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Median lethal dose (LD50) and cytotoxicity of Adriamycin in female albino mice

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ABSTRACT

The median lethal dose of Adriamycin and its cytotoxicity in three vital organs (heart, liver and kidney) was studied. The median lethal dose (LD50) was 56.875mg/kg body. Weight (b.wt). High doses of Adriamycin induced significant increases in heart tissues enzymes (lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and Creatine Kinase-MB isoenzyme (CKMB)). Hepatotoxicity appeared in significant increment in liver function enzymes (aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities) and lipid profile (cholesterol and triglycerides) and significant reduction in total protein and albumin. Moreover, urea and creatinine have recorded significant increases in kidney tissues after interpretoneal administration of high doses of Adriamycin (100, 90 and 80 mg/kg b.wt.).

INTRODUCTION

Adriamycin is a quinine containing anticancer and antibiotic that is used to treat many solid malignancies and lymphomas (Quiles *et al*., 2002). Adriamycin have severe toxic effects towards heart, kidney and liver so that its clinical efficacy is restricted(Quiles *et al*., 2002; Chae *et al*., 2005; Kim *et al*., 2006; Zahraei and Rabbani-Chadegani, 2007; Othman *et al*., 2008). The toxicity effect of Adriamycinon heart and liver are condensation, myofibrils, mitochondrial swelling and loss disruption in heart and lipid droplets accumulation, polymorphic mitochondria, and vacuolization of cytoplasm in liver. A nephrotic syndromeis induced by a single high-dose injection of Adriamycin (ADR). Heavy proteinuria, hypoalbuminemia, hypercoagulability, and hyperlipemia with hypercholesterolemia were used to characterize ADR(Deepa and Varalakshmi, 2005b).

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Many mechanisms are consider to illustrate the cytotoxic effects of Adriamycin such as inhibition of DNA synthesis, alkylation and binding of DNA, interference of DNA strand separation and helicase activity, effects of direct membrane, cross-linking of lipid peroxidation DNA and free radical formation (Deepa and Varalakshmi, 2006).

The aim of the present study isto determine the (Median lethal dose) LD_{50} of Adriamycin intraperitoneal injection and cytotoxicity of 80, 90, and 100 mg/kg b.wt. in female albino mice.

MATERIAL AND METHODS

Experimental animals

84 adult female albino mice were obtained from the animal house, veterinary division, NRC. Their weights ranged between (20 and -30 g). Mice were housed in plastic cages, each cage contained eight mice. Animals were kept under controlled temperature of 25 ± 2 ^{o}C for12 hours under light and 12 hours dark cycle throughout the experiment. A commercial pelleted diet was used during the experiment. Food and water were available *ad libitum*.

Drugs

Adriamycin as Adricin was purchased from EIMC United Pharmaceuticals, Badr City, Cairo, A.R.E.

Treatment and dosage

An approximate LD50 was initially determined in pilot study by a so called "staircase method" using a small number of animals (2 for each dose) with increasing doses of Adriamycin. Five doses of 80, 90, 100, 110 and 120mg/kg b.wt., were given to 5 groups of mice (8 in each)for the determination of intraperitoneal LD50 in female mice (Table 1).

Animals were observed for the 2, 6and 24 hours for any toxic symptoms. After 24 hours, number of died animals was counted in each group and transformed to probits and then LD50 determined by the method of Karber (Shetty Akhila and Alwar, 2007). The cytotoxicity of 80,90 and 100 mg/kg b.wt., was determined in three tissues (heart, Liver and kidney).

Determination of biochemical parameters:

After 24hrs the surviving animals were quickly sacrificed by decapitation and heart, liver and kidney organs were collected for determination the biochemical parameters. The organs were weighed and homogenized in 10 m mol/L phosphate buffer saline (10% W/V) of PH 7.4 and centrifuged .The supernatant used for determination of LDH, ALP, CKMB in heart tissues, ALT, AST, TP, albumin, cholesterol, triglycerides in liver tissues andurea and creatinine in kidney tissues.

In heart tissue, lactate dehydrogenase (LDH) was determined according to (Young, 2000; Young and Friedman, 2001)and ALP was measured according to (Belfield and Goldberg, 1971) while creatine kinase-MB isoenzyme (CKMB) was measured in heart according to (Stein, 1998). aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities In liver tissue were measured spectrophotometrically according to the method of (Murray, 1984; Murray, 1989), Total Protein concentration was measured by the method of (Gornall *et al*., 1949), Albumin levels were measured according to the method of (Doumas *et al*., 1971), The measurement of triglycerides levels using assay kit according to (Fossati and Prencipe, 1982) and cholesterol was measured according to (Richmond, 1973; Allain *et al*., 1974). Moreover, urea content was performed in kidney tissue according to the procedure of (Fawcett and Scott, 1960) and creatinine concentration measured according to (Schirmeister *et al*., 1964).

Statistical analysis:

Reported values for liver, heart and kidney parameters represented in the table 2 expressed as means ± SE. Statistical analysis evaluated by one-way ANOVA. Once a significant F test obtained, LSD comparisons performed to assess the significance of differences among various treatment groups. Statistical Processor System Support "SPSS" for Windows software, Release 12.0 (SPSS, Chicago,IL) was used.

RESULTS AND DISCUSSION

Among the treated animals with high doses of Adriamycin, it was found that some animals were died immediately after few seconds. But the rest of the treated animals were drowsy and less responsive. The severity of these effects were related to the dose concentration. However, few numbers of survivors had recovered from these symptoms after 24 hours.

Table 1: Results of the lethal doses of Adriamycin for the determination of the LD50 after intraperitoneal injection in female mice (n=8)

group	Dose mg/kg	No. of animal dead	dose difference (a)	mean mortality (b)	probity (a*b)
1	control	0			
2	80	1	0		
3	90	2	10	1.5	15
4	100	4	10	3	30
5	110	8	10	6	60
6	120	8	10	8	80

LD50 = the apparent least dose lethal to all in a group- sum (a*b)/n
Where n is the number of animals in each group, (a) is the dose difference and (b) is the mean mortality =mortality in second group +1st group /2 (Table 1)

LD_{50}=80-185/8= 80-23.125=56.875 mg/kg b.wt.

The LD_{50} of Adriamycin in adult female mice was found to be 56.875 mg/kg b.wt. after intraperitoneal injection (Table 1).

Adriamycin, is anthracycline isolated Streptomyces, used as antibiotic and antitumor (Eliaz *et al*., 2004; Al-Ghamdi, 2008). Waldemar *et al*. (1993) indicated that LD50 of Adriamycin was 25.7 mg/kg. While, the LD50 reported by Bertezzoli *et al*. (1985) was 11.98 mg/kg in mice treated intraperitoneally. Also**,** (Kratz *et al*., 2007) indicted that the LD50 of intravenous administration of Adriamycin in CD-1 mice was 12mg/kg, while it was 23.4 and 45.9 mg/kg for males and females Sprague Dawley rats respectively.

A single dose of the encapsulated form of Adriamycin was less toxic (LD50 of 32mg/kg) than free Adriamycin (LD_{50} of 17 mg/kg). In addition, The LD50 of Adriamycin hydrochloride nanoliposome was 31.69 mg/kg in kuming mice (Xie *et al*., 2013). This difference in the reported results might be due to rout of administration, animal sex, method used for the estimation of LD50 and different derivative of Adriamycin.

Adriamycin as antibiotic and anticancer was successful in solid tumors, lymphomas and leukemia's treatment (Pal and Sil, 2012; Elsherbiny and El-Sherbiny, 2014). The therapeutic efficacy of Adriamycin have been severely limited by the toxic side effects of the drug on heart as well as other organs including the liver, heart, brain and kidney (Elsherbiny and El-Sherbiny, 2014; Wang *et al*., 2014).

The data in this study present the effect of i.p. administration of three acute doses (80, 90 and 100 mg/kg b.wt.) of Adriamycin on heart, liver and kidney functions of female albino mice after 24hrs of one dose injection.

In our study, Adriamycin produced significant increases in heart tissues lactate dehydrogenase, alkaline phosphatase and Creatine Kinase-MB isoenzyme(CKMB) Table (2).

Table 2: Effect of i.p. administration of single dose Adriamycin(80, 90 and 100mg/kg b.wt.) on the heart, liver and kidney functions of female albino mice.

	Control	Adriamycin (80mg/kg b.wt.)	Adriamycin (90mg/kg b.wt.)	Adriamycin (100mg/kg b.wt.)
LDH U/g tissue	1.610±0.0368	3.389±0. 125a	5.451±0. 104ab	4.777±0. 168ab
Alk.U/g tissue	0.062±0.002	0. 172±0. 006a	0. 271±0. 005ab	0. 252±0. 016ab
CKMB U/g tissue	2.284±0.079	4.414±0.171a	5.710±0. 236ab	5.667±0. 246ab
ALT U/gtissue	0.0149±0.0004	0. 232±0. 010a	0. 255±0. 011a	0. 349± 0. 015abc
AST U/g.tissue	0.065±0.003	0. 244±0. 028a	0. 302±0. 030a	0. 436±0. 008ab
TP mg/g tissue	6.005±0.195	3.198±0. 170a	2.799±0. 173a	2.124±0. 145a
Album.mg/g tissue	14.285±0.701	10.672±0.173a	9.160±0.278a	7.228±0.163ab
TG.mg/g tissue	11.862±0.386	25.208±0.410a	25.154±0.481a	26.064±1.316a
Chol.mg/g tissue	125.570±3.782	211.76±4.646a	496.45±17.55ab	406.898±11.286abc
Urea mg/g tissue	0.424±0.016	0. 551±0. 019a	0. 583±0. 019a	0. 599±0. 019a
Creat.mg/g tissue	0.162±0.006	0.238±0. 010a	0. 374±0. 011ab	0. 375±0. 005ab

Values are means of 8 mice ± SE. in group (1), 7 mice ± SE in group (2), 6 mice± SE in group (3) and means of 4 rats± SE in group (4) a = significant change from control, b = significant change from Adriamycin (80mg/kg) rats and c = significant change from Adriamycin (90mg/kg) rats at $p \leq 0.05$.

Ibrahim *et al.* (2009) examined the effect of accumulative Adriamycindose (15mg/kg) and they found a significant rise in LDH and CK-MB enzyme activities which act as important markers of heart disease(Ibrahim *et al*., 2009).

There are several mechanisms that illustrate cardiotoxicity results from Adriamycin, including membrane lipid peroxidation, mitochondrial damage, free radical generation, lipid peroxidation (LPO),and iron-dependent oxidative damage to macromolecules (Singh *et al*., 2015).There are significant rise in malondialdehyde level (MDA) accompanied with glutathione (GSH) activity inhibition in heart followed by the activity of catalase (CAT) with a significant elevation in lactate dehydrogenase (LDH) and aspartate transaminase (AST)(Nazmi *et al*., 2013).

Adriamycin myocardial impairment involve myocyte apoptosis via oxidative free radical formation (Deepa and Varalakshmi, 2006). Also Adriamycin increase radicals of superoxide and NO in heart tissue then form toxic free radicals their(Prahalathan *et al*., 2005).

Adriamycincan impair normal liver by generating oxidative stress, decreases in the antioxidation capacity of the liver and the liver weight (Wang *et al*., 2014).

In the present study, Adriamycin induced significant increases ($p<0.05$) in liver tissues enzymes (ALT and AST) and significantly decreases in liver total protein and albumin contents. In addition, the treatment of single different doses of Adriamycin showed significant increases in liver triglycerides and cholesterol contents (Table (2).

Single i.p. dose of 15mg/kg of Adriamycin caused liver toxicity appeared in an increased in alanine transaminase (ALT) and aspartate transaminase (AST) which attributed to oxidative stress that appears in significant decrease in GSH level and increase in MDA (El-Moselhy and El-Sheikh, 2014). Moreover, a single i.p. dose of 10mg/kg b.wt. into male rats cased elevations in amino transferases and these changes were associated with increases in peroxidation of lipid and protein oxidation as well as decrease in glutathione levels and glutathione-s-transferase while glutathione peroxidase and catalase activity were elevated in liver Adriamycin treated rat (Othman *et al*., 2008). Adriamycin intravenous injection dose of (7.5 mg/kg) rise the lipids levels on heart, liver and kidney (cholesterol, triglycerides and LDL cholesterol), and decrease in HDL cholesterol levels (Deepa and Varalakshmi, 2005a; Deepa and Varalakshmi, 2006).

In kidney tissues, the data showed significant increases in urea and creatinine content after the treatment with 15 mg/kg Adriamycin i.p. single dose for 5 days, which cause renal toxicity.(El-Moselhy and El-Sheikh, 2014).Repeated injection doses from Adriamycin lead to renal dysfunctions involving elevation in levels of urea and creatinine in blood and albumin in urine which attributed to oxidative stress mechanisms.(Elsherbiny and El-Sherbiny, 2014). Also, Adriamycin induced renal toxicity was monitored in kidney via increase in MDA and decrease in GSH levels(Ibrahim *et al*., 2009; Nazmi *et al*., 2013). Decreases in GSH cause cellular electrocytes disrupt which lead to renal dysfunctions and renal toxicity(Pal and Sil, 2012). The high levels of oxygen free radicals produced by Adriamycin metabolism cause pathologic alterations in kidneys including, increased level of lipid peroxidation products and apoptosis (Dzięgiel *et al*., 2002; Chmielewska *et al*., 2015).

CONCLUSION

In this paper, the median lethal dose (LD_{50}) and cytotoxicity of Adriamycin were evaluated in female albino mice. The median lethal dose was 56.875 mg/kg. Cytotoxicity of 80, 90 and 100mg/kg were examined by the biochemical analysis of heart, liver and kidney tissues. After 24 hrs of one dose, the data recorded sever effects as significant changes in heart and liver enzymes, hepatic total protein and albumin, hepatic lipid profile and kidney function.

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