The possible protective effect of antioxidant Alpha-lipoic acid on the liver of pregnant albino rat and its offspring treated with Doxorubicin

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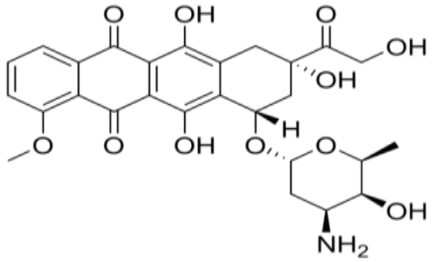
Abstract: The paucity of data on the fetal effects of prenatal exposure to chemotherapy prompted us to study transplacental transport of chemotherapeutic agents and its effect on liver. The liver was used because of the predominantly high penetration of almost all compounds to this organ. Doxorubicin (DOX) an anthracycline antibiotic, which is widely used as an antineoplastic drug in the treatment of various solid tumors, has been shown to induced hepatotoxicity. The aim of the present study was to investigate the protective efficacy of Alpha- lipoic acid (ALA) on DOX-induced hepatotoxicity in female albino rats and their offspring. The pregnant mothers were divided into four main groups G1 (control) i.p. injected with distilled water, after delivery divided into four subgroups:G1A (one day old offspring) G1B (seven days old offspring), G1C (mothers one day after delivery). G1D (mothers seven days after delivery). G2 orally treated with ALA at 1.3mg/kg.b.wt. via gastric tube for four successive days at 11^{th}, 12^{th},13^{th}, and 14^{th} day of gestation. After delivery divided to G2A, G2B, G2C, G2D. G3 (DOX treated group) i.p. injected with DOX at 1.782mg/kg b.wt. for three successive days at 12^{th}, 13^{th}, and 14^{th} days of gestation (2^{nd} trimester). After delivery divided to G3A, G3B, G3C, G3D. G4 (ALA+DOX treated group) i.p. injected with DOX at 12^{th}, 13^{th}, 14^{th}days of gestation and were given orally ALA 24h prior to and during treatment with DOX from the day11^{th} to 14^{th} day of gestation. After delivery divided to G4A, G4B, G4C, G4D. DOX hepatotoxicity and beneficial effects of ALA were monitored by micronucleus and chromosomal aberrations assays in rat liver. DOX –induced a highly significant percentage of micronucleated hepatocytes in mothers and offspring one and seven days after delivery, and the percentage of chromosomal aberrations reached after excluding gaps 4% and 8.53% for one and seven days old albino rats respectively. Groups treated with ALA+DOX showed a significant reduction in the frequency of micronuclei formation as well as of chromosomal aberrations. In conclusion, this study shows limited fetal exposure after maternal administration of DOX and illustrate the protective efficacy of ALA against hepatotoxicity induced by DOX in rat liver. [Researcher. 2012;4(2):25-35]. (ISSN: 1553-9865).

Keywords: Doxorubicin, Alpha-lipoic acid, hepatotoxicity, micronucleus, chromosomal aberrations.

Introduction

Pregnancy complicated by cancer is relatively rare but, there is a growing number of women who delay pregnancy to advanced age **(**Fitzgerald et al., 1998). Since older age is associated with malignant diseases (Yancik et al., 1994), expected that the incidence of cancer in pregnancy will rise also. Thus, there is the potential for more frequent exposure of women, embryos and fetuses to cytotoxic and/or immunosuppressive agents during pregnancy. Antineoplastic drugs may affect the placenta directly or may cross the placenta to enter fetal circulation, carrying a risk for fetal maldevelopment and malformations (Zemlickis et al., 1992; Pacifici et al., 1995). Doxorubicin (DOX) or Adramycin (ADR) (Fig.1) is an anthracycline, antineoplastic antibiotic. It acts by forming stable complexes with DNA and by interfering with the synthesis of nucleic acids. DOX displays linear pharmacokinetics after intravenous administration. It is widely distributed in the plasma and tissues, and plasma protein binding ranging from 50 to 85%. The drug is extensively metabolized in the liver by aldo-keto reductase, to yield the dihydrodiol derivative doxorubicinol, which retains antitumor activity (Danesi et al., 2002). Aldo-keto reductase activity is present also in the placenta (Grimshaw and Lai, 1996). DOX is often administered to patients with lymphoma or solid tumors (such as breast cancer), which are the leading malignancies in pregnant women. (Montvale, 1993; Weisz et al., 2001). DOX is teratogenic in laboratory animals, as demonstrated by a number of in vivo and in vitro experiments. It produced increased malformation rates when tested in rats (Menegola et al., 2001; Gillick et al., 2002). Hence, it is vital to determine the frequency and severity of genotoxicity following treatment with chemotherapy and develop strategies to reduce its occurrence in normal cells. The measurement of micronucleated hepatocytes and analysis of chromosomal aberrations in liver are important tools in assessment of hepatotoxicity (Injac et al., 2008).



Fig(1) Tetracycline ring Daunosamine Chemical structure of doxorubicin.

Alpha-lipoic acide (ALA), a naturally occurring nutraceutical, functions as an essential cofactor in metabolic reactions involved in energy utilization. ALA and its reduced form dihydrolipoic acid are effective against conditions in which oxidative stress has a role (Packer et al., 1995). It shows beneficial effects in oxidative stress conditions because of its synergistic action with other antioxidants (Suzuki et al., 1993). ALA, which is universal antioxidant functions both in aqueous and membrane phases. (Kagan et al., 1992)ALA supplementation has been shown to be beneficial in treating a number of clinical disorders (Greenamyre et al., 1994; Somani et al., 2000). (Fig,2).

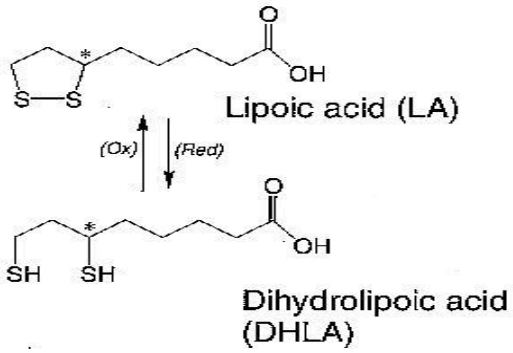


Fig. (2): Redox couple formed by lipoic acid and dihydrolipoic acid.

The aim of this study was to investigate the potential protective efficacy of ALA on DOX – induced hepatotoxicity using pregnant female and their offspring albino rats monitored by micronucleus assay in liver hepatocytes in both modles and chromosomal aberration assay in liver offspring after one and seven days of delivery.

2-Materails and methods:

2.1. Drugs and chemicals:

Doxorubicin was purchased from Pharmacia and Upjohn-Milan-Italy (Trade name Adriaplastina). Alpha-lipoic acid was purchased from Eva-Pharma Cairo, Egypt (Trade name Thiotacid).

2.2. Animals:

Random bred 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.

2.3. Doses and treatments:

According to Paget and Barnes (1964), the dose of doxorubicin per rat: 99x18/1000=1.782mg/kg b.wt.Alpha lipoic acid per rat: 75x18/1000=1.3mg/kg.b.wt.

The pregnant mothers were divided into four main groups (20 for each). G1(control) i.p. injected with distilled water, after delivery divided into four subgroups: G1A (one day old offspring). G1B (seven day old offspring) G1C (mothers one day after delivery), G1D (mothers seven day after delivery). G2 orally treated with ALA via gastric tube for four successive days at 11^{th}, 12^{th},13^{th}, and 14^{th} day of gestation. After delivery divided to G2A, G2B, G2C, G2D. G3 (DOX treated group) i.p. injected with DOX for three successive days at 12^{th}, 13^{th}, and 14^{th} days of gestation(2^{nd} trimester). After delivery divided to G3A, G3B, G3C, G3D. G4 (DOX with ALA treated group) i.p. injected with DOX at 12^{th}, 13^{th}, 14^{th}days of gestation and were given orally ALA 24h prior to and during treatment with DOX from the day11^{th} to 14^{th} day of gestation. After delivery divided to G4A, G4B, G4C, G4D.

Table (1) ANOVA test Tukey's test with multiple comparisons between all groups regarding mean % of micronucleated hepatocytes (MNHS) in one and seven days old albino rat.

Groups	No.Of MNHs	Mean%+ S.D.	ANOVA		RMN%
			F	P-value	
IA	87	0.58+ 0.20	33.124	0.001***	0.20%
IIA	63	0.42+ 0.37			
IIA	462	3.08+ 2.45			
IVA	162	1.08+ 1.15			
IB	93	0.62+ 0.20	25.431	0.001***	0.19%
IIB	66	0.44+ 0.22			
IIB	489	3.26+ 2.32			
IVB	168	1.12+ 1.35			

Tukey's test					
IA&IIA	IA&IIIA	IA&IVA	IIA&IIIA	IIA&IVA	IIIA&IVA
0.750	0.001***	0.05*	0.001***	0.05*	0.003**
IB&IIB	IB&IIIB	IB&IVB	IIB&IIIB	IIB&IVB	IIIB&IVB
0.632	0.001***	0.01*	0.001***	0.01*	0.01**

Each group consisted of 15 offsprings, 3 offsprings from each mother. The total number of scored cells is 15000 /group. A: one day. B: seven days Group IA: control one day. Group IIA: ALA. Group IIIA: DOX Group IVA: ALA+ DOX Group IB: control 7 days Group IIB: ALA. Group IIIB: DOX. Group IVB: ALA+DOX. SD: Standard deviation.Significance level: P< 0.05. * P<0.01**. P< 0.001***. RMN: Reduction of micronuclus level.

2.4. Cytogenetic studies:

2.4.1. Micronucleus test in hepatocyte:

The liver of mothers and their offspring were used, according to Tates et al. (1980) with some modifications. Hepatocytes were isolated from anaesthetised rats by the collagenase perfusion method (collagenase, 45^{O}C; 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 suspention 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 min 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. (ValentinSeverin 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)

2.4.2. Chromosomal aberrations in hepatocyte:

Metaphases were prepared according to Yosida and Amano (1965) with some modifications. The liver of albino rat offspring used only, Chromosomal aberrations assay described here utilises the autonomous proliferation of hepatocytes of young rats. Rat offspring were injected i.p. with colchicines at a final concentration of 3mg/kg b. Wt. 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.

2.4.3.Statistical analysis:

The significance of the results from control data was calculated using ANOVA and Tukey's test.

3. Results:

3.1. Micronucleus test:

Table (1) represented the number and mean % of micronucleus hepatocytes in one and seven days old albino rats. ALA (groups IIA & IIB) showed no significant elevation in the frequency of MNHS, indicating its safety profile. The results revealed that DOX (groups IIIA & IIIB) recorded a highly significant frequency (p< 0.001) of MNHs in one and seven days respectively comparing with control

groups (IA & IB). The protective effect of ALA administered to the pregnant mothers before and during gestation for four successive days reduced MN frequency 0.20% and 0.19% one and seven days respectively ($p<0.05$). Fig.4 (a, b, c).

Table (2) showed statistical analysis between one and seven days after delivery mothers groups IC, IIC, IIIC, IVC and ID, IID, IIID, IVD as regards means % ± S.D. of MNHs**.** Groups of DOX at 1.782mg/kg b.wt. treated for three successive days during second trimester were statistically highly significant ($p<0.001$) at one and seven days and declined when treated with ALA at 1.3mg/kg. b.wt. before and during DOX treatment to reach low significant levels ($p<0.025$ & 0.011) respectively, indicating ALA protective effect and safety for pregnant mothers.

3.2. Chromosomal aberrations in hepatocyte offsprings::

Table (3) and (4) represented the mean% ± SD of the percentage of chromosomal aberrations scored in one and seven days old albino rats. Groups IIIA & IIIB (their mothers treated with DOX) recorded a highly significant percentage of aberrations in hepatocytes ($p<0.001$)***. Groups IVA & IVB (their mothers treated with (ALA+DOX) recorded a lower significant level, at one day old was no significant ($p<0.404$), at seven days old was significant at ($p<0.003$)** level comparing to treated groups IIIA&IIIB respectively. The percentage after seven days was more than that of one day indicating the accumulative effect of anthracyclines in liver cells. (fig3:d, e, f).

4. Discussion:

Animal and human data suggest that most antineoplastic drugs may have deleterious effects on the fetus, including increasing incidence of prematurity, intrauterine growth retardation and low birth weight. Animal models have also show a high risk of congenital anomalies, which in human occur mainly when the drugs are administered during the first trimester (Zemlickis et al., 1993). DOX affect pregnancy outcome in human and animals (Gillick et al., 2002), the drug may damage DNA structure, inhibit proliferation or induce apoptosis of different cells that are important during placental and embryonic development in the first trimester of pregnancy (Matalon et al ., 2004). Anthracycline was found in the maternal and fetal parts of the placenta of women with lympho-proliferative disorders were treated with DOX-containing regimens (Karp et al.,

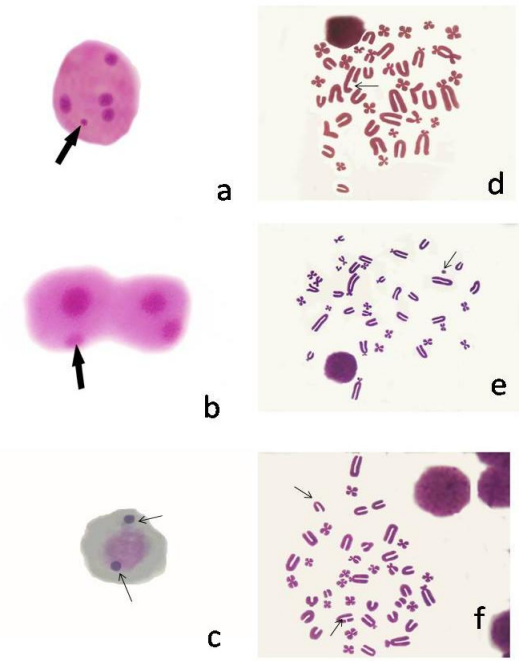


Fig. (3):
a- A mother hepatocyte with multinucleated and micronuclei.
b- Two offspring hepatocytes with micronuclei.
c- An offspring hepatocyte with two micronuclei.
d- A metaphase of offspring hepatocyte with chromosome break.
e- A metaphase of offspring hepatocyte with fragment.
f- A metaphase of offspring hepatocyte with chromatid gap and chromosome deletion.

1983). Moreover, DOX was found in the liver, kidney and lung, but not in the amniotic fluid of a woman with Hodgkin's lymphoma, who aborted at the 17th week of gestation (D' Incalci et al., 1983). Those findings suggest that DOX may cross the placenta. In a recent study, Van Calsteren et al., 2010, in a baboon model, a limited fetal exposure was found after maternal administration of DOX, epirubicin, vinblastine and 4-hydroxy-cyclophosphamide. The limited transfer and undetectable levels in brain and cerebrospinal fluid (CSF) are reassuring findings.

Voulgaris et al., 2011, a comprehensive review about cancer and pregnancy illustrated that anthracycline antibiotics are agents specific for the G2/S phase that interact with the topoisomerase II enzyme. No adverse outcomes have been reported for the use after the 1st trimester for DOX or daunorubicin (Germann et al., 2004; Ring et al.,

Table (2): ANOVA test and Tukey's test with multiple comparisons between all groups regarding mean % of micronucleated hepatocytes (MNHS) of albino rat mothers one and seven days after delivery.

Groups	NO.of MNHs	Mean%+ S.D.	ANOVA		RMN%
			F	P-value	
IC	**44**	0.88± 0.58	23.154	0.001***	
IIC	**33**	0.66± 0.60			
IIIC	**134**	2.68± 0.92			
IVC	**77**	1.54± 0.75			0.37**%**
ID	**41**	0.82± 0.37	25.1231	0.001***	
IID	**31**	0.62± 0.20			
IIID	**154**	3.08± 1.24			
IVD	**60**	1.20± 0.95			0.19**%**

Tukey's test					
IC&IIC	**IC&IIIC**	**IC&IVC**	**IIC&IIIC**	**IIC&IVC**	**IIIC&IVC**
0.215	0.001***	0.001**	0.001***	0.001**	0.025*
ID&IID	**ID&IIID**	**ID&IVD**	**IID&IIID**	**IID&ID**	**IIID&IVD**
0.351	0.001***	0.05*	0.001***	0.05*	0.011**

Each group consisted of 5 mothers. The total number of scored cells is 5000 /group. C: one day after delivery. D: 7 days after delivery Group IC: control group. Group IIC: ALA .Group IIIC: DOX. Group IVC: ALA+DOX .Group ID: control group. Group IID: ALAGroup IIID: DOX. Group IVD: ALA+DOX.SD: Standard deviation. Significance level: P< 0.05.* P<0.01** P<0.001. *** RMN: Reduction in micronuclus level**.**

2005; Loibl et al., 2006). DOX and epirubicin seem to display similar toxicity profiles and transplacental transfer during pregnancy. Peccatori et al., 2009, recently reported the feasibility and safety of weekly low dose single agent epirubicin after the 1st trimester in 20 patients with gestational breast cancer. DOX is teratogenic in laboratory animals, as demonstrated by a number of in vivo and in vitro experiment, it affects almost every organ system in the body, the cell populations that typically exhibit rapid cell turnover, such as those of the bone marrow and gastrointestinal mucosa are the most sensitive to its deleterious side effects **(**Kocak et al., 2004).

The liver micronucleus assay was performed in mothers and their offspring to investigate the inducibility of micronucleated hepatocyte. The present study demonstrated that, DOX gave up to ~ 2mg/kg b.w. /day, i.p. treatment for three consecutive days, cumulative dosage ~ 6 mg/kg b.w. during second trimester of gestation produced increased frequencies of micronuclei in albino rat hepatocytes of offsprings and their mothers. The results recorded a significant (p< 0.01) elevation in the percentage of micronuclei at seventh day of delivery than the first day, for the mothers and offspring. The liver was used in this study as it is extremely important for the risk assessment of the potential aberrations because of the predominantly high penetration of almost all compounds to this organ (Igarashi et al., 2007).

According to the unique nature of hepatocyte micronuclei were scored in mono-,bi- and multinucleated cells . Since micronuclei are the result of either chromosome breaks or disturbances of the mitotic spindle (Heddle, 1977). The formation of micronuclei is a good example of drug induced chromosomal alterations that do not kill the cell. Stopper et al., 1999**,** reported that, topo II inhibitors from different chemical classes are able to induce micronuclei in concentrations of low toxicity which are in the clinically relevant dose range. Micronuclei might be induced without apoptosis under certain conditions. If the micronucleus containing cells survive and divide they may be precursors of malignant cells (Stopper and Mũller, 1997). Even if they are not precursors themselves they are an indication of the amount of genotoxic damage induced in healthy tissue by therapy.

DOX induces mutations and chromosomal aberrations in normal and tumor cells.DNA damage can have a variety of biological ramifications including inhibition of DNA replication and/ or RNA

Table (3): ANOVA test and Tukey's test with multiple comparisons between all groups regarding chromosomal aberrations in one day old albino rat liver cells.

Groups (One day)		Gaps	Breaks and / or fragments	Deletions	More than one aberration	Total aberrations with gaps	Total aberrations without gaps
		Mean%±SD	Mean%±SD	Mean% SD	Mean%±SD	Mean%±SD	Mean%±SD
Group IA		1.53±1.06	0.53±0.64	0.20±0.41	0.00±0.00	2.27±1.39	0.73±0.88
Group IIA		1.60±1.06	0.60±0.51	0.53±0.52	0.00±0.00	2.73±0.96	1.13±0.64
Group IIIA		4.00±1.19	1.80±1.01	1.53±1.36	0.67±0.82	8.00±1.36	4.00±1.60
Group VIA		3.60±1.59	1.60±1.12	1.00±0.93	0.60±0.51	6.80±2.04	3.20±2.01
N O V	F	16.33	8.85	6.43	8.73	55.97	19.28
	P-value	0.000***	0.00***	0.00***	0.00***	0.00***	0.00***
Tuky's test	IA&IIA	0.999	0.997	0.732	1	0.826	0.861
	IA&IIIA	0.000***	0.001***	0.001***	0.002**	0.000***	0.000***
	IA&IVA	0.000***	0.007**	0.075	0.006**	0.000***	0.000***
	IIA&IIIA	0.000***	0.002**	0.016**	0.002**	0.000***	0.000***
	IIA&IVA	0.000***	0.012**	0.478	0.006**	0.000***	0.001***
	IIIA&IVA	0.816	0.919	0.359	0.981	0.134	0.404

Each group consisted of 15 offsprings, 3 offsprings from each mother. The total number of scored cells is 1500 /group. Group IA: One day old albino rat liver of the control group. Group IIA: One day old albino rat liver of the ALA treated group. Group IIIA: One day old albino rat liver of the DOX treated group. Group IVA: One day old albino rat liver of ALA+DOX treated group . significant level: P<0.05, * P<0.01, ** P<0.001. ***

transcription and ultimately leads to cell death (Gewirtz, 1999; Olsen et al.,2005). DOX intercalates with DNA and partially uncoils the double-strand helix, it has a high affinity for cell nuclei as much as 60% of the total intracellular amount of DOX found in the nucleus. Once binding to DNA occurs several consequences may issue. DOX binds to DNA polymerase and inhibits nucleic acid synthesis. In addition, anthracyclines are known to stabilize the otherwise cleavable complex between DNA and homodimeric top II enzyme subunits, resulting in formation of protein-linked DNA double strand breaks (Evert et al., 2001), and undergo redox reactions that generate reactive oxygen species (ROS). (El-makawy and El-Ashmaoui, 2003; Kurz et al.,2004; Pommier et al., 2010). ADR induced myocardial, hepatic lipide peroxidation and enhanced DNA damage as single strand breaks in rat hepatocytes 24h after treatment. (Bagchi etal., 1995).

The micronuclei-induction by ADR is mainly due to DNA strand breaks and chromosome aberration in Hela cells (Jagetia and Nayak,1996) human lymphocytes (Amara-Mokrane et al., 1996), and in human cancer cells (Dhawan et al.,2003; Tan and Porter, 2009). In addition, DOX can also react with cellular formaldehyde to form DNA adducts (Swift et al., 2006).

It is believed that oxidative stress and the formation of free radicals play a crucial role in the mechanism of DOX toxicity in liver. Two different mechanisms have been identified. The first implicates the formation of semiquinone-type free radical molecules, which are produced via the NADPH-depended reductase enzyme pathway. Derivatives originating from DOX give rise to superoxide radicals by reacting with oxygen. The second pathway includes a non-enzymatic reaction, which involves a reaction of DOX with iron. Semiquinone metabolites delocalized Fe^{2+} DOX from ferritin and generate H_2O_2, hence causing hydroxyl radical formation and oxidant injury in cellular systems (Injac et al.,2008).

Patel et al., 2010 suggest that DOX metabolism triggered production of ROS and reactive intermediates in liver resulting in oxidative stress and genomic injury followed by cell death.

Chromosomal aberrations assay described here utilises the autonomous proliferation of hepatocytes of young rats. DOX induced 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..Another concern was the presence of high ploidy and two nuclei in hepatocyes, i.e. the binucleated tetraploid

Table (4): ANOVA test and Tukey's test with multiple comparisons between all groups regarding chromosomal aberrations in seven days old albino rat liver cells.

Groups(seven days)		Gaps	Breaks and / or fragments	Deletions	More than one aberration	Total aberrations with gaps	Total aberrations without gaps
		Mean%±SD	Mean%±SD	Mean%±SD	Mean%±SD	Mean%±SD	Mean%±SD
Group IB		1.27±1.10	0.67±0.62	0.13±0.35	0.00±0.00	2.07±1.49	0.80±0.86
Group IIB		2.07±0.88	0.47±0.52	0.53±0.52	0.00±0.00	3.07±1.03	1.00±0.76
Group IIIB		5.93±1.98	4.27±1.67	2.87±1.25	1.40±1.18	14.47±2.20	8.53±1.88
Group VIB		3.53±1.30	1.60±1.40	1.00±0.53	0.47±0.52	3.07±1.53	6.47±2.20
ANOVA	F	33.13	33.99	39.33	15.68	198.573	94.21
	P-value	0.000***	0.00***	0.00***	0.00***	0.000***	0.00***
Tuky's test	IB&IIB	0.393	0.965	0.464	1	0.337	0.985
	IB&IIIB	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***
	IB&IVB	0.000***	0.136	0.012**	0.208	0.337	0.000***
	IIB&IIIB	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***
	IIB&IVB	0.026*	0.047*	0.327	0.208	1	0.000***
	IIIB&IVB	0.000***	0.000***	0.000***	0.001***	0.000***	0.003**

Each group consisted of 15 offsprings, 3 offsprings from each mother. The total number of scored cells is 1500 /group. Group IB: Seven days old albino rat liver of the control group. Group IIB: Seven days old albino rat liver of the ALA treated group. Group IIIB: Seven days old albino rat liver of the DOX treated group. Group IVB: Seven days old albino rat liver of the ALA +DOX treated group. Significant level :. P<0.05, * P<0.01**, P<0.001. ***

and mononucleated triploid seen commonly in mature rats **(**Igarashi et al., 2007).

In this context, 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 etal., 2004) chromosomal aberration and comet assays in human lymphocytes (Leite-Silva et al., 2007). 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 and genotoxicity in bone marrow cells of albino mice (Yalcin et al., 2010).

The aim of the present study was to investigate the protective efficacy of Alpha-lipoic (ALA) acid against DOX clastogenicity and hepatotoxicity in liver of albino rats (mothers and offspring). The beneficial effects of ALA were monitored by the same assays micronucleus and chromosomal aberrations in hepatocytes after pre- and concurrent treatment of ALA with DOX in a regimen for four consecutive days during second trimester of gestation. The results revealed a significant reduction in the frequency of micronucleus as well as chromosomal aberrations in hepatocytes of mothers after delivery (one and seven days) and for one and seven days old albino rats. ALA reduced the frequency of aberrant cells to approach the control levels.

AlA a naturally occurring nutraceutical, functions as an essential cofactor in metabolic reactions involved in energy utilization.ALA and its reduced form dihydrolipoic acid (DHALA) are effective against conditions in which oxidative stress has a role **(**Packer et al., 1995).So it shows beneficial effects in oxidative stress conditions for its synergistic action with other antioxidants (Suzuki et al.,1993), Dozio et al.,2010 ,reported that ,ALA a naturally occurring ROS scavenger, has been shown to possess anticancer activity due to its ability to suppress proliferation and to induce apoptosis in different cancer cell lines.

The dithiol nature of lipoate renders this compound highly reactive against a number of ROS and it also has the ability to regenerate oxidized antioxidant.ALA and its reduced form have the ability to scavenge the singlet oxygen, hydrogen peroxide, hydroxyl radicals, superoxide radicals and also chalate the ferrous ion involved in the production of hydroxyl radical **(**Navari-Izzo et al., 2002). Specifically, the hydroxyl radical react with DNA and cause the

hydroxylation of deoxyguanosine at C_8 (Palmeira et al., 1997). Scavenging of hydroxyl radical prevents hydroxylation of deoxyguanosine and thus associated DNA damage. Furthermore, Devasagayam et al. (1993) reported that ALA exerted effective action against singlet oxygen-induced DNA single strand breaks and inhibited the site specific degradation of deoxyribose by pro-oxidants. ALA and DHALA both inhibit the apoptosis of rat thymocytes after exposure to either methyl prednisolone or etoposide, this inhibition was manifested at an early stage in the apoptosis as cell shrinkage, chromatin fragmentation (Bustamante et al., 1995). Prahalathan et al., 2005 have reported the protective role of ALA on ADR – induced testicular toxicity, Selvakumar et al (2006) demonstrated the chemoprotective effect of ALA in cyclophosphamide-induced clastogenicity in rat bone marrow cells**.** Prahalathan et al. (2006) investigate the protective efficacy of ALA against ADR– induced genotoxicity in erythropoietic system of rats. Single i.p. of ALA prior to ADR administration showed significant reduction in the frequency of chromosomal aberrations, DNA strand breaks and apoptosis in bone marrow cells as well as decreased the micronuclei formation in bone marrow and peripheral blood, indicating the dose dependent effect of ALA. The protection rendered by ALA in the present study, well corroborates with their findings.

In this concern, Saad et al. (2010) illustrated the role of oxidative stress and nitric oxide in the protective effect of alpha-lipoic acid and aminoguanidine against isoniazid- rifampicin-induced hepatotoxicity in rats and Tabassum et al.(2010) reported about the protective effect of ALA aganist (antitumor drug) methotrexate-induced oxidative stress in liver mitochondria.

In conclusion, in the current study a limited transplacental transfer of DOX was demonstrated in rats hepatocytes and causes liver injury. The improvement in liver cytotoxicity of mothers and their offspring treated with ALA against DOX hepatotoxicity may focus attention on the beneficial effect of ALA to overcome one of the most serious problems in cancer chemotherapy. ALA was effective in reducing DNA damage and may decrease the risk of secondary malignancy.

5-References:

[1] Aly F A, Donya S M, and Abo-Zeid M A M. The protective role of folic acid, vitamin B12 and vitamin C on the mutagenicity of the anticancer drug daunorubicin. Researcher. 2009; 1(60) :16-26.

[2] Amara-Mokrane Y A, Lehucher-Michel MP ,Balansard G, Duménil and Botta A. Protective effects of α-hederin, chlorophyllin and ascorbic acid towards the induction of micronuclei by doxorubicin in cultured human lymphocytes. Mutagenesis. 1996; 11 (2): 161-167.

[3] Bagchi D, Bagchi M, Hassoun E A , Kelly J and Stohs S J . Adriamycin-induced hepatic and myocardial lipid peroxidation and DNA damage and enhanced exretion of urinary lipid metabolites in rats. Toxocology. 1995; 95: 1-9.

[4] Bustamante J, Slater A F and Orrenius S. Antioxidant inhibition of thymocyte apoptosis by dihydrolipoic acid. Free Radic. Biol. Med. 1995; 19: 339-347.

[5] Danesi R, Fogli S, Gennari A, Conte P and Del Tacca M. Pharmacokinetic- pharmacodynamic relationships of the anthracycline anticancer drugs. Clin Pharmacokinet . 2002; 41: 431-344.

[6] Devasagayam T P , Subramanian M, Pradhan D S and Sies H. Prevention of singlet oxygen-induced DNA damage by lipoate. Chem. Biol. Int .1993; 86: 79-92.

[7] Dhawan A, Kayani M A, Parry J M, Parry E and Anderson D. Aneugenic and clastogenic effects of doxorubicin in human lymphocytes. Mutagenesis. 2003; 18: 487-490.

[8] D'Incalci M, Broggini M and Pardi G. Transplacental passage of doxorubicin. Lancet. 1983;1:75.

[9] Dozio E, Ruscica M, Passafaro L, Dogliotti G, Steffani L, Pagani A, Demartini G, Esposti D, Fraschini F and Magni P. The natural antioxidant alpha-lipoic acid induced p27 ^{kip1}-dependent cell cycle arrest and apoptosis in MCF-7 human breast cancer cells. European Journal of Pharmacology. 2010; 641: 29-34.

[10] El-Makawy A and El-Ashmaoui H M .Evaluation of the protective activity of beta-carotene and L-carnitine on doxorubicin-induced genotoxicity in rats.Arab.J.Biotech. 2003; 6(2): 289-296.

[11] Evert L, De Beer, Antonio E B and Emile E V. Doxorubicin and mechanical performance of cardiac trabeculae after acute and chronic treatment: a review. European Journal of Pharmacology. 2oo1; 415 (1):1-11.

[12] Fitzgerald C,Zimon AE and Jones EE. Aging and reproductive potential in women. Yale J. Biol. Med.1998; 71: 367-381.

[13] Germann N, Goffinet F and Goldwasser F. Anthracyclines during pregnancy : embryo-fetal outcome in 160 patients. Ann Oncol . 2004; 15(1): 146-150.

[14] Gewirtz D A .A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics

adriamycin and daunorubicin. Biochem. Pharmacol. 1999; 57: 727-741.
[15] Gillick J, Giles S, Bannigan S and Puri P. Midgut atresias result from abnormal development of the notochord in an Adriamycin rat model. J Pediatr Surg .2002; 37: 719-722.
[16] Greenamyre JT, Garcia-Osuna M and Green JG. The endogenous cofactors, thioctic acid and dihydrolipoic acid, are neuroprotective against NMDA and malonic acid lesions of striatum. Neurosci. Lett. 1994; 171:17-20.
[17] Grimshaw CE and Lai CJ. Oxidized aldose reductase: in vivo factor not in vitro artifact. Arch Biochem Biophys 1996; 327: 89-97.
[18] Gűlkac M D, Akpinar G, Ŭstũn H and Kanli A Ŏ. Effects of vitamin A on doxorubicin-induced chromosomal aberrations in bone marrow cells of rats.2004; 19: 231-236.
[19] Heddle J A. A rapid in vivo test for chromosomal damage.Mutation Res. 1977; 18: 63-69.
[20] Igarashi M, Setoguchi M, Takada S, Itoch S and Furuhama K. Optimum conditions for detecting hepatic micronuclei caused by numerical chromosome aberration inducers in mice. Mutation Res. 2007; 632: 89-98.
[21] Injac R, Perse M, Obermajer N, Djordjevic-Milic V, Prijatelj M, Djordjevic A, Cerar A and Strukelj B. Potential hepatoprotective effects of fullerenol C_{60} $(OH)_{24}$ in doxorubicin-induced hepatotoxicity in rats with mammary carcinomas. Biomaterials. 2008; 29: 3451-3460.
[22] Jagetia G C and Nayak V. Micronuclei- induction and its correlation to cell survival in Hella cells treated with different doses of adriamycin. Cancer Lett. 1996; 110: 123-128.
[23] Kagan VE, Shvedova A, Serbinova E, Khan S, Swanson C and Powell R et al., Dihydrolipoic acid-a universal antioxidant both in the membrane and in the aqueous phase. Biochem Pharmacol. 1992; 44 :1637-1649.
[24] Karp GH I, von Oeyen P, Valone F, Khetarpal V K, Israel M, Mayer R J,et al. Doxorubicin in pregnancy: possible transplacental passage. Cancer Treat Rep. 1983; 67:663-667.
[25] Kocak N, Ustun H, Gulkac M D , Kanli AO, Borazan A and Yilmaz A .Effects of10alpha, 25-dihydroxyvitamin D3on doxorubicin-induced chromosomal aberrations in rat bone marrow cells . Acta Oncol . 2004; 43: 204-248.
[26] Kurz E U, Douglas P and Lees-Miller S P. Doxorubicin activates ATM-dependent phosphorylation of multiple downstream targets in part through the generation of reactive oxygen species. Journal Biol. Chem. 2004; 279:53272-53281.
[27] Leite-Silva C, Gusmão C L S and Takahash C S. Genotoxic and antigenotoxic effects of Fucus vesiculosus extract on cultured human lymphocytes using the chromosome aberration and Comet assays. Genet. Mol. Biol. 2007; 30: 1-11.
[28] Loibl S, von Minckwitz G, Gwyn K, Ellis P, Blohmer J U, Schlegelberger B et al.,Breast carcinoma during pregnancy. International recommendations from an expert meeting. Cancer .2006; 106(2): 237-246.
[29] Matalon S T, Ornoy A and Lishner M. Review of the potential effects of three commonly used antineoplastic and immunosuppressive drugs (cyclophosphamide, azathioprine, doxorubicin on the embryo and placenta).Reproductive Toxicology . 2004; 18: 219-230.
[30] Menegola E, Broccia ML and Renzo FD. Teratogenic effects of doxorubicin in rats at midgestation and at term. Teratog Carcinog Mutagen .2001; 21: 283-293
[31] Mokrane A, Michel L, Balansard G, Dumbnil G and Botta A.Protive effects of a-hederin, chlorophyllin and ascorbic acid towards the induction of micronuclei by doxorubicin in cultured human lymphocytes. Mutagenesis .1996; 11(2): 161-167.
[32] Montvale; physicians' desk reference. Medical economic data ,a division of medical economics company.1993.
[33] Navari-Izzo F, Quartacci M F, and Sgherri C. Lipoic acid a unique antioxidant in the detoxification of activated oxygen species. Plant Physiol. Biochem. 2002; 40: 463-470.
[34] Olsen A K, Lindeman B, Wiger R, Duale N and Brunborg G. How do male germ cells handle DNA damage? Toxicol. Appl. Pharmacol. 2005; 207: 521-531.
[35] Pacificl GM and Nottoli R. Placental transfer of drugs administered to the mother, Clin. Pharmacokinet. 1995; 28: 235-269.
[36] Packer L, Witt E H and Trirschler H J. Alpha-lipoic as biological antioxidant. A review, Free Radic. Biol.Med. 1995; 19: 227-250.
[37] Paget GE and Barnes JM. Evaluation of drug activities. In Pharmacometrics, Vol I, Edited by :Laurence, D R and Bacharach A L, London, Academic Prss . 1964; 50.
[38] Palmeira CM, Serrano J, Kuehl D W and Wallace K B. Preferential oxidation of cardiac mitochondrial DNA following acute intoxication with doxorubicin. Biochem. Biophys. Acta. 1997; 1321: 101-106.
[39] Patel N, Joseph C, Corcoran G B and Ray S D. Silymarin modulates doxorubicin-induced oxidative stress, BcI-xL and p53 expression

while preventing apoptotic and necrotic cell death in the liver. Toxicology and Applied Pharmacology. 2010; 245: 143-152.

[40] Peccatori FA, Azim Jr HA,Scarfone G, Gadducci A Bonazzi C, Gentilini O et al., Weekly epirubicin in the treatment of gestational breast cancer (GBC).Breast Cancer Res. Treat. 2009; 115(3):591-594.

[41] Pommier Y, Leo E, Zhang H L and Marchand C. DNA Topoisomerases and their poisoning by anticancer and antibacterial drugs. Chemistry & Biology. 2010; 17: 421-433.

[42] Prahalathan C, Selvakumar E and Varalakshmi P . Protective effect of lipoic acid on adriamycin-induced testicular toxicity. Clinical chimica Acta.2005; 360: 160-166.

[43] Prahalathan C , Selvakumar E, Varalakshmi P, Kumarasamy P, and Saravanan R. Salubrious effects of lipoic acid against adriamycin-induced clastogenesis and apoptosis in Wistar rat bone marrow cells.Toxicology. 2006; 222: 225-232.

[44] Ring A E, Smith IE, Jones A, Shannon C, Galani E and Ellis PA. Chemotherapy for breast cancer during pregnancy: an 18-year experience from five London reaching hospitals J. Clin Oncol .2005; 23(18): 4192-4197.

[45] Saad E I, El-Gowilly S M, Sherhaa M O and Bistawroos A E. Role of oxidative stress and nitric oxide in the protective effects of α-lipoic acid and aminoguanidine against isoniazid-rifampicin-induced hepatotoxicity in rats. Food and chemical toxicology. 2010; 48: 1869-1875.

[46] Selvakumar E, Prahalathan C, Sudharsan P T, and Varalakshmi P. Protective effect of lipoic acid on micronuclei induction by cyclophosphamide. Arch. Toxicol. 2006; 80: 115-119.

[47] Somani SM, Husain K, Whitworth C, Trammell GL, Malafa M and Rybak LP. Dose dependent protection by lipoic acid against cisplatin-induced nephrotoxicity in rats: antioxidant defense system. Pharmacol Toxicol .2000; 86:234-241.

[48] Stopper H and Műller S O. A minireview : micronuclei as biological endpoints for genotoxicity. Toxicol. Vitro. 1997;

[49] Stopper H, Boos G, Clark M and Gieseler F .Are topoisomerase II inhibitor-induced micronuclei in vitro a predictive marker for the compounds ability to cause secondary leukemias after treatment? Toxicology Letters. 1999; 104: 103-110.

[50] Suzuki Y J, Tsuchiya M and Packer L. Antioxidant activities of dihydrolipoic acid and its structural homologues. Free Radic. Res. Commun. 1993; 18 :115-122.

[51] Swift L P, Rephaeli A, Nudelman A, Phillips D R and Cuees SM. Doxorubicin-DNA adducts induce a non-topoisomerase II-mediated form of cell death. Cancer Res. 2006; 66: 4863-4871.

[52] Tabassum H, Parvez S, Pasha S T, Banerjee B D and Raisuddin S. Protective effect of lipoic acid against methotrexate-induced oxidative stress in liver mitochondria. Food and Chemical Toxicology. 2010; 48: 1973-1979.

[53] Tan H H and Porter A G. DNA methyltransferase I is a mediator of doxorubicin-induced genotoxicity in human cancer cells. Biochemical and Biophysical Research Communications .2009; 382: 462-467.

[54] Tates A D, Neuteboom I, Hofker M and den Engelse I . A micronucleus technique for detecting clastogenic effects of mutagens/carcinogens (DEN, DMN) in hepatocytes of rat liver in vivo. Mutation Res. 1980; 107: 131-151.

[55] Tohamy A A, EI-Ghor A A, EI-Nahas S M and Noshy M M. ß-Glucan inhibits the genotoxicity of cyclophosphamide , adramycin and cisplatin .Mutation Res. 2003; 541 :45-53.

[56]Valentin-Severin I, Le Hegarat L, Lhuguenot J-C, Le Bon A-M and Chagnon M-C. Use of HepG2 cell line for direct or indirect mutagens screening: comparative investigation between comet and micronucleus assays. Mutation Research. 2003; 536: 79-90.

[57] Van Calsteren K, Verbesselt R, Beijnen J, Devlieger R, De Catte L , Chai D C ,Van Bree R, Heyns L, de Hoon J and Amant F. Transplacental transfer of anthracyclines, vinblastine and 4-hydroxy-cyclophosphamide in a baboon model. Gynecologic Oncology. 2010; 119: 594-600.

[58] Voulgaris E, Pentheroudakis G and Pavlidis N. Cancer and pregnancy : A comprehensive review. Surgical Oncology . 2011;10 :1-11.

[59] Weisz B, Schiff E and Lishner M. Cancer in pregnancy: maternal and fetal implications. Hum Reprod Update 2001 ; 7 :384-393.

[60] Yalcin E,Oruc E, Cavusoğlu K and Yapar K. Protective role of grape seed extract against doxorubicin-induced cardiotoxicity and genotoxicity in albino mice. Journal Med Food. 2010 ; 13: 17-25.

[61] Yancik R and Ries LA. Cancer in older persons. Magnitude of the problem-how do we apply what we know? Cancer. 1994 ; 74: 1995-2003.

[62] Yongjia Y U, Zhidong X U and Abraham W H. Adriamycin induces large deletions as a major

type of mutation in CHO cells.Mutation Res. 1994 ; 325: 91-98.
[63] Yosida H and Amano K. Autosomal polymorphism in laboratory bred and wild Norway rats, Rattus norvegicus. Misima Chromosoma . 1965; 16: 658-666.
[64] Zemlickis D, Lishner M, Erlich R and Koren G.Teratogenicity and carcinogenicity in a twin exposed in utero to cyclophosphamide. Teratog Carcinog Mutagen. 1993; 13: 139-143.
[65] Zemlickis D, Lishner M,Degendorfer P, Panzarella T, Sutcliffe SB and Koren G. Fetal outcome after in utero exposure to cancer chemotherapy. Arch Intern. Med .1992; 152:573-576.

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