EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 6
22-31
Year: 2019

ATTENUATION OF AMIODARONE-INDUCED LUNG, LIVER AND KIDNEY TOXICITY BY NITRIC OXIDE SYNTHASE INHIBITOR, AMINOGUANIDINE IN RATS

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Article Received on 23/03/2019 **Article Revised on 14/04/2019** **Article Accepted on 05/05/2019**

ABSTRACT
Amiodarone (AM) is one of the most important antiarrhythmic drugs. Despite the use of this drug is widespread, it is associated with unwanted systemic effects. The present study was conducted to investigate whether aminoguanidine (AG), an inhibitor of nitric oxide synthase (iNOS) can protect against amiodarone-induced lung, liver and kidney toxicity in rats. Rats divided into four groups, control group: rats received saline. AG- treated group: rats received AG (100 mg/kg, i.p) daily for 15 days. AM- treated group: rats received saline daily for 5 days, followed by AM (100 mg/kg, i.p) for 10 days. AG+AM- treated group: rats received AG (100 mg/kg i.p.) daily for 5 days before and 10 days concomitant with AM (100 mg/kg, i.p). In this study, AM- treated group showed significant decrease in the body weight and significant increase in organs/body weight ratio compared to the control group. Furthermore, there was significant increase in serum AST, ALT, bilirubin, urea and creatinine levels, with a significant decrease in serum albumin level. Moreover, total leukocyte count and protein levels in BALF were significantly increase. In addition, there was significant increase in TNF-α , NO and MDA levels in lung, liver and kidney tissues and, there were histopathological abnormalities in them. The present study demonstrated that AG has a protective effect against AM induced lung, liver and kidney toxicity via its iNOS inhibition, antioxidant and anti-inflammatory effects.

KEYWORDS: Amiodarone, Aminoguanidine, Lung Toxicity, Liver Toxicity, Kidney Toxicity, Tumor Necrosis Factor, Antioxidant.

INTRODUCTION
Amiodarone (AM) is one of the most effective Class III antiarrhythmic drugs frequently used in patients with atrial fibrillation.[1] Moreover it is one of the most important antiarrhythmic therapy for the termination and prevention of ventricular arrhythmia in different clinical settings because of its proven efficacy and safety.[2] AM is an iodine-rich drug that is metabolized in the liver to produce the active metabolite desethylamiodarone. Because of its lipophilic nature, it has strong tissue affinity and a large volume of distribution. Amiodarone and its metabolite accumulate in several tissues such as liver, lung, pancreas, thyroid gland, kidney, brain and heart producing multiple well-established side effects during long-term therapy.[3]

Amiodarone induced pulmonary toxicity is a complex and multi-factorial, involving several mechanisms including direct toxicity to lung tissue, hypersensitivity reaction to amiodarone, and enhanced oxidative stress.[4] Furthermore, it causes idiosyncratic, drug-induced liver injury in human especially after long-term oral intake and acute intravenous administration.[5] The liver is the central organ for drug metabolism and removal and any imbalance in the activity of drug metabolizing enzymes leads to a free radical generation which is harmful to macromolecules and causes liver toxicity.[6] Furthermore, Cumulative effects of reactive oxygen species (ROS) may result in significant damage to cell structures leading to harmful effects such as lipid peroxidation and DNA damage.[7] Nitric oxide (NO) is an important mediator of hepatotoxicity. As it reacts with superoxide radical, forming peroxynitrite, an even more potent oxidizing agent that can react directly with sulfhydryl residues in the cell membranes leading to lipid peroxidation as well as with DNA resulting in cytotoxicity.[8,9] Amiodarone is not an obvious nephrotoxic, but few previous reports show increases in serum creatinine associated with amiodarone therapy.[10,11,12,13]

Aminoguanidine (AG), a compound structurally similar to L-arginine (the substrate for nitric oxide) is a known specific inhibitor of iNOS, a potent antioxidant and a free radical scavenger. There is growing evidence for the role of aminoguanidine as an antioxidant preventing the

loss of antioxidant enzyme activities as well as cellular damage in rats.[14] It has also been described that compounds, such as aminoguanidine, that act as iNOS inhibitors and peroxynitrite scavengers may be useful anti-inflammatory agents.[15]

This study was planned to investigate the possible protective effect of aminoguanidine on amiodarone-induced lung, liver and kidney toxicity in adult male albino rat.

2. MATERIALS AND METHODS

2.1. MATERIALS

Amiodarone was purchased from AK Scientific, Inc. (USA). Aminoguanidine Was purchased from Merck, Germany. Kits for determination of tumor necrosis Factor (TNF-α) was Obtained from Wuhan EIAab Science Co. Ltd (China). Kits for determination of Malondialdehyde (MDA), Nitric Oxide were obtained from Bio-Diagnostic Company, Egypt. While, Kits for determination of total protein was obtained from Sigma-Aldrich (USA).

2.2. Animals: Forty adult male albino rats, weighing 150-200g were purchased from the animal house, Faculty of Medicine, Sohag University, Egypt, and were housed in animal facility, Faculty of Medicine, Sohag University, maintained in a controlled environment under standard conditions of temperature (25±2^{o}C). A time controlled system provided 12 hours of light and 12 hours of dark was applied. All rats were given ad libitum access to rodent chow diet. The Medical Research Ethics Committee of Faculty of Medicine, Sohag University, Egypt approved the experimental protocol. (Approval No. 45/2018).

2.3. Experimental protocol

After acclimatization for one week, animals were divided into four groups, ten animals each.

Control Group: rats received normal saline intraperitonially (i.p.) daily for 15 days.

Aminoguanidine treated group (AG- treated group): rats received AG in normal saline (100 mg/kg, i.p) daily for 15 days.[16]

Amiodarone treated group (AM- treated group): rats received normal saline (i.p.) daily for 5 days, followed by AM (100 mg/kg, i.p) for 10 days.[17]

AG+AM- treated group: rats received AG (100 mg/kg i.p.) daily for 5 days before and 10 days concomitant with AM (100 mg/kg, i.p).

2.4. Samples collection

At the end of experimental period, investigated rats of all groups were weighed and were anaesthetized using ether. Blood samples were obtained from the heart and serum was separated by centrifugation for estimation of liver functions and kidney functions. Moreover, the lungs were lavaged two to three times with 5 ml phosphate-buffered saline, pH 7.4. Pooled bronchoalveolar lavage fluid (BALF) was kept on ice and were used immediately to measure total leukocyte counts and total protein level. Moreover, lungs, liver and kidneys from each animal were removed and weighed, and organs/total body weight ratio were calculated. Then, tissues samples were obtained from them for assessment of the level of TNF-α, NO and the degree of lipid peroxidation and for histopathological examination.

2.5. Measurements: of liver and kidney function

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin (total and direct) and albumin (liver function tests) as well as the levels of urea and creatinine (kidney function tests). They were measured spectrophotometrically (Jenway 6051 colorimeter spectrophotometer) using standardized commercially available kits (Egyptian Company for Biotechnology, Cairo, Egypt).

2.6. Measurements of total leukocyte count and protein levels in BALF

Total leukocyte counts were measured according to the method of Barbara and Stanley, (1980).[18] Lung cells viability were estimated using the trypan blue dye exclusion technique. Equal volumes of lung cell suspension and trypan blue stain (0.047%) were mixed. A hemocytometer (Neubauer chamber) was used to count the viable cells (unstained). Bronchoalveolar lavage fluid was centrifuged at 300 rpm for 10 min to remove cells, and the supernatant was recentrifuged for 20 min at 48,000 rpm to remove particulate materials. Protein concentration in the supernatant was determined by the method Bradford (1976) by using bovine serum albumin as standard, the protein level was expressed as mg/ml.[19]

2.7. Measurements of TNF-α, NO and the degree of lipid peroxidation: Lung, liver and kidney samples were homogenized in 10ml ice-cold potassium phosphate buffer (50 mM, pH 7.4) per gram tissue (v/w). The homogenates were centrifuged at 4000 rpm for 15 min at 4 C for the determination of. TNF-α, NO and the degree of lipid peroxidation.

Tumor necrosis factor -α was determined in lung, liver and kidney tissues using enzyme-linked immunosorbent assay (ELISA) specific for rat. The TNF-α contents was expressed as pg/g tissue. Nitric Oxide was determined in lung, liver and kidney tissues by a colorimetric method according to the method of Montgomery and Dymock, 1961.[20] The level of Nitrite in the sample was expressed in µmol/L. Lipid peroxidation was estimated by the measurement of MDA levels in lung, liver and kidney tissues by a colorimetric method according to the method of Ohkawa et al., (1979).[21] MDA level was expressed in nmol/g tissue.

2.8. Histopathological studies

Samples of lung, liver and kidney tissues was excised, fixed in 10% formal saline, and dehydrated in ascending grades of ethanol, cleaned in xylene and embedded in paraffin. Sections (5 µm thick) were cut and stained with hematoxylin and eosin (H&E).

2.9. Statistical analysis of data

Data were expressed as mean±standard error (SE). Statistical difference between studied groups was analyzed using one-way analysis of variance (ANOVA). followed by Tukey post hoc test to judge the difference between groups. The difference was regarded as significant when P<0.05. All statistical analyses were performed using SPSS statistical version 20 software package.

3. RESULTS

The results of the present study revealed that daily intraperitoneal administration of AG (100 mg/kg) for 15 days resulted in an insignificant change (*P*>0.05) in body weight and organs/body weight ratio compared to the control group. On the other hand, administration of AM in a dose of 100 mg/kg, i.p. for 10 successive days resulted in significant decrease *(p< 0.05)* in the body weight and significant increase *(p< 0.05)* in organs/body weight ratio compared to the control group. Administration of AG in a dose of 100 mg/kg i.p. five days before administration of AM and concomitant with it exhibited a significant increase *(p< 0.05)* in the body weight and significant decrease *(p< 0.05)* in organs/body weight ratio compared AM- treated group (Table 1).

Table. 1: Effect of treatment with 100mg/kg aminoguanidine intraperitoneally on body weight and organs weight/ body weight ratio in rats given amiodarone 100 mg/kg intraperitoneally.

Groups	Body weight	Organ weight/Body weight (ratio x 10^{-2})		
		lung	Liver	kidney
control group	267±15.71	0.41±0.04	3.19±0.19	0.35±0.02
AG- treated group	265±15.29	0.45±0.04	3.20±0.23	0.35±0.02
AM- treated group	181±5.86*	1.66±0.07*	7.91±0.21*	1.06±0.04*
AG+AM- treated group	256±19.16 [a]	0.49±0.05 [a]	3.64±0.38 [a]	0.38±0.02 [a]

Values are expressed as mean±SE, (n = 10), Data were analyzed by one-way ANOVA followed by Tukey post hoc test. AG = aminoguanidine, AM = Amiodarone. *Significantly different from control value at *p<0.05*. [a] Significantly different from AM- treated group value at *p<0.05*.

3.1. Changes in serum AST, ALT, bilirubin and albumin levels

Table (2) showed that administration of AG (100 mg/kg i.p.) daily for 15 days produced insignificant change (*P*>0.05) in serum AST, ALT, bilirubin (total and direct) and albumin levels compared to the control group. On the other hands, administration of AM in a dose of 100 mg/kg, i.p. for 10 successive days caused hepatotoxicity in rats as indicated by significant increase *(p<0.05)* in serum AST, ALT and bilirubin (total and direct) levels and a significant decreased in serum albumin level (*P*<0.05) compared to the control group. Whereas, animals treated with AG in a dose of 100 mg/kg i.p. five days before administration of AM and concomitant with it exhibited a significant decrease *(p<0.05)* in the levels of AST, ALT and bilirubin (total and direct) and significant increase *(p< 0.05)* in serum albumin level compared to AM- treated group.

Table. 2: Effect of treatment with 100mg/kg aminoguanidine intraperitoneally on liver function in rats given amiodarone 100 mg/kg intraperitoneally.

Parameters	Control Group	AG- Treated Group	AM- Treated Group	AG+AM- Treated Group
Serum AST (U/L)	41.45± 0.94	41.3± 0.82	167± 15.81*	48.5±1.51[a]
Serum ALT (U/L)	39.9± 1.08	38.9± 0.77	216± 20.54*	55.5± 1.04[a]
T.Bil (mg/dl)	1.04± 0.02	1.03± 0.02	3.07± 0.06 *	1.11± 0.03[a]
Direct. Bil (mg/dl)	0.24± 0.01	0.25± 0.01	1.78± 0.04 *	0.53± 0.04[a]
albumin (g/ dL)	3.49± 0.04	3.58± 0.06	2.16± 0.09 *	3.25± 0.05[a]

Values are expressed as mean±SE, (n = 10), Data were analyzed by one-way ANOVA followed by Tukey post hoc test. AG = aminoguanidine, AM = Amiodarone. *Significantly different from control value at p<0.05. [a] Significantly different from AM- treated group value at p<0.05.

3.2. Changes in serum urea and creatinine levels

Administration of AG (100 mg/kg i.p.) daily for 15 days produced insignificant change (*P*>0.05) in serum urea and creatinine levels compared to the control group. On the other hand, a significant increase *(p< 0.05)* in serum urea and creatinine levels were observed after treatment of the rats with AM (100 mg/kg, i.p.) for 10 successive days compared to the control group. However, animals treated with AG (100 mg/kg ip.) for 5 days before and concomitantly with AM exhibited significant decrease *(p< 0.05)* in the levels of serum urea and creatinine compared to AM- treated group (Table 3).

Table. 3: Effect of treatment with 100mg/kg aminoguanidine intraperitoneally on kidney function in rats given amiodarone 100 mg/kg intraperitoneally.

Parameters	Control group	AG- treated group	AM- treated Group	AG+AM- treated Group
Urea	40.50±1.75	41.6±1.97	53.7±1.27*	44.6±0.93 [a]
Creatinine	0.81±0.05	0.85±0.04	1.99±0.06*	0.96±0.06 [a]

Values are expressed as mean±SE, (n = 10), Data were analyzed by one-way ANOVA followed by Tukey post hoc test. AG = aminoguanidine, AM = Amiodarone. *Significantly different from control value at p<0.05. [a] Significantly different from AM- treated group value at p<0.05.

3.3. Changes in total leukocyte count and protein levels in BALF

Total leukocyte count and protein levels in BALF showed insignificant changes (*P*>0.05) in AG- treated group compared to the control group. Conversely, in AM- treated group there was significant increase *(p< 0.05)* in total leukocyte count and protein levels. Whilst, by addition of aminoguanidine in AG+AM- treated group there were significant decrease *(p< 0.05)* in the total leukocyte count and protein levels in BALF compared to AM- treated group (Table 4).

Table. 4: Effect of treatment with 100mg/kg aminoguanidine intraperitoneally on total leukocyte count and protein levels in BALF in rats given amiodarone 100 mg/kg intraperitoneally.

Parameters	Control Group	AG-Treated Group	AM-Treated Group	AG+AM-Treated Group
Total leukocyte count	22.70±1.92	24.00±2.33	69.50±4.04*	23.50±2.59[a]
Protein	2.02±0.18	2.04±0.19	7.39±0.36*	2.91±0.26[a]

Values are expressed as mean±SE, (n = 10), Data were analyzed by one-way ANOVA followed by Tukey post hoc test. AG = aminoguanidine, AM = Amiodarone. *Significantly different from control value at p<0.05. [a] Significantly different from AM- treated group value at p<0.05.

3.4. Changes in lung, liver and kidney TNF-α levels

It was observed that the level of TNF-α was insignificantly change in the AG- treated group compared to the control group in lung, liver and kidney tissues (*P*>0.05). But administration of AM in a dose of 100 mg/kg, i.p. for 10 successive days produced a significant increase *(p< 0.05)* in the TNF-α level in lung, liver and kidney tissues compared to the control group. On the other hands, TNF-α was significantly decrease *(p< 0.05)* in the AG+AM treated group compared to the AM- treated group (Fig. 1).

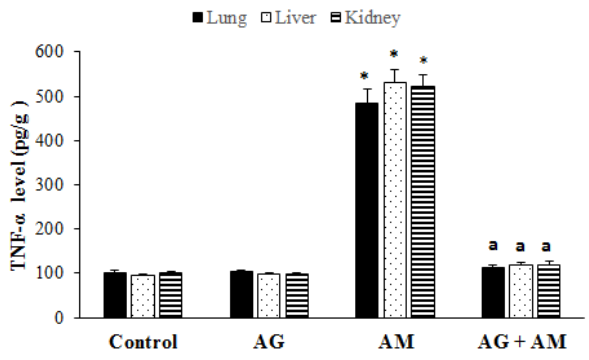

Figure. 1: Effect of treatment with 100mg/kg aminoguanidine intraperitoneally on lung, liver and kidney TNF-α levels in rats given amiodarone 100 mg/kg intraperitoneally.

Values are expressed as mean±SE, (n = 10), Data were analyzed by one-way ANOVA followed by Tukey post hoc test. AG = aminoguanidine, AM = Amiodarone. *Significantly different from control value at *p<0.05.* [a] Significantly different from AM- treated group value at *p<0.05.*

3.5. Changes in lung, liver and kidney NO levels

As shown in Figure 2, NO level in the lung, liver and kidney homogenate in the AG- treated group were insignificantly changed (*P*>0.05) compared to the control group. However, AM- treated group showed a significant increase *(p< 0.05)* in the NO level in lung, liver and kidney tissues compared to the control group. Administration of AG (100 mg/kg i.p.) for 5 days before and 10 days concomitantly with AM produced a significant decrease *(p< 0.05)* in the NO level in the lung, liver and kidney tissues compared to the AM-treated group.

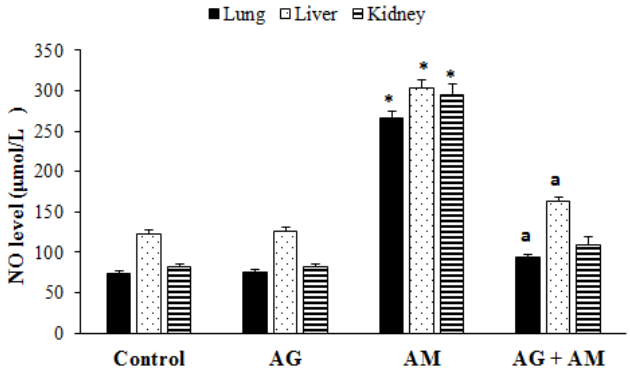

Figure 2: Effect of treatment with 100mg/kg aminoguanidine intraperitoneally on lung, liver and kidney NO levels in rats given amiodarone 100 mg/kg intraperitoneally.

Values are expressed as mean±SE, (n = 10), Data were analyzed by one-way ANOVA followed by Tukey post hoc test. AG = aminoguanidine, AM = Amiodarone. *Significantly different from control value at p<0.05. [a] Significantly different from AM- treated group value at p<0.05.

3.6. Changes in lung, liver and kidney lipid peroxidation levels

Compared to the control group, the AG- treated group showed insignificant change (P>0.05) in the lung, liver and kidney tissues MDA level. On the other hand, the lung, liver and kidney tissues MDA level in AM- treated group showed a significant increase *(p< 0.05)* in its level compared to the control group. Whilst, AG+AM treated group exhibited a significant decrease *(p< 0.05)* in the MDA level in the lung, liver and kidney tissues compared to the AM- treated group (Fig. 3).

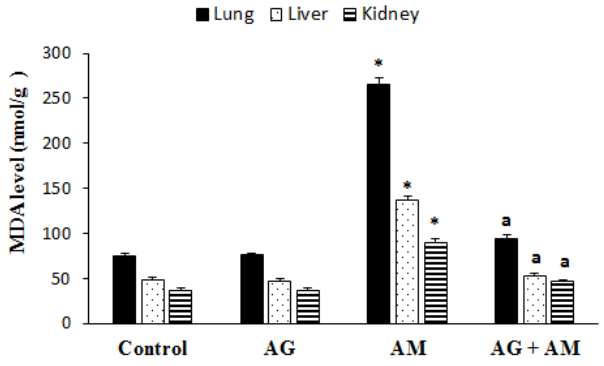

Figure. 3: Effect of treatment with 100mg/kg aminoguanidine intraperitoneally on lung, liver and kidney lipid peroxidation levels in rats given amiodarone 100 mg/kg intraperitoneally.

Values are expressed as mean±SE, (n = 10), Data were analyzed by one-way ANOVA followed by Tukey post hoc test. AG = aminoguanidine, AM = Amiodarone. *Significantly different from control value at p<0.05. [a] Significantly different from AM- treated group value at p<0.05.

3.7. Histopathological changes: Microscopically, examination of lung, liver and kidney tissues of the control group showed normal histological architecture (Figs.4A, 5A and 6A).in addition, no histopathological alterations were observed in AG- treated groups (Figs.4B, 5B and 6B). In the AM-treated group, the lung, liver and kidney tissues showed severe histopathological alterations as there were severe intra alveolar hemorrhage, decreased alveolar spaces marked thickening in alveolar wall and large number of inflammatory cells infiltrates in the lung tissue (Figs.4C, D). Moreover, there was lymphocytic aggregation, nucleomegaly and congested vein in the liver tissue (Figs.5C, D, E). In addition, the kidney tissue showed cloudy swelling (hydropic degeneration) in the tubular epithelial cells with congested glomerulus (Fig.6C). Pretreatment with aminoguanidine in AG+AM treated group attenuate the histopathological lesions observed in the lung, liver and kidney tissues of the rats treated with AM alone (Figs. 4E, 5F, 6D).

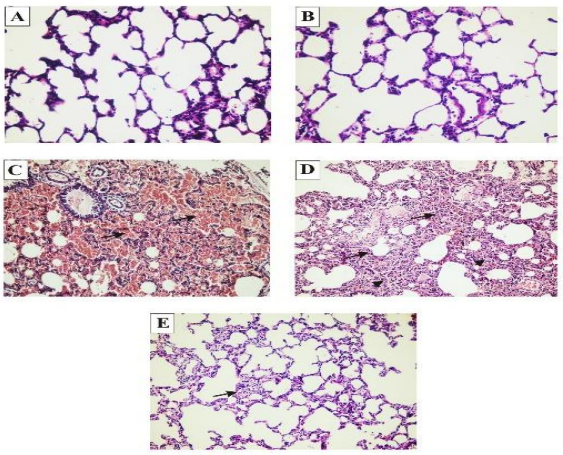

Figure 4: Photomicrographs of the lung tissue showing effects of treatment with 100mg/kg aminoguanidine intraperitoneally in amiodarone induced lung toxicity in rats.

A. Lung tissue of the control group showing normal lung architecture (H&E x200).
B. Lung tissue of the AG- treated group showing normal lung architecture (H&E x200).
C. Lung tissue of the AM- treated group showing severe intra alveolar hemorrhage (arrow) (H&E x200).
D. Lung tissue of the AM- treated group showing decreased alveolar spaces, marked thickening in alveolar wall (arrow) and large number of inflammatory cells infiltrates (arrow head) (H&E x200).
E. Lung tissue of AG+AM- treated group showing normal lung tissue, except minimal thickening in alveolar wall (arrow) (H&E x200).

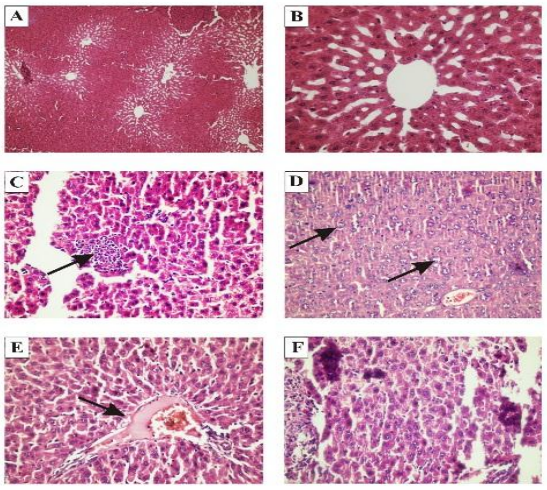

Figure. 5: Photomicrographs of the liver tissue showing effects of treatment with 100mg/kg aminoguanidine intraperitoneally in amiodarone induced liver toxicity in rats.

A. Liver tissue of the control group showing normal liver architecture (H&E x200).
B. Liver tissue of the AG- treated group showing normal liver architecture (H&E x400).
C. Liver tissue of the AM- treated group showing lymphocytic aggregation (arrow) (H&E x400).
D. Liver tissue of the AM- treated group showing nucleomegaly (arrow) (H&E x400).
E. Liver tissue of the AM- treated group showing congested vein (arrow) (H&E x400).
F.Liver tissue of AG+AM- treated group showing normal liver tissue (H&E x200).

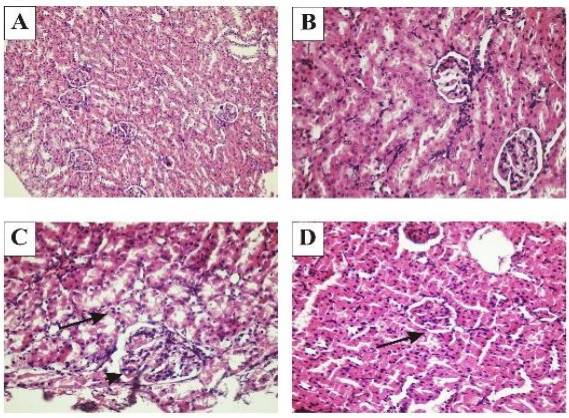

Figure. 6: Photomicrographs of the kidney tissue showing effects of treatment with 100mg/kg aminoguanidine intraperitoneally in amiodarone induced kidney toxicity in rats.

A. Kidney tissue of the control group showing normal kidney architecture (H&E x200).
B. Kidney tissue of the AG- treated group showing normal kidney architecture (H&E x400).
C. Kidney tissue of the AM- treated group showing cloudy swelling (arrow) and congested glomerulus (arrow head) (H&E x400).
D. Kidney tissue of AG+AM- treated group showing normal kidney tissue, except minimal congested glomerulus (arrow) (H&E x400).

4. DISCUSSION

In the present study, administration of AM in a dose of (100 mg/kg i.p) for 10 successive days produced a significant decrease in the body weight compared to the control group indicating that the rats reacted adversely to AM treatment. This result was in a good agreement with other result obtained by Wilson et al., (1989)[22] that showed that AM treated animals weighed significantly less than control rats at all experimental time points. Wilson et al., (1989)[22] explained that the drug caused a derangement of lipid metabolism, since AM has been shown to be an extremely potent inhibitor of phospholipases.

On the other hand, the significant increase in organs/body weight ratio of AM- treated group compared to the control group in the present study may be attributed to the accumulation of oedema as a result of inflammation and oxidative stress in these studied organs. These results were in harmony with other results obtained by Al-Shammari et al., (2016)[23] who showed a significant decrease in body weight with an increase in both lung weight and lung/body weight coefficient in the first week in AM treated rats. In addition, Gado and Aldahmash., (2013)[24] showed a significant increase in lung/body coefficient in AM treated group compared to the control group. Lung/body coefficient is a marker of pulmonary oedema and is a conventional toxicological method successfully used to determine the level of lung injury.

In the present study, the administration of AG (100 mg/kg i.p) daily for 5 days before and 10 days concomitant with AM improved the effect of AM on the body weight and on organs/body weight ratio, indicating the positive effect of AG in prevention of AM- induced toxicities in the studied organs.

To evaluate the role of AG in preventing organs damage induced by AM administration, this research was conducted for the analysis of liver and kidney functions, total leukocyte count and protein levels in BALF, TNF-α, NO and MDA levels in tissue homogenates of the studied organs. Moreover, a histopathological examination of the studied organs was performed.

In our study, administration of AM in a dose of (100 mg/kg i.p) for 10 successive days produced hepatic toxicity to the rats as evidenced by significant increase in the serum activities of ALT, AST and both total and direct bilirubin levels with a significant decrease in the serum albumin level. These findings are consistent with previous reports.[25,26] Moreover, Riaz et al., (2016)[27] found that administration of AM in a dose of (200 mg/kg i.p) three times a day for three days produced significant elevation of serum ALT and AST activities and significant decrease in serum albumin, but with insignificant effect on serum bilirubin. On the other hand, study of Kikkawa et al., (2006)[28] showed no change of ALT levels in normal rats treated with AM.

Amiodarone is a lipophilic drug and has a large volume of distribution so accumulates in the lipid rich reservoirs which may induce injury to the liver tissues and altered the permeability of membranes by the formation of ROS and oxidative stress lead to the leakage of enzymes from the cells.[29] Therefore, the elevation of serum ALT and AST enzyme activities in AM-treated group indicates damage to the liver.[30] In addition, decreased albumin level in the present study is due to reduced synthesis of albumin as a result of injury to hepatocytes. Moreover, Dara et al., (2011)[31] reported that in drug induced liver injury various intracellular signaling mediators are released leading to endoplasmic reticulum stress and dysfunction leading to decreased albumin synthesis.

In the present study, animals treated with AG five days before administration of AM and concomitant with it exhibited a significant decrease in the levels of AST, ALT and bilirubin (total and direct) and significant increase in serum albumin level compared to AM-treated group. These findings are in accordance with Fahmy Ahmed et al., (2011)[32] who reported that AG caused significant decrease in the elevated activities of ALT and AST when compared to CCl_4-treated rats.

In this work, significant elevation in serum urea and creatinine levels were recorded in AM- treated rats. Our results are in accordance with Sakr and El-Gamal., (2016)[13] who showed significant elevation in creatinine and serum urea levels in AM- treated animals. Moreover, Jacobs., (1987), Pollak et al., (1993) and Morales et al., (2003)[10,11,12] also reported that rats treated with AM had a higher serum creatinine than the controls.

Sakr and El-Gamal., (2016)[13] explained these changes by decreased glomerular filtration which occurred due to atrophy of glomeruli or decrease in the renal tubule reabsorption due to degeneration of tubular epithelial cells and their desquamation.

On the other hand, the findings of this study strongly indicate that AG is important in protecting the kidney from AM- induced injury. This is evidenced by significant decrease in the levels of serum urea and creatinine in animals treated with aminoguanidine 5 days before and 10 days concomitantly with AM. These results are in agreement with Abo-Salem., (2012)[33] as AG administration significantly decreased the serum levels of urea and creatinine as compared to doxorubicin-treated group. Polat et al., (2006)[34] also in consistence with our results.

The protective effect of AG on serum urea and creatinine levels can be attributed to its antioxidant properties as it has been found that ROS may be involved in the impairment of glomerular filtration rate.[35]

In our study, there was significant increase in total leukocytic count and protein levels in AM- treated group compared to the control group. Our results are consistent with previous studies that demonstrated elevated levels of total leukocytic count and protein levels in AM-treated rats.[22,23,36] The observed increase in total leukocytic count and protein levels in BALF is an informative indication of development of pulmonary inflammation and damage of the alveolar integrity.

In our study, addition of aminoguanidine in AG+AM-treated group results in significant decrease in the total leukocyte count and protein levels in BALF compared to AM- treated group. These findings agreed with Lanzetti et al., (2012).[37]

Tumor-necrosis factor-α is considered one of the major hepatotoxicity mediators in liver injury. When an inflammatory reaction occurs, TNF-α is expressed by both infiltrating inflammatory cells such as macrophages in blood and hepatocytes in case of liver injury.[38]

In our study, administration of AM for 10 successive days produced a significant increase in the TNF-α level in lung, liver and kidney tissues compared to the control group. This is in accordance with Madkour and Ahmed., (2013)[39] who showed marked increase in serum level of TNF-α in AM-treated rats. On the other hand, our results disagree with the results of Lu et al., (2012)[40] , who showed insignificant change in the serum TNF- α level in AM-treated rats.

Tumor-necrosis factor-α caused overproduction of nitric oxide in liver which reacts with superoxide radical, forming peroxynitrite, which is a potent oxidizing agent. Peroxynitrite can react directly with sulfahydryl residues in cell membrane as well as with DNA leading to lipid peroxidation and cytotoxicity.[8]

Our study demonstrated that TNF-α was significantly decreased in the AG+AM treated group compared to the AM- treated group. This is in a good agreement with Fahmy Ahmed et al., (2011)[32] who reported that prophylactic administration of AG with CCL_4 in rats show significant reduction in serum TNF-α compared to CCL_4 treated group as AG has anti-inflammatory properties. Also, Abosalem., (2012)[33] is in accordance with our results in which pretreatment of animals with TNF-α inhibitor, aminoguanidine, ameliorated the increased level of plasma TNF-α in the doxorubicin-treated group.

In the present study, AM- treated group showed a significant increase in the NO level in lung, liver and kidney tissues compared to the control group. This is in accordance with Gado and Aldahmash., (2013)[24] and Al-shammari et al., (2016)[23]. The increased NO observed is indicative of oxidant related tissue injury by formation of highly reactive nitrogen intermediates which is responsible for most of the adverse effects of excessive generation of NO.[41]

In our study, administration of AG for 5 days before and 10 days concomitantly with AM produced a significant reduction in the NO level in the lung, liver and kidney tissues compared to the AM- treated group. Our findings are in consistence with the previous studies.[32,33,34]

The enzyme iNOS is expressed in hepatocytes and inflammatory cells during the development of cirrhosis. Therefore, inhibition of iNOS expression may have an important role in both cirrhosis and endotoxaemia. AG inhibits iNOS in a selective manner, leading to decreased generation of NO.[42,43]

In the present study, lung, liver and kidney tissues MDA level in AM- treated group showed a significant increase compared to the control group. This is agreed with study

by Zaeemzadeh et al., (2011), Gado and Aldahmash., (2013), Madkour and Ahmed., (2013) and Gawad et al., (2018)[24,39,44,45] verifying that amiodarone induces excessive production of ROS that leads to serious oxidative damage.

On the contrary, Al-Shammari et al., (2016)[23] showed that AM given to rats in a dose of (80mg/kg/day i.p) decreased the lung content of MDA compared to the control group explaining their results as a part of the antioxidant effect of amiodarone.

In our study, rats treated with AG five days before administration of AM and concomitant with it exhibited a significant decrease in the MDA level in the lung, liver and kidney tissues compared to the AM- treated group. This is consistent with previous reports.[32,33,34]

Aminoguanidine acts as an antioxidant by preventing ROS formation and lipid peroxidation in cells and tissues, thus preventing oxidant-induced apoptosis. In addition, AG may also inhibit lipid peroxidation by inducing glutathione peroxidase and SOD or scavenging and inactivating H_2O_2.[46,47]

Generally, in our study the present biochemical findings were strongly supported by histopathological changes, our results revealed that AM caused histopathological alterations in the lung, liver and kidney tissues of the rats in AM-treated group compared to the control group. Our results agreed with the previous reports.[12,13,45,48,49] On the other hand, pretreatment of the rats with AG (100 mg/kg i.p) daily for 5 days before and 10 days concomitant with AM attenuate the histopathological lesions observed in the lung, liver and kidney tissues compared to the rats treated with AM alone. These results agreed with Lanzetti et al., (2012)[37]. Moreover, Fahmy Ahmed et al., (2011)[32] reported that AG- treated rats revealed marked reduction in the hepatic lesions caused by CCL_4.

Our results are also consistent with Abo Salem., (2012)[33] in which renal lesions were reduced significantly in animals which received AG prior to doxorubicin-treatment. In addition, Polat et al., (2006)[34] reported that pretreatment with AG afforded significant protection against nephrotoxicity induced by gentamycin treatment.

CONCLUSION

According to our biochemical findings, which are supported by histopathological evidence, pretreatment with aminoguanidine has protective effect against amiodarone induced lung, liver and kidney toxicity via its iNOS inhibition, antioxidant effect and anti-inflammatory effect.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. Ahmed R. Hamed, Ass. Prof. of pathology, Faculty of Medicine, Sohag University, for help in histopathological studies. Also, our thanks go to H. Sleem, Zoology Dept., Faculty of Science, Sohag University for her help in BALF collection.

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