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# PROTECTIVE EFFECTS OF HYDROALCOHOLIC EXTRACT OF RED GRAPE SEED (*VITIS VENIFERA)* IN NEPHROTOXICITY INDUCED BY AMIKACIN IN MICE

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**Abstract**

Amikacin is one of aminoglycosides that is used as an antibiotic in infections caused by gram-negative microorganisms. Amikacin adverse effects such as nephrotoxicity and ototoxicity limit its application and use. Most reports have shown that aminoglycosides can cause reduction of antioxidant capacity of enzymes. In this study hydroalcoholic extract of red grape seed *(Vitis venifera*) as a natural antioxidant was used to protect nephrotoxicity induced by amikacin. The hydroalcoholic extract of red grape seeds was prepared by maceration method. Five groups of mice (each group consisted of 10 mice) were used. The negative control group received distillated water and the positive control group were administered with amikacin in doses of 250 mg/kg intraperitoneal (ip.).

The treated groups received orally 100, 200 and 400 mg/kg of the red grape seed extract and after one hour amikacin was administered ip. 250 mg/kg. This experiment was carried out for seven days and then, 24 hours after the last administration of crude extract and amikacin, (on 7$^{th}$ day) blood was taken from the jugular vein of mice for Blood Urea Nitrogen (BUN) and Creatinine measurements. Then kidneys were removed for histopathological examinations. Results obtained in this study indicated that group received red grape seed extract in dose of 400 mg/kg showed significant kidney protection and the crude extract was dose dependent as compared with the positive control group. The histopathological studies also have confirmed the results mentioned above.Thus, *Vitis venifera* extract can be a good protective agent against nephrotoxicity induced by amikacin but it requires further toxicological studies.

**Keywords:**

Amikacin, Nephrotoxicity, *Vitis vinefera.*

## Introduction

The glory of grapes was once relegated to enjoying a glass of wine but today *Vitis venifera* of the family of Vitaceae, native of Asia minor and Caspian sea region is becoming known as a source of dietary supplement, exclusive of the health benefits attributed to red wine obtained from red grape seed. Agronomists thought they were doing consumers a favour when they developed seedless grape but as it turns out, seed extract may be the most valuable part of the grape. As the most important economy plant, the chemistry of grape has been extensively studied (1).

Functional ingredients of grape seeds include several flavonoid with a phenolic

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nature such as monomeric flavonols (catechin and epicatechin), dimeric, trimeric and polymeric procyanidins, and phenolic acids (gallic acid and ellagic acid) have been reported to exhibit antioxidant activity *in vivo* and *in vitro* in a number of studies. The antioxidant activity of flavonoids is closely associated with activity against various cancer types, cardiovascular diseases and several dermal disorders (2,3). The preventive or therapeutic medicinal properties of flavonoids of the red grape seed make it desirable for human to consume fruits, vegetables and leaf, bark or root infusions that contain these compounds. Aminoglycoside antibiotics are widely used as antibacterial agents for the treatment of severe aerobic gram-negative infections and act synergistically against certain gram-positive organisms and also for prophylaxis especially against endocarditis gram-negative bacteria. Amikacin may be particularly effective against resistant organisms. It is well established that aminoglycosides generate free oxygen radicals, leading to an increased oxidoreductase production, which in turn increases tissue toxicity. However, aminoglycosides induced nephrotoxicity and ototoxicity by oxygen free radicals are the limiting factors for their clinical use (4,5,6). Most reports showed that aminoglycosides can cause reduction of antioxidant capacity of enzymes. It is well known that antioxidants are beneficial for their role in protecting against cell damage, premature aging and degenerative diseases caused by free radical occurring in human body. Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged (7,8) . In this study, we presented the hydroalcoholic extract of grape seeds with emphasis on its protective effect in nephrotoxicity induced by amikacin.

## Materials and methods

Amikacin sulphate was purchased from Exir pharmaceutical company Iran. BUN and Creatinine kits were obtained from Zist Shimi (Tehran Iran). Albino Swiss male mice weighing 20-25 g were obtained from Razi Research Center, (Hasarak Karaj Iran). Red grapes were purchased from local market in Iran and taxonomically were identified at dept. of agriculture at Shahid Chamran University. The other materials were prepared at our toxicology laboratory. The method is based on the modification of Kalantari et al. (9). From grapes the seeds were removed and dried in shadow according to drying process (10). Then, the dried seeds were grinded to a uniform powder and weighed. The most important and essential part of extraction of plant material is the selection of a proper organic solvent which depends on the part and constituents of the plant. In this study, a mixture of ethanol and water in the ratio of 7:3 was prepared. Then, 500 g of the red grape seed powder were extracted by maceration method for three days. The extracted material was filtered and the filtrated material was concentrated under vacuum evaporator until dryness. Mice were divided in five groups (each group consisted of 10 mice) and were given rodent chow and water *ad libitum* and allowed to acclimatized in an environment of controlled temperature, humidity and 12 hours light /12 hours dark cycle. Doses of 150, 200 and 250 mg/kg of amikacin solution were prepared and administered intraperitoneal (ip.) to mice to get the nephrotoxic dose of amikacin. According to the dose response relationship procedure dose of 250 mg/kg was the most damaging to kidney (dose dependent); therefore this dose was selected as positive control. The negative control was the vehicle of amikacin (distilled water). Then, from the dried crude extract of red grape seed doses of 100, 200, 400mg/kg were administered to

mice orally and after one hour amikacin was adminstered in toxic dose of 250 mg/kg for seven days, respectively. Then, 24 hours after the last administration of crude extract and amikacin (day 7), blood was withdrawn from the jugular vein of the mouse to prepare serum for Creatinine and Blood Urea Nitrogen (BUN) concentration measurement. Kidneys were removed and kept in 10% buffered formalin solution and stained with Hematoxylin and Eosin for histopathological studies. The nephrotoxicity was determined by measuring the Blood Urea Nitrogen (BUN) and Creatinine levels. The obtained data were analyzed by using analysis of variance for significance ($P<0.005$).

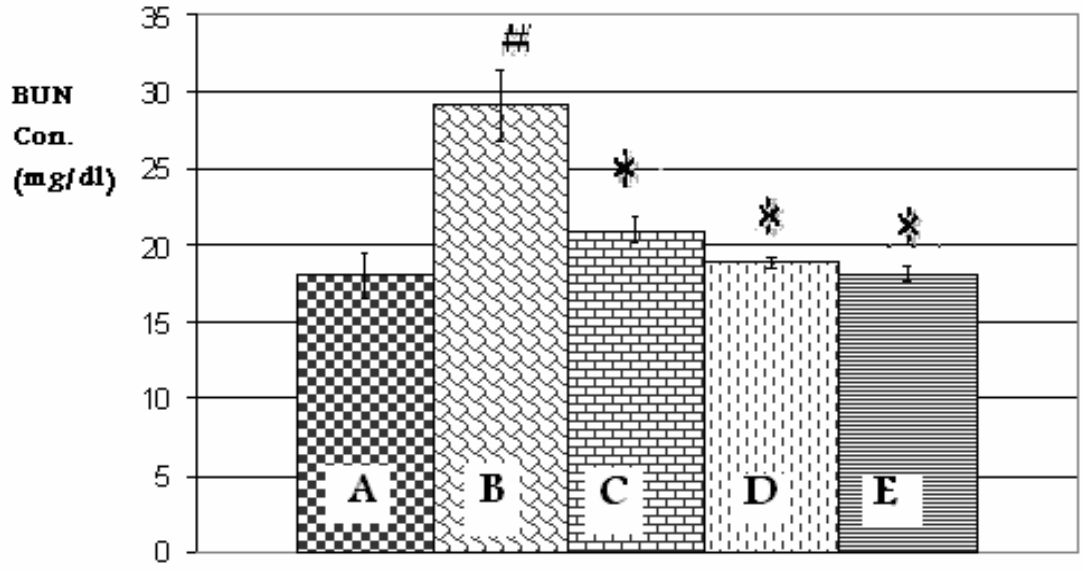
## Results

In Table 1 the dose schedule, its application and biochemical results in different groups are summarized .In this study, the results showed that the means and standard deviation of Blood Urine Nitrogen (BUN) and Creatinine concentration in positive control group after seven days were 29.40 ± 2.3 and 1.680 ± 0.02 mg/dL respectively; thus, the P value ($P<0.001$) was significantly different compared to the treated groups.

Table 1: Dose schedule, its application and biochemical results in different groups

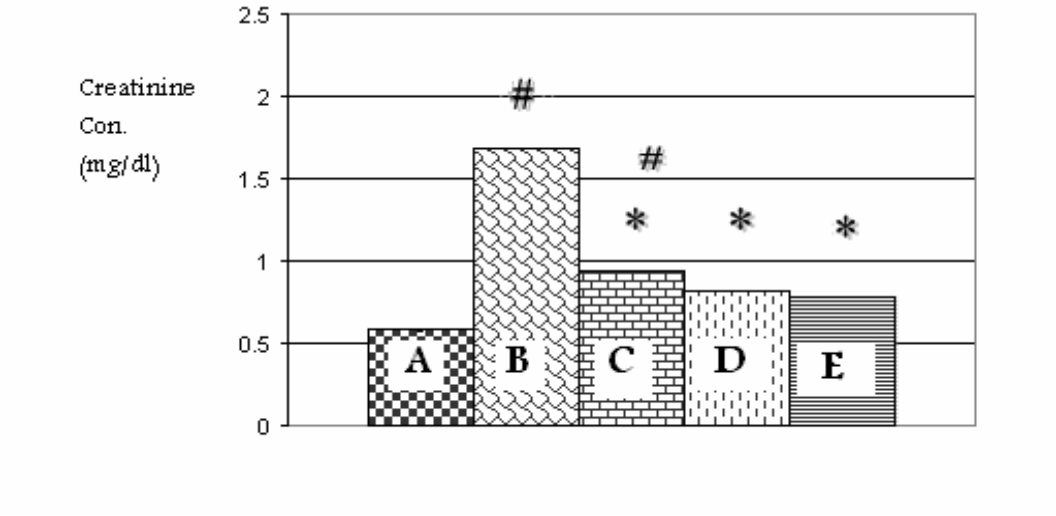
| Group/time | 0 time | After 1 hr. | BUN after 7 days mg/dL ( Mean ± SD) | Creatinine concentration after 7 days mg/dL (Mean ±SD) |
|---|---|---|---|---|
| - ve control | Red grape seed vehicle (d.water) | Amikacin vehicle (d.water) | 18.08±1.40 | 0.580±0.002 |
| + ve control | Red grape seed vehicle(d.water) | 250 mg/kg Amikacin | 29.14±2.3 | 1.680±0.002 |
| Test | 100 mg/kg of grape seed extract | 250 mg/kg Amikacin | 21.0±0.9 | 0.94±0.04 |
| Test | 200 mg/kg of grape seed extract | 250 mg/kg Amikacin | 18.88±0.40 | 0.82±0.02 |
| Test | 400 mg/kg of grape see extract | 250 mg/kg Amikacin | 18.52±0.4 | 0.780±0.001 |

The amounts of Blood Urea Nitrogen (BUN) and Creatinine concentration after seven days in groups received 100 mg/kg of red grape seed extracts were 21.00 ± 0.9 and 0.94 ± 0.04 mg/dL respectively. The amount of Blood Urea Nitrogen (BUN) and Creatinine concentrations in group received 200 mg/kg of red grape seed extracts after seven days were 18.88 ± 0.40 mg/dL for BUN and 0.82±0.02 mg/dL for Creatinine. The amount of Blood Urea Nitrogen (BUN) and Creatinine concentrations in group received 400 mg/kg of red grape seed extract were 18.52± 0.40 mg/dL for Blood Urea Nitrogen (BUN) and 0.780 ± 001 mg/dL for Creatinine. The histopathtological studies and paraclinical findings indicated that most of the kidney tissue in the toxic group had extensive tubular necrosis and there were congestion of blood cells as elucidated in Fig. 1. The significant differences of Blood Urea Nitrogen (BUN) and Creatinine concentrations in different groups are elucidated in Figs. 1 and 2. The histopathological findings are shown in microscopic photographs of Fig. 3.




A) Negative control C) 100 mg/kg E) 400 mg/kg
B) Positive control D) 200 mg/kg

Fig. 1: Blood Urea Nitrogen concentration in different groups after 7 days administration of *vitis vinifera* extract ($^{*}P<0.05$).




A) Negative control C) 100 mg/kg E) 400 mg/kg
B) Positive control D) 200 mg/kg

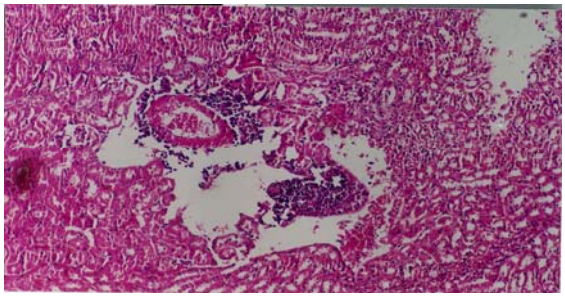
Fig. 2: Creatinine concentration in different groups after 7 days administration of *vitis vinifera* ($^{*}P<0.05$).

## Discussion

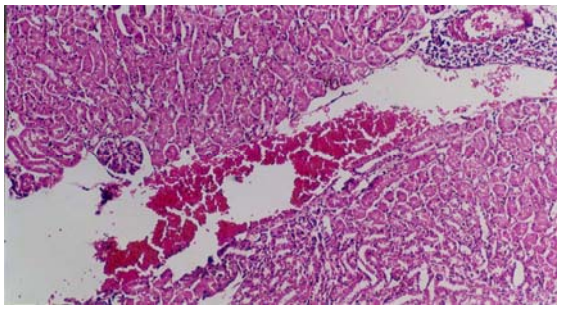
In this study, we have demonstrated a link between the natural herbal products (red grape seed) and the toxicity caused by conventional synthetic medicine aminoglycoside amikacin. Flavonoids of the red grape seed make it desirable for human to consume fruits and vegetable. During the last two decades the importance of the red grape seed due to its flavonoids content, the antioxidant property and use for cardiovascular health has become apparent (11). Most reports have shown that aminoglycosides can cause reduction of antioxidant capacity of enzymes. Therefore in this study red grape seed (*Vitis venifera*) as a natural source of antioxidant was used to protect nephrotoxicity induced by amikacin .The antioxidant activity of flavonoids is closely associated with activity against various degenerative diseases caused by free radical occurring in human body. In this work, the extent of nephrotoxicity and kidney damage was assessed by the level of BUN and Creatinine in the test and control groups and the obtained results were compared with the positive control.

Results obtained in this study indicated that group received red grape seed extract in dose of 400 mg/kg showed significant kidney protection and the crude extract was dose dependent as compared with the positive control group. The histopathological studies also have confirmed the results mentioned above as it is clear from the microscopic photograph in which massive cetribular necrosis, accumulation of inflammatory cells and congestion are lower than positive control group.

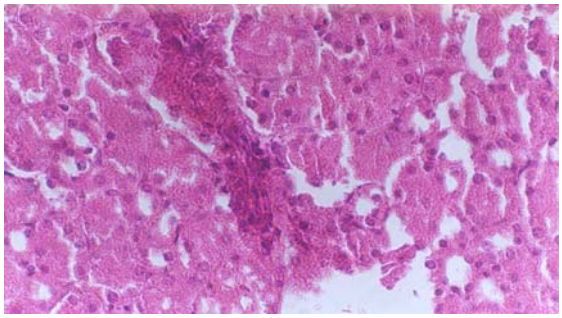
a)



b)



c)



d)

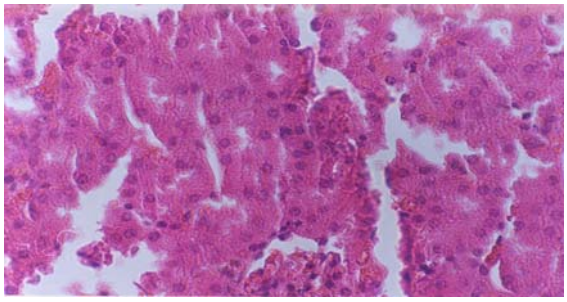


Fig. 3: Microscopic photograph of kidney in different groups after 7 days (H&E 40X); (a)positive control (250 mg/kg amikacin), (b) group reseived 100mg/kg grape seed extract, (c) group reseived 200 mg/kg grape seed extract and (d) group reseived 400 mg/kg grape seed extract.

We supposed that it may inhibit lipid peroxidation by scavenging of glutathione. Previous studies have indicated that flavonoides as antioxidant can protect kidney from toxic effects of free radicals (12,13). The histopathtological studies and paraclinical findings indicated that most of the kidney tissue in the toxic group had extensive tubular necrosis and there were congestion of blood cells as elucidated in microscopic photographs. From this findings it is clear that the pretreatment of mice with red grape seed extract had a good protective effect against amikacin-induced nephrotoxicity and this protection was dose dependent.

From the results mentioned above it is clear that *Vitis venifera* extract can be a good protective agent against nephrotoxicity induced by amikacin, but it requires further toxicological studies.

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