Protective Role of Pomegranate and Sodium bicarbonate on Acute Renal Failure Induced by Gentamicin In Rabbits Model

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ABSTRACT

Acute renal failure ARF is common condition can be categorized as prerenal, intrinsic or postrenal causes in which the glomerular filtration rate decreases GFR over days to weeks. The aim of this study was to test whether the administration of pomegranate and sodium bicarbonate minimize the adverse health effect of acute renal failure. Methods: twenty female rabbits were assessed in to five groups (n = 4). Group 1: control group (C). Group 2: Gentamicin group (G): animals were injected with a single dose of Gentamicin (80 mg/Kg/bw) intramuscular, daily for 10 days to induce renal failure. Group 3: sodium bicarbonate group (G+S), animals were treated as in group 2 plus they received sodium bicarbonate (1 ml/Kg/bw) of 8.4% by gavage daily for 10 days. Group 4: pomegranate group (G+P), animals were treated as in group 2 plus they received pomegranate 500 (mg/Kg/bw) by gavage. Group 5: combination group (G+S+P), animals were treated as in group 2 plus group 3 and group 4 for 10 days. Results: serum creatinine and blood urea levels were decreased in both groups (G+P) and (G+S+P), p < 0.05. However in group (G+S) they increased significantly than the (G) group. Serum electrolytes concentrations and serum (pH) shows different response, they were improved mostly in the (G+S+P) group, and they fluctuated in (G+S) group showing significant decreasing in serum (Cl and Ca), p < 0.05. Although the results for the hematological parameters were also improved in the combination group (G+S+P) but the differences were non significant between all others measured parameters and control group. Conclusions: the administration of sodium bicarbonate in Gentamicin model to induced acute renal failure induction has adverse biological effects. But those effects were minimized by the ameliorative effects of the pomegranate administration which was helpful in speeding recovery.

1. INTRODUCTION

Renal failure disease a case characterized by the impairment of the kidney to filter waste products from the body (Gansevoort et al., 2013). The two main types of kidney diseases, could be reversed with suitable treatment, while chronic kidney disease, is not reversible. In both if these cases, there is usually an underlying cause. Renal failure is diagnosed by a decline in glomerular filtration rate GFR. This is achieved by a decrease in or nonproduction of urine or estimation of renal
parameters creatinine and urea in the blood. Depending on the cause, blood loss in the urine due to the leaked red blood cells known as hematuria and proteinuria the losses of the protein from the blood to the urine may be distinguish. In renal failure, there may be problems with increased fluid in the body which leads to the swelling, academia, increased potassium levels, fall in calcium levels, the levels of phosphate rise up and in the later stages of kidney disease anemia produced. Bone health may also be affected renal bone diseases can cause low bone density and fragility fractures; however, drugs used to treat osteoporosis may make renal-related bone diseases worse, increasing fracture risk rather than reducing it. Long-term kidney disease are associated with an increased the symptoms of the cardiovascular disease (Liao et al., 2012, Amerling et al., 2010).

Acute kidney injury AKI previously known as acute renal failure ARF usually occurs when the blood supply to the kidneys is suddenly interrupted or when the kidneys become overloaded with toxins. Causes of acute kidney disease include sudden accident, trauma, or side effects from surgical operations in which the kidneys may are not supplied with normal blood flow for a long periods of time. Heart-bypass surgery is an example of one such procedure (Chertow et al., 1998). Unlike chronic kidney disease, however, the kidneys can often recover from acute kidney injury, allowing the patient to resume a normal life. People suffering from acute kidney injury require supportive treatment until their kidneys recover function, and they often remain at increased risk of developing future kidney failure (Amerling et al., 2010). Acute renal failure is characterized by a rapid, potentially reversible, decline in renal function including rapid fall in GFR and retention of nitrogenous waste products over a period of hours or days. The mortality rate of patients with ARF has remained 25–70% despite the use of various pharmacologic agents. Therefore, it continues to be a frequent threatening complication following trauma, complex surgical procedures, and in patients hospitalized in intensive care units (Lee et al., 2009).

Pomegranate (*Punica granatum*), an ancient fruit-bearing deciduous shrub, is the predominant member of two species comprising the Punicaceae family (Lansky and Newman, 2007). It is a native of the Himalayas in northern India, but it has been cultivated and naturalized throughout the Middle East, the entire European Mediterranean region, the drier parts of southeast Asia, northern and tropical Africa, and to some extent the United States, specifically California and Arizona (Viuda-Martos et al., 2010). Pomegranate’s fruit is a large berry characterized by the presence of thin membranes in its interior, which allow the suspension of numerous arils, each surrounded by juice-containing sacs. The fruit can be divided into three parts: the seeds and the juice, which represent about 3 and 30% of the fruit weight, respectively, and the peels, which include the inner membranes with distinct chemical compositions and potential medical benefits (Lansky and Newman, 2007). Pomegranate extracts have been used since ancient times to treat several conditions including parasitic and microbial infections, diarrhea, ulcers, aphthae, hemorrhage, and respiratory complications (Naqvi et al., 1991, Cáceres et al., 1987). Modern applications include hormone replacement therapy and oral hygiene as well as the treatment immune suppression and cardiovascular complications (de Nigris et al., 2007). Moreover, other therapeutic properties such as antitumor, anti-inflammatory, antiviral, antibacterial, antidiarrheal, and antiobesity are currently under investigation.
Although pomegranate has been consumed and used as a medicinal food in the Middle East for thousands of years, it has recently gained popularity in the United States (Johanningsmeier and Harris, 2011) leading to clinical studies of pomegranate juice or fruit extracts for efficacy against several diseases. In 2010, 23 clinical trials were registered with the National Institutes of Health to examine effects of pomegranate extracts or juice consumption on many chronic and acute diseases for instance prostate cancers, prostatic hyperplasia, diabetes mellitus, lymphoma, rhinovirus infection, common cold, oxidative stress in diabetic, hemodialysis, atherosclerosis, coronary artery disease, infant brain injury and hemodialysis for kidney disease (Adams et al., 2010). Obesity is associated with a number of chronic diseases such as type II diabetes, cardiovascular disease, chronic kidney disease, Non-alcoholic fatty acid liver disease, and various types of cancers. In this regard, the effects of pomegranate in some of these disorders are also under investigation (Bassaganya-Riera et al., 2010). New research by British scientists (Fortino) 2013 at the Royal London Hospital shows that sodium bicarbonate can dramatically slow the progress of chronic kidney disease (Fortino).

From previous research information the aim of this study was to test whether the administration of pomegranate which is a natural occurrence plants riches with many antioxidative substance which proved by many researches and sodium bicarbonate minimize the adverse health effect of acute renal failure.

2. MATERIALS AND METHODS

2.1. Animals

The experimental animals, young local female rabbits, were bred from the animal house of the biology Department, University of Salahaddin. The animals were maintained under standard conditions of temperature (20 ± 5°C), with a regular 12-h light/12-h dark cycle. They were allowed free access to standard food and water ad libitum.

2.2. Experimental design

Young local female rabbits (average body weight range, between 1300 to 110 grams) were randomly assigned to five groups:

Group 1: Control group (C): -The animals of this group received standard rat chow and tap water ad libitum and were injected with single dose of (1ml) Normal saline intra- muscular (n=4) for 10 days.

Group 2: Gentamicin treated group (G), Animals were injected with single dose of Gentamicin intra- muscular (80 mg/Kg / b.w) (n=4) daily for ten days. The drug was purchased from The Arab Pharmaceutical Manufacturing Co. LTD, Sult- Jordan

Group 3: Gentamicin plus Sodium bicarbonate treated group (G + S), Animals treated as in group 2 plus they received a single dose of (1 ml / Kg / b.w) of (8.4% w/v) Sodium bicarbonate (by oral route (gavage) (n=4) daily for ten days.

Group 4: Gentamicin plus pomegranate treated group (G+P), Animals treated as in group 2 plus they received a single dose of (2 ml / Kg / b.w) of (100% pure Pomegranate 500 mg powder capsules) dissolved in (D.W) were purchased from Club natural dietary supplements, USA, by oral route (gavage) (n=4) daily for ten days.

Group 5: Gentamicin plus sodium bicarbonate plus pomegranate treated group (G+S+P), Animals treated as in group 2 plus received a single dose of (1 ml / Kg / b.w) of (8.4% w/v) Sodium bicarbonate and a single dose of (2 ml / Kg / b.w) of (100% pure Pomegranate 500 mg powder capsules) dissolved in (D.W)
as a combination by (gavage) (n=4) daily for ten days.

2.3. Gentamicin-induced acute renal failure (ARF)

Bledsoe et al. reported the development of acute renal failure (ARF) in rats by administration of gentamicin at a dose of 80 mg/kg, sc for ten days with the assessment of renal failure on the eleventh day (Bledsoe et al., 2008). But in our study we injected the animals with gentamicin at a dose of 80 mg/kg, im for ten days with the assessment of renal failure on the eleventh day.

2.4. Collection of blood samples

At the end of each experiment the rabbits were anesthetized with (16 mg ketamine, 0.8 mg xylazine) given subcutaneously. Blood samples were taken by cardiac puncture into chilled tubes with or without ethylene diamine tetraacetic acid (EDTA-K₃) (4.5 mM) as anticoagulant for hematological parameters determinations and other blood specimen without EDTA were allow to stand at room temperature for 15-30 and centrifuged at 2,500 revolutions per minute (rpm) for 5-10 minutes; then serum were stored at (–80°C) (Sony Ultra low, Japan).

2.5. Biochemical determination

Serum Creatinine and blood nitrogen Urea were estimated after 10 days of the experiments using Random Access biochemistry analyzer (KINZA-240 TX-), BIOLABO diagnostics - France.

2.6. Serum electrolytes, calcium and serum pH determination

Serum electrolytes and serum calcium determination were determined by blood gas analyzer (GASTAT-602i) Techno-Medica, Japan.

2.7. Measurement of hematological parameters

Complete blood count were performed after 10 days of the experiments using hematology analyzer – three differential (celltac α) MEK 6400K NIHON KOKDEN (Japan).

2.8. Statistical analysis

All data are expressed as means ± standard error means (M± SEM) and statistical analysis was carried out using statistically available software (SPSS Version 11.5). Data analysis was made using one-way analysis of variable (ANOVA). The comparison between groups were done depending on $P<0.05$ that considered as statistical significant.

3. RESULTS AND DISCUSSION

Table (1) and figures (1) showing the obtained results for serum creatinine and blood urea. Gentamicin treated group (G) and Gentamicin plus Sodium bicarbonate treated group (G+S) shows the highest significant increasing in the serum creatinine levels (8.54 ± 0.26, 11.23 ± 1.73) compared with the control group (C), (0.88 ± 0.03) at the level ($P<0.05$). While the other two treated groups, Gentamicin plus pomegranate (G+P) and Gentamicin plus sodium bicarbonate plus pomegranate (G+S+P) in both of them the serum creatinine levels shows no significant differences when compared with the control group (C).

At the same time the results which obtained from the estimation of blood urea levels as shown in the table (1) and figure (2), the
blood urea levels in Gentamicin treated group (G), Gentamicin plus Sodium bicarbonate treated group (G+S) and Gentamicin plus pomegranate treated group (G+P), (212.00 ± 23.20, 237.00 ± 15.38 and 122.38 ± 38.70) consecutively where increased significantly at the level (p < 0.05) when compared with the result of the control group (C), (31.75 ± 3.56).

In the other hand the blood urea level in Gentamicin plus sodium bicarbonate plus pomegranate treated group (G+S+P) shows no significant differences in the blood urea concentration (62.85 ± 14.03) when compared with the result of the control group (C) (31.75 ± 3.56).

In our study the results which obtained from the estimations of serum electrolytes (Na, K, Cl), serum (Ca) and serum (pH) listed in the table (2) and figures (3,4,5,6 and 7).

The results shows that the concentrations of the serum sodium (Na), serum chloride (Cl) and serum calcium (Ca) only in the Gentamicin plus Sodium bicarbonate treated group (G+S) all of them where significantly decreased (118.50 ± 5.86) for (Na), (85.75 ± 4.13) for (Cl) and (1.26 ± 0.16) for (Ca) when compared with the control group (C) at the level of (p<0.05) (133.25 ± 1.10) for (Na), (106.75 ± 0.47) for (Cl) and (1.59 ± 0.07) for the (Ca) consecutively. while the others treated groups doesn't shows any significant differences for the serum (Na, Cl and serum Ca) when compared with the control group.

The results for serum potassium (K) concentrations in the Gentamicin treated group (G) (8.97 ± 0.24), Gentamicin plus Sodium bicarbonate treated group (G+S) (8.92 ± 0.33), Gentamicin plus pomegranate treated group (G+P) (6.92 ± 0.56) and in the Gentamicin plus sodium bicarbonate plus pomegranate treated group (G+S+P) (6.22 ± 0.43) all these results where increased significantly at the level of (p<0.05) when compared with the control group (C) (3.57 ± 0.13).

But the results which obtained from the estimation of serum (pH) showed that the (pH) values in the Gentamicin plus Sodium bicarbonate treated group (G+S) (7.72 ± 0.05) and Gentamicin plus pomegranate treated group (G+P) (8.07 ± 0.10) where increased significantly at the level of (p<0.05) when compared with the control group (C) (7.50 ± 0.02). Meanwhile serum (pH) in the Gentamicin treated group (G) (7.72 ± 0.05) and in the Gentamicin plus sodium bicarbonate plus pomegranate treated group (G+S+P) (7.76 ± 0.07) doesn't shows any significant differences compared with control group.

The data which obtained from the previous study for the some of the hematological parameters where listed in the table (3) and (8,9 and 10).

The data which obtained from the total white blood cells count (WBCs) and total platelets count (PLTs) doesn't shows any significant differences between all treated groups and the control group.

The total red blood cells count (RBCs), where decreased significantly in the Gentamicin treated group (G) (3.98 ± 0.45), Gentamicin plus Sodium bicarbonate treated group (G+S) (4.67 ± 0.18) and in Gentamicin plus pomegranate treated group (G+P) (5.01 ± 0.31) at the level of (p<0.05) when compared with the control group (C) (6.08 ± 0.17), and no significant differences obtained between Gentamicin plus sodium bicarbonate plus pomegranate treated group (G+S+P) (5.75 ± 0.26) and control group (C). Although the same results were obtained for the hemoglobin concentration (HGB) and the hematocrit percentage (HCT) in the above mentioned groups (G), (G+S) and (G+P) in such a manner the values of the (HGB) where decreased significantly (8.8000 ± 0.91924), (10.0 ± 0.43), (10.52 ± 0.55).
consecutively and the values of the (HCT) were also decreased significantly (27.92 ± 3.30), (30.80 ± 1.3459), (31.87 ± 1.78) consecutively at the level of (p< 0.05) compared with the control group (C) (12.92 ± 0.50) for the (HGB) and (39.20 ± 1.70) for the (HCT).

Once again there were no significant differences obtained for the results in the values of the (HGB) (12.42 ± 0.66) and (HCT) (39.0 ± 2.50) between Gentamicin plus sodium bicarbonate plus pomegranate treated group (G+S+P) and control group (C).

In the current study the high dose of Gentamicin, like other aminoglycosides, causes nephrotoxicity by inhibiting protein synthesis in renal cells. The most finding mechanism which obtained from researches confirming the causes due to the necrosis of cells in the proximal tubule, which resulting in acute tubular necrosis and may be lead to acute renal failure (SUNDIN et al., 2001). The adverse effects represented with increased serum levels of creatinine and blood urea significantly. From the obtained data in our study in the Gentamicin treated group. The interaction of pomegranate minimize the adverse effects of Gentamicin on serum levels of blood Urea and creatinine in process white rat acute renal failure (Chory Pudji. 2013). In other hand, the pretreatment with the hydroalcoholic extract of pomegranate flowers in glycerol-induced renal dysfunction model with a concentration between (125 and 250 mg/kg) significantly attenuated renal dysfunction. The juice of pomegranate has positive protective effect in ethylene glycol-induced nephrolithiasis in rats (Tugcu et al., 2008). To explore the possible mechanism of renoprotective effect of pomegranate, a Peroxisome proliferator-activated receptor gamma PPAR-γ antagonist (Rinwa et al., 2010).

The action of PPAR-γ regulating glucose to fatty acid storage and metabolism and has also been played a role in regulating renal functions. Many other reports suggested that activation of PPAR-γ has protection action in different models of renal failure like chronic renal and allograft damage with renal ischemia-reperfusion injury (Kiss et al., 2010, Doi et al., 2007). Even though a number of researches suggested that methanolic extract of pomegranate flowers activates PPAR-γ receptors (Huang et al., 2005a). Furthermore, the beneficial effects of pomegranate was studied in sensitivity Zucker diabetic fatty rats they showed improvement in insulin receptors activities (Huang et al., 2005a). Inhibiting postprandial hyperglycemia and in diminishing cardiac fibrosis have been shown to be mediated through activation of PPAR-γ receptors (Huang et al., 2005b).

New studies have reported that endothelial PPAR-γ regulates vascular NO nitric oxide production. Activation of PPAR-γ induced increased NO synthesis has been linked to Protein kinase B (AKT/PKB) and endothelial nitric oxide synthase (eNOS), Akt-eNOS signaling pathway (Liang et al., 2009). Furthermore, NO has also been shown to activate PPAR-γ and increase its binding to PPRE (PPAR response element) (Ptasinska et al., 2007, Borniquel et al., 2010). Before it may be proposed that these two signaling pathways are interlinked and constitute an integrated mechanism to produce protective effects including renoprotection in Gentamicin-induced acute renal failure in rats.

In the oxidative stress rats model induced by toxic level of lead The natural Pomegranate juice has protective effect against the damage in liver and kidney cells (Aviram et al., 2002). Pomegranate juice has also shown significant anti-hypertensive, anti-atherosclerotic, antioxidant, and anti-inflammatory effects in human subjects and Rabbit models. Pomegranate juice minimize the oxidative destruction of nitric oxide and enhance its
antioxidant and anti-inflammatory functions (Ignarro et al., 2006).

The exact mechanism to the renal protective effect of sodium bicarbonate in humans has not been established till now. Studies in experimental animal models of chronic kidney disease suggested that alkali therapy decreasing tubulointerstitial inflammation and may slow the progression to kidney failure (Nath et al., 1985, Wesson and Simoni, 2010).

In the current study the administration of sodium bicarbonate to the acute renal failure. Animal models had high risks because both serum creatinine and blood urea levels were elevated significantly more than obtained in the Gentamicin treated Group this reveals that the probability of adverse effects interactions between the both chemicals in such a manner they speed up the production of acute renal failure. This unexpected results could be related to the serum bicarbonate level and sodium bicarbonate administration, which has not been clarified yet.

There are some evidence from other study showed that the patients with a serum bicarbonate level lower than 25.5 mEq/l had a high risk of chronic kidney disease progression. The patients with serum bicarbonate levels from 26 to 40 mEq/l had a lower risk of death or kidney failure than patients with serum bicarbonate levels from 11 to 25 mEq/l (Menon et al., 2010).

However, the target serum bicarbonate level in the upper limit has not been established. A cohort study showed that patients with serum bicarbonate levels higher than 32 mEq/l showed a higher risk of death than patients with serum bicarbonate levels from 23 to 32 mEq/l (Navaneethan et al., 2011). The effect of a high serum bicarbonate level on mortality may be is related to complications such as hypokalemia, hypocalcemia, or hypomagnesemia, with resultant cardiac arrhythmias (Kovesdy et al., 2007).

Unfortunately in this study we did not measure the serum bicarbonate levels and this is one of the limitations that we faced made the obtained results required farther investigation. Meanwhile the combination of pomegranate and sodium bicarbonate administration showed synergistic effects in reducing the elevated serum creatinine and blood urea in ARF model, these levels are near the levels in control group. This action may be attributed to the free-radical scavenging properties of the Pomegranate juice, that help in reducing the levels of of both serum creatinine and urea. The antioxidant effects of Pomegranate juice was attributed to its constituents like antioxidant trace elements and flavonoids therefore Pomegranate juice has been suggested to be able to decrease lipid peroxidation (Rosenblat and Aviram, 2006). Also the antioxidant activity of Pomegranate juice is due to phenolic compounds and enzymes (glucose oxidase, catalase and peroxidase) (Seeram et al., 2004, Gil et al., 2000).

The results of this study showed many fluctuation which obtained from the estimation of the serum electrolyte showed many fluctuations in the serum electrolytes in the sodium bicarbonate treated in such a manner the levels of (Na, Cl and Ca ) all of them decreasing significantly, while the level of (K) increased more than what we obtained in the Gentamicin treated group. this finding confirming that the reaction between sodium bicarbonate and Gentamicin promoting the development of sever acute renal failure. Hyperkalemia is a common result of clinical problem that is most often a result of impaired urinary potassium excretion due to acute or chronic kidney disease (CKD) and/or disorders or drugs that inhibit the renin-angiotensin-aldosterone axis. Therapy for hyperkalemia due to potassium retention is ultimately aimed potassium loss (Hine et al., 2017, Kamel and Wei, 2003).
At least two circulating factors are required for the regulation of renal K+ secretion by the cortical collecting duct (CCD), the main site of renal potassium excretion. Aldosterone is considered as a second factor that increases the number of conducting K+ channels (Biner et al., 2002). Aldosterone as key regulator of renal potassium homeostasis binds to the nuclear mineralocorticoid receptor within the distal tubule and the principal cells in the CCD. It activates the basolateral Na+/K+-ATPase, thereby increasing Na+ and water reabsorption into the blood and secretion of K+ into the urine. Aldosterone also upregulates the amiloride sensitive sodium channels (ENaCs) in the apical membrane of CCD and stimulates H+ secretion by intercalated CCD cells, thereby influencing acid/base balance (Palmer and Frindt, 2000).

Effective excretion of potassium is dependent on aldosterone and sufficient distal delivery of sodium and water within the nephron. Hyperkalemia may occur when one of these mechanisms is impaired because of renal failure, renal hypoperfusion (e.g., volume depletion, congestive heart failure), or hypoaldosteronism. Hypoaldosteronism may be the cause of hyperkalemia in patients who do not have advanced renal failure or hypoperfusion (DeFronzo, 1980).

Hyponatremia and hyperkalemia are the two major electrolyte abnormalities of primary adrenal insufficiency. Hyponatremia is mediated by increased release of antidiuretic hormone (ADH) which results in water retention and a reduction in the plasma sodium concentration (Oelkers, 1989, Ishikawa and Schrier, 1982). The hypersecretion of ADH induced by aldosterone deficiency is caused by renal salt wasting with resultant volume depletion. Hypovolemia increases ADH levels by reducing the osmotic threshold for ADH release from the hypothalamus and by increasing the magnitude of ADH release for a given change in plasma osmolality (Robertson and Aycinena, 1982, Mount, 2009). The low calcium levels hypocalcemia may be due to the effect of a high serum bicarbonate level as we mentioned previously (Kovesdy et al., 2007). The serum pH become more alkaline which is a normal response due to the administration in the sodium bicarbonate.

Pomegranate contain many alkaloids It was indicated that alkaloids were present at the rate of 0.35 to 0.60% in the body rinds, and over 3% in the roots; but none was found in the fruit rinds (Mohammad and Kashani, 2012, Cáceres et al., 1987). It was also indicated that pseudopelletierine, pelletierine, isopelletierine, methylpelletierine 1-pelletierine, dl-pelletierine and methyl isopelletierines were found in composition of the root, body and branch rinds of *P. granatum* (Chidambara Murthy et al., 2002, Dean et al., 1971). Those alkaloids may be responsible to increasing the serum pH in this study in pomegranate treated group. But in the combination (G+S+P ) group the acidic compounds in the pomegranate seems to be reacts with the sodium bicarbonate and minimize the acidity action for the pomegranate extracts.

Depending on the hematological parameters in acute renal failure group with Gentamicin anemia was expected because renal diseases are associated with a variety of haemopoietic changes. The cause belongs to the failure of renal erythropoietin secretion also chronic blood loss, hemolysis and bone marrow suppression by retained uremic factors (Hasan, 2015). In renal disease the impaired erythropoietin secretion, increased destruction of red blood cells and fall in red blood cell count, reduces Hb and hematocrit HCT concentration in patients with mild to moderate renal insufficiency (Emmanuel et al., 2004).

Iron content of pomegranate plays crucial roles in haemopoiesis, control of infection and cell mediated immunity (Bhaskaram, 2001,
Because of their high content of polyphenols. Cranberry and pomegranate extracts rich in polyphenols have now been shown to have potent iron-chelating capabilities, by suppressing iron-catalyzed oxidant reactions (Guo et al., 2007, Mladěnka et al., 2011). All the hematology parameters return back near to the control group obtained values because of the bioactivity of the pomegranate, because in recent publication mentioned that iron deficiency anemia will be prevented by adequate dietary intake fruits such as pomegranate (Swaminathan, 2008).

4. CONCLUSIONS

From the results of current study, concluded that the administration of sodium bicarbonate in Gentamicin model to induced acute renal failure has adverse biological effects. but those effects were minimized by the ameliorative effects of the pomegranate administration which is helpful in speeding recovery due to the high antioxidant activities, bioactive chemical compositions, and high rich minerals, pomegranate extract could be useful for reducing the nephrotoxic effects of Gentamicin. And as a recommendation further investigations required to find out the toxic effects of the interaction between sodium bicarbonate and Gentamicin.

Table 1. shows the results for the administration of pomegranate, sodium bicarbonate and their combination on some kidney function test parameters in gentamicin induced renal failure.

| Treated Groups | Parameters obtained results ( Mean ± S.E ) |
|----------------|-------------------------------------------|
|                | Creatinine mg /dl | Urea mg /dl |
| Control        | 0.88 ± 0.03<sup>a</sup>              | 31.75 ± 3.56<sup>a</sup> |
| G              | 8.54 ± 0.26<sup>b</sup>               | 212.00 ± 23.20<sup>c</sup> |
| G+S            | 11.23 ± 1.73<sup>c</sup>              | 237.00 ± 15.38<sup>c</sup> |
| G+P            | 2.37 ± 0.51<sup>a</sup>               | 122.38 ± 38.70<sup>b</sup> |
| G+S+P          | 1.18 ± 0.08<sup>a</sup>               | 62.85 ± 14.03<sup>ab</sup> |

Data presented as mean ± S.E
The same letters mean no statistical differences
The different letters mean statistical differences. The level of significance was set at p < 0.05.

Table 2. Shows the results for the administration of pomegranate, sodium bicarbonate and their combination on serum electrolyte, serum calcium and serum (pH) in gentamicin induced renal failure.

| Treated Groups | Parameters obtained results (Mean ± S.E.) |
|----------------|------------------------------------------|
|                | (Na) mmol/L  | (K) mmol/L  | (Cl) mmol/L | (Ca) mmol/L | Serum (pH) |
| Control        | 133.25 ± 1.10 b | 3.57 ± 0.13 a | 106.75 ± 0.47 b | 1.59 ± 0.07 b | 7.50 ± 0.02 a |
| G              | 138.75 ± 3.01 b | 8.97 ± 0.24 c | 111.00 ± 2.44 b | 1.78 ± 0.01 b | 7.72 ± 0.05 ab |
| G+S            | 118.50 ± 5.86 a | 8.92 ± 0.33 c | 85.75 ± 4.13 a | 1.26 ± 0.16 a | 7.84 ± 0.14 bc |
| G+P            | 135.75 ± 1.25 b | 6.92 ± 0.56 b | 106.00 ± 3.58 b | 1.81 ± 0.09 b | 8.07 ± 0.10 c |
| G+S+P          | 136.50 ± 0.86 b | 6.22 ± 0.43 b | 105.75 ± 0.47 b | 1.78 ± 0.03 b | 7.76 ± 0.07 ab |

Data presented as mean ± S.E

The same letters mean no statistical differences
The different letters mean statistical differences. The level of significance was set at p < 0.05.
Table 3. Shows the results for the administration of pomegranate, sodium bicarbonate and their combination on some hematological parameters in gentamicin induced renal failure.

| Treated Groups | Parameters obtained results (Mean ± S.E) |
|----------------|------------------------------------------|
| Control        | WBCs $\times 10^3$ 6.08 ± 0.17$^a$      |
| G              | RBCs $\times 10^6$ 12.92 ± 0.50$^c$      |
| G+S            | HGB - gm/dL 39.20 ± 1.70$^b$             |
| G+P            | HCT % 5.80 ± 1.50$^a$                    |
| G+S+P          | PLT $\times 10^3$ 8.59 ± 3.07$^a$        |

Data presented as mean ± S.E

The same letters mean no statistical differences

The different letters mean statistical differences. The level of significance was set at $p < 0.05$. 

Figure 1. shows the results for the administration of pomegranate, sodium bicarbonate and their combination on serum creatinine levels.

Figure 2. shows the results for the administration of pomegranate, sodium bicarbonate and their combination on blood urea levels.
Figure 3. Shows the results for the administration of pomegranate, sodium bicarbonate and their combination on serum Sodium levels.

Figure 4. Shows the results for the administration of pomegranate, sodium bicarbonate and their combination on serum Potassium levels.

Figure 5. Shows the results for the administration of pomegranate, sodium bicarbonate and their combination on serum Chloride levels.

Figure 6. Shows the results for the administration of pomegranate, sodium bicarbonate and their combination on serum Calcium levels.
Figure 7. shows the results for the administration of pomegranate, sodium bicarbonate and their combination on serum pH levels.

Figure 8. shows the results for the administration of pomegranate, sodium bicarbonate and their combination on total Red blood cells count.

Figure 9. shows the results for the administration of pomegranate, sodium bicarbonate and their combination on blood hemoglobin levels.

Figure 10. shows the results for the administration of pomegranate, sodium bicarbonate and their combination on blood hematocrit levels.
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