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Protective Effect of Metformin on Cardiac and Hepatic Toxicity Induced by Adriamycin in Swiss Albino Mice*

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Abstract: Diabetes Mellitus (DM) is a chronic disease that is characterized by deterioration of glycemic control. The disease is known to be caused by imbalance between Reactive Oxygen Species (ROS) and antioxidant defense systems. Hyperglycemia is commonly observed in a wide variety of diseases, including cancer. Although, therapy against glycemic control is used in all these diseases, the diabetic cancer patients are on additional therapy with anticancer drugs. The objective of present study was to investigate if metformin, a very popular antidiabetic agent can avert the cardiac and hepatic toxicity caused by Adriamycin (ADR), which is a commonly used cytotoxic drug. The experimental protocol included oral treatment of mice with different doses (62.5, 125 and 250 mg kg^{-1} day^{-1}) of metformin for 7 days. Some mice in each group were injected i.p. with ADR (15 mg kg^{-1}), 24 h prior to sacrifice. In each case animals were killed, 24 h after the last treatment, blood sample was collected and plasma was separated for analysis of AST, ALT and CK-MB. Liver and heart from the same animals were excised for analysis of proteins, nucleic acids, MDA and NP-SH. The results obtained revealed that pretreatment with metformin (i) reduced the ADR-induced increase in the concentrations of AST, ALT and CK-MB (ii) protected against the ADR-induced increase of MDA and decrease of DNA and NP-SH in both cardiac and hepatic tissues. These results demonstrate that the treatment with metformin might be useful to protect cardiac and hepatic toxicity. The exact mechanism of action is not known, however; the inhibition of ADR-induced increase of plasma enzymes and MDA and depletion of DNA and NP-SH by metformin may be attributed to its antioxidant potentials, which are well known for the reduction of glycotoxins and general improvement in cellular dysfunction. The use of Metformin by cancerous diabetic patients on cytotoxic therapy will be a boon to avert the cardiac and hepatic toxicity.

Key words: Metformin, adriamycin, biochemical changes, heart, liver, mice

INTRODUCTION

An imbalance between Reactive Oxygen Species (ROS) and antioxidant defense is known to cause deterioration of glycemic control in a number of conditions, including Diabetes Mellitus (DM) and other diseases (Anderson *et al*., 1997). Hyperglycemia is known to be a major risk factor for lymphocytic leukemia, β-cell lymphoma and solid organ cancers of breast, pancrease, liver, colon, bladder, prostate and oral cavity (Weiser *et al*., 2004; Breidert *et al*., 2000; Oshiro *et al*., 2003; Suba and Ujpal, 2006). The cytotoxic treatment regimen (cyclophosphamide, vincristine, doxorubicin, dexamethasone) is often prescribed to diabetic patients suffering from cancer. However, these drugs are also known to cause hyperglycemia and a high incidence of mortality, in addition to severe cardiac and liver toxicity (Weiser *et al*., 2004; Zeidan *et al*., 2002; Yagmurca *et al*., 2007).

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Adriamycin (ADR), an anthracycline antibiotic has been used for more than 30 years for the treatment of a wide variety of cancers. It is one of the many cytotoxic drugs used in the clinical management of a wide variety of cancers, including breast and esophageal carcinomas, osteosarcoma, Kaposi's sarcoma, soft-tissue sarcomas and Hodgkin's and non Hodgkin's lymphomas, gastric, liver, bile-duct, pancreatic and endometrial carcinomas (Singal *et al*., 2000; Schneider *et al*., 2001; Quiles *et al*., 2002). The antitumor effects of ADR involve the production of free radicals (Gewirtz, 1999; Singal *et al*., 2000) and it is the oxidant action of ADR which has been described to cause cardiotoxicity (Cole *et al*., 2006; Bast *et al*., 2006) and hepatotoxicity (Kalender *et al*., 2005; Yagmurca *et al*., 2007). Thus in a situation where the diabetic cancer patients have to use cytotoxic drugs, it is imperative to find antidiabetic treatment strategies, with antioxidant activity to avert the toxicity of anticancer drugs.

The cardiotoxicity-induced by ADR has been shown to be protected by flavonoids (Bast *et al*., 2006) selenium (Danesi *et al*., 2006) nitric oxide and superoxide dismutase (Cole *et al*., 2006). The hepatotoxicity caused by ADR is protected by Erdosteine (Yagmurca *et al*., 2007), vitamin E and catechin (Kalender *et al*., 2005) and L-carnitine (Zeidan *et al*., 2002). Most of the antidiabetic agents (both natural and synthetic) have been reported to be the scavengers of ROS (Signorini *et al*., 2002; Bellin *et al*., 2006; Murugesh *et al*., 2006), however; there is a paucity of an agent which can control blood glucose, inhibit lipid peroxidation and revert the cardiotoxicity and hepatotoxicity caused by a cytotoxic drug.

Metformin [1-(diaminomethylidene)-3,3-dimethyl-guanidine] is the most commonly prescribed oral anti-hyperglycemic drug used in the management of DM. It is reported to have several properties, including (i) reducing the formation of advanced glycation end products (AGE) that are involved in the pathogenesis of the secondary complications of DM (Tanaka *et al*., 1997) (ii) replenishing the deficient levels of glutathione in DM (Arnalich *et al*., 2001) (iii) potentiate the antioxidant defense (Onaran *et al*., 2006) and (iv) decrease the cellular oxidative reactions associated with DM and establish favorable effect on lipid parameters (Tankova, 2003; Anisimov *et al*., 2005). In addition metformin has been found to inhibit the development of a number of cancers, including mammary adenocarcinomas and pancreatic carcinogenesis in hamsters (Anisimov *et al*., 2005). Nevertheless, there is a paucity of literature to show the protective effect of metformin, against the cardiotoxicity and hepatotoxicity induced by ADR. Since, ROS is described as the basis of cardiotoxicity and hepatotoxicity and metformin is a proven antioxidant, it was found worthwhile to study the protective effects of metformin against cardiotoxicity and hepatotoxicity-induced by ADR in order to analyze the relevance of metformin to avert the toxicity of ADR.

MATERIALS AND METHODS

The present study on protective effect of metformin on cardiac and hepatic toxicity induced by ADR in Swiss albino mice was conducted in the Department of Pharmacology, College of Pharmacy, King Saud University. The experimental part was undertaken during the period October to December 2006.

Chemicals

ADR was obtained from Farmitalia Carlo Erba, Italy. Metformin was purchased from Merck Sante, France. All the laboratory reagents were obtained from Sigma Chemical Company, St. Louis, MO, USA and the kits for estimating the biochemical indices from Randox Laboratories, USA.

Animals

Swiss albino male mice (SWR, home bred), aged 6-8 weeks and weighing 26-30 g, provided by Experimental Animal Care Center, College of Pharmacy, King Saud University were used in the study. All experimental mice were provided with Purina chow and free access to water. The animals were

maintained under controlled conditions of temperature, humidity and light. The conduct of experiments and the procedure of sacrifice (using ether) were approved by the Ethics Committee of the Experimental Animal Care Society, College of Pharmacy, Riyadh, Kingdom of Saudi Arabia.

Dose Selection, Preparation of Drugs and Route and Duration of Administration

The dose of ADR was based on an earlier study in our laboratory (Al-Shabanah, 1993). The dose range of metformin used was based on some studies (Pari and Ashokkumar, 2005; Gras *et al*., 2006) and human therapeutic dose with reference to surface area rule. The doses of metformin selected in the present study were 62.5, 125 and 250 mg kg^{-1} day^{-1}). The prescribed dose of this drug for an average weight of human being is 500 mg day^{-1}. According to the rule of surface area, the ratio of mice (20 g) and man (60 kg), was calculated to be 0.0026 and hence the dose of metformin per kg mice would be ($0.0026\times500\times50 = 65$ mg kg^{-1}). The lower dose used in the present study was 62.5 mg kg^{-1} day^{-1} and the medium dose (125 mg kg^{-1} day^{-1}) was double and the high dose (250 mg kg^{-1} day^{-1}) was quadruple. The increased medium and high doses were used in view of the fact that the metabolic rate in mice is more as compared to human beings (Al-Yahya *et al*., 2006). ADR was soluble in water and hence its aqueous solution was given intraperitoneally in tap water (0.1 mL/10 g body weight of mice). Aqueous suspension of metformin was administered (24 h apart for 7 days) by gavage in tap water (0.1 mL/10 g body weight of mice). The animals in the control group were administered the vehicle by gavage (0.1 mL/10 g body weight of mice).

Experimental Groups

Twenty male mice were used in each group for study on DNA, RNA, proteins, MDA, NP-SH in both heart and liver and plasma biochemical indices. The experimental groups of mice consisted of: (1) untreated control (tap water); (2) Metformin 62.5 mg kg^{-1} day^{-1}; (3) Metformin 125 mg kg^{-1} day^{-1}; (4) Metformin 250 mg kg^{-1} day^{-1}; (5) ADR 15 mg kg^{-1} i.p.; (6) Metformin 62.5 mg kg^{-1} day^{-1} pretreatment (7 days) + ADR 15 mg kg^{-1} i.p.; (7) Metformin 125 mg kg^{-1} day^{-1} pretreatment (7 days) + ADR 15 mg kg^{-1} i.p.; (8) Metformin 250 mg kg^{-1} day^{-1} pretreatment (7 days) + ADR 15 mg kg^{-1} i.p., Aqueous suspension of metformin was administered orally to groups 2, 3, 4, 6, 7 and 8 for 7 days. ADR (aqueous solution) was injected (15 mg kg^{-1}, i.p., group 5) 24 h before sacrifice. In each case animals were killed 24 h after the last treatment, liver and heart were quickly excised and stored at −70°C. Blood from each mouse was collected from heart, plasma separated and preserved for analysis of Alanine transaminase (ALT), Aspartate transaminase (AST) and Creatinine phospho kinase (CK-MB). Proteins, Deoxyribose nucleic acid (DNA), Ribose nucleic acid, RNA, Nonprotein Sulph-hydryl groups (NP-SH)` and malondialdhyde (MDA) were determined in both cardiac and hepatic cells of the mice.

Determination of Blood Biochemistry

The blood plasma samples were used to determine the activity of AST, ALT, CK-MB according to the methods described for the particular kit. The estimations were carried out by using the specific commercial kits (Randox diagnostic reagents). The measurements were done with a UV-visible spectrophotometer, Ultrospec III (LKB).

Estimation of Total Proteins

Total proteins were estimated by the modified Lowry method of Schacterle and Pollack (1973). Bovine serum albumin was used as standard.

Determination of Nucleic Acids

The method described by Bregman (1983) was used to determine the levels of nucleic acids. Tissues were homogenized and the homogenate was suspended in ice-cold trichloroacetic acid (TCA). After centrifugation, the pellet was extracted with ethanol. DNA was determined by treating the

nucleic acid extract with diphenylamine reagent and reading the intensity of blue color spectrophotometrically at 600 nm. For quantification of RNA, the nucleic acid extract was treated with orcinol and the green colour was read spectrophotometrically at 660 nm. Standard curves were used to determine the amounts of nucleic acids present.

Malondialdehyde Estimation

The method described by Ohkawa *et al*. (1979) was used. Malondialdehyde (MDA) was measured as an indicator of lipid peroxidation. Cardiac and liver tissues were homogenized in potassium chloride solution and incubated with thiobarbituric acid. After centrifugation the pink clear layer was read spectrophotometrically at 532 nm. Malondialdehyde bis (dimethyl acetal) was used as an external standard.

Determination of Nonprotein Sulfhydryl Groups (NP-SH)

The estimation of NP-SH levels in different organs was undertaken according to the method of Sedlak and Lindsay (1968). The tissues were homogenized in ice cold 0.02 M ethyleneodiaminetetraacetic acid disodium. The homogenate was treated with 50% w/v trichloroacetic acid and centrifuged. Supernatant fractions were mixed with tris buffer, 5-5'-dithiobis-(2 nitrobenzoid acid) (DTNB) was added. After shaking the contents, its absorbance was determined spectrophotometrically at 412 nm within 5 min of the addition of DTNB against reagent blank with no homogenate.

RESULTS

The treatment with metformin for seven days failed to induce any significant changes in the plasma levels of AST, ALT and CK-MB, while single dose of ADR caused significant rise in the levels of AST and ALT ($p<0.01$) and CK-MB ($p<0.05$) as compared to the values obtained in the control. The pretreatment with metformin was found to significantly ($p<0.05$) inhibit the levels of AST and ALT (250 mg kg^{-1} day^{-1}) and CK-MB (125 and 250 mg kg^{-1} day^{-1}), as compared to the values obtained in the ADR group (Table 1).

Metformin treatment for 7 days failed to show any significant changes in the cardiac levels of proteins, RNA and DNA, while ADR treatment significantly decreased the proteins and RNA ($p<0.05$) and DNA ($p<0.01$) as compared to the values obtained in the control. The pretreatment with metformin for 7 days failed to protect the ADR-induced depletion of proteins and RNA, whereas, DNA concentrations were significantly protected at 125 ($p<0.05$) and 250 mg kg^{-1} day^{-1} ($p<0.01$) of metformin against the treatment with ADR (Table 2).

Table 1: Effect of metformin on adriamycin-induced changes in some biochemical indices in blood plasma of Swiss albino mice

Treatment and dose (mg kg^{-1} body weight)	Biochemical indices and their values (Mean±SE)		
	AST (U L^{-1})	ALT (U L^{-1})	CK-MB (U L^{-1})
Control (tap water, 0.3 mL $mouse^{-1}$)	29.78±3.48	23.22±2.32	95.82±9.18
Metformin (62.5)	31.86±3.60	21.56±2.86	80.06±7.45
Metformin (125)	35.64±2.68	22.98±4.98	91.34±6.89
Metformin (250)	40.46±8.42	24.75±2.90	70.89±4.68
Adriamycin (15)	58.45±6.64**	36.56±1.90**	130.25±7.56*
Metformin (62.5) + Adriamycin (15)	50.54±4.96	33.67±2.80	120.45±6.46
Metformin (125) + Adriamycin (15)	45.64±8.65	30.97±3.56	100.56±8.78*
Metformin (250) + Adriamycin (15)	37.86±4.89*	26.88±3.56*	90.55±10.20*

Five mice were used in each group; Groups 2, 3 and 4 were statistically compared with group 1 and groups 6, 7 and 8 were statistically compared with group 5; *: $p<0.05$; **: $p<0.01$; (One way ANOVA and Post hoc Tukey-Kramer multiple comparison test was done)

Table 2: Effect of metformin on adriamycin-induced changes in Protein and Nucleic acid concentrations of cardiac tissue in Swiss albino mice

Treatment and dose (mg kg^{-1} body weight)	Cardiac tissue (Mean±SE)		
	Proteins (mg/100 mg tissue)	RNA (µg/100 mg tissue)	DNA (µg/100 mg tissue)
Control (tap water, 0.3 mL $mouse^{-1}$)	14.11±0.73	578.65±49.02	222.56±15.59
Metformin (62.5)	13.68±0.82	607.67±39.65	221.09±21.17
Metformin (125)	14.03±0.66	625.45±40.08	236.66±18.60
Metformin (250)	13.87±0.62	592.17±51.19	256.74±19.58
Adriamycin (15)	11.50±0.86*	434.73±34.44*	150.26±4.52**
Metformin (62.5) + Adriamycin (15)	12.53±0.81	462.37±41.63	164.72±11.26
Metformin (125) + Adriamycin (15)	13.49±0.97	515.59±52.30	193.92±12.96*
Metformin (250) + Adriamycin (15)	13.38±0.49	500.09±60.25	218.45±16.21**

Five mice were used in each group; Groups 2, 3 and 4 were statistically compared with group 1 and groups 6, 7 and 8 were statistically compared with group 5; *: $p<0.05$; **: $p<0.01$ (One way ANOVA and Post hoc Tukey-Kramer multiple comparison test was done)

Table 3: Effect of metformin on adriamycin-induced changes in Protein and Nucleic acid concentrations of hepatic tissue in Swiss albino mice

Treatment and dose (mg kg^{-1} body weight)	Hepatic tissue (Mean±SE)		
	Proteins (mg/100 mg tissue)	RNA (µg/100 mg tissue)	DNA (µg/100 mg tissue)
Control (tap water, 0.3 mL $mouse^{-1}$)	14.59±0.42	709.99±20.07	206.98±7.02
Metformin (62.5)	13.52±0.29	718.48±15.20	214.16±5.41
Metformin (125)	14.22±0.58	724.20±11.34	223.58±7.07
Metformin (250)	14.44±0.66	731.55±16.80	208.21±4.98
Adriamycin (15)	13.45±0.23*	650.05±10.69**	185.58±4.99*
Metformin (62.5) + Adriamycin (15)	13.44±0.37	641.74±15.63	199.70±4.28
Metformin (125) + Adriamycin (15)	13.33±0.56	670.58±13.01	202.48±13.54
Metformin (250) + Adriamycin (15)	13.62±0.57	660.74±17.93	227.80±9.69**

Five mice were used in each group; Groups 2, 3 and 4 were statistically compared with group 1 and groups 6, 7 and 8 were statistically compared with group 5; *: $p<0.05$; **: $p<0.01$ (One way ANOVA and Post hoc Tukey-Kramer multiple comparison test was done)

Treatment with metformin for 7 days failed to show any significant changes in the concentrations of proteins, RNA and DNA, while ADR treatment significantly decreased the hepatic levels of proteins ($p<0.05$), RNA ($p<0.01$) and DNA ($p<0.05$) as compared to the values obtained in the control. The pretreatment with metformin for 7 days failed to protect the ADR-induced depletion of proteins and RNA, whereas, the DNA concentrations were significantly ($p<0.01$) protected at the high dose (250 mg kg^{-1} day^{-1}) of metformin against the treatment with ADR (Table 3).

The cardiac levels of MDA were decreased significantly ($p<0.05$) after treatment with metformin (250 mg kg^{-1} day^{-1}) for seven days, while the single dose treatment with ADR was found to significantly ($p<0.05$) increase these levels, as compared to the values obtained in the control. Pretreatment with metformin was found to significantly ($p<0.05$) decrease the MDA levels at 125 and 250 mg kg^{-1} day^{-1}, against the increase after treatment with ADR. There was no effect of metformin on the cardiac levels of NP-SH, while the levels were significantly ($p<0.05$) decreased after treatment with ADR, as compared to the values obtained in the control. Pretreatment with metformin showed significant ($p<0.05$) protection at the higher doses (125 and 250 mg kg^{-1} day^{-1}) against the ADR-induced depletion of NP-SH concentrations in the heart (Table 4).

Table 4: Effect of Metformin on Adriamycin-induced changes in MDA and NP-SH concentrations of cardiac tissue in Swiss albino mice

Treatment and dose	Cardiac tissue	
(mg kg^{-1} body weight)	MDA (nmole mL^{-1})	NP-SH (µmol mL^{-1})
Control (tap water, 0.3 mL $mouse^{-1}$)	200.65±16.14	74.31±7.54
Metformin (62.5)	194.82±11.53	70.23±4.32
Metformin (125)	170.59±15.84	85.50±7.85
Metformin (250)	155.50±11.44*	95.46±8.29
Adriamycin (15)	254.69±16.25*	51.71±5.05*
Metformin (62.5)+Adriamycin (15)	214.55±27.41	60.48±6.79
Metformin (125)+Adriamycin (15)	199.48±16.19*	75.57±8.51*
Metformin (250)+Adriamycin (15)	180.62±21.29*	90.65±11.45*

Five mice were used in each group; Groups 2, 3 and 4 were statistically compared with group 1 and groups 6, 7 and 8 were statistically compared with group 5; *: $p<0.05$ (One way ANOVA and Post hoc Tukey-Kramer multiple comparison test was done)

Table 5: Effect of metformin on adriamycin-induced changes in MDA and NP-SH concentrations of hepatic tissue in Swiss albino mice

Treatment and dose	Hepatic tissue	
(mg kg^{-1} body weight)	MDA (nmole mL^{-1})	NP-SH (µmol mL^{-1})
Control (tap water, 0.3 mL $mouse^{-1}$)	258.65±7.57	69.99±5.95
Metformin (62.5)	240.47±24.73	65.36±2.81
Metformin (125)	220.46±18.39	71.00±5.80
Metformin (250)	201.41±17.76*	79.83±6.47
Adriamycin (15)	319.72±19.08*	49.13±4.46*
Metformin (62.5)+Adriamycin (15)	270.41±27.26	52.55±2.75
Metformin (125)+Adriamycin (15)	258.53±26.03	58.28±4.08
Metformin (250)+Adriamycin (15)	220.59±17.91**	69.87±4.72*

Five mice were used in each group; Groups 2, 3 and 4 were statistically compared with group 1 and groups 6, 7 and 8 were statistically compared with group 5; *: $p<0.05$; **: $p<0.01$ (One way ANOVA and Post hoc Tukey-Kramer multiple comparison test was done)

The hepatic levels of MDA were decreased significantly ($p<0.05$) at the high dose (250 mg kg^{-1} day^{-1}), after treatment with metformin for seven days, while these levels were significantly ($p<0.05$) increased after a single dose of ADR, as compared to the values obtained in the control. Pretreatment with metformin was found to significantly ($p<0.01$) protect the MDA levels at the high dose (250 mg kg^{-1} day^{-1}) against the increase after treatment with ADR. There was no effect of metformin on the hepatic levels of NP-SH, while these levels were significantly ($p<0.05$) decreased after treatment with ADR, as compared to the values obtained in he control. Pretreatment with metformin failed to cause any significant changes in the hepatic levels of NP-SH after treatment at the lower doses (62.5 and 125 mg kg^{-1} day^{-1}), while the high dose (250 mg kg^{-1} day^{-1}) showed a significant ($p<0.05$) protection against the ADR-induced depletion of NP-SH concentrations in the liver (Table 5).

DISCUSSION

The treatment with metformin did not show any significant changes in the plasma concentrations of AST, ALT and CK-MB. These changes demonstrate lack of any effect of the treatment with metformin for 7 days. Present data are confirmed by lack of any effect on the cardiac and hepatic levels of proteins, RNA, DNA and glutathione observed in cardiac and hepatic tissues. It is interesting to note that the treatment with metformin was found to significantly reduce the MDA concentrations in both cardiac and hepatic tissues. These results support the literature reports which showed metformin to potentiate antioxidant defense (Arnalich *et al*., 2001; Onaran *et al*., 2006). The treatment with ADR induced significant increase of AST, ALT and CK-MB of the blood plasma indicating cardiac and

hepatic toxicity. Present data are in confirmation of the known cardiac (Zeidan *et al*., 2002) and hepatic toxicity (Yagmurca *et al*., 2007) of ADR. The treatment with ADR also caused inhibition of proteins and nucleic acids in cardiac and hepatic tissues. The data on inhibition of nucleic acids by ADR support literature reports on clastogenicity and cytotoxicity of ADR (Dhawan *et al*., 2003; Prahalathan *et al*., 2006). The exact mode of the inhibition of nucleic acids is not known, however; present results support the earlier reports (Cullinane *et al*., 2000) which showed ADR to form adducts or breaks of DNA. The depletion of DNA observed in the present study might be attributed to the influence of topoisomerase II activity (Duca *et al*., 2006). Another possibility is the involvement of the genesis of ROS. Literature reports suggest that ROS are implicated in various pathological conditions, including cancer, apoptosis and clastogenicity (Liu *et al*., 2001; Wang *et al*., 2002; Kiningham *et al*., 2004) activation of NF-kB (Faux and Howden, 1997), changes in mitochondrial function (Li *et al*., 2002) and organ toxicity including the cardiac and hepatic toxicity (Zeidan *et al*., 2002; Yagmurca *et al*., 2007). ADR-induced production of free radicals has been reported to be the major cause of DNA fragmentation, cell damage and organ toxicity (Zeidan *et al*., 2002; Yagmurca *et al*., 2007; Quiles *et al*., 2002; Onaran *et al*., 2006; Prahalathan *et al*., 2006; Satoh *et al*., 2000). Thus the genesis of ROS and the cascade of related events might be responsible for the ADR-induced increase of MDA and depletion of proteins, RNA, DNA and NP-SH in both the tissues.

Pretreatment with metformin was found to protect the ADR-induced increase in AST, ALT, CK-MB and MDA and depletion of proteins, nucleic acids and NP-SH in heart and the liver. There is a paucity of literature on the prevention of ADR-induced cardiac and hepatic toxicity by metformin. Nevertheless, it is found to prevent the beta-aminopropionitrile fumarate-induced formation of atheromatous lesions in the aorta and minimized the level of lipids in the aortic wall in rats (Bouissou *et al*., 1980). Stern (1998) showed metformin and troglitazone to protect against the macrovascular and atherosclerosis complications related with DM. The exact mechanism of inhibition of ADR-induced changes in the plasma enzymes, DNA and lipid peroxidation in the present study is not known. However, previous reports showed that metformin ameliorates the cellular oxidative reactions, mitochondrial dysfunction, formation of AGEs, activation of NF-kB and apoptosis (Wang *et al*., 2002; Bonnefont-Rousselot *et al*., 2003). Furthermore, since, the intracellular GSH is known to regulate the activation of NF-kB (Arnalich *et al*., 2001), it is possible that the influence of metformin on GSH might also be instrumental in regulating the NF-kB. These reports support our finding on protection of ADR-induced changes and the impact of metformin on increasing the antioxidant defense. Present study support the literature reports which showed metformin to reduce the formation of glycation products (Forbes *et al*., 2005) and to potentiate antioxidant defense (Arnalich *et al*., 2001; Onaran *et al*., 2006). There are no parallel experiments conducted on other antidiabetic agents, except rosiglitazone (Avandia) which has been found to reverse the acetaldehyde (Jung *et al*., 2006) and MPP+ (Jung *et al*., 2007) induced apoptosis in human neuroblastoma SH-SY5Y cells. However, in a recent report, Nissen and Wolski (2007) suggested an increased risk of myocardial infarction and death from cardiovascular causes to be linked with rosiglitazone. Consequent upon the publication of this report, there has been a widespread patient panic and rampant media furor, which claimed irresponsible behavior of the regulatory agencies. Thus the safety concerns of rosiglitazone might edge over its benefits.

Most of the antioxidants are known to have protective effects against cardio-toxicity and hepatotoxicity-induced by cytotoxic drugs (Zeidan *et al*., 2002; Yagmurca *et al*., 2007), however; the treatment with metformin, in the present study was found to increase antioxidant defense and was devoid of any toxicity, by itself, in mice. Present study demonstrates that metformin might be useful to reduce the accumulation of ROS and related biochemical changes and may be useful to avert such toxicities in diabetic patients who are on cytotoxic therapy. Further studies are suggested on antidiabetic agents (thiazolidinediones and biguanides) that are known to possess anticarcinogenic potentials, in order to find compounds which can simultaneously be beneficial for glycemic control and inhibition of toxic effects.

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