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Ameliorative effect of free radical scavenger aminoguanidine hemisulfate on amikacin induced biochemical alteration in Wistar rats

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Abstract
The present study was aimed to investigate the biochemcal alterations induced by subacute administration of amikacin and the effect of aminoguanidine hemisulfate alone and their combination in wistar rats of either sex. Twenty-four healthy wistar rats were randomly divided into 4 groups (I, II, III and IV) and each group comprises of 6 animals. Group-I served as control to which normal saline was administered. For sub-acute study rats of group-II and group-III were treated with amikacin (15mg/Kg BW) and aminoguanidine hemisulfate (20mg/Kg BW) daily for 28 days intra-peritoneally, respectively. In group-IV rats, amikacin and aminoguanidine hemisulfate were co-administered at their respective doses. A significant increase in the biochemical-parameters such as ALT, plasma-creatinine, total bilirubin, plasma uric acid and plasma urea nitrogen were found as compared to control group after 28 days of intra-peritoneal administration of amikacin. However, a significant decrease in plasma albumin was found as compared to control group.

Keywords: amikicin; aminoguanidine hemisulfate; biochemical alterations; wistar rats

1. Introduction
Amikacin, a semi-synthetic aminoglycoside derived from Kanamycin-A (*Streptomyces kanamyceticus*) was introduced in 1972 (Gilbert *et al*., 1995) [9]. It is primarily used against gram-negative-aerobic-organisms (Edson and Terrell, 1999) [8] and also against gram-positive-pathogens (Isaksson *et al*., 1991) [11]. Amikacin can also be used in combination with beta-lactam-antibiotics to produce synergistic-effect and also broaden the activity against both gram positive and gram negative bacteria (Sandhu *et al*., 2007) [22]. Most often it is used to treat severe hospital-acquired infections of gram-negative-bacteria with multidrug resistance and also as a second-line drug for anti-tuberculosis drugs (Edson and Terrell, 1999) [8]. The pharmacokinetic behavior of the drug is known to be influenced by pathophysiological conditions (Zaske *et al*., 1992) [24]. During severe sepsis and septic shock, amikacin disposition is altered by an increased volume of distribution and a reduced total body clearance, decreased protein binding, and organ failure (Roberts and Lipman, 2006) [21]. Its nephrotoxicity and ototoxicity have widely guided attempts to rationalize the drug dosage strategy (Barclay and Begg, 1994) [3]. It produces free-radicals/reactive oxygen species (ROS) which participate in the patho-physiology of amikacin-induced-nephrotoxicity (Parlakpinar *et al*., 2004) [19]. Aminoguanidine-hemisulfate is an effective antioxidant (Ihm *et al*., 1999) [10] and a free radical scavenger (Szabo *et al*., 1997) [23] which is well-known to protect nephrotoxicity (Parlakpinar *et al*., 2004) [19]. It inhibits inducible nitric oxide synthase (iNOS) in a selective and competitive manner, leading to decreased generation of nitric-oxide (Misko *et al*., 1993) [19] and free-radicals. Aminoguanidine is a nucleophilic reagent that can inhibit the formation of advanced glycation end products by reacting with reactive carbonyl groups of proteins to form relatively non-toxic adducts. This way, it stops the aging of the body and prevents thickening of the arteries, senile cataracts, age-related yellowing and toughening of the skin, some cancers, and damage to the immune system (Li *et al*., 1996). Paucity of literature on ameliorative effect of aminoguanidine hemisulfate on amikacin induced biochemical alteration in wistar rats prompted this study.

2. Materials and Methods
In the present investigation, twenty-four healthy wistar rats were taken for the study. After acclimatization, rats were randomly divided into 4 groups (I, II, III and IV) and each group comprised 6 animals. Group-I served as control to which normal saline was administered. For sub-acute study rats of group-II and group-III were treated with amikacin (15mg/Kg BW) and

aminoguanidine hemisulfate (20mg/Kg BW) daily for 28 days intra-peritoneally, respectively. In group-IV rats, amikacin and aminoguanidine hemisulfate were co-administered at their respective doses (Table 1).

Table 1: The experimental design

Sl. No.	Treatment Group(s) (n=6)	Dose (mg/kg)	Exposure-Period	Routes
1.	Group-I (Control)	Normal saline	28 days	I.P.
2.	Group-II (Amikacin sulphate)	15mg/kg/day	28 days	I.P.
3.	Group-III (Aminoguanidine hemisulfate)	20mg/kg/day	28 days	I.P.
4.	Group-IV (Amikacin sulphate + Aminoguanidine hemisulfate)	15mg/kg + 20mg/kg/day	28 days	I.P.

Blood samples of about 2-4ml were collected from retro-orbital sinus of rats on zero, 15th and 29th day using capillary-tubes in aliquots containing anticoagulant heparin (strength 10 IU/ml of blood) and sodium EDTA for estimaton of haematological parameters. The blood samples were centrifuged at 3000 rpm for 15 min to harvest the plasma and kept in clean sterile-glass-test-tubes and stored at -20ºC for further biochemical analysis.

Statistical analysis

A standard statistical procedure was followed. The data collected during the experiment was subjected to analysis of variance under completely randomized design (CRD) and the level of significance was tested using Duncan Multiple Range Test (Duncan, 1955) at 5% ($P < 0.05$) level.

3. Results and Discussion

3.1 Plasma albumin

Daily administration of amikacin in rats induced significant decreased in plasma albumin levels as compared to control rats (group-I). Similar observations were also reported by Chaudhary *et al*. (2008) [4] in amikacin treated rats. Such decrease may be due to amikacin induced nephrotoxicity resulting from excessive generation of free radicals (Table 2). Aminoguanidine treatment produced a significant increase in albumin levels in plasma as compared to control rats (group-I) as reported by Preedy and Hammond (1991) [20] with daily administration of aminoguanidine in rats for 3 weeks. It may be due to minor hepatic perturbations or impairment in kidney function. However, amikacin and aminoguanidine co-administered rats (group-IV) exhibited a significant decrease in albumin levels in plasma as compared to control rats (group-I). Contrary to our observations, no changes in albumin levels in plasma were reported by Parlakpinar *et al*. (2004) [19] in rats after co-administration of amikacin and aminoguanidine. Significant decrease in albumin levels in plasma may be due to ameliorative effect of aminoguanidine on amikacin induced nephrotoxicity.

Table 2: Showing the effect of amikacin, aminoguanidine and their co-administration on plasma albumin (g/dl) after intra-peritoneal administration in wistar-rats.

Treatments Groups	Treatment-Period		
	Day zero	Day fifteen	Day twenty-nine
Group-I (Control)	3.16 ± 0.04^{aA}	3.19 ± 0.03^{aB}	3.28 ± 0.06^{aB}
Group-II (Amikacin)	3.22 ± 0.06^{aA}	2.68 ± 0.06^{bC}	2.53 ± 0.04^{bD}
Group-III (Aminoguanidine)	3.23 ± 0.04^{bA}	3.46 ± 0.06^{aA}	3.49 ± 0.06^{aA}
Group-IV Amikacin + Aminoguanidine)	3.29 ± 0.07^{aA}	2.80 ± 0.07^{cC}	3.02 ± 0.04^{bC}

Values are in Mean± SE, Similar superscript do not differ significantly at 5% ($P<0.05$)
Capital superscripts represent significance between the groups
Small superscripts represent significance within the groups

3.2 Plasma uric acid

Daily administration of amikacin in rats (group-II) induced a significant increase in plasma uric acid as compared to control rats (group-I), as was reported by by Yazar *et al*. (2003) in amikacin treated mice. It may be due to injury to renal tissues leading to improper excretion. Aminoguanidine treatment produced a significant decrease in plasma uric acid as compared to control rats (group-I) (Table 3). Similar findings were also found by Mansour *et al*. (2002) [16] in aminoguanidine pretreated rats. It may be due to free radical scavenging property of aminoguanidine resulting in alleviation of any stress to rats. However, in amikacin and aminoguanidine co-adminstered rats (group-IV), no significant change in plasma uric acid was observed as compared to control rats (group-I). Similar observations were also reported by Mansour *et al*. (2002) [16] in cisplatin induced nephrotoxic rats after aminoguanidine administration. It may be due to ameliorative effect of aminoguanidine on amikacin induced nephrotoxicity and, therefore, reduced damage to renal tissues.

Table 3: Showing the effect of amikacin, aminoguanidine and their co-administration on plasma uric acid (mg/dl) after intra-peritoneal administration in wistar-rats.

Treatments Groups	Treatment-Period		
	Day zero	Day fifteen	Day twenty-nine
Group-I (Control)	1.54 ± 0.02^{aA}	1.55 ± 0.08^{aC}	1.54 ± 0.06^{aC}
Group-II (Amikacin)	1.53 ± 0.05^{cA}	2.51 ± 0.11^{bA}	2.63 ± 0.08^{aA}
Group-III (Aminoguanidine)	1.55 ± 0.04^{aA}	1.22 ± 0.09^{bD}	1.14 ± 0.06^{cD}
Group-IV(Amikacin + Aminoguanidine)	1.52 ± 0.09^{cA}	1.95 ± 0.08^{aB}	1.81 ± 0.10^{bB}

Values are in Mean± SE, Similar superscript do not differ significantly at 5% ($P<0.05$)
Capital superscripts represent significance between the groups
Small superscripts represent significance within the groups

3.3 Plasma urea nitrogen and creatinine

Daily administration of amikacin in rats (group-II) induced a significant increase in plasma creatinine and plasma urea nitrogen as compared to control rats (group-I). Similar to our observations, significant increase in these parameters was reported in rats and rabbits (Kose *et al*., 2012**;** Khalaf *et al*., 2011**;** Martines *et al*., 1988) [14, 13, 15]. The high levels of plasma urea nitrogen results from either increased breakdown of tissue or dietary protein or impaired excretion (Table 4). Creatinine is present in tissues (muscles, brain, blood etc) as a high energy compound phospho-creatinine. Increase in creatinine concentration might be due to loss of kidney function and is considered as functional evidence of amikacin-induced nephrotoxicity. Creatinine is obtained from phospho-creatinine in muscles and is excreted in small quantities in urine. Metabolism of xenobiotics being energetic process would utilize phospho-creatinine for energy generation and therefore, it may possibly be one of the reasons for increases level of creatinine observed in the present study. Aminoguanidine treated rats (group III) produced a significant decrease in plasma urea nitrogen and creatinine as compared to control rats (group-I) (Table 5). Similar observations were reported by Preedy and Hammond (1991) [20] with daily administration of aminoguanidine in rats for 3 weeks. The significant decrease in this parameter may be due to the protective effect of aminoguanidine on kidney via inhibiting diamine oxidase. Also in amikacin and aminoguanidine co-adminstered rats (group-IV), no significant change in plasma urea nitrogen and creatinine was observed as compared to control rats (group-I). Similar observations were reported by Mansour *et al.* (2002) [16] in rats administered single dose of cisplatin, pretreated with aminoguanidine. This may be due to diamine oxidase inhibitor property of aminoguanidine and its ameliorative effect on amikacin induced nephrotoxicity.

Table 4: Showing the effect of amikacin, aminoguanidine and their co-administration on plasma urea nitrogen levels (mMol/L) after intra-peritoneal administration in wistar-rats.

Treatments Groups	Treatment-Period		
	Day zero	Day fifteen	Day twenty-nine
Group-I (Control)	$60.65+1.67^{aA}$	$62.91+1.20^{aC}$	$62.74+1.43^{aB}$
Group-II (Amikacin)	$60.21+1.41^{cA}$	$81.95+1.28^{bA}$	$87.31+0.81^{aA}$
Group-III (Aminoguanidine)	$61.20+1.27^{aA}$	$53.14+1.31^{bD}$	$50.33+0.81^{bC}$
Group-IV (Amikacin + Aminoguanidine)	$63.02+1.55^{bA}$	$71.52+0.53^{aB}$	$62.96+1.11^{bB}$

Values are in Mean± SE, Similar superscript do not differ significantly at 5% (P<0.05)
Capital superscripts represent significance between the groups
Small superscripts represent significance within the groups

Table 5: Showing the effect of amikacin, aminoguanidine and their co-administration on on plasma creatinine levels (mg/dl) after intra-peritoneal administration in wistar-rats.

Treatments Groups	Treatment-Period		
	Day zero	Day fifteen	Day twenty-nine
Group-I (Control)	$0.53±0.03^{\mathbf{aA}}$	$0.53±0.04^{\mathbf{aC}}$	$0.54±0.03^{\mathbf{aB}}$
Group-II (Amikacin)	$0.51±0.04^{\mathbf{bA}}$	$1.01±0.04^{\mathbf{aA}}$	$1.10±0.05^{\mathbf{aA}}$
Group-III (Aminoguanidine)	$0.51±0.03^{\mathbf{aA}}$	$0.46±0.03^{\mathbf{abC}}$	$0.40±0.02^{\mathbf{bC}}$
Group-IV(Amikacin + Aminoguanidine)	$0.53±0.03^{\mathbf{cA}}$	$0.78±0.03^{\mathbf{aB}}$	$0.64±0.03^{\mathbf{bB}}$

Values are in Mean± SE, Similar superscript do not differ significantly at 5% (P<0.05)
Capital superscripts represent significance between the groups
Small superscripts represent significance within the groups

3.4 Total bilirubin

Amikacin treatment in rats (group-II) exhibits a significant increase in total bilirubin as compared to control rats (group-I) (Table 6). Similar to our observations significant increase in total bilirubin was reported by Singhal and Prajapati, (2011) in rats after amikacin and cefepime administration. It may be due to hepatotoxic and nephrotoxic effect of amikacin which resulted in disturbance in the ratio of free radical generating and free radical scavenging enzymes and leading to disruption of signal transduction pathway and increased cellular permeability by acting on membrane phospholipids. The binding of the amikacin with cellular membrane also causes impairment of phospholipid catabolism, changes in membrane aggregation and reduces the activities of phospholipases. However, aminoguanidine treatment (group III) produced a significant decrease in total bilirubin as compared to control rats (group-I). Similar to our observations significant decrease in total bilirubin was reported by Ahmed *et al*. (2011) [1] in aminoguanidine treated rats. It may be due to hepatoprotective and nephroprotective effect of aminoguanidine by inhibiting iNOS pathway. Also in amikacin and aminoguanidine co-adminstered rats (group-IV), no significant change in total bilirubin was found as compared to control rats (group-I). Similar findings were also reported by Abo-Salem, (2012) [2] in doxorubicin-induced nephropathy in rats after aminoguanidine treatment. It may be due to ameliorative effect of aminoguanidine on amikacin induced hepatitis and nephrotoxicity.

Table 6: Showing the effect of amikacin, aminoguanidine and their co-administration on total plasma bilirubin (mg/dl) after intra-peritoneal administration in wistar-rats.

Treatment groups	Treatment period		
	Day zero	Day fifteen	Day twenty-nine
Group-I (Control)	$0.235±0.02^{aA}$	$0.233±0.02^{aC}$	$0.238±0.03^{aC}$
Group-II (Amikacin)	$0.240±0.01^{cA}$	$0.328±0.01^{bA}$	$0.390±0.02^{aA}$
Group-III (Aminoguanidine)	$0.243±0.01^{aA}$	$0.195±0.01^{bC}$	$0.176±0.02^{bD}$
Group-IV(Amikacin + Aminoguanidine)	$0.253±0.03^{bA}$	$0.293±0.01^{aB}$	$0.283±0.01^{aB}$

Values are in Mean± SE, Similar superscript do not differ significantly at 5% (P<0.05)

Capital superscripts represent significance between the groups
Small superscripts represent significance within the groups

3.5 Total Plasma protein

Daily administration of amikacin in rats (group-II) produced a non-significant increase in total protein as compared to control rats (group-I) (Table 7). Similar observations were also reported in amikacin treated rats and goats (Chaudhary *et al*., 2008**;** Dinev *et al*., 2005) [4, 6]. However, aminoguanidine treatment (group III) produced non-significant decrease in total protein as compared to control rats (group-I). Similar observations were also observed by Preedy & Hammond, (1991) [20] in aminoguanidine treated rats. However, in amikacin and aminoguanidine co-adminstered rats (group-IV), non-significant decrease in total protein was observed as compared to control rats (group-I). Similar observations were also observed by Parlakpinar *et al*., (2004) [19] in amikacin and aminoguanidine co-administered rats.

Table 7: Showing the effect of amikacin, aminoguanidine and their co-administration on Total plasma proteins (gm/dl) after intra-peritoneal administration in wistar-rats.

Treatment groups	Treatment period		
	Day zero	Day fifteen	Day twenty-nine
Group-I (Control)	$6.75+0.17^{aA}$	$6.70+0.21^{aA}$	$6.73+0.18^{aA}$
Group-II (Amikacin)	$7.13+0.21^{aA}$	$6.60+0.11^{bA}$	$6.95+0.12^{abA}$
Group-III (Aminoguanidine)	$6.72+0.09^{aA}$	$6.42+0.11^{aA}$	$6.58+0.12^{aA}$
Group-IV(Amikacin + Aminoguanidine)	$6.92+0.12^{aA}$	$6.62+0.11^{aA}$	$6.72+0.14^{aA}$

Values are in Mean± SE, Similar superscript do not differ significantly at 5% (P<0.05)
Capital superscripts represent significance between the groups
Small superscripts represent significance within the groups

4. Conclusion

The study revealed that a significant increase in the biochemical-parameters such as ALT, plasma-creatinine, total bilirubin, plasma uric acid and plasma urea nitrogen were found as compared to control group after 28 days of intra-peritoneal administration of amikacin. However, a significant decrease in plasma albumin was found as compared to control group.

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