http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 4 (5): 604-607, 2001

Antagonistic Role of Silymarin Against Cardiotoxicity and Impaired Antioxidation Induced by Adriamycin and/or Radiation Exposure in Albino Rats

[1]Ahmed Rashad M.Abu Ghadeer, [2]Samia E.Ali, [1]Soheir A.A. Osman,
[3]F.A.AbuBedair, [1]M.M. Abbady and [1]Maha R.El-Kady[1]
[1]Radiation Biology Department, National Centre for Radiation Research and Technology, P.O. Box 29 Nasr City, Cairo, Egypt
[2]Nuclear Research Centre, Egypt [3]National Cancer Institute, Cairo University, Egypt

Abstract: Adriamycin (ADR) is one of the most potent antineoplastic agents known, however, its clinical use is limited due to its cardiotoxicity. In this investigation, the role of silymarin (flavonoid found in most plants) against the action of ADR and radiation was evaluated. ADR was injected to rats in four equal doses (3 mg/kg body weight twice a week) and after the last dose rats were exposed to whole body (6 Gy) gamma irradiation. Blood samples were taken 1 and 10 days after radiation exposure. Silymarin was orally administered 10 days before ADR injection. ADR and radiation have dramatic effects on the blood level of lactate dehydrogenase (LDH), creatinine phosphokinase (CPK), indicating heart toxicity, as well as on the content of malondialdehyde (MDA) & glutathione (GSH) and the activity level of gluathione peroxidase (GSH-Px) & superoxide dismutase (SOD), indicating depression in the antioxidant system. The effect was more deleterious on the 10th day than the 1st day of investigation (indicating late and continuos action of ADR and radiation). Production of free radicals of both ADR and radiation may be the main cause of the altered biochemical levels of the assayed parameters. Daily intubation of silymarin reduced the alterations in the assayed biochemical parameters induced by ADR and/or radiation in rats.

Key words: Adriamycin, cardiotoxicity, CPK, flavonoid, gamma radiation, MDA, rats, silymarin, SOD.

Introduction

Radio- and/or chemotherapy are known to be the two major modalities in cancer treatment. Nevertheless, radiotherapy is known to encounter severe side effects whereas many drugs exhibiting effective control of cancer tumors showed to exert cumulative toxicity that limits their clinical use or disrupts the continuity of treatment.

Previous studies indicated that gamma irradiation of experimental animals results in deleterious biochemical alterations in the antioxidant system of rats accompanied by significant increase in blood content of lipid peroxides (Abbady *et al.*, 1999).

On the other hand, adriamycin (ADR) being an anthracycline glycoside antibiotic, is one of the most effective drugs used in cancer chemotherapy. However, its clinical usefulness is still limited in view of its severe, cumulative and dose-dependent cardiotoxicity (Singal *et al.*, 1987and Booser & Hortobagyi, 1994). In spite of the fact that the precise mechanism of cardiotoxicity exerted by the drug is still not clear, yet certain mechanisms have been proposed to account for the drug cardiotoxicity including production of cytotoxic free radicals species and lipid peroxidation of cardiac microsomal membranes (Mimnaugh *et al.*, 1983 and Rajagopalan *et al.*, 1988).

Among several hypotheses put forward to explain the mechanism of radiation and chemical toxicity, is the one involving the generation of free radicals. Radical mediated lipid peroxidation plays a crucial role in many physiological and pathological processes including carcinogenesis and degenerative diseases. Oxidative stress is known to be a disparity between the rates of free radical production and elimination . The living body is injured by excess oxygen and oxidative stress. Antioxidant defense in the body protects living subjects against most of the toxic effects of oxygen. Additional protection is provided by dietary antioxidants such as α-tocopherol (vitamin E), ascorbate (reduced vitamin C), flavonoids and carotenoids. The physiological importance of some of these has been proved in delaying and preventing certain human diseases, particularly cardiovascular disease and some types of cancer.

Epidemiological studies have suggested that certain microchemicals present in several herbs and plants with diversified pharmacological properties, are useful dietary agents for the prevention of a wide variety of human cancers. Silymarin, a flavonoid isolated from milk thitle, has been clinically used in Europe and Asia, as an antihepatotoxic agent, largely due to its strong antioxidant activity (Lahiri-Chatterjee *et al.*, 1999).

Both radio- and chemotherapy practices are confronted by generation of free radicals and propagation of oxidative processes. Since most of the antioxidants afford protection through scavenging of free radicals, the present study has been carried out to investigate the protective effect of silymarin against the toxicity of both ADR and/ or ionizing radiation. In the course of the present investigations, LDH and CPK have been assayed as sensitive parameters for cardiac dysfunction, MDA has been evaluated as a marker of lipid peroxidation whereas GSH, GSH-Px and SOD have been determined in view of their role as antioxidants in the biological tissues.

Materials and Methods

Eighty-four male albino rats (140-150 g) were categorized into seven groups (G_1-G_7), each of 12 rats and provided with standard diet and water *ad libitum*.

- **G_1:** Control rats.
- **G_2:** Rats were i.p. injected with ADR of 4 equal doses (3 mg/kg body weight, twice a week,). The drug was purchased from Pharmacia &Upgohn S.P.A. Milan, Italy.
- **G_3:** Rats were exposed to whole body gamma irradiation (6 Gy), the radiation source was $^{137}C_s$, Gamma cell-40 biological irradiator belonging to the National Centre for Radiation Research & Technology (NCRRT), Egypt, giving a dose rate of 0.64 Gy/min at the time of experimentation.

G_4: Rats were injected with ADR as in G_2 and after the last dose ,animals were exposed to irradiation as in G_3.

G_5: Animals were orally treated (by means of gastric tube) with Silymarin daily (8 mM/kg body weight) for 10 days and treated with ADR as in G_2. Silymarin was kindly granted by Seideco for Drug Comp., Egypt.

G_6: Animals were orally treated with Silymarin for 10 days and then exposed to ionizing radiation as in G_3.

G_7: Rats of this group were treated with Silymarin and exposed to both ADR and radiation as in G_4.

In groups G_5, G_6 & G_7, administration of Silymarin was continued for ten days after the above mentioned treatment. Blood samples were taken from all groups by heart puncture after slight anaesthesia with ether. The samples were taken at two time intervals: one and ten days after the last dose of ADR or after radiation exposure.

Lactate dehydrogenase (LDH) and creatinine phosphokinase (CPK) were freshly assayed colorimetrically in the serum according to the method described by Rec. (1970) and Rec. (1977) respectively. Lipid peroxidation was ascertained by measuring malondialdehyde (MDA) content according to the method described by Yoshioka *et al.* (1979). Glutathione (GSH) content; glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities in the blood were estimated according to Tietz (1986); Paglia & Valentine, (1967) and Minami & Yoshikawa (1979) respectively.

Results

Table 1 presents the activity level of LDH in the serum of rats injected with 4 equal doses of ADR, over two weeks or rats exposed to gamma radiation (6 Gy). The data showed significant elevation in the enzyme activity one day after the experiment in G_2 , G_3 , & G_4 with percentage increase from control amounting to 74.73; 33.17 & 74.8% respectively. On the 10^{th} day after termination of the experiment, the percentages increases in LDH from control level were 90.13; 50.21 & 97.13 in rats of G_2, G_3 & G_4 respectively.

Table 1 presents the effect of oral administration of silymarin on the level of serum LDH of rats injected by ADR and/or exposed to gamma irradiation. The data obtained revealed that Silymarin tended to normalize the effects of ADR and radiation; the serum LDH level increased in rats of groups G_5, G_6 & G_7 on the 1^{st} day with percentage change from the control equal to 48.87; 18.32 & 46.73% respectively.

On the 10^{th} day (after last dose of ADR and radiation) serum LDH increased significantly (with continued injection with silymarin) in G_5, G_6 & G_7 with percentage changes from the control equal to 55.12; 23.46 & 53.39% respectively.

As shown in Table 1, the activity of CPK in the serum of rats in different experimental groups significantly increased from control in the two time intervals of experiment (1 and 10 days) while the highest increase occurred in animals of G_4 with a percentage of 120.01 and 99.04% respectively.

The increase in the enzyme level (indicating cardiac dysfunction) in rat groups treated with silymarin (G_5, G_6 & G_7) was less intensified than in those treated with ADR or irradiated with no silymarin treatment (G_2, G_3 & G_4).

The data presented in Table (2) showed highly significant increase in lipid peroxides activity as MDA content in serum in all groups of animals under investigation (G_2-G_7) one and ten days after treatment .The highest MDA content was shown in animals of group 4 which were injected with ADR and irradiated. The percentage increase from control amounted to 82.76% after one day and 110.88% after 10 days.

Table 2 presented the SOD activity in different animal groups under examination. The data obtained showed significant decrease in SOD activity after one day in groups G_2, G_3 and G_4 (23.69; 29.04 & 32.29%) respectively. While the percentage decreases in SOD from control level after 10 days were 22.73; 16.95 and 23.71 in G_2, G_3 and G_4 respectively .

Table 3 demonstrated the results of reduced glutathione content (GSH). The data showed significant decreases from control in GSH of all groups under investigation throughout the experimental period except for G_5 on the 1^{st} and 10^{th} day and G_6 & G_7 on the 10^{th} day indicating the role of silymarin in the protection of antioxidant cycle.

The data summarized in Table 3 showed significant depletion in the activity of glutathione peroxidase (GSH-Px) in serum of all treated groups. The more decreased values were in G_4 (ADR + Rad.) with percentage decrease from control amounting to -36.76 & -38.87% on the first and tenth day of treatment respectively.

Table 1: Activity levels of LDH and CPK in the serum of rats treated with ADR and/or irradiated with or without Silymarin administration

Groups	LDH (U/l)		CPK (U/l)	
	Ist day	10th day	Ist day	10th day
Control G_1	193.4±18.7 (100%)	199.3 ± 26.7 (100%)	82±16.1 (100%)	86.9 ±16.4 (100%)
ADR G_2	337.9 ± 23.2** (174.2)	378.9 ± 18.4** (190.13)	174.7 ± 19.1** (213.01)	168.1 ± 17.9** (193.38)
Irrad. G_3	257.5 ± 18.3** (133.17)	229.4 ± 25.4** (150.21)	127.1 ± 14.1** (155.02)	165.7 ± 24.4** (190.56)
ADR + Irrad. G_4	338 ± 20.2** (174.80)	392.8 ± 23.8** (197.13)	180.4 ± 16.4** (220.01)	173.0 ± 15.4** (199.04)
Sil + ADR G_5	287.9 ± 33.5** (148.87)	309.1 ± 42.1** (155.12)	138.6 ± 19.5** (169.0)	145.9 ± 21.0 ** (167.92)
Sily + Irrad G_6	228.8 ± 19.1* (118.32)	246 ± 15.6* (123.46)	113.2 ± 12.45* (138.01)	132.0 ± 21.7* (151.87)
Sily + ADR + Irrad. G_7	283.8 ± 33.9** (146.73)	306.7 ± 18.8** (153.39)	142.7 ± 13.9** (174.00)	149.3 ± 15.1** (171.69)

Each value is the mean of 6 animals ± SD *Significant difference from control at $P<0.05$; **$P<0.001$

Table 2: Lipid peroxides as MAD level and activity of SOD in blood of rats treated with ADR and/or irradiated with or without Silymarin administration

Group	MAD (μ mol/ml)		SOD(a) (U/ml)	
	1st day	10th day	1st day	10th day
Control G_1	64.4 ± 5.4 (100.00%)	61.1 ± 5.4 (100.00%)	8.6 ± 1.0 (100.00%)	8.1 ± 0.9 (100.00%)
ADR G_2	106.6 ± 6.9** (165.50)	98.9 ± 9.1** (161.81)	6.6 ± 1.5* (76.31)	6.3 ± 1.2* (77.27)
Irrad. G_3	113.8 ± 9.5** (176.71)	117.8 ± 7.2** (192.71)	6.1 ± 0.9* (70.96)	6.8 ± 1.1* (83.05)
ADR + Irrad. G_4	117.8 ± 8.6** (182.76)	128.9 ± 9.3** (210.88)	5.8 ± 1.0* (67.71)	6.2 ± 1.3* (76.29)
Sil + ADR G_5	87.7 ± 8.1** (136.11)	81.7 ± 9.6* (133.63)	8.1 ± 1.3 (93.49)	7.6 ± 0.7 (93.24)
Sily + Irrad G_6	94.9 ± 16.7* (147.41)	88.3 ± 10.3** (144.53)	7.7 ± 1.4 (89.19)	7.5 ± 1.0 (92.01)
Sily + ADR + Irrad. G_7	98.9 ± 15.0** (153.44)	92.2 ± 12.4** (150.89)	7.4 ± 0.9 (86.06)	7.2 ± 1.6 (88.69)

Legends as in Table 1. (a) U = 50% inhibition of nitroblue tetrazolium.

Table 3: GSH content and activity level of GSH-Px in blood of rats treated with ADR and/or irradiation with or without Silymarin administration

Groups	GSH (mg/dl)		GSH-Px (U/l)	
	1st day	10th day	1st day	10th day
Control G_1	67.8 ± 6.9 (100%)	73.5 ± 6.5 (100%)	88.2 ± 2.7 (100%)	87.7 ± 3.2 (100%)
ADR G_2	54.9 ± 5.9* (80.94)	49.3 ± 6.6** (67.08)	56.7 ± 3.1** (64.32)	54.6 ± 2.5** (62.23)
Irrad. G_3	48.2 ± 5.5** (71.07)	48.7 ± 5.5** (66.32)	60.1 ± 2.6** (68.11)	56.9 ± 2.6** (64.94)
ADR + Irrad. G_4	48.7 ± 3.5** (71.85)	47.6 ± 4.9** (64.84)	55.8 ± 3.4** (63.24)	53.6 ± 3.3** (61.13)
Sil + ADR G_5	61.1 ± 3.9 (90.08)	69.5 ± 5.1 (94.65)	76.5 ± 1.5** (86.75)	77.2 ± 2.0** 88.04)
Sily + Irrad G_6	57.7 ± 5.8* (85.07)	68.41 ± 7.27 (93.12)	70.8 ± 7.2** (80.27)	75.5 ± 1.9** (85.86)
Sily + ADR + Irrad. G_7	48.2 ± 5.5** (71.07)	66.2 ± 4.1 (90.10)	74.6 ± 2.1** (84.59)	73.9 ± 1.7** (84.23)

Legends as in Table (1). U = μg consumed gultathion/min.

Discussion

In the present study, the elevated level activities of LDH&CPK in rat's serum after ADR injection, is a good indicator of cardiotoxicity. The present findings are in accordance with the study of Kimura *et al.* (2000) who recorded an elevation in plasma LDH & CPK as a measure of myocardiac damage following ADR ingection in mice. Although several mechanisms have been suggested to explain this toxicity, the exact mechanism is still not clear. Recently, it has been suggested that ADR may exert at least a part of its cardiotoxicity by inhibition of long chain fatty acid oxidation in the heart (Abdel-Aleem *et al.*, 1997 and Sayed Ahmed *et al.*, 2000). This inhibition causes several deleterious effects in cardiac tissues due to deficiency in energy supply and accumulation of toxic intermediates (Corr *et al.*, 1985), which are usually associated with cardiotoxicity and congestive heart failure similar to those reported by ADR administration (Bohles *et al.*, 1986). Toxicity of ADR may be attributed to its alchohol metabolite (Doxorubicinol) and free radical production. Inhibition of aldoketoreductase could decrease this toxicity by reducing alcohol metabolite (Behnia & Boroujerdi, 1999).

The observed high levels of blood MDA after ADR injection as recorded in the present study, being associated with low levels of GSH, GSH-Px and SOD, may be attributed to the free radicals produced by the drug. The production of cytotoxic free radicals by ADR is one mechanism proposed to account for the drug cardiotoxicity (Rajagopalan *et al.*, 1988).

ADR i.p. administration to rats in six equal (2.5 mg/kg) doses over a 2-week period, caused injury and was monitored by measurement of aldehydic lipid peroxidation products (Luo *et al.*, 1999). They reported that, animals sacrificed 2 h after the sixth dose, had significantly higher aldehyde concentration than 2 h after a single dose of ADR. They also found that, aldehydes in plasma and heart remained elevated for 3 weeks after the final dose of ADR suggesting cumulative damage.

The data obtained in the present study, revealed similar action of ionizing radiation to ADR thereby production of superoxide radicals. The antioxidant enzyme (SOD) catalyses the conversion of superoxide radiclas to H_2O_2 which can

subsequently lead to the formation of hydroxyl radicals in the presence of metal ion catalysts (Alegria *et al.*, 1989). The latter can react with polyunsaturated fatty acids yielding lipid hydroperoxides, thereby initiating a lipid-radical chain reaction and oxidative damage to the cell membranes (Singal *et al.*, 1995).

The results indicated that after ADR and radiation exposure, free radicals mediated lipid peroxidation is initiated (MDA) and antioxidant system (GSH, GSH-Px & SOD) is depressed. These showed to be very sensitive parameters to monitor the biological damage affected by the drug and radiation exposure. Silymarin administration before ADR and/or radiation exposure as shown in the present study, exhibited some normalization. Several studies have shown that the human diet contains various mutagens and carcinogens, along with many antimutagens and anticarcinogens (Ames *et al.*, 1995). Dietary intake of naturally occurring antioxidants therefore, has been suggested to be a useful strategy against the toxic effects of the mutagenic and carcinogenic agents. Silymarin has high protection against tumour promotion, primarily targeted against stage I tumours, and that the mechanism of such effects may involve inhibition of promoter-induced edema, hyperplasia, proliferation index and oxidant state (Lahiri-Chatterjee *et al.*, 1999). As a therapeutic agent, silymarin is well tolerated and largely free of adverse effect (Mourelle *et al.*, 1989).

Large group of flavonoids from all major structural subclasses were investigated on their ability to inhibit ADR induced lipid peroxidation and to chelate Fe^{2+} (Van Acker *et al.*, 1996). Most flavonoids tested chelated Fe^{2+} with a different chelating capacity. Chelation can raise the activity to the level of most active scavengers, possibly by site-specific scavenging.

Mechanistic studies have shown that silymarin is a strong antioxidant that is capable of scavenging both free radicals and reactive oxygen species in rodents and in cell cultures and that it results in a significant increase in cellular antioxidant defense machinary by ameliorating, the deleterious effects of free radical reactions (Muzes *et al.*, 1991).

References

Abbady, M.M., A.A. Osman-Soheir and A.R.M. Abu-Ghadeer, 1999. Parsley oil boosting antioxidation capacity in irradiated rats. Egypt. J. Rad. Sci.Applic., 12:27-35.

Abdel-Aleem, S., M.M. El-Merzabani, M.M. Sayed-Ahmed, D.A. Taylor and J.E. Lowe, 1997. Acute and chronic effects of adriamycin on fatty acid oxidation in isolated cardiac myocytes. J. Mol. Cell. Cardiol., 29:789-797.

Alegria, A.E., A. Samuni, J.B. Mitchell, P.Riesz and A. Russo, 1989. Free radicals induced by adriamycin-sensitive and adriamycin-resistant cells: a spin-trapping study. Biochem., 28: 8653-8658.

Ames, B.N., L.S. Gold and W.C. Willelt, 1995. The causes and prevention of cancer. Proc. Natl. Acad. Sci. USA, 92: 5258-5265.

Behnia,K. and M. Boroujerdi, 1999. Inhibition of aldo-keto reductases by phenobarbital alters metabolism , pharmacokinetics and toxicity of Doxorubicin in rats. J. Pharm Pharmacol., 51: 1275-1282.

Bohles, H., T. Noppeney, Z.Akcetin, J. Rein and J. Vonder Embey, 1986. The effect of preoperative L-carnitine supplementation on myocardial metabolism during aorto-coronary bypass surgery. Curr. Ther. Res., 39:429-435.

Booser, D.J. and G.N.Hortobagyi, 1994. Anthracycline antibiotics in cancer therapy focus on drug resistance. Drugs, 47: 223-258.

Corr, P.B., R.S. Gross and B.E. Sobel, 1985. Amphipathic metabolites and membrane dysfunction in ischemic myocardium. Cir. Res., 55:135-154.

Kimura ,T., I.Fujita, N.Itoh, N.Muto, T. Nakanishit, K.Takhashi, J. Azuma and K.Tonaka, 2000 : Metallothionein acts as a cytoprotectant against doxorubicin toxicity. J. Pharmacol. Exp.Ther., 292: 299-302.

Lahiri-Chatterjee, M., S.K. Katiyar, R.R. Mohan and R. Agarwal, 1999. A flavonoid antioxidant, silymarin, affords exeptionally high protection against tumor promotion in the Sencar mouse skin tumorigenesis model. Cancer Res., 59: 622-632.

Luo, X., B. Reichetzer, J.Trines, L.N. Benson and D.C. Lehoty, 1999. L-carnitine attenuates doxorubicin-induced lipid peroxidation in rats. Free Rad. Biol.Med., 26:1158-1165.

Mimnaugh, E.G., M.A.Trush and T.E. Gram, 1983. Enhancement of rat heart microsomal lipid peroxidation following doxorubicin treatment in vivo. Cancer Treat. Rep., 67: 731-732.

Minami, M. and H.Yoshikawa, 1979. A Simplified assay method of superoxide dismutase (SOD) activity for clinical use. Clinica Chimica Acta, 92: 337- 342.

Mourelle, M., P.Muriel, L.Favari and T. Frano, 1989. Prevention of CCl_4- induced liver cirrhosis by silymarin. Fund. Clin. Pharmacol., 3:183-191.

Muzes, G., G. Deak, L.Lang, K. Nekman, P.Gergely and J.Feber, 1991. Effect of the bioflavonoid silymarin on the in vivo activity and expression of superoxide dismutase (SOD) enzyme. Acta Physiol. Hung., 78:3-9.

Paglia, D.E. and W. N. Valentine, 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J .Labor. Clin. Med., 70: 158-169.

Rajagopalan, S., P.M. Politi, B.K.Sinha and C.E. Myers, 1988. Adriamycin-induced free radical formation in the perfused rat heart: Implication for cardiotoxicity. Cancer Res., 48:4766-4769.

Rec. Gscc (DGKC), 1970. J.Clin. Chem. Clin.Biochem., 8:658.

Rec. Gscc (DGKC), 1977. J.Clin. Chem. Clin.Biochem., 15:255. C/F. Diamond, Diagnostics Kit.

Sayed-Ahmed, M.M., S.S. Shouman, B.M. Rezk, M.H. Khalifa, A.M. Osman and M.M. El-Merzabani, 2000. Propionyl-L_carnitine as potential protective Aagent against adriamycin induced impairment of fatty acid B-oxidation in isolated heart mitochondria. Pharmacol. Res., 41:143-150.

Singal, P.K., C.M.R. Deally and L.E.Weinberg, 1987. Subcellular effects of adriamycin in the heart : a concise review. J. Mol. Cell. Cardiol., 19:817-828.

Singal, P.K., N.Siveski-Iliskovic, M.Hill, T.P.Thomas and T.Li, 1995. Combination therapy with probucal prevents adriamycin-induced cardiomyopathy. J. Mol. Cell. Cardiol., 27: 1055-1063.

Tietz, N.W., 1986. Text book of clinical chemistry. WB. Saunders Comp. Philadelphia.

Van-Acker, S.A., D.J. Vandan Berg, M.N.Tramp, D.H. Griffioen, W.P.Van-Bennekam, W.J.Vander-Vijgh and A.Bast, 1996. Structural aspects of antioxidant activity of flavonoids. Free Radic. Biol. Med., 20: 331-34.

Yoshioka, T., K.Kawada, T.Shimada and M.Mori, 1979. Lipid peroxidation in maternal and cord blood and Protective mechanism against activated oxygen toxicity in the blood. Am. J. Obstet. Gynecol., 135:372- 376.