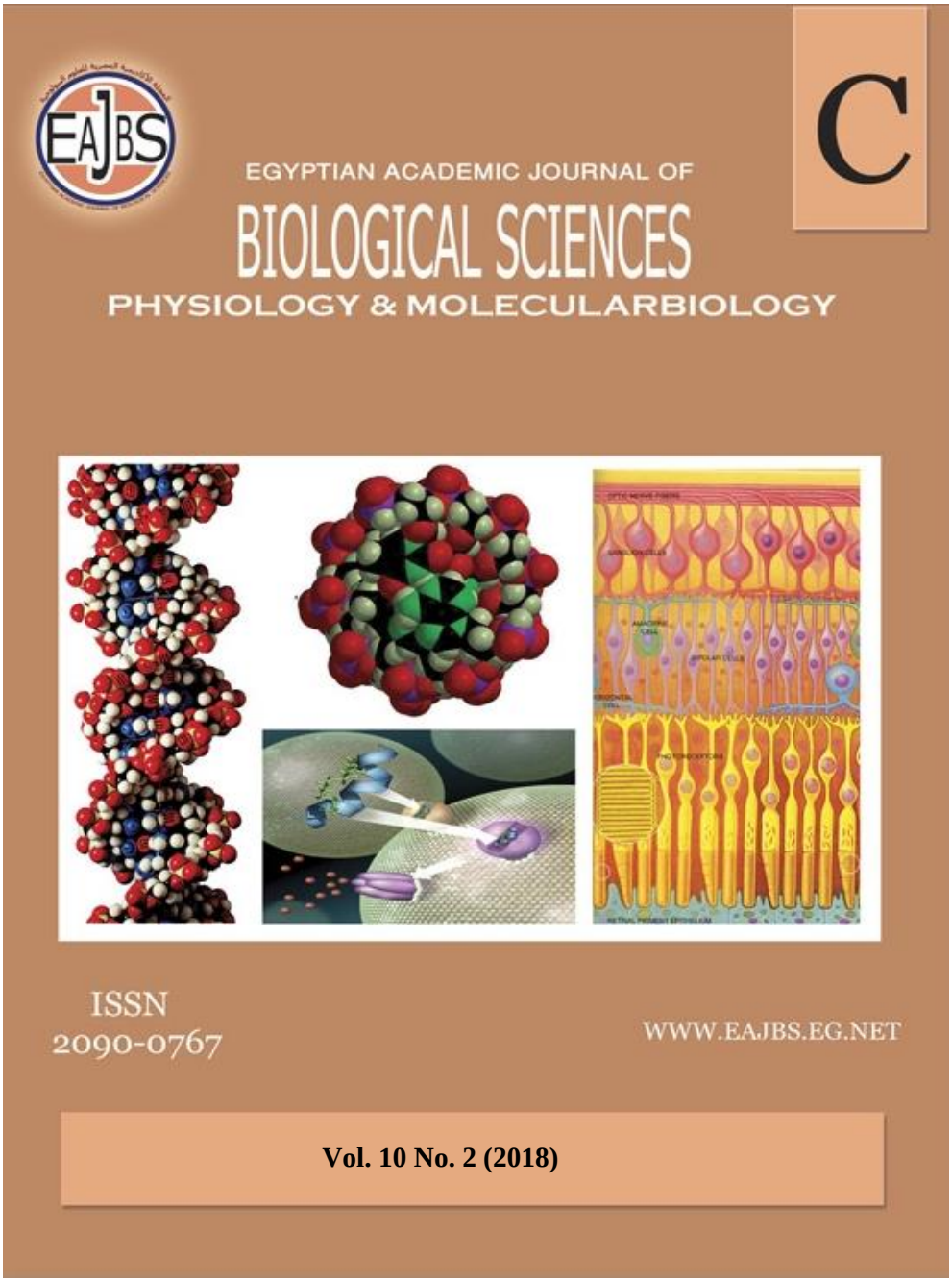

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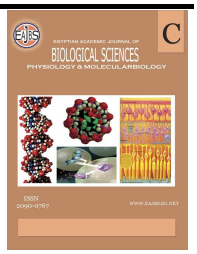

Comparative Biochemical Studies on the Effect of Nano-Magnetic Particles (Nps) and Graviola Leaves Extract on Adriamycin Induced-Gonadotoxicity in Male Albino Rats

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Adriamycin, iron oxide nanoparticles, graviola leaves extract, gonadotoxicity, antioxidant enzymes, serum hormones, rats.

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ABSTRACT

Adriamycin (ADR) is an effective antineoplastic agent commonly used to treat different types of cancer. The use of ADR in clinical chemotherapy is limited due to diverse toxicities, including gonadotoxicity. The present study was designed to evaluate the effect of nano-magnetic particles (NFe3O4) and Graviola leaves extract and (NFe3O4 + Graviola leaves extract) against ADR -induced gonadotoxicity. The rats divided into 5 groups (10 rats per group). The first group (normal g.), the second group received ADR alone (5 mg/kg body weight, i.p.) as (control g.), the third group received ADR followed by NFe3O4 (5 mg/kg body weight, i.p.), the fourth group received ADR followed by Graviola (200mg/kg body weight, orally), and the fifth group received ADR followed by NFe3O4(5 mg/kg body weight, i.p.) and Graviola leaves extract(200mg/kg body weight, orally). Animals were sacrificed 36 days after treatment and sex hormones (Testosterone and F.S.H) in serum. Also, the activities of antioxidants such as (SOD), (CAT), (GSH) and the level of (MDA) as a marker of lipid peroxidation were measured. In the ADR-exposed rats, Testosterone, F.S.H, GSH, SOD and CAT were significantly decreased, while MDA was significantly increased. When compared with normal animals. In the Group NFe3O4, graviola, and (NFe3O4 +graviola), Testosterone, F.S.H, GSH, SOD and CAT enzymes levels were significantly increased, while MDA level was significantly decreased when compared with ADR treated animals. The results showed that there is a possibility that the ethanolic extract of graviola leaves and NFe3O4 ameliorate the gonadotoxicity induced by ADR in rats.

INTRODUCTION

The anthracycline antibiotic Adriamycin, is an anticancer drug, it used for the treatment of many types of tumors, and is known to produce reactive oxygen species (ROS) in various tissues. In spite of its high antitumor efficacy. Though gonadal damage by anticancer drugs like ADR is commonly observed, but it has been relatively less studied compared to the other possible side effects (Sridevi *et al.*, 2012).

(Zanetti *et al.,* 2007) Showed that treatments with ADR cause an important decrease in testicular weight resulted in reduced spermatogenic cell number. The number of sperm was significantly decreased and low sperm motility was also detected in rats (Gamal, 2012). In addition, there is an increased apoptosis in spermatogonia and spermatocytes following ADR treatment (Baumgartner *et al.*, 2004). Acceptance of apoptosis by this medication is one of the soonest indications of genotoxic harm to the grown-up testis. In any case, the systems by which this chemotherapeutic operator harms spermatogenesis not fully elucidated (Hou *et al.,* 2005). ADR produces persistent damage to the spermatogenic cells as well as the increase in the testicular oxidative stress (Saalu *et al.,* 2010). ADR toxicity can be intervened by collaboration with topoisomerase II, a compound that is plenteous in cells with fast expansion (Brilhante *et al.*, 2012).

The use of nanoparticle (NP) materials offer many advantages due to their unique size and physical properties (Faraji *et al.,* 2010) some nanoparticles, such as nickel, cobalt and iron are known as magnetic nanoparticles because of magnetic properties and stability (Lu *et al.,* 2007). One of these magnetic nanoparticles is iron oxide (IO) which has special attention because of its various scientific and technological applications such as hyperthermic cancer treatments, cell sorting and targeted drug delivery (Gupta and Gupta, 2005 and Lida *et al,.* 2005) Besides, it has additionally been broadly utilized as a part of biomedical research on account of its biocompatibility and magnetic properties (Sudhanshu *et al.*, 2012).

One of the fundamental reasons that made magnetic nanoparticles intriguing for biomedical applications is their biocompatibility. As these particles are being utilized as drug delivery vehicles. These particles have been shown to have low toxicity in the human body by several in vitro and in vivo studies.

Previous studies carried by (Lucian *et al.,* 2011) Founded that the dose less than 10 mg/kg of nanoparticles is safe for biomedical applications while the dose more than 20 mg/kg is toxic.

(Kristin *et al.,* 2015) Indicated that the small size of NPs enables greater absorption and potentially greater bioavailability than bulk materials and they indicated that the use of a single, low dose (10 mg/kg.b.w.) of NPs was chosen to investigate the impact of a biomedically-relevant dosage of iron oxide NPs given amid development. No indications of toxicity were watched. Truth be told, it gives some advantage to the mother and developing the fetus.

(Shi *et al.,* 2012) Indicated the use of magnetic iron oxide nanoparticles for the treatment of osteoporosis and loosening of prostheses. The accomplishment of such a treatment requires, to the point that the particles are cytocompatible with osteoblasts. Some investigations demonstrated that a few NPs demonstrate a helpful or nontoxic influence on spermatogenesis (Shi *et al.,* 2010).

(Park *et al.,* 2010) illustrated that the species of animal, route of administration, the dose of NP and NP characteristics (e.g., size, shape, chemical composition, surface area and surface charge) show an important role in determining the influence of NPs on spermatogenesis.

With regard to the low toxicity of magnetic NPs, iron oxide Nano Particles covered with polyvinyl alcohol (Fe3O4-PVA) spontaneously binds to the sperm cell membrane and penetrates sperm cells without affecting sperm motility (Makhluf *et al.,* 2006).

(Sundarraj *et al.,* 2017) indicated that repeated exposure to iron oxide

nanoparticles (Fe2O3-NPs) could be toxic to mice testis. On the other hand (Yoshida *et al.,* 2009) indicated that the mice instilled intratracheally with diverse doses of carbon NPs. They showed no change in body weight, epididymis weight or testes weight in the carbon NP-treated animals compared to the control group.

Everywhere the herbal medicine needs being utilized for those medications of ailment since in the recent past recorded historical backdrop. The bioactive compounds of plants such as flavonoids, tannins, alkaloids and phenolic compounds were reflected to be most important agents in treatment of some disease (Duraipandiyan *et al.*, 2006).

Graviola is a genus of tropical fruit trees belonging to the family Annonaceae, of which there are approximately 119 species. Graviola is known as soursop in English-talking nations and is referred to by numerous common names (Blench and Dendo, 2006). It is more known as soursop, Blanda , Nangka, Guanabana, durian belanda or prickly custard apple. Graviola contains the chemical compound which displays antitumor, pesticidal, antiviral and germicide effects, thus suggesting many potentially beneficial applications. Traditionally, the leaves are used for headaches, insomnia, cystitis, liver problems, diabetes and hypertension and as an inflammatory, spasmolytic and antidysenteric (Sushmita *et al.,* 2012)

Many of the health benefits of graviola are thought to be derived from its antioxidant properties (George *et al.*, 2015). In animal studies graviola leaves extracts showed anti-inflammatory, analgesic (De Sousa *et al.,* 2010), and antiulcer effects (Moghadamtousi *et al.*, 2014). Recent investigations propose that utilizing plant-derived chemopreventive operators in the mix with chemotherapy can upgrade the productivity of chemotherapeutic specialists and lower their toxicity to tissues (Orsˇolic *et al.*, 2010). Studies done on the leaves of Graviola has been resulted in the isolation of eight cytotoxic acetogenins (Kim *et al.,* 1998). Acetogenins are the chemicals which have different natural properties including the cytotoxic influence against the neoplastic cells which recommends their potential use as the antitumoral agents. Acetogenins additionally have the ability to diminish the mouse colon crypts that is incited by azoxymethane (Azo) and was discovered 50% reduction in the number of crypts in the animals treated with acetogenin when compared with the level determined in mice treated with Azo (Padmaa *et al.*, 2009). The leaves of graviola are also hepatoprotective against carbon tetrachloride and acetaminophen-induced liver damage (Adewole and Ojewole, 2008).

MATERIALS AND METHODS

Animals:

50 adult male albino rats at age (2-3 months) 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 for 2weeks before the starting the experimental steps 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 into 5 groups of 10 animals in each group.

Drugs and chemicals:

- **Adriamycin (ADR)** (5 mg/kg body weight, i.p.) (Kelleni *et al.,* 2015), was obtained from Ebewe Pharma co. Austria in tablets form.
- **Graviola (*Annona muricata*)** were obtained from the farm (Deshna, Qena, Egypt) in January 2014. They were taxonomically identified by the Botany Department, Faculty of Science, South

Valley University. Graviola fresh leaves were air-dried at room temperature. The air-dried leaves of the plant were milled into the fine powder in a commercial blender; 150 g of the powdered leaves were extracted with 500 ml ethanol for three days .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 4 °C (Gavamukulya *et al.*, 2015), and subsequently used in this study. 200 mg/kg b.w. (Offor *et al.*, 2015) of this extract was dissolved in dist. water and administered to the animals.

- **Magnetic Iron Oxide nanoparticles** were used in this experiment at dose (5 mg/kg body weight, i.p.) (Yu *et al.*, 2008) 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.

- **Preparation of iron oxide nano particles (NFe3O4) suspension:**

NFe3O4 particles were suspended in deionized water, the solution was affected by ultrasonic continuously for 60 min, then cooled rapidly to below 10ºC. When the solution was centrifuged for 20 min and 104rpm, 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 (Zefeng *et al.*, 2005).

Experimental Design:

Fifty male albino rats with the body (180-200 g) were involved in the current study. Animals were divided into 5 groups (10 rats /group).

Group 1(normal group): Rats received saline solution by i. p. injection for 36 days.

Group 2(control group): Received ADR alone by i. p. injection at a dose of 5 mg/ kg body weight for 3 days day by day and then received saline solution for the remaining of 36 days.

Group3: Received ADR (5 mg/kg b. w.) by i. p. injection for 3 days day by day and after one day, rats received NFe3O4 (5mg/kg/day) injection i.p for 3 days day by day, and then received saline solution for the remaining of 36 day.

Group 4: Received ADR (5 mg/kg b. w.) by i. p. injection for 3 days day by day, one day later, they received graviola extract (200 mg/kg/day) orally for 28 consecutive days and then received saline solution for the remaining of 36 day.

Group 5: Received ADR (5 mg/kg b. w.) by i. p. injection for 3 days day by day, and after one day, rats received NFe3O4 (i.p.) (5mg/kg/day) for 3 days day by day and then received graviola (200 mg/kg/day) orally for 28 consecutive days

At the end of the experiment, blood samples of all animals obtained from retro-orbital eye vein, Samples were collected in clean tubes at room temperature to clot then after an hour; sera were separated by centrifugation for 30 minutes at 3000 rpm. The sera were collected in labeled Eppendorf tubes and stored at -20 °C until used for biochemical analysis. and then the animals were sacrificed by decapitation, one testis was removed and taken 0.5gm from it and 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 and another testis was removed and fixed in a suitable fixative for the histopathological examination.

Biochemical Analysis:

Testosterone was measured by (CLSI EP5-A2), and F.S.H was described by (Wu, 2006) immunoassay for the in vitro quantitative determination of testosterone

and F.S.H in human serum and plasma. The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobras immunoassay analyzers.

The antioxidant enzymes Kits for determination of GSH, SOD, CAT and MDA were brought from bio-diagnostic co. Giza. Egypt. GSH was determined by the colorimetric method described by (Beutler *et al.,* 1963), SOD and CAT were determined by colorimetric methods described by (Nishikimi *et al.,* 1972 and Aebi, 1984) respectively. Determination of MDA was carried out according to the method of (Ohkawa *et al.,* 1979)

Histopathological Preparation:

All experimental rats were examined daily during the period of the experiment, the clinical signs and post mortem changes were recorded specimens were collected from Testis which fixed in neutral buffer formalin for 3 days, washed in distilled water, then dehydrated in ascending series of ethyl alcohol (70%, 80%, 90%, 95% and 100%), impregnated in methyl benzoate three days through three changes and in paraffin wax.The embedded tissues were the section at 5 µ. Thickness then stained with Harris haematoxalin and eosin (H&E), (Bancroft *et al.,* 1990)

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 considered statistically significant when $p < 0.05$.

RESULTS

A) Serum Sex hormones

The ADR treated animals showed a highly significant decrease in the activities of Testosterone and F.S.H at ($p<0.05$) when compared with normal animals. Testosterone and F.S.H concentration in rats treated with NFe3O4, graviola and (NFe3O4 + graviola leaves extract) showed a significant increase as compared to The ADR treated animals, but it was still less than normal animals. (Table1).

B) Testis homogenate biochemical analysis.

In the present study ،the ADR- treated animals showed the significant decrease at ($p<0.05$) in the activities of CAT, SOD and GSH level as compared with normal animals. While the treatment with NFe3O4 ،graviola leaves extract or (NFe3O4 + graviola leaves extract) of ADR-exposed rats showed a significant increase in the activities of CAT, SOD and GSH level when compared with control group, but when compared with the normal rats the results indicated that the activities of CAT, SOD and GSH level were improved but not approached to the normal level Table (2).While the ADR treated animals showed a significant increase in the levels of MDA as compared with normal animals. But the treatment with NFe3O4, graviola leaves extract or (NFe3O4 + graviola leaves extract alone) showed a significant decrease at ($p<0.05$) in MDA level compared to control group, While the level of MDA still higher than the normal level (Table 2).

Table 1: Effects of NFe_3O_4, graviola leaves extract and (NFe_3O_4 + graviola leaves extract) on ADR-induced alterations in serum testosterone and F.S.H. activities in male albino rats.

Groups / **Parameters**	G1 Normal group Mean ± S.D.	G2 Control group (ADR only) Mean ± S.D.	G3 (ADR+ NFe_3O_4) Mean ± S.D.	G4 (ADR+ graviola) Mean ± S.D.	G5 (ADR + NFe_3O_4+graviola) Mean ± S.D.
Testosterone (ng/dl)	5.47 ± 0.2	3.3± 0.1 $^{-a}$	4.11± 0.18$^{-a +b}$	4.36 ± 0.17^{-a+b}	5.06± 0.13^{-a+b}
F.S.H (mlU/ml)	3.27± 0.21	1.41± 0.07^{-a}	1.92 ± 0.05^{-a+b}	2.55 ± 0.11^{-a+b}	2.76 ± 1.2^{-a+b}

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

Table 2: Effects of NFe_3O_4, graviola leaves extract and (NFe_3O_4 + graviola leaves extract alone) on antioxidant enzymes CAT, GSH, SOD and MDA in testis tissues of male albino rats injected with Adriamycin (ADR).

Groups / **Parameters**	G1 Normal group Mean ± S.D.	G2 Control group (ADR only) Mean ± S.D.	G3 (ADR+ NFe_3O_4) Mean ± S.D.	G4 (ADR+ graviola) Mean ± S.D.	G5 (ADR + NFe_3O_4+graviola) Mean ± S.D.
CAT (U / g.tissue)	1.466± 0.22	1.265± 0.025 $^{-a}$	1.34± 0.026 $^{-a+b}$	1.32± 0.016 $^{-a+b}$	1.40± 0.018 $^{-a+b}$
SOD (U / g.tissue)	592.77±.04	504.1 ±0.07 $^{-a}$	542.3 ±5.39^{-a+b}	526.4 ±4.30 $^{-a+b}$	566.4 ±3.76 $^{-a+b}$
GSH (mmol/ g.tissue)	3.78± 0.12	1.20 ± 0 .07 $^{-a}$	3.05 ± 0.11^{-a+b}	2.80 ± 0.10^{-a+b}	3.24 ± 0.06^{-a+b}
MDA (nmol/ g.tissue)	15.12 .27	18.52 .24^{+a}	16.19 .35 $^{+a -b}$	16.30 .26 $^{+a -b}$	15.72 .18 $^{+a -b}$

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

c- Histopathological results:

Testis:

Group1 (normal rats) showed the normal structure of testis formed coiled tubules called seminiferous tubules which manifested by each tubule lined with germ cells (spermatogonia), which progress to spermatocytes and ended to become sperm, in addition to Sertoli cells which support the lining epithelium of tubules (Fig.1)

Testis in Group2 (the ADR- treated animals) showed complete destruction in seminiferous tubules with edema in Tunica vaginalis leading to atrophy in testis (Fig. 2). Degeneration and necrosis in spermatocytes resulting in loss in spermatogonia in seminiferous tubules (Figs.3). Inflammatory edema was detected around the congested blood vessels in the interstitial tissue with complete loss of germ cells in tubules (Fig. 4). Edema in interstitial tissue compressed the seminiferous tubules leading to atrophy and necrosis in most tubules (Fig. 5). Seminiferous tubules suffered from degeneration in germ cells in and round spermatids with condensed and marginated nuclear chromatin and no spermatids inside the lumen (Fig. 6). Destruction and necrosis in most tubules with destruction basement membrane and irregular in shape resulting in the debris of germ cells in interstitial tissue (Fig. 7). The tubules detected the increase of ledge cells in interstitial tissue and sertoli cell vacuolation and germ cell degeneration (Fig. 8). Spermatids with excessive lightly basophilic granular cytoplasm formed multinucleated giant cell beside abnormal spermatids (Fig. 9).

Testis in Group 3 (treatment with NFe3O4 of ADR-exposed rats) showed moderate degeneration and necrosis in the spermatocytes in seminiferous tubules with edema in interstitial tissue in some rats (Fig.10). Recovery in some seminiferous tubules with production in normal sperm cells in some rats (Fig. 11).

Testis in Group 4 (treatment with graviola leaves extract of ADR-exposed rats)showed production of sperm in some tubules but the others with mild necrosis and degeneration in spermatogenesis with no sperm production (Fig. 12).

Testis in Group 5 (treatment with NFe3O4 + graviola leaves extract of ADR-exposed rats) showed the seminiferous tubules in normal structure, in with sperm production (Fig.13).

DISCUSSION:

ADR is a quinone-containing anthracycline chemotherapeutic and 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). Is known it has many side effects on some organs function which it produces reactive oxygen species (ROS) in various tissues.

Results of biochemical investigations showed that ADR induced a marked gonadotoxicity through induction of oxidative stress that is ameliorated by iron oxide nanoparticles and Graviola leaves plant extract administration.

In this study, ADR caused a significant decrease in the testosterone and F.S.H activities and this decrease is due to reduced number of germ cells, atrophy of Leydig cells and the lower rate of spermatogenesis.

Similar observations were obtained by Ait *et al.*, (2009) and Gamal, (2012), who reported that the ADR-administered rats showed the significant decrease of serum testosterone and LH concentrations. It has been reported that antineoplastic agents can destroy Leydig cells directly.

Howell and Shalet, (2001) reported that the incidence of male infertility following ADR chemotherapy resulted from changes in sperm count, motility, dead sperm and abnormal sperm. Germ cells in testes are vulnerable to ADR-induced oxidative stress (Hrdina *et al.*, 2000). This increased oxidative stress effects on the sperm membranes, proteins and DNA (Kalender *et al.*, 2005), DNA damage may be liable for the increased level of abnormal spermatozoa forms.

Therefore, a possible reason for the disruption of spermatoiogenesis in the ADR-treated rats is the failure of

testosterone dependent attachment of spermatids to Sertoli cells (Rezvanfar *et al.,* 2008).

From the previous studies ADR was found to significantly decreased serum testosterone level (Atessahin *et al.*, 2006). Degeneration of spermatocytes by ADR exposure may be due to the low intratesticular concentrations of testosterone, as high level of testosterone in testis is essential for the ordinary spermatogenesis as well as for the maintenance of the structural morphology and the typical physiology of seminiferous tubule (Sharpe *et al.*, 1992). In the present study, we also observed decreased serum testosterone level in ADR-exposed animals and investigated the possible effect of some antioxidants to improve the testosterone level.

The results of the present work indicated that group of rats administered iron oxide nanoparticles showed a significant increase of Testosterone and F.S.H and the same result for Graviola leaves extract which has ameliorated effect where it increases the levels of Testosterone and F.S.H. in the toxicity rats with ADR. That indicated the Graviola leaves extracts have antioxidant properties (Baskar *et al.,* 2007).

Data of the present study indicated that, treatment with ADR produced marked oxidative impact as evidenced by a significant increase in the level of Malondialdehyde (MDA) in testis tissue homogenate as one of the main end products of lipid peroxidation. GSH, SOD and CAT levels reduction were detected in testis tissue in ADR treated rats. Ihab and Ahmed, (2009) Found out MDA increasing and SOD activity reduction in response to ADR administration, all together support an oxidative mechanism of ADR-toxicity. Also, Su *et al.,* (2009) explained that ADR administration to rats significantly increased lipid peroxide expressed as TBARS, and decreased both glutathione peroxidase and superoxide dismutase activities in cardiac tissues.

The significant decrease in the activity of SOD and CAT might be, also, attributed to the excess of ROS, which interacts with the enzyme molecules causing their denaturation and partial inactivation.

Saalu *et al.,* (2010) showed that the testicular oxidative status of ADR treated rats was severely decrease the activities of SOD, CAT and the significant reduced the GSH level as well as the significantly enhanced lipid peroxidation measured as MDA. We observed that ADR exposure produced pronounced Testis histopathology evidenced by histological alternations in testis, including degeneration and necrosis in spermatocytes leading to the loss in spermatogonia in seminiferous tubules and complete destruction in seminiferous tubules with edema in Tunica vaginalis leading to atrophy in testis.

The aim of the present study is to evaluate the effects of ADR on the antioxidant defense system and the protection afforded by iron oxide nanoparticles, Graviola leaves plant extract and (iron oxide nanoparticles + Graviola leaves plant extract).

The results of the present work showed that group of rats administered iron oxide nanoparticles showed a significant increase of GSH, SOD and CAT and a significant decrease of MDA in Testis when compared to ADR treated group

One of the main causes that made nanoparticles interesting for biomedical applications is their biocompatibility. As these particles are being used as drug delivery vehicles, these particles have been shown to have low toxicity in the human body by several in vitro and in vivo studies (Seyda *et al.,* 2012).

Results of the present study agree with Dawei *et al.,* (2009) explained that Zinc oxide nanoparticles (NZnO) is able

to protect cell membrane integrity against oxidative stress damage and increase antioxidant enzyme levels (CAT, SOD, GSH) and decrease MDA level. It can improve antioxidant activity, enhance the activities of antioxidases, and decrease the levels of free radicals (Badkoobeh *et al.,* 2013).

Also, in agreement with Badkoobeh *et al.,* (2013) who indicated that the protective role of zinc oxide nanoparticles against ADR- induced male gonadtoxicity.

Shi *et al.,* (2012) reported that the serum activity of GSH-Px, SOD and catalase was higher in goats fed nano-Se compared with goats fed selenite or Se-yeast diets. In addition, they found that the MDA levels in the goats fed nano-Se were the lowest compared with goats fed selenite or Se yeast diets or controls. This indicates that nano-Se has strong antioxidant properties

The results of the present work showed that group of rats administered graviola leaves plant extract showed a significant increase of GSH, SOD and CAT and a significant decrease of MDA in testes.

The previous studies, declare that ethanolic leaves extract of graviola showed antioxidant activities and due to that, the results showed a significant increase of GSH, SOD and CAT and a significant decrease of MDA in Testes.

The results of the present work showed that group of rats administered Iron oxide nanoparticles + Graviola leaves extract showed a significant increase of GSH, SOD, CAT and a significant decrease of MDA in Testes, this group represent the best in improvement due to the presence of both antioxidant making a reducing of free radicals. The ameliorative effect of Iron oxide nanoparticles and Graviola leaves extract and the combination of them confirmed by histopathological observations where showing recovery in seminiferous tubules with production in normal sperm cells and seminiferous tubules return in normal structure.

Conclusion

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

Acknoledgment:

We are grateful to Prof. Dr. Khaled Ebn EL-Waleed, professor of physics, Faculty of science, South Valley University for his kind help in performing Nano studies and co-operation of the results.

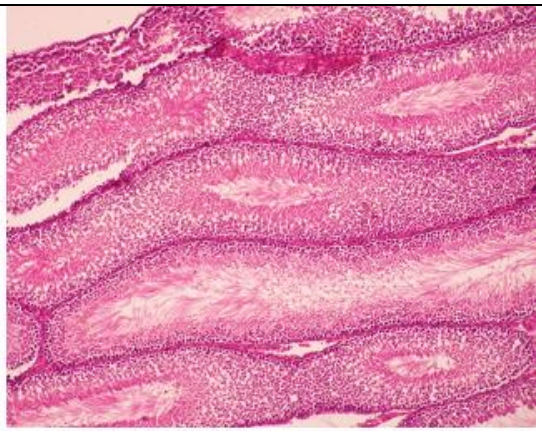

Fig. (1): Testis in the group (1) showing the normal structure of testis formed coiled tubules called seminiferous tubules. (H&E., x 200)

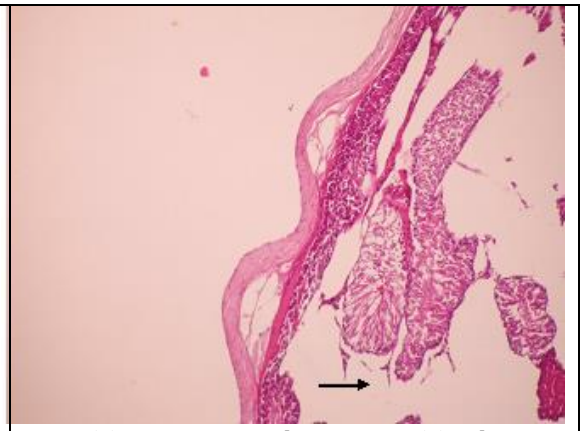

Fig. (2): Testis in the group (2) showing complete destruction in seminiferous tubules with edema in Tunica vaginalis leading to atrophy in testis. (H&E., x 200)

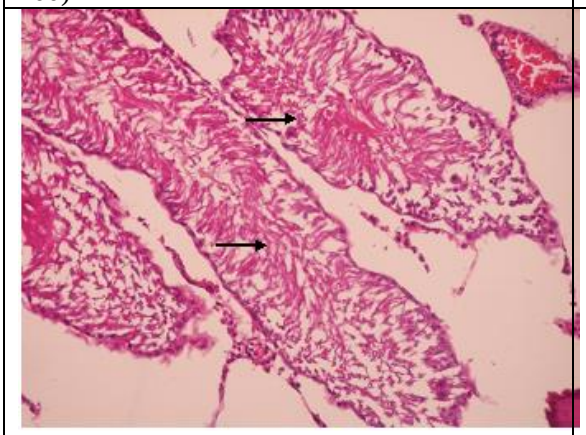

Fig. (3): Testis in the group (2) showing degeneration and necrosis in spermatocytes leading to the loss in spermatogonia in seminiferous tubules. (H&E., x 200)

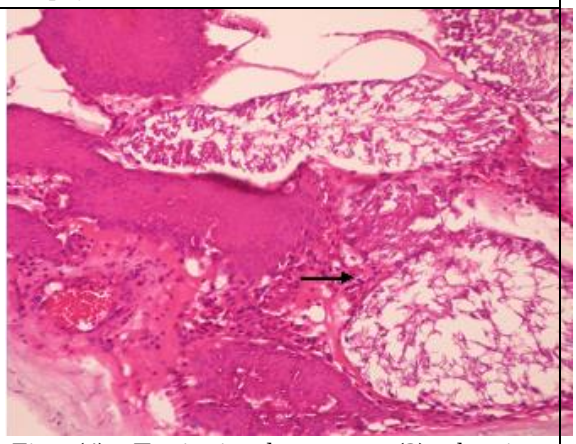

Fig. (4): Testis in the group (2) showing inflammatory edema in the interstitial tissue replaced the necrotic tubules which appeared completely loss of germ cells (H&E., x 400)

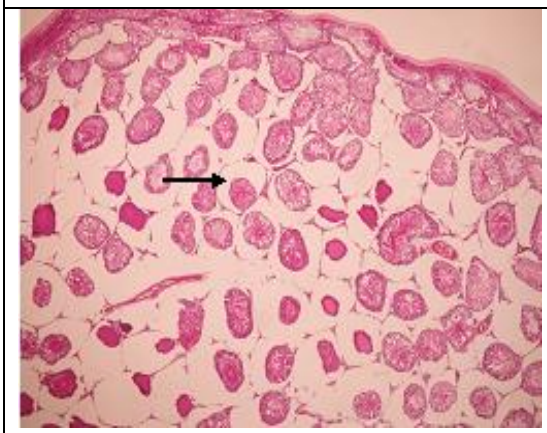

Fig. (5): Testis in the group (2) showing edema in interstitial tissue compressed on the seminiferous tubules leading to atrophy and necrosis in most tubules.(H&E., x 100)

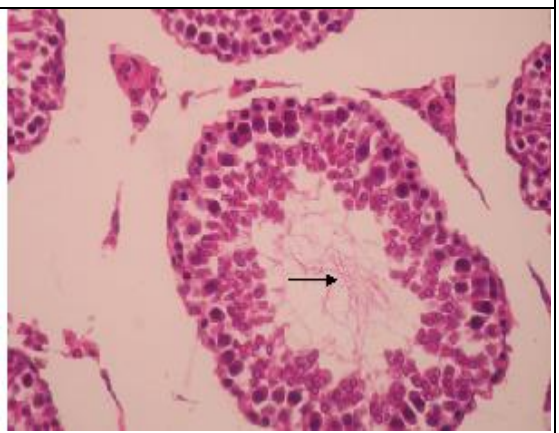

Fig. (6): Testis in the group (2) showing degeneration in germ cells in seminiferous tubules and round spermatids with condensed and marginated nuclear chromatin with no spermatids inside the lumen.
(H&E., x 400)

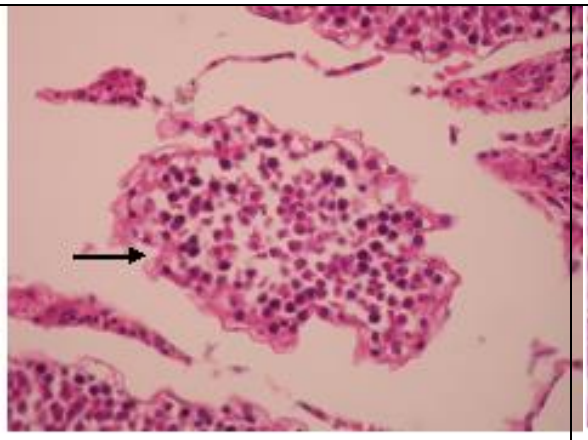

Fig. (7): The high power of Testis in the group (2) showing severe destruction in seminiferous tubules with the loss in basement membrane. (H&E., x 400)

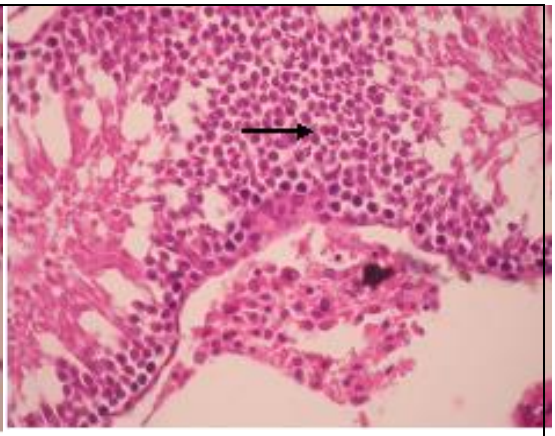

Fig. (8): Testis in the group (2) showing increase of leyding cells in interstitial tissue and Sertoli cell vacuolation and germ cell degeneration. (H&E., x 400)

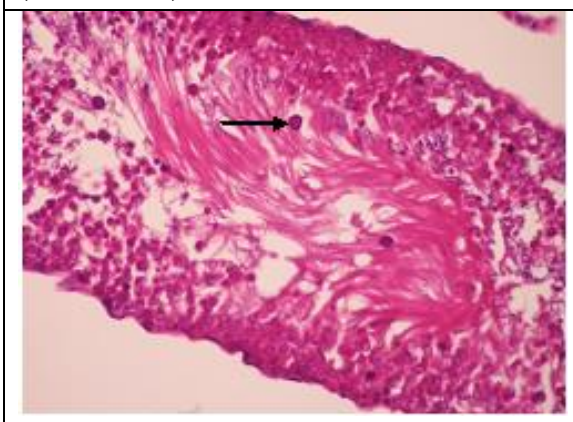

Fig. (9): Testis in the group (2) showing spermatids with excessive lightly basophilic granular cytoplasm formed multinucleated giant cell beside abnormal spermatids. (H&E., x 400)

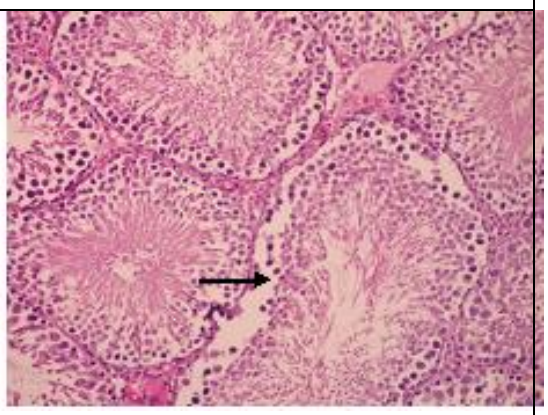

Fig. (10): The high power of Testis in the group (3) showing degeneration and necrosis in the **seminiferous tubules. (H&E., x 400)**

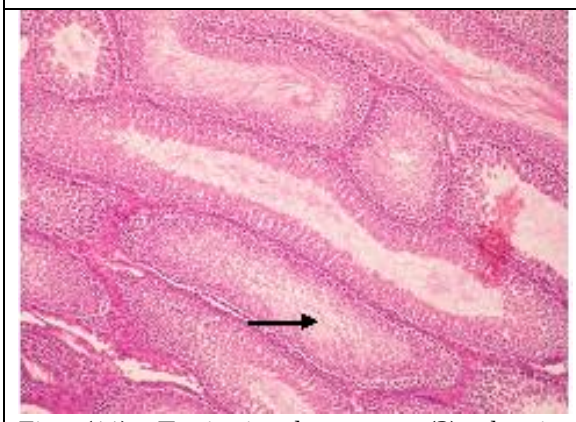

Fig. (11): Testis in the group (3) showing recovery in some seminiferous tubules with production in normal sperm cells in some rats. (H&E., x 200)

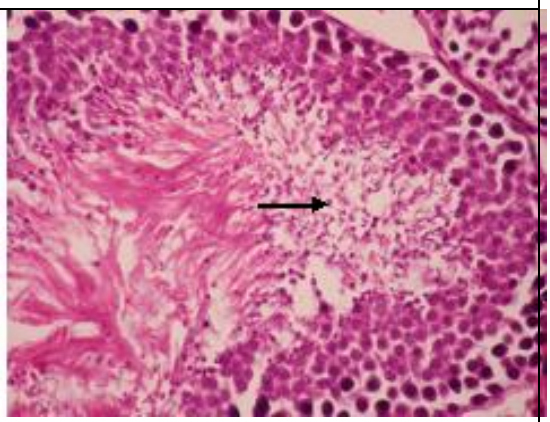

Fig. (12): The high power Testis in the group (4) showing to show degeneration in spermatogenesis with no sperm production. (H&E., x 400)

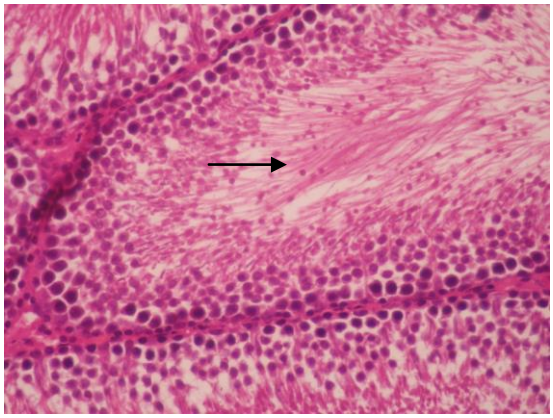

Fig. (13): The high power of Testis in the group (5) showing seminiferous tubules in normal structure, in with sperm production. (H&E., x 400)

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