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NEPHROCURATIVE AND NEPHROPROTECTIVE EFFECTS OF NIGELLA SATIVA OIL IN COMBINATION WITH VITAMIN-C IN GENTAMICIN-INDUCED RENAL TOXICITY

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ABSTRACT

In our present research, we investigated the nephrocurative and nephroprotective effects nigella sativa oil [NSO] and vitamin-C [vit-C] both alone and in combination form on gentamicin-induced nephrotoxicity, and compared the effects of NSO and vit-C with combined effects of NSO and vit-C. Biochemical analysis of serum and histopathological examination of kidney were done for all groups. Nephrotoxicity was confirmed by comparing the serum levels of creatinine, urea and antioxidant activity in gentamicin-treated group with that of normal saline treated groups. NSO and vit-C both alone and in combined forms considerably ameliorated the toxic effects of gentamicin on kidneys. NSO and vit-C both alone and in combined forms showed the ability to avert the elevated serum creatinine and urea levels, and augmented the antioxidant activity of the body along with improved histopathological changes in the tubular parts of kidneys. On comparing with NSO in combination with vit-C with NSO and/or vit-C, combined form of NSO and vit-C showed a considerably significant [$P<0.01$] improvement in biochemical parameters and morphological changes of kidneys in gentamicin-induced nephrotoxicity.

Keywords: Gentamicin; Nephrotoxicity; Nigella sativa; vitamin-C; combination therapy.

INTRODUCTION

Although, aminoglycosdies are being used for the treatment of several gram-negative bacterial infections now a day, but their large quantity of consumption may cause the nephrotoxicity. Among them, gentamicin is an effective bactericidal agent against various gram-negative bactericidal infections [1]. Although, gentamicin may cause nephrotoxicity in wide range but still it is the drug of choice for the treatment of various gram-negative bacterial infections [2,3]. Usually, gentamicin-induced nephrotoxicity arises when gentamicin accumulates in the proximal convoluted tubules [4]. This accumulation of gentamicin involves the production of free radicals in these tubules and diminishes the antioxidant defense mechanism of the body which may cause the acute tubular necrosis and glumerular congestion [5,6]. These phenomenons ultimately decrease the glumerular filtration rate along with renal dysfunctions. Gentamicin-induced nephrotoxicity may also causes proteinuria with increased levels of serum creatinine and urea. These altered levels of serum creatinine and urea lead to acute kidney damage [7].

Even though, many pharmacological interventions have been studied to stop the gentamicin-induced nephrotoxicity [8], and various agents might also be used in concomitant with gentamicin in order to ameliorate its deterioted effects on kidney [9,10] but no specific therapy has been investigated to protect the deleterious effects of gentamicin on kidneys.

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Vitamin-C is an antioxidant supplement that exhibits its powerful scavenging effects against activated oxygen species and various free radicals by neutralizing ROS and decreasing oxidative damage to cell membranes [7]. That is why; vitamin-C has been used as protective antioxidant agent against numerous kinds of deteriorations caused by oxidative stress.

Although, various studies have been conducted to investigate the nephrocurative and nehoroprotective effects of nigella sativa [11,12] and vitamin-C [7,13] separately, but to the best of our knowledge, there is no online data available in which NSO together with vit-C have been studied to investigate their nephrocurative and nephroprotective effects in drug-induced nephrotoxicity. Here in our present study, we have determined the combined effects of NSO and vitamin-C in gentamicin-induced nephrotoxicity. These findings of our present study may lead to further potentiate the research to evaluate the nephroprotective effects of this combination therapy.

Materials and methods

Materials

Nigella sativa oil [NSO] and ampoules of Gentamicin sulphate were purchased from care pharmacy Lahore, Pakistan. Vitamin-C [vit-C], $Fe[NH_4]_2SO_4$, uric acid, sodium benzoate, sodium hydroxide, EDTA, H_2O_2, acetic acid, sodium phosphate and thiobarbituric acid were at least of analytical grade and purchased from Merck, Germany. The Randox Standard Creatinine Kit and Urea Kit were obtained from Pak Chemicals, Lahore, Pakistan.

Animals

The present research was conducted on 50 male albino rabbits with average body weight 2.5± 0.25 Kg which were purchased from local market. These rabbits were placed in the animal house of University College of pharmacy in temperature controlled room with 12 hrs light/dark cycle. The standard food was given to them with water *ad libatum*. The rabbits were familiarized by placing them in stainless steel cages one week before the start of treatment. The approval for animal experiments was obtained from institutional ethic committee.

Experimental design

Rabbits were divided into two groups i.e. group A [for nephrocurative study], and group B [for nephroprotective study] as briefly described in scheme-I. 80mg/kg/day GS, 200mg/kg/day vit-C and 2ml/kg/day NSO were administered. Blood samples were collected from marginal vein of rabbits with sterilized disposable needles before the start of treatment, and then at day 10^{th}, 14^{th}, 18^{th}, 22^{nd} and 26^{th} during the treatment period. After coagulation, the blood samples were centrifuged at 4000 RPM for 15 min to obtain the serum for further biochemical analysis.

Biochemical and histopathological analysis

To investigate the nephrocurative and nephroprotective effects of NSO and vit-C, various biochemical parameters such as serum Creatinine level [SCL], blood urea nitrogen [BUN] level, and serum antioxidant activity [SAA] were measured. By following the colorimetric method, SCL and BUN were measured using Randox standard Creatinine and urea kits. SAA was assessed following spectrophotometric method as previously described [14]. For histopathological evaluations, rabbits were sacrificed and their kidney tissues were removed, washed and then fixed with 10% formalin. These tissues were further processed using various concentrations of ethanol and then embedded in paraffin wax for 6 hrs. Thin slices of these embedded tissues were obtained with microtoming and fixed on glass slides with gelatin. These slides were then placed in oven at 58 °C for 12hrs. Finally, these slides were stained with hematoxylin and eosin, and observed for histopathological alterations in kidneys.

Statistical analysis

The results were represented as mean ± SD with 5 replicates. These results were interpreted following one way ANOVA using SPSS version 13.0. The level of significant difference between the results was adjusted with P values at 0.05

Results

To investigate the nephrocurative and nephroprotective effects of vit-C and NSO, we measured body weights of rabbits at day 0, 10^{th} and 26^{th} of the study period and the rate of mortality during whole studies period. In both nephrocurative and nephroprotective studies, the body weights of all groups were non-significantly [$p>0.05$] reduced at the day 10 of study period but however, the group A-5 and B-5 considerably regained their body weights till the last day of treatment [Table 1]. Similarly, the groups A-1 and B-1 also showed a pronounced decrease in their body weights as compared to the body weights of treated groups with either, vit-C, NSO or both [table 1]. The mortality rate of rabbits in both studies was only 2%. In nephrocurative study, no rabbit died while, in nephroprotective study, one rabbit died on day 14^{th} and other one on day 23^{rd} of the study period.

Biochemical analysis

We measured various biochemical parameters [SCL, BUN level and SAA] in order to determine the effects of NSO, vit-C, and combination of NSO with vit-C. From the results of nephrocurative study, it had been clearly found that the SCL [Table 3] and BUN levels [Table 4] were significantly [$p<0.05$] augmented as

compared to the control group [A-1] at the day 10 of study. However, these values in group A-3, A-4 and A-5 were significantly [$p<0.05$] going to decline on day 18^{th} and 22^{nd} when compared with the values of these parameters in GS treated control group [A-2]. While, at the last day of this study period, the values of these parameters were nearly reached to the base line values of same group measured before the start of treatment whereas, the values of SCL and BUN in GS treated control group [A-2] were persistently high as compared to the values of group A-1. Similarly, we also compared the nephrocurative effects of NSO with vit-C [combination therapy] with either vit-C and/or NSO to determine that either this combination therapy showed synergistic effects or not. From the results, it had been observed that the SCL [Table 3] and BUN levels [Table 4] in group A-5 treated with NSO in combination with vit-C were more profoundly and significantly [$P<0.01$] declined as compared to those treated with either vit-C [group A-3] and/or NSO [group A-4] alone.

It has been found that the serum antioxidant activity is an important parameter to determine the defending ability of the body against various mechanisms that may harmful to the body [15]. The antioxidant activity of body is decreased considerably due to the increased production of free radicals in various diseases like Nephrotoxicity. In our present study, we also measured the antioxidant activity of the rabbits against GS induced Nephrotoxicity, and treated with either NSO or vit-C alone or NSO in combination with vit-C. The antioxidant activity of GS treated rabbits were significantly [$P<0.05$] diminished when compared with control group [A-1] at day 10 [Table 5] but when these rabbits were treated with either NSO, vit-C or combination of NSO with vit-C, then the antioxidant activity were considerably increased [Table 5]. We also compared the antioxidant activity of NSO in combination with vit-C with either NSO and/or Vit-C alone and found the NSO with vit-C showed highest antioxidant activity [$P<0.01$] as compared to NSO and/or vit-C alone.

We also investigated the nephroprotective effects of NSO and vit-C by the concomitant administration of NSO and vit-C with GS for 26 days. In this study, the levels of serum Creatinine [Table 6] and urea [Table 7] in GS-treated control group [B-2] were significantly [$P<0.05$] much higher than that of control group [B-1] at day 10. Whilst, serum levels of Creatinine and urea in vit-C [B-3], NSO [B-4] and/or combination of NSO with vit-C [B-5] treated groups were considerably lesser than that of GS-treated group [B-2] at same day. The serum levels of Creatinine and urea in these treated groups [B-3, B-4, and B-5] were persistently lesser than that in the GS-treated group [B-2] till the last day [26^{th}] of nephroprotective study while these levels in GS-treated group [B-2] were almost higher as compared to control group [B-1] till the last day of this study period. We this study, we also compared the nephroprotective effects of NSO in combination with vit-C with either NSO and/or vit-C. From the results [Table 6 and 7], it was observed that NSO in combination with vit-C had ability to decrease the serum levels of Creatinine and urea induced by gentamicine sulphate as compared to the NSO or vit-C alone. Instead of measuring the serum Creatinine and urea levels, we also measured the antioxidant activity of rabbits in this study. From the results [Table 8], the antioxidant activity in GS-treated croup [B-2] was significantly [$P<0.05$] decreased as compared to the control group [B-1] at day 10 and remained persistently decreased till the end of treatment, while in case of NSO and vit-C treated groups [B-3, B-4 and B-5], there was not a considerably significantly [$P<0.05$] difference with that of control group [B-1]. We also measured that NSO with vit-C had better ability to restore the antioxidant activity [$P<0.01$] rabbits treated with gentamicine as compared to NSO and/or vit-C alone [Table 8].

Histopathological analysis

Histopathological examination of kidneys treated with gentamicin confirmed the occurrence of gentamicin-induced renal damage in kidneys [Figure 2 and 3]. After staining the tubular section of kidneys, histopathological observation illustrated remarkable tubular necrosis and cellular infiltrations along with sloughing of cells in tubular lumen of the kidneys of gentamicin treated groups in both nephrocurative and nephroprotective studies. Nevertheless, the administration of NSO and vit-C in combined form ameliorated the nephrotoxic effects of gentamicin on kidneys as clearly verified by the normalization of histopathological variations in A-5 and B-5 groups.

Although, NSO and vit-C alone showed better nephroprotective and nephrocurative effects but we performed the histopathological analysis of only NSO in combination with vit-C groups only in both studies. From histopathological results of group A-2 treated with GS and group A-5 treated with NSO and vit-C [Figure 1], it was observed that an increased amount of cellular infiltrations and along with extensive renal hemorrhage was occurred in GS-treated group [A-2]. Meanwhile, Sloughing of cells in tubular lumen was also occurred in this group. But these histopathological conditions were not observed in group A-5 treated with NSO and vit-C [Figure 1] which demonstrated an incredible fortification of renal tissues. Such type histopathological results [Figure 2] were also observed during nephroprotective study in GS-treated group [B-2] and NSO with vit-C treated group [B-5].

Table 1. Body weights [Kg] of rabbits in nephrocurative effects of vit-C and NSO. Data are represented as mean±SD [n=5].

Day	Nephrocurative study				
	A-1	**A-2**	**A-3**	**A-4**	**A-5**
0	2.35±0.11	2.20±0.16	2.30±0.19	2.35±0.15	2.35±0.11
10	2.25±0.13	2.10±0.19	2.25±0.45	2.15±0.14	2.20±0.14
26	2.25±0.08	1.95±0.19	2.15±0.25	2.25±0.11	2.30±0.15

Table 2. Body weights [Kg] of rabbits in nephroprotective effects of vit-C and NSO. Data are represented as mean±SD [n=5].

Day	Nephroprotective study				
	B-1	**B-2**	**B-3**	**B-4**	**B-5**
0	2.30±0.14	2.26±0.20	2.22±0.14	2.30±0.07	2.32±0.13
10	2.20±0.15	2.22±0.14	2.20±0.07	2.32±0.10	2.22±0.14
26	2.24±0.11	2.24±0.18	2.14±0.11	2.24±0.11	2.22±0.11

Table 3. Serum Creatinine levels [mg/dl] of rabbits during nephrocurative study. Data are represented as mean±SD [n=5].

Groups	Days					
	1	**10**	**14**	**18**	**22**	**26**
A-1	1.80±0.57	1.88±1.09	2.29±1.43	2.02±0.68	1.74±0.39	2.05±0.90
A-2	2.00±0.87	5.76±2.20$^{\Phi}$	4.84±1.26	4.82±1.04	4.68±0.66	4.57±0.35$^{\Phi}$
A-3	1.87±0.73	5.88±1.71$^{\Phi}$	3.20±0.72	3.16±0.83*	2.99±0.55*	2.81±0.57*
A-4	2.09±1.09	5.82±2.18$^{\Phi}$	3.18±0.62	3.04±0.37*	2.68±0.60*	2.26±0.44*
A-5	1.82±0.58	6.09±1.49$^{\Phi}$	3.11±0.43	2.74±0.45*^{o}	2.14±0.80*^{o}	1.81±0.26*^{o}

$^{\Phi}$ P< 0.05, when compared with values of Control [Group A-1]
* P< 0.05, when compared with values of GS Control [Group A-2]
^{o} P<0.01, when compared with values of vit-C [A-3] and/or NSO [A-4] treated groups.

Table 4. Serum Urea levels [mmol/L] of rabbits during nephrocurative study. Data are represented as mean±SD [n=5].

Groups	Days					
	1	**10**	**14**	**18**	**22**	**26**
A-1	6.59±1.13	6.70±1.54	6.86±1.44	6.88±1.22	6.95±1.45	6.75±1.58
A-2	7.16±1.04	16.24±1.50$^{\Phi}$	17.06±1.45	17.21±1.70	16.41±1.65	16.42±3.39
A-3	7.10±1.39	15.87±0.95$^{\Phi}$	15.30±2.85	13.55±1.65*	11.40±1.80*	11.55±2.09*
A-4	5.75±2.30	14.10±3.80$^{\Phi}$	13.05±1.05	11.10±1.156*	10.45±1.55*	9.80±2.11*
A-5	6.05±1.60	14.85±1.55$^{\Phi}$	12.40±1.90	10.30±1.25*^{o}	8.95±1.15*^{o}	8.50±0.65*^{o}

$^{\Phi}$ P < 0.05, when compared with values of Control [Group A-1]
* P < 0.05, when compared with values of GS Control [Group A-2]
^{o} P<0.01, when compared with values of vit-C [A-3] and NSO [A-4] treated groups.

Table 5. Serum antioxidant activity of rabbits in nephrocurative study. Data are represented as mean±SD [n=5].

Days	Nephrocurative group				
	A-1	**A-2**	**A-3**	**A-4**	**A-5**
1	2.35±0.22	2.30±0.16	2.15±0.25	2.19±0.32	2.35±0.31
10	1.75±0.15	0.85±0.25$^{\Phi}$	0.88±0.33$^{\Phi}$	0.55±0.15$^{\Phi}$	1.10±0.19$^{\Phi}$
26	1.95±0.55	0.94±0.53$^{\Phi}$	1.75±0.44	1.72±0.45	2.04±0.22 ^{o}

$^{\Phi}$ P < 0.05, when compared with values of Control [Group A-1]
^{o}P<0.01, when compared with values of vit-C [A-3] and NSO [A-4] treated groups.

Table 6. Serum Creatinine levels [mg/dl] of rabbits during nephroprotective study. Data are represented as mean±SD [n=5].

Groups	Days					
	1	10	14	18	22	26
B-1	2.11±0.65	2.30±0.65	2.32±0.75	1.93±0.84	2.16±0.96	1.99±0.45
B-2	2.29±0.54	6.08±1.17$^{\Phi}$	6.05±1.50	6.55±1.25	6.70±1.45	6.40±0.85$^{\Phi}$
B-3	2.45±0.56	2.88±1.30*	2.80±1.45*	2.20±0.65*	2.35±0.85*	2.15±0.68*
B-4	2.72±0.75	2.65±0.95*	2.49±0.89*	1.95±0.76*	2.25±0.82*	2.09±0.42*
B-5	2.38±0.75	2.06±0.45*	2.17±0.85*	1.96±0.76*	2.06±0.67*	1.17±0.38* ^{o}

Φ P < 0.05, when compared with values of Control [Group B-1]
* P < 0.05, when compared with values of GS Control [Group B-2]
^{o}P<0.01, when compared with values of vit-C [B-3] and NSO [B-4] treated groups.

Table 7. Serum Urea levels [mmol/L] of rabbits during nephroprotective study. Data are represented as mean±SD [n=5].

Groups	Days					
	1	10	14	18	22	26
B-1	5.74±2.50	5.42±1.92	6.17±1.90	5.92±2.18	6.12±2.45	6.24±2.25
B-2	4.57±2.15	18.85±2.71$^{\Phi}$	19.44±3.76	19.95±5.61	19.15±1.56	19.35±2.56
B-3	6.10±2.18	6.45±1.67*	6.22±1.43*	5.75±1.15*	5.45±0.96*	4.38±0.85*
B-4	4.04±1.35	5.94±1.08*	5.35±1.96*	4.97±0.77*	4.40±0.81*	3.59±1.08*
B-5	5.58±2.41	5.85±1.25*	5.52±0.92*	5.10±0.43*	4.46±0.66*	3.44±0.87* ^{o}

Φ P < 0.05, when compared with values of Control [Group B-1]
* P < 0.05, when compared with values of GS Control [Group B-2]
^{o}P<0.01, when compared with values of vit-C [B-3] and NSO [B-4] treated groups.

Table 8. Serum antioxidant activity of rabbits in nephroprotective study. Data are represented as mean±SD [n=5].

Days	Nephroprotective group				
	B-1	B-2	B-3	B-4	B-5
1	2.32±1.08	2.10±0.92	1.96±1.16	1.82±0.78	2.28±1.16
10	1.98±0.87	0.72±0.71	1.88±0.45	2.12±0.93	2.39±0.78
26	2.15±0.56	0.41±0.69$^{\Phi}$	2.13±0.69	2.05±0.46	2.30±0.65 ^{o}

Φ P < 0.05, when compared with values of Control [Group B-1]
^{o}P<0.01, when compared with values of vit-C [B-3] and NSO [B-4] treated groups.

Figure 1. Nephrocurative effects of NSO in combination vit-C. A; sloughing of tubular lumen and cellular infiltration in damaged glumerulus and tubular necrosis in GS treated group [A-2]. B; improvement of glumerulus and renal tubules after treated with NSO in combination with vit-C.

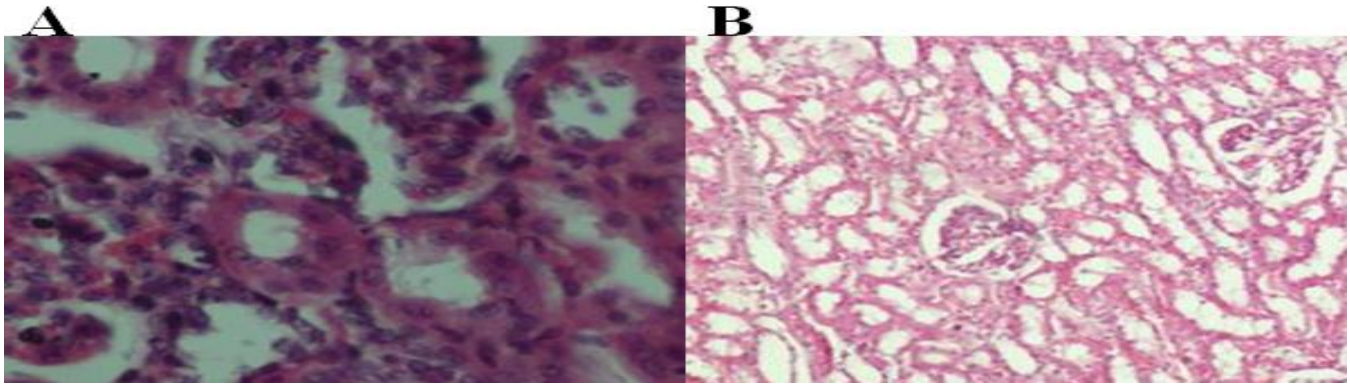
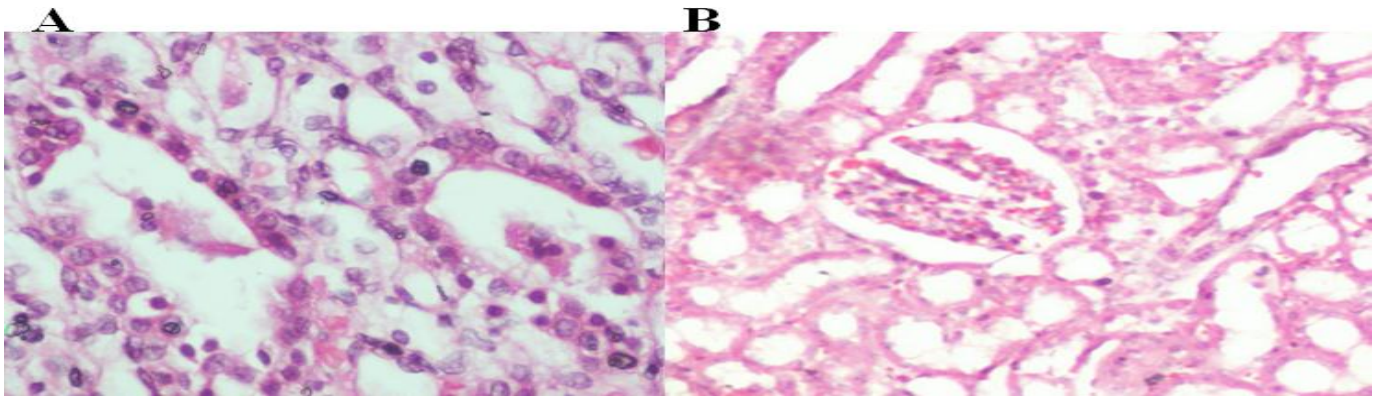
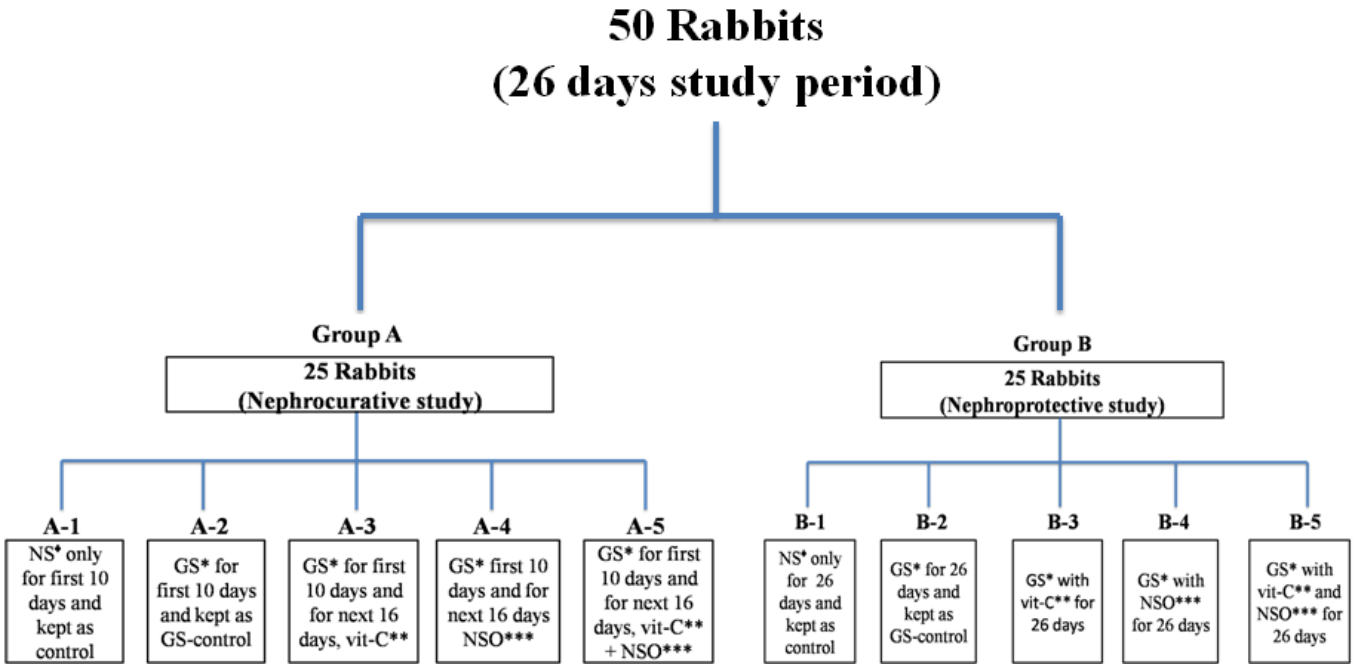


Figure 2. Nephroprotective effects of NSO in combination with vit-C. A; tubular necrotic and damaged renal tubules of GS treated group [B-2]. B; protective effects of NSO in combination with vit-C.



Scheme 1. Schematic representation of experimental plan for nephrocurative and nephroprotective effects of NSO and vit-C. Φ normal saline; * gentamicine sulphate [80mg/kg/day]; ** vitamin-C [200mg/kg/day]; *** nigella sativa oil [2ml/kg/day]



Discussion

The main purpose of our present research was designed to find out the nephrocurative and nephroprotective effects of NSO and vit-C alone and as well as in combination form. Biochemical and histopathological results show a considerably significant nephrocurative and nephroprotective effects of these two antioxidants either alone or in combination form on gentamicin-induced nephrotoxicity.

The main findings or our present research are that NSO and vit-C exerted both nephrocurative and as well as nehoroprotective effects against gentamicin-induced morphological damages in kidneys. Gentamicin induced nephrotoxicity is considered to be the conditioned by increased production of free radicals and ROS along with initiation of necrosis and apoptosis in tubular parts of kidneys due to the accumulation of gentamicin in these parts [5,6].

The serum levels of creatinine and urea in GS-Treated groups [A-2 and B-2] were considerably high [$P<0.05$] along with the decreased serum antioxidant activity as compared to control groups [A-1 and B-1].

Similarly, the levels of serum creatinine and blood urea in nephrocurative and nehoroprotective groups were also statistically significantly decreased [P<0.05] as compared to their GS-treated control groups respectively. Our results are highly supported by already published data [7]. The main reason for altered amounts of serum creatinine and urea is due to the kidney damage in which the retention of nitrogen products and electrolytes occur in the form of hyponatremia and hypokalemia [7].

Here in our present findings, we found that the combined effects of NSO and vit-C were also statistically significantly [P<0.01] high as compared to the individual effects of NSO and/or vit-C alone. The reason for these augmented effects of NSO in combination with vit-C may be due to synergistic effects that resulted in the decreased levels of serum creatinine and blood urea with increased antioxidant activity of body was due to the cytoprotective effects NSO and vit-C by the inhibition of ROS.

From the results of histopathological examinations, it was clearly found that there was a significant tubular spoilage in the kidneys of gentamicin treated groups in both nephrocurative and nephroprotective studies and these results confirmed that kidneys are very sensitive to gentamicin-induced nephrotoxicity. When histopathological observations were assessed then it was found that NSO and vit-C protected kidneys from gentamicin-induced nephrotoxicity. Our observations are highly supported by the other studies which suggest that NSO and vit-C exert their protective effects against gentamicin-induced nephrotoxicity [7,11].

From the results of our present findings, it has been clearly found that NSO [2ml/kg/day] in combination with vit-C [200mg/kg/day] considerably ameliorates the deleterious effects of gentamicin on histological and morphological damages of kidneys. Although, our results are correlated with some other similar studies [7,16,17] but the nephrocurative and nehoroprotective effects of NSO and vit-C in combined form are considerably high as compared to that with NSO and/or vit-C alone. As it has been found that gentamicin is believed to induce oxidative stress during nephrotoxicity that initiates various biochemical reactions along with cell damage by the generation of free radicals. Here, we have observed that when NSO concomitantly administered with vit-C curatively and protectively repairs the morphological and histopathological changes to tubular parts of kidneys by potentiating the existing antioxidant defense mechanism at the levels of tubular parts of kidneys along with the reduction of serum creatinine and urea levels. Our biochemical and histopathological findings foretell that NSO in combination with vit-C exhibit excellent nephrocurative and nephroprotective effects in gentamicin-induced nephrotoxicity by augmenting the antioxidant defense mechanism of the body along with the reduction of serum creatinine and urea levels as compared to NSO and/or vit-C alone.

CONCLUSION

To the best of our knowledge, this is the first study, in which the nephrocurative and nephroprotective effects of NSO and vit-C was studies together in combination form for the prevention of gentamicin-induced nephrotoxicity. The combination form of NSO and vit-C provides more encouraging results by lowering the serum creatinine and urea levels, and augmenting the antioxidant activity of the body more quickly as compared to NSO and/or vit-C alone.

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