The Possible Protective Effect of Antioxidant Alpha-Lipoic Acid on the Postnatal Developing Liver of Albino Rats Treated with Doxorubicin (Histological and Cytogenetic Study)

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Abstract: Delaying childbearing to a later age has increased the number of women with cancer in pregnancy. Little information on the effects of prenatal exposure to chemotherapy on the fetus, prompted us to study the transplacental transport of chemotherapeutic agents and its effect on the liver of offsprings. Doxorubicin (DOX) an anthracycline antibiotic, which is widely used as an antineoplastic drug in the treatment of various solid tumors, especially breast cancer, has been shown to induced hepatotoxicity. The aim of the present study was to investigate the possible protective effect of Alpha- lipoic acid (ALA) on the postnatal developing liver of albino rats whose mothers treated with DOX. The pregnant mothers were divided into four main groups. G1 (control), G2 (ALA treated group), G3 (DOX treated group) and G4 (ALA+DOX treated group). After delivery, liver of newborn from all groups at the 1st and 7th days were collected and processed for histological examination. Monitoring of micronuclei formation and chromosomal aberrations assays in rat liver were also done. The liver from G3, showed various degenerative changes especially on the liver of the 7th day. The hepatotoxicity of the offspring suggested transplacental passage of doxorubicin. The pretreatment with ALA in G4 showed ameliorating effect on the hepatotoxicity induced by DOX. The cytogenetic results revealed that groups treated with ALA+DOX showed a significant reduction in the frequency of micronuclei formation as well as of chromosomal aberrations. Conclusion: from the results of the present study, it could be concluded that the pretreatment by alpha-lipoic could attenuate the DOX induced hepatotoxicity of newborn albino rat.

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Key words: development structure of liver, doxorubicin, alpha lipoic acid, micronucleus test, chromosomal aberrations

1. Introduction

Pregnancy-associated malignancy presents a significant dilemma in management as a result of conflict between maternal therapy and effects of treatment on fetal well-being. Due to lack of large studies, there is a need for guidelines for most of these conditions. Delaying childbearing to a later age has increased the number of women with cancer in pregnancy (Shah and Shafi, 2008). Chemotherapy stops and eliminates the growth of cancer cells, however it does not differeniate between cancer and normal cells (Bonadonna et al., 1995 and Abraham, et al. 1996).

Doxorubicin (DOX) or adriamycin (ADR) is a broad-spectrum antineoplastic agent which is commonly used in the treatment of uterine, ovarian, breast and lung cancers, Hodgkin's disease and soft tissue sarcomas as well as in several other cancer types (Quiles et al., 2002). DOX has already proved to have hepatotoxic activity (Ganey et al., 1988). When DOX is given to adult individuals in therapeutical doses, it causes some changes not only in neoplastic cells, but also in liver, where it is metabolized (Pedrycz et al., 2004). Strategies to attenuate toxicity include dosage optimization, synthesis and the use of analogues or a combined therapy with antioxidants (Quiles et al., 2002). Biological compounds with antioxidant properties may contribute to the protection of cells and tissues against deleterious effects of reactive oxygen species (ROS) and other free radicals induced by DOX. Biological compounds with antioxidant properties may contribute to the protection of cells and tissues against deleterious effects of reactive oxygen species (ROS) and other free radicals induced by DOX (Kagan et al., 1992 and Deepa and Varalakshmi, 2003).

Alpha-lipoic acid (ALA), is a universal antioxidant. Its administration has been shown to be beneficial in various pathologies in which ROS have been implicated (Midaoui et al., 2003 and Bjelakovic, 2007). ALA through its powerful antioxidant activity causes a general systemic improvement including the

liver health (Pari and Murugavel, 2004 and Li et al. 2014)).

Aim of the work: since, genetic study on the effect of ALA on the liver of the mothers and their offsprings treated with DOX was mentioned that groups treated with ALA+DOX showed a significant reduction in the frequency of micronuclei formation in the liver cells as well as of chromosomal aberrations (Aboul-Mahasen, et al., 2012), thus, promote us to investigate the histological alterations by DOX and the possible protective effect of alpha -lipoic acid on the structure of the liver of the 1st and 7th new born rats whose mothers treated with DOX. The results of micronucleus test and chromosomal aberrations in the 1st and 7th day rats were statistically analysis by unpaired test, which was used to compare between two ages.

2. Material and Methods

Ethical approval

The procedures were conducted in accordance with guidelines and protocols reviewed and approved by the ethical committee for animal care and use in Faculty of Medicine for Girls, Al-Azhar University and National Research Centre, Cairo, Egypt.

A. Animals

Eighty adult albino rats (weighing 180-200 gm) obtained from the National Research Centre, Cairo, Egypt, were used. Animals were housed in groups and maintained under standard food and water ad libitium. Each adult three females were kept overnight in a cage with a single male rat. In the next morning, the presence of vaginal plug was considered as an evidence of mating, this day was considered to be the first day of pregnancy. Each pregnant female was kept in a separate cage to be observed until delivery. The pregnant rats were divided into main four groups (twenty pregnant albino rats in each group). After delivery, their offsprings were divided into A and B subgroups.

Group I (Control group): the pregnant albino rats were given orally distilled water at 11th, 12th, 13th and 14th days of gestation and were injected intraperitoneally with distilled water at 12th, 13th and 14th days of gestation. The offsprings were divided into:

Subgroup IA: consisted of one day old albino rats.

Subgroup IB: consisted of seven days old albino rats.

Group II (Alpha-lipoic acid treated group): the pregnant albino rats were given alpha-lipoic acid orally by a gastric tube at the 11th, 12th, 13th and 14th days of gestation. The offsprings were divided into:

Subgroup IIA: consisted of one day old albino rats.

Subgroup IIB: consisted of seven days old albino rats.

Group III (Doxorubicin treated group): the pregnant albino rats were injected with doxorubicin intraperitoneally at the 12th, 13th and 14th days of gestation. The offsprings were divided into:

Subgroup IIIA: consisted of one day old albino rats.

Subgroup IIIB: consisted of seven days old albino rats.

Group IV (Doxorubicin with alpha-lipoic acid treated group): the pregnant albino rats were injected with DOX intraperitoneally at the 12th, 13th and 14th days of gestation and were given orally alpha-lipoic acid 24 hours prior to and during treatment by DOX, at the11th, 12th, 13th and 14th days of gestation. The offsprings were divided into:

Subgroup IVA: consisted of one day old albino rats.

Subgroup IVB: consisted of seven days old albino rats.

B. Drugs:

Doxorubicin (is manufactured by Pharmacia and Upjohn –Milan- Italy (Trade name, Adriaplastina). It was supplied as vials, each one of them containing 10 mg of doxorubicin hydrochloride as a freeze – dried powder. This powder is dissolved by distilled water.

Calculation of the dose: the human therapeutic dose ranged from 50 mg up to 75 mg /m² (Brunton et al., 2006).

Average therapeutic dose= 60 mg /m².

A person weight 70Kg has 1.65 m² surface areas. So the average human therapeutic dose: 60 x1.65/100=99 mg. According to Paget and Barnes (1964), the dose of doxorubicin per rat: 99x18/1000=1.782 mg.

Alpha-lipoic acid (is manufactured by Eva-Pharma (Trade name, Thiotacid).

It was supplied as light yellow tablets (each tablet contains 300 mg alpha-lipoic acid). The tablets were dissolved in distilled water.

Calculation of the dose: the human therapeutic dose 75 mg /kg body wt /day (Anandakumar et al., 2007). According to Paget and Barnes (1964), the dose of alpha- lipoic acid per rat: 75x18/1000=1.3 mg.

C. Histological technique:

The newborn albino rat, one and seven days after delivery were anaesthetized lightly by diethyl ether inhalation The abdominal cavity was exposed by a midline incision. The liver was identified in the abdominal cavity and was collected, immediately fixed in 10% buffered formalin and processed for preparing histological sections 5µm thick. They were stained with hematoxylin and eosin (H & E), Masson's trichrome stains (Drury and Wallington, 1980) and

Periodic Acid Schiff's reaction (PAS) (Bancroft and Stevens, 1996).

Table 1: Unpaired T-test with comparison between one and seven days old albino rat liver regarding mean % of micronucleated hepatocytes (MNHS).

Groups	No. of counted MNHS/1000	Mean%± S.D.	P-value
Group I			
IA	87	0.58± 0.20	0.58
IB	93	0.62± 0.20	
Group II			
IIA	63	0.42± 0.37	0.858
IIB	66	0.44± 0.22	
Group III			
IIIA	462	3.08± 2.45	0.837
IIIB	489	3.26± 2.32	
Group IV			
IVA	162	1.08± 1.15	0.931
IVB	168	1.12± 1.35	

D. Cytogenetic studies:

I. Micronucleus test in hepatocyte:

The liver of the offspring were used, according to Tates et al. (1980) with some modifications. Hepatocytes were isolated from anaesthetised rats by the collagenase perfusion method (collagenase, 45OC; room temperature ~25^{o}C), rinsed with 10% neutral formalin three times and centrifuged at 42xg for 1 min. The hepatocyte pellets were suspended in 10 % neutral buffered formalin and stored under refrigeration. Approximately 10-20 µl of hepatocyte suspension was dropped onto a glass slide, after air-dried, slides were rinsed in absolute methanol. After air-dried slides stained with May-Grũnwald diluted 1:1 with distilled water for 5 min and followed by 7% Giemsa stain for 15 minutes. Micronucleated hepatocytes (MNHEPs) were scored in 1000 cells for each animal. MNs were defined as round, with a diameter 1/4 or less than that of the nucleus and stained like the nucleus. (Valentin Severin et al., 2003). The results were expressed as the percentage of micronucleated hepatocytes (%) for micronuclei level (RMN) was determined for ALA treatment according to Mokrane et al. (1996) as following:

RMN=(micronuclei of clastogen and anticlastogen - micronuclei of negative control) / (micronuclei of clastogen -micronuclei of negative control)

II. Chromosomal aberrations in hepatocyte:

Metaphases were prepared according to Yosida and Amano (1965) with some modifications. Chromosomal aberrations assay was described. Rat offsprings were injected intraperitoneally with colchicine at a final concentration of 3mg/kg. B.W. 2 hrs prior to sacrifice. Slides were stained with 7% Giemsa stain in phosphate buffer (pH6.8). 100 well spread metaphases per animal were analyzed for chromosomal aberrations. The types of aberrations in hepatocytes included gaps, breaks, fragments and deletions.

III. Statistical analysis:

The significance of the results from control data was calculated using unpaired test to compare between the two ages. It was done to test the significance of the difference between means of a pair of groups (Mould, 1989). If the degree of probability (P) is more than 0.0500, the results will be insignificant statistically. The lower the (P) value was (0.01, 0.001, 0.0001….etc.) the higher the degree of significance.

3. Results

Histological results:

A) Liver of one day old rat Control (SubG1A) and alpha-lipoic acid maternally treated groups (Sub GIIA):

Histological examination of transverse sections of one day old albino rat liver of the control group (subgroup IA) and alpha-lipoic acid maternally treated group (subgroup IIA) stained by Hx & E showed a normal hepatic architecture. The liver was nearly developed and was covered by a very thin capsule (Fig.1a). Under the capsule, the liver was formed of irregular groups of hepatocytes which appeared irregularly radiating from the central vein and were separated by irregular shaped primitive blood sinusoids and numerous hemopoietic cells. The central veins were more or less rounded in shape. They were irregularly lined by a single layer of flat endothelial cells with flat nuclei (Fig.1a). Some of the blood sinusoids were lined with endothelial cells and Von-Kupffer cells.

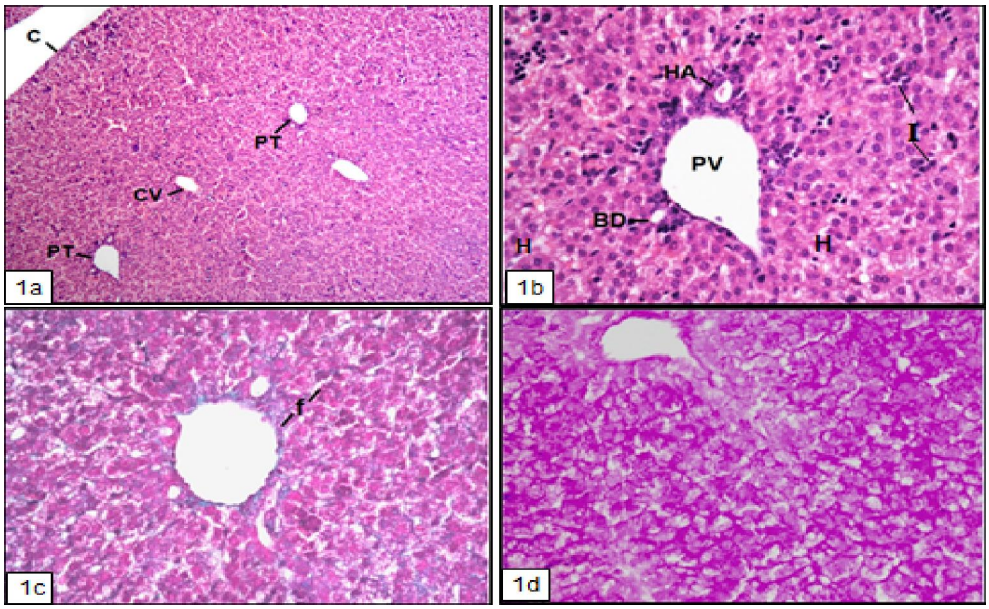


Fig. 1: Photomicrographs of transverse sections of one day old albino rat liver of the control subgroup (IA) and ALA subgroup (IIA) showing:

a. a normal hepatic architecture. Liver is covered by a very thin capsule (C). It is formed of irregular groups of hepatocytes around the central vein (CV). Notice the primitive portal tract (PT). H & E X100.

b. the primitive portal tract is formed of a branch of portal vein (PV), a branch of hepatic artery (HA) and a branch of bile duct (BD). Notice the hepatocytes and some hemopoietic islets (I) H & E X400).

H & E X 400.

c. a mild amount of collagen fibers (f) deposition in portal tract and in walls of the blood sinusoids. Masson's trichrome X400.

d. showing a severe PAS positive reaction of hepatic parenchyma PAS X 400.

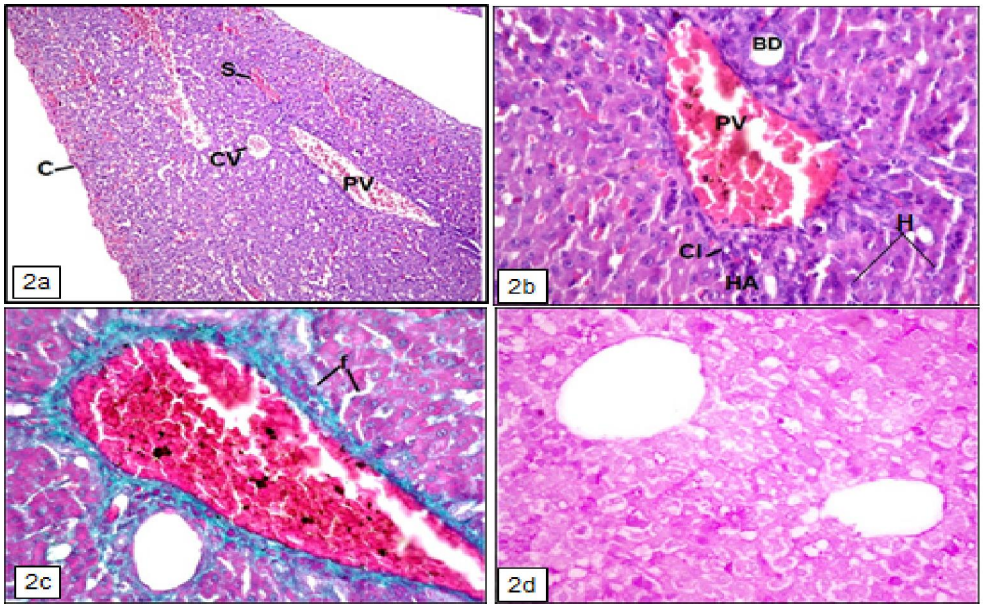


Fig. 2. Photomicrographs of transverse sections of one day old albino rat liver of the doxorubicin maternally treated group (subG. IIIA) showing:

a. the liver is covered by a thin irregular capsule (C). The groups of hepatocytes were separated by dilated blood sinusoids (S). Notice congestion and dilation of central vein (CV) and portal vein (PV) H & E X100.

b. a dilated congested portal vein (PV),. The bile duct (BD) is dilated and the hepatic artery (HA) is poorly developed. Notice inflammatory cell infiltrations (CI) around the portal tract. Showing some hepatocytes (H) have small pyknotic nuclei (V) with vacuolated cytoplasm. H & E X400.

c. a severe amount of collagen fibers (f) deposition in portal tract and in walls of the blood sinusoids. Masson's Trichrome X 400.

d a mild PAS positive reaction of the hepatic parenchyma. PAS X 400.

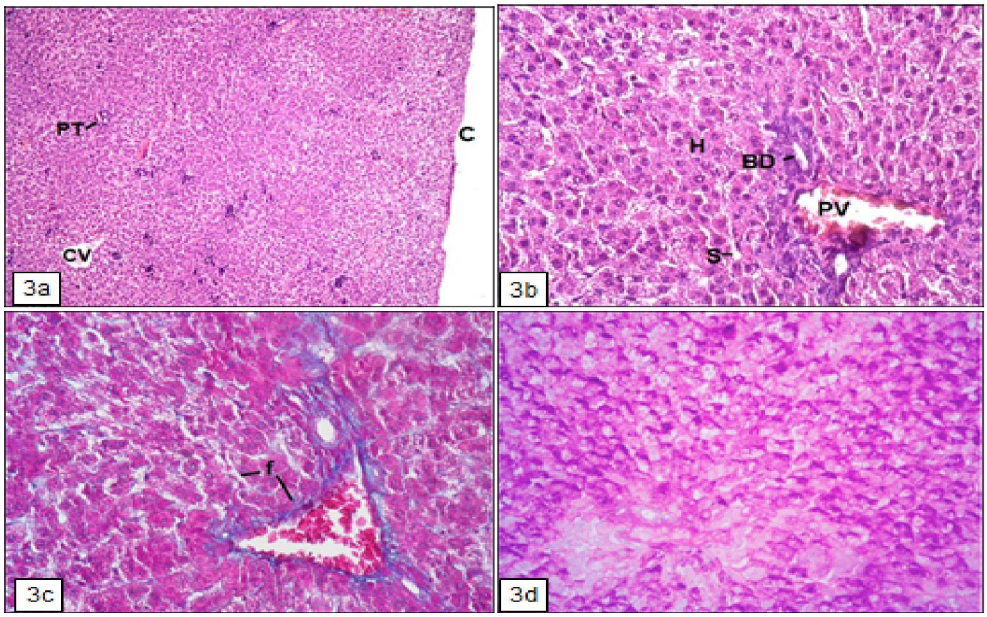


Fig.3. Photomicrographs of transverse sections of one day old albino rat liver of the doxorubicin and alpha lipoic acid maternally treated group (subG IVA) showing:

a. the liver is covered by a thin capsule (C) and is formed of irregular groups of hepatocytes. The cells have not a definite arrangement around the central vein (CV). Notice the presence of the primitive portal tract (PT). H & E X 100.

b. the portal tract contains slightly congested portal vein (PV). No dilation in bile duct (BD) is observed. Some blood sinusoids (S) appear to be dilated. Most of hepatocytes (H) around the portal area appear nearly of normal shape and size. H & E X 400.

c. a moderate amount of the collagen fibers (f) deposition in the portal tract and in the walls of the blood sinusoids. Masson's Trichrome X 400.

d. a moderate PAS positive reaction in some areas of the hepatic parenchyma and a nearly PAS negative reaction in other areas. PAS X 400.

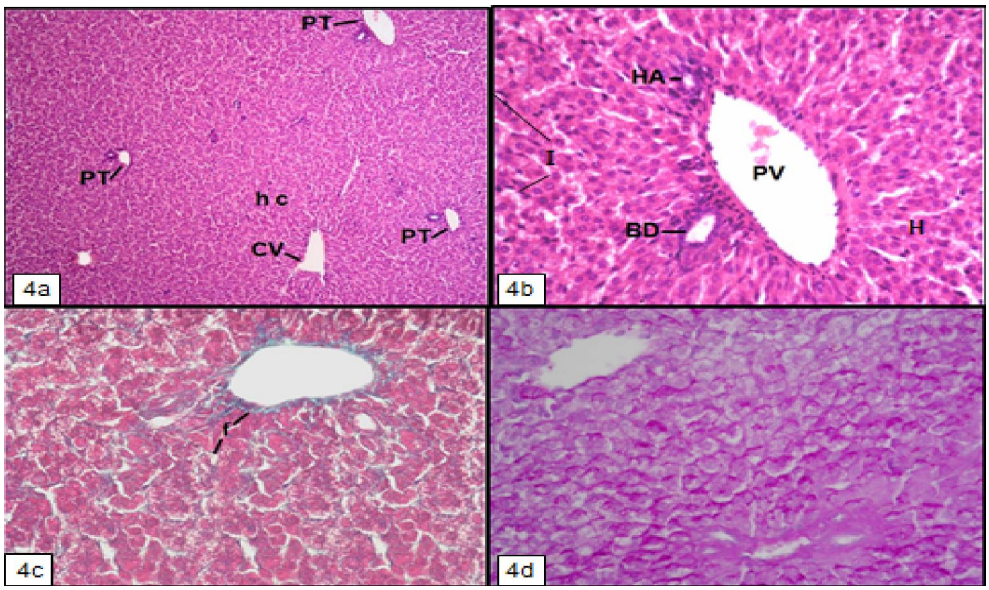


Fig. 4: Photomicrographs of transverse sections of seven day old albino rat liver of the control subgroup (IB) and ALA subgroup (IIB) showing:

a. showing the liver is formed of ill- defined hepatic lobules. The hepatic cords (h c) are radiating from the central vein (CV) towards the periphery of the lobule. Notice presence of many portal tracts (PT).
H & E X 100.

b. a portal tract (PT) becomes more developed and contains a branch of the portal vein (PV), a branch of the hepatic artery (HA) and a branch of the bile duct (BD). The hepatocytes (H) are polyhedral in shape with eosinophilic granular cytoplasm. Notice few islets (I) of hemopoietic cells between the hepatic cords. H & E X 400.

c. a moderate amount of collagen fibers (f) deposition in the portal tract and in the walls of the blood sinusoids. Masson′s trichrome X400.

d. a severe PAS positive reaction of the hepatic parenchyma. PAS X 400.

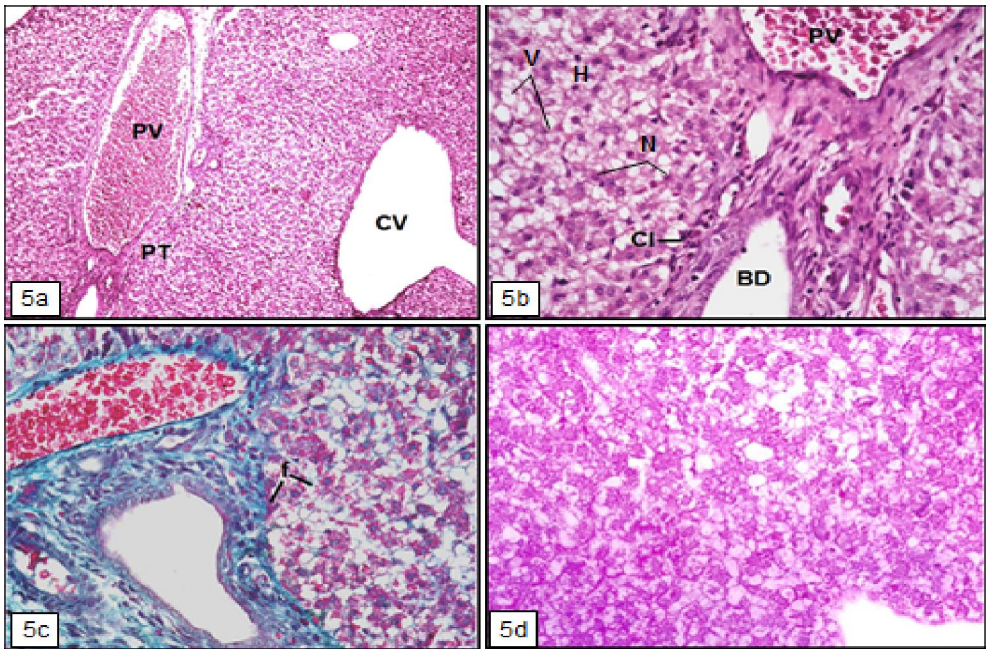


Fig.5. Photomicrographs of transverse sections of one day old albino rat liver of the doxorubicin maternally treated group (subgroup IVA) showing:
a. a marked dilation of the central vein (CV). Notice the portal tract (PT) contains a widely dilated congested branch of the portal vein (PV). H & E X 100.
b. the portal tract (PT) contains a widely dilated congested portal vein (PV) and a dilated bile duct (BD). Also the portal tract is infiltrated with inflammatory cells (CI). The hepatocytes (H) have vacuolated cytoplasm (V) and pyknotic darkly stained basophilic nuclei (N). H & E X 400.
c. showing a severe amount of collagen fibers (f) deposition in the portal tract and in between the hepatocytes. Masson's Trichrome X 400.
d. showing a moderate PAS positive reaction of the hepatic parenchyma. PAS X 400.

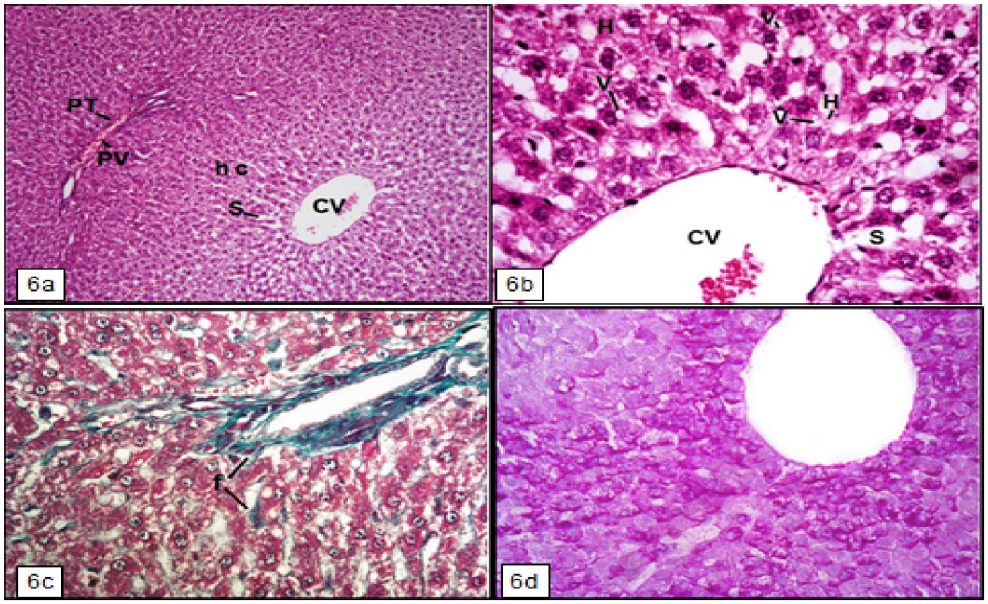


Fig.6. Photomicrographs of transverse sections of one day old albino rat liver of the doxorubicin & alpha lipoic acid maternally treated group (subgroup IVA) showing:
a. the hepatic cords (h c) are irregularly radiating from the dilated central vein (CV) towards the periphery. They are separated by a dilated blood sinusoids (S) surrounding the central vein. The portal tract (PT) contains slightly congested portal vein (PV). H & E X 100 b. dilated central vein (CV) and blood sinusoids (S). Some hepatocytes contain vacuoles (V) in their cytoplasm. H & E X 400.
c. showing a moderate amount of collagen fibers (f) deposition in the portal tract and in the walls of the blood sinusoids. This amount less than the amount in control group (Fig. 1c).
d. a severe PAS positive reaction in some areas and a nearly moderate PAS reaction in other areas of the hepatic parenchyma. PAS X 400.

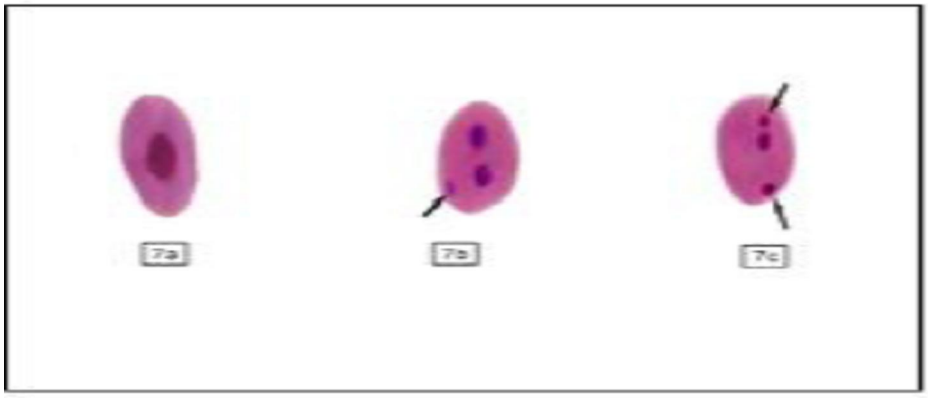


Fig. 7: Photomicrographs of hepatocytes of one day and seven days albino rats
a. Normal hepatocyte of one day old albino rat.
b. Binucleated hepatocytes with micronucleus (arrow) of one day old albino.
c. Micronucleated hepatocytes (arrow) of seven days old albino rat.
May- Grũnwald stain X1250 oil.

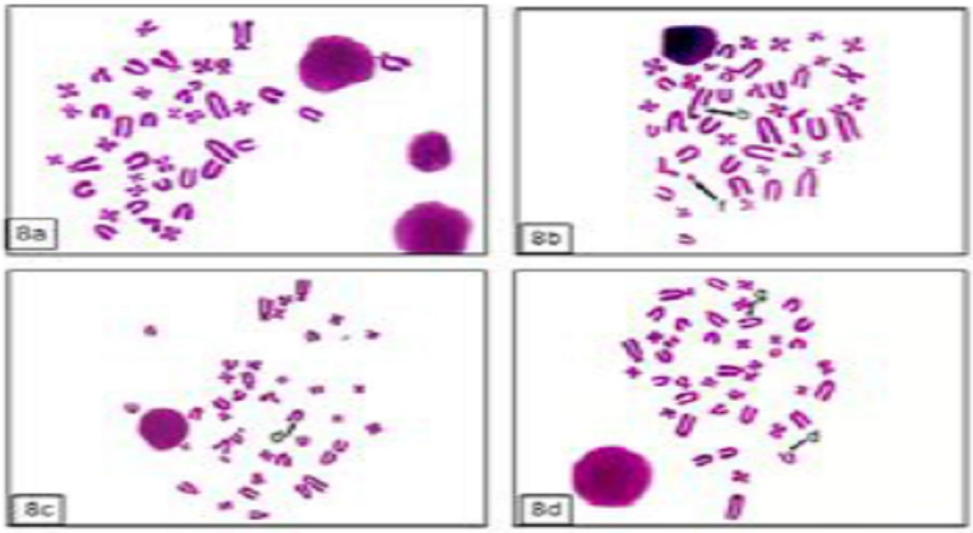


Fig. 8: photomicrographs of metaphase spread of offspring albino rat liver.
a. A metaphase of normal hepatocyte
b. A metaphase of hepatocyte with chromosome break (b) and fragment (f)).
c. A metaphase of hepatocyte with deletion (d).
d. A metaphase of hepatocyte with chromatid gap (g) and chromosome deletion (d).
Giemsa stain X1250 oi.

The hepatocytes were polyhedral in shape with eosinophilic granular cytoplasm. Each cell had one or two large rounded vesicular basophilic (Fig.1b). The hemopoietic cells had intense eosinophilic cytoplasm with deeply stained nuclei. They were found widely dispersed throughout the liver and some of them were arranged into small islets (Fig.1b). Areas of nearly developed portal tracts were appeared. Each portal tract was formed of a branch of the portal vein, a branch of the hepatic artery and a branch of the bile duct. The portal vein was lined with a single irregular layer of flat endothelial cells with flat nuclei. The hepatic artery was lined with a single layer of flat endothelial cells resting on a nearly developed basal lamina. The bile duct was lined with a single layer of cubical epithelium (Fig.1b). Sections stained with Masson's trichrome showed a mild amount of collagen fibers deposition in the portal tract and in the walls of the blood sinusoids (Fig.1c). Sections stained with Periodic acid Schiff showed a severe PAS positive reaction of the hepatic parenchyma (Fig. 1d).

Doxorubicin treated groups (SubG. IIIA)

Histological examination of transverse sections of one day old albino rat liver of the doxorubicin maternally treated group (Subgroup IIIA) stained with Hx & E showed that the liver had variable histological changes as losting of its normal architecture, dilation and congestion of the blood sinusoids and central

veins, degeneration and necrosis of the hepatocytes especially in the subcapsular and pericentral areas (Fig. 2a). The hepatocytes showed vacuolation of their cytoplasm and pyknosis of their nuclei. There was a decrease in hemopoietic islet cells within the parenchyma. The portal vein was congested and full with hemolysed red blood cells. The bile duct was dilated. Marked inflammatory cell infiltration around the portal tract was appeared (Fig.2b). Sections stained with Masson's trichrome showed a large amount of collagen fibers deposition around the portal tracts and in the wall of the blood sinusoids (Fig. 2c). Sections stained with Periodic acid Schiff showed a decrease of PAS reaction in the hepatic parenchyma (Fig.2d).

Doxorubicin with alpha –lipoic acid treated groups (SubG. IVA)

Histological examination of transverse sections of one day old albino rat liver of the doxorubicin with alpha-lipoic acid maternally treated group (Subgroup IVA) stained with Hx & E showed a marked recovery of the hepatic parenchyma (Figs. 3a & 3b) as compared with that of doxorubicin treated groups (Figs.2a & 2b). The liver was nearly similar to that of the control group (Figs.1 a & 1b).

Sections stained with Masson´s trichrome showed a decrease in the amount of the collagen fibers deposition in the portal tract area and in the walls of the blood sinusoids (Fig. 3c). Sections stained with PAS stain showed restoration of the gluconeogenesis by a gradual increase of PAS reaction (Fig. 3d).

B) Liver of seven days old rat

Control (SubG1B) & alpha-lipoic acid maternally treated groups (Sub GIIB):

Histological examination of transverse sections of seven days old albino rat liver of the control group (subgroup IB) and the alpha-lipoic acid maternally treated group (subgroup IIB) stained by Hx & E showed the normal hepatic architecture. The liver became more developed (Fig. 4a) as compared with the liver of one day old (Fig.1a). The irregular groups of the hepatocytes became arranged into ill-defined hepatic lobules. In each lobule, the hepatocytes became nearly arranged into hepatic cords. These hepatic cords were irregularly radiating from the central vein towards the periphery. The central veins and the portal tracts were numerous (Fig.4a) as compared with the liver of one day old (1a). The blood sinusoids appeared well developed and lined with flat endothelial cells and Von-Kupffer cells. The hepatocytes were polyhedral in shape with eosinophilic granular cytoplasm. Each cell had one or two large rounded vesicular basophilic nuclei (Fig. 4b). The hemopoietic cells and the hemopoietic islets were less numerous (Fig.4b) as compared with those of one day old liver (Fig. 1b). The portal areas appeared more developed (Fig. 4b) as compared with the liver of one day old (Fig.4b), it was formed of a branch of the portal vein, a branch of the hepatic artery and a branch of bile duct. The portal vein was lined with a regular single layer of flat endothelial cells with flat nuclei. The hepatic artery was lined by a single layer of flat endothelial cells resting on a prominent basal lamina. The bile duct was lined with a single layer of cubical epithelium (Fig. 4b). Sections.

stained with Masson's trichrome showed a moderate amount of collagen fibers deposition in the portal tract and in the walls of the blood sinusoids (Fig. 4c).

Sections stained with Periodic acid Schiff showed a severe PAS positive reaction of the hepatic parenchyma (Fig. 4d).

Doxorubicin treated groups (SubG. IIIB)

Histological examination of transverse sections of seven days old albino rat liver of the doxorubicin maternally treated group (Subgroup IIIA) stained with Hx & E showed that the normal hepatic architecture was lost. The arrangement of the hepatocytes into cords was lost (Fig. 5a). The central vein was markedly dilated with damage of its endothelial lining. Most of the blood sinusoids were hardly detected due to disorganization and disruption of the hepatic cords. Some of the blood sinusoids appeared to be dilated. There were signs of massive degeneration and necrosis in the hepatocytes. The hepatocytes lost their normal shape, size and arrangement. Most of the hepatocytes had vacuolated cytoplasm and their nuclei were pyknotic (Figs. 5b). The hemopoietic cells and islets could not be detected. The portal areas showed marked congestion and dilatation of the portal vein and a dilation of the bile duct. The portal tract was infiltrated with inflammatory cells (Fig. 5b). Sections stained with Masson's trichrome showed a large amount of collagen fibers deposition in the portal tract and in between the hepatocytes (Fig. 5c). Sections stained with Periodic acid.

Schiff showed a mild PAS positive reaction of the hepatic parenchyma (Fig.5d).

Doxorubicin with alpha –lipoic acid treated groups (SubG. IVB)

Histological examination of transverse sections of seven days old albino rat liver of the doxorubicin with alpha-lipoic acid maternally treated group (Subgroup IVB) stained with Hx & E showed marked improvement of the hepatic parenchyma (Fig. 6a) as compared with doxorubicin treated group (Fig.5a) but it was still not as the control group (Fig. 4a). The hepatic cords were irregularly radiating from dilated central vein towards the periphery and were separated by dilated blood sinusoids (Figs. 6a & 6b). While the central veins and the blood sinusoids were dilated, the portal veins became less dilated (Figs. 6a & 6b).

There was an improvement in the hepatocytes which became of normal size and shape, and their cytoplasm appeared to be less vacuolated especially the hepatocytes around portal tract area. There were few hemopoietic cells scattered within the parenchyma (Figs. 6a & 6b). The portal areas showed slightly congested portal vein. The dilation of the bile duct disappeared. There was some inflammatory cells infiltration around the portal tract (Fig.6b).

Sections stained with Masson's trichrome showed a moderate amount of collagen fibers deposition in the portal tract and in the walls of the blood sinusoids (Fig. 6c). This amount was more than that of control group (Fig. 4c) and less than that of doxorubicin treated group (Fig. 5c). Sections stained with Periodic acid Schiff showed a severe PAS positive reaction in some areas of the hepatic parenchyma and a nearly mild PAS positive reaction in other areas (Fig. 6d).

Cytogenetic results

1. Micronucleus test (MNH):

The number and means % of micronucleated hepatocytes in control and treated albino rat offsprings in the different subgroups were studied by examining 1000 cells per animal. Table (1) and graph (1) using unpaired statistical analysis, represented the number and mean % of micronucleus hepatocytes in one and seven days old albino rats. ALA (subgroups IIA & IIB) showed no significant elevation in the frequency of MNHS, indicating its safety profile. The results revealed that DOX (subgroups IIIA & IIIB) recorded a highly significant frequency ($p< 0.001$) of MNHs in one and seven days (Fig. 7) comparing with control (subgroups IA & IB). An extensive presence of necrotic and apoptotic cells (having more than five nuclei or no nuclei) was observed in offspring hepatocytes.

II. Chromosomal aberrations assay

Table (2) using unpaired statistical analysis, represented the mean% ± SD of the percentage of chromosomal aberrations (Fig. 8) scored in one and seven days old albino rats. Subgroups IIIA & IIIB (whose their mothers treated with DOX) recorded a highly significant percentage of chromosomal aberrations in hepatocytes ($p<0.001$). ***. The more frequent aberrations were gaps, breaks and /or fragments and deletions (Fig. 8). The percentage after seven days old rats was more than that of one day old rats indicating the accumulative effect of DOX in liver cells.

Subgroups IVA & IVB (whose their mothers treated with (ALA+DOX) recorded that at one day old was at lower significant level or no significant ($p<0.404$) while at seven days old was significant at ($p<0.003$) ** level comparing to treated groups IIIA & IIIB respectively. (graphs 2a, b, c & d).

4. Discussion

In the present histological study, the normal development of the liver in the rats at one and seven days old was in agreement with Le Bouton (1974), Chiasson (1983); Snell (1984) and Gartner and Hiatt (2007).

In the present study, the liver of albino rat offspring was affected by DOX injection. The hepatotoxicity in the offspring suggested transplacental passage of doxorubicin. These results were in agreement with many researchers, Pommier et al. (1996) and Garcia et al. (1999) suggested that fetal tissues were more susceptible to the effect of doxorubicin since topoisomerase II was overexpressed in rapidly growing tissues. Gillick et al. (2002) found that doxorubicin affected pregnancy outcome in human and animals. Also, Germann et al. (2004); Matalon et al. (2004) and Pedrycz et al. (2005) stated that topoisomerase II inhibitors (as DOX) was a potential source of major damage for the embryo. Calstern et al. (2010) ensured the transplacental passage of anthracycline in the experimental baboon model.

In the present histological studies, the results were more severe and pronounced in seven days old albino rats than in one day old albino rats. These results were supported by Pedrycz et al. (2006) who suggested that in the body of mother, DOX or its metabolites could be still present long time after finished treatment. It was also probable that free radicals, which arose during DOX biotransformation were also hepatotoxic and genotoxic in the body of fetus. Esterbauer et al. (1986), and Luo et al. (1997) stated that DOX produced lipid peroxidation that resulted in the production of a great variety of stable, diffusible saturated and unsaturated aldehydes such as malondialdehyde, alkanals and alkadienals. These cytotoxic aldehydes were extremely active, and they could diffuse within or even escaped from the cell and attacked targets far from the initial site of the free radical event, and there for acted as secondary cytotoxic messengers.

In the present study, histological examination of one day old albino rat liver of the DOX maternally treated group revealed that the normal hepatic architecture was lost. The liver was covered with an irregular thin capsule. The irregular groups of hepatocytes were widely separated by dilated blood sinusoids. Their radiation from the central veins was difficulty detected. These results were in agreement with Molodykh et al. (2006) who studied changes in the liver caused by a single injection of DOX in a sublethal dose (10mg/kg). They found severe dilatation of the sinusoids, central and portal veins which gave the hepatic parenchyma a "honey comb" appearance. Also, Yagmurca et al. (2007) and Sakr et

al. (2011) stated that the liver after DOX treatment in rats reflected signs of injury as congestion of intrahepatic veins, central and portal veins with dilatation of sinusoids.

In the present study, seven days old albino rat liver showed that the normal hepatic architecture was still lost. The degeneration became more advanced and marked than in one day old albino rats. The arrangement of the hepatocytes into cords was lost. These results were in agreement with El-Sayyad et al. (2009) who demonstrated the toxic effect of DOX on the rat liver, they revealed massive hepatotoxicity including dissolution and degeneration of hepatic cords with vacuolar degeneration and apoptotic cell death.

In the present study, the hepatocytes of rat offsprings revealed signs of marked degeneration and necrosis especially in the subcapsular and the pericentral areas. In support, Saad et al. (2001) and Molodykh et al. (2006) mentioned that the number of vacuolated hepatocytes increased significantly in the pericentral zone of the lobules and in the subcapsular area. The hepatocytes in these areas were exposed to the lowest oxygen and nutrient concentrations, so they were more affected than other hepatocytes when exposed to any toxic substance (Mescher, 2010).

In the present study, the dilatation and congestion of the portal areas with leucocytic infiltrations were agreed with Yagmurca et al. (2007) and Sakr et al. (2011), they stated inflammation in portal area, hemorrhage, vascular congestion and remarkable abundance of leucocytic infiltrations after DOX injection to the rats.

In the present study, a marked fibrosis was detected in the portal areas of liver of one and seven days old albino rats. Bacon and Britton (1989) and Ross and Pawlina (2003) explained the increased collagen formation as they reported that formation of hydroxyl reactive oxidizing molecules in biological system led to lipid peroxidation. The later caused damage of proteins and nucleic acids. The end results of these reactions increased the collagen formation. El-Sayyad et al. (2009) and Sakr et al. (2011) stated that DOX showed higher tendency for liver fibrosis manifested by remarkable abundance of collagen fibers deposition around the portal tracts, in the walls of the blood sinusoids or in between the hepatocytes.

In the present study, testing glycogen content of hepatic parenchyma by PAS reaction stain revealed its marked depletion in one and seven days old albino rats after maternal treatment with DOX. These results were correlated with finding of Pedrycz et al. (2006) and Yagmurca et al. (2007), they mentioned that DOX induced cell death and a moderate to extensive decrease in glycogen staining intensity. Most injury was confined to the centrilobular zone and characterized by generalized depletion of glycogen.

In the present study, there was a marked improvement in the hepatic parenchyma of one and seven days old albino rats whose maternally treated with doxorubicin and alpha lipoic acid. In one day old albino rats, the hepatic parenchyma was a nearly similar to control. It was covered with a thin capsule. Under the capsule, the liver was formed of irregular groups of hepatocytes. These cells had not definite arrangement. They were nearly irregularly radiating from the central veins. In seven days old albino rat, there was a marked improvement as compared with DOX treated group but it was not as the control group. The hepatic cords were irregularly radiating from dilated central veins towards the periphery and were separated by dilated blood sinusoids. These results were in agreement with Balachandar et al. (2003) demonstrated that prttreatment with ALA offered a significant cardioprotection from doxorubicin in the rat heart. Malarkodi et al. (2003) stated a significant improvement of DOX induced nephrotoxicity upon pretreatment with ALA. Also Malarkodi et al. (2004) and El Batsh et al., (2015) demonstrated that ALA had the ability to protect the liver from poisons and the ability to regenerate the liver if it has already been damaged.

As regards the cytogenetic study. the liver micronucleus assay was performed in the 1st and 7th day offsprings to investigate the formation of micronucleated hepatocyte. The present study demonstrated that DOX increased the frequencies of micronuclei in albino rat hepatocytes. The results recorded a significant ($p< 0.01$) elevation in the percentage of micronuclei at 7th day old than the 1st day old.

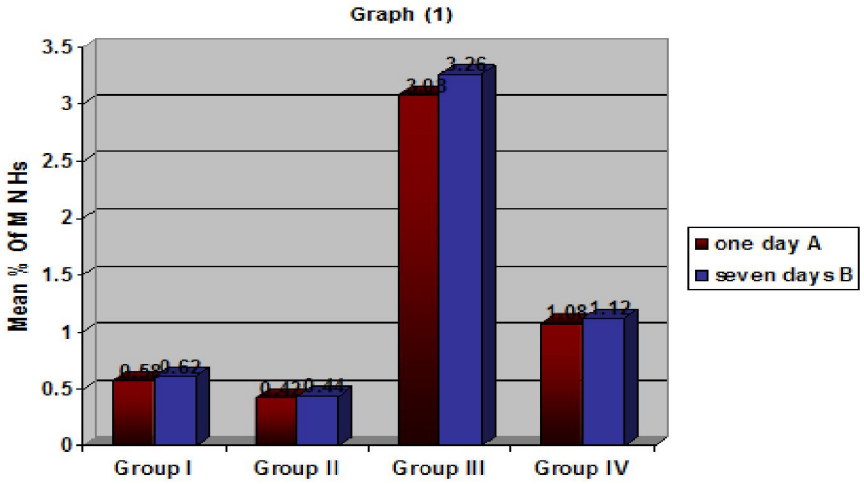
These results are in agreement with (Heddle, 1977 and Stopper et al., 1999). They mentioned that micronuclei are the result of either chromosome breaks or disturbances of the mitotic spindle, so formation of micronuclei is a good example of drug induced chromosomal alterations. Patel et al., 2010 suggested that DOX metabolism triggered production of ROS and reactive intermediates in liver resulting in oxidative stress and genomic injury followed by cell death.

Regarding the chromosomal aberrations assay, DOX induced a highly significant percentage of structural chromosomal aberrations in hepatocytes of one and seven days old albino rats. The more frequent aberrations were gaps, breaks and /or fragments and deletions.

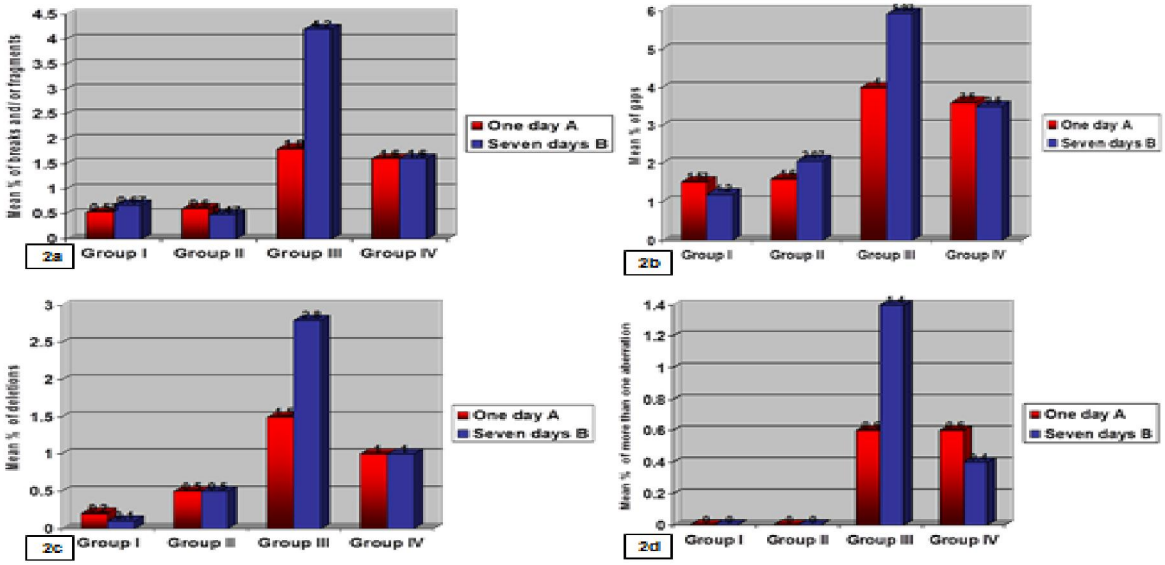
These results supported by many authors. ADR induces cytogenetic damage e.g. large deletions as a major type of gene mutation in mammalian cells, suggesting the involvement of ROS as one mutagenic

pathway (Yongjia et al., 1994), chromatid breaks are the most frequent aberrations induced in rat bone marrow cells (Tohamy et al., 2003; Gũlkac et al., 2004). Aly et al. (2009) reported that daunorubicin (DOX analogue) produced a significant percentage of chromosomal aberrations and sister-chromatid exchanges in somatic and germ cells of mice. DOX induced cardiotoxicity, hepatotoxicity and genotoxicity in bone marrow cells of albino mice (Yalcin et al., 2010 and Aboul-Mahasen et al. 2012)).

In the present study, the beneficial effects of ALA were monitored by the same assays micronucleus and chromosomal aberrations in hepatocytes. The results revealed a significant reduction in the frequency of micronucleus as well as chromosomal aberrations in hepatocytes of one and seven days old albino rats. ALA reduced the frequency of aberrant cells to approach the control levels. These results were in agreement with Pedrycz et al. (2006), Anandakumar et al. (2007), Saad et al. (2010), Tabassum et al. (2010), Aboul-Mahasen et al. (2012) and Li et al., 2014), they found that pretreatment with ALA was ameliorating the hepatotoxicity induced by DOX. ALA played a role as an antioxidant and also improved the degenerative state of liver cells and DOX induced hepatotoxic side effects.



Graph (1): Relationship regarding means % of micronucleated hepatocytes (MNHS) in one and seven days old albino rat liver in all groups.



Graph (2): Relationship between the means% of chromosomal aberrations in all subgroups with comparison between one and seven days old albino rat liver

a. means % of gaps
b. means % of breaks and/ or fragments
c. means % of deletions
d. means % of more than one aberration

Table 2: Unpaired T-test s with multiple comparisons between all groups regarding chromosomal aberrations between one and seven days old albino rat liver.

Types of chromosomal a aberrations		Groups A		Groups B		T-test	
		Mean% ± SD		Mean% ± SD		T	P-value
Gaps	Group I	1.53	± 1.06	1.27	± 1.10	0.659	0.515
	Group II	1.60	± 1.06	2.07	± 0.88	1.321	0.197
	Group III	4.00	± 1.19	5.93	± 1.98	3.236	0.003
	Group VI	3.60	± 1.59	3.53	± 1.30	0.132	0.895
Breaks and / or fragments	Group I	0.53	± 0.64	0.67	± 0.62	0.609	0.547
	Group II	0.60	± 0.51	0.47	± 0.52	0.691	0.495
	Group III	1.80	± 1.01	4.27	± 1.67	4.902	0.001
	Group VI	1.60	± 1.12	1.60	± 1.40	0.000	1.000
Deletions	Group I	0.20	± 0.41	0.13	± 0.35	0.503	0.619
	Group II	0.53	± 0.52	0.53	± 0.52	0.000	1.000
	Group III	1.53	± 1.36	2.87	± 1.25	2.810	0.008
	Group VI	1.00	± 0.93	1.00	± 0.53	0.000	1.000
More than one aberration	Group I	0.00	± 000	0.00	± 0.00		
	Group II	0.00	± 0.00	0.00	± 0.00		
	Group III	0.67	± 0.82	1.40	± 1.18	1.968	0.059
	Group VI	0.60	± 0.51	0.47	± 0.52	1.968	0.059
Total aberrations with gaps	Group I	2.27	± 1.39	2.07	± 1.49	0.380	0.706
	Group II	2.73	± 0.96	3.07	± 1.03	0.935	0.357
	Group III	8.00	± 1.36	14.47	± 2.2	9.688	0.001
	Group VI	6.80	± 2.04	3.07	± 1.53	5.665	0.001
Total aberrations without gaps	Group I	0.73	± 0.88	0.80	± 0.86	0.220	0.827
	Group II	1.13	± 0.64	1.00	± 0.76	0.507	0.616
	Group III	4.00	± 1.60	8.53	± 1.88	7.107	0.001
	Group VI	3.20	± 2.01	6.47	± 2.20	4.250	0.001

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