Research Article

Evaluation of Antioxidant, Nephroprotective and Immunomodulatory Activity of Vitamins C and E -Sodium Selenite in Mice Intoxicated with Sodium Nitrate

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Abstract | A major worldwide problem that impairs our bodies' physiological systems is nitrate toxicity. Evaluation of antioxidant, nephroprotective and immunomodulatory activity of vitamins C and E -sodium selenite in mice intoxicated with sodium nitrate. Thirty healthy adult male mice of 25-30gm body weight divided randomly into six groups (5 mice/each) administered the following daily for 2 weeks, respectively: Vitamin E -sodium selenite 0.5 ml/ LDW(T1); vitamin C (0.5 gm/L (T2); NaNO3 0.5gm/L (T3); NaNO3 (70 mg/kg) and vitamin E -sodium selenite 0.5 ml/L (T4); NaNO3 [70 mg/kg and vitamin C 0.5gm/ L], (T5); Control receive distilled water (T6). Urea level increased obviously among T3 followed by T2 and seriously decreased in T4. Creatinine level increased obviously among T3 and T2. Statistical difference was reported in urea level among T2 and T6(P Value=.002), T3 and T6(*P value*=.000). Statistical difference in urea level among T1 and T3(*P value* <0.0001), T2 and T3(P Value=0.003089), T4 and T3(*P value*<.0001), T5 and T3(*P value* <0.0001). Considerable contrariety among T2 and T6, T1 and T3; T4 and T3, T5 and T3 were noticed in creatinine levels. Histopathological changes of mouse kidney for T1 and T2 revealed a congestion of renal blood vessels, perivascular cuffing, and infiltration of inflammatory cells in the dilated blood vessels. Congestion of glomerular capillaries. While (T3) revealed sever congestion of renal blood vessels, perivascular cuffing, and infiltration of inflammatory cells in the dilated blood vessels. Extensive distension of Bowman's space with sever congestion of glomerular capillaries and extensive cellular swelling of proximal and distal convoluted tubules with vacuolar degeneration surrounding proximal and distal convoluted tubules and tubule necrosis with dilation and vacuolar degeneration of proximal and distal convoluted tubules. Nitro blue tetrazolium activity for monocytes and macrophages do not correlate with blood urea and creatinine concentrations. Vitamin E -sodium selenite and vitamin C have important role in enhancement for renal functional activity and clearance of urea and creatinine; They considered potent antioxidant that ameliorates the histopathological effects of sodium nitrate on renal tissue. Concentrations of blood urea and creatinine have no effect on potent scavenging activity of monocytes and macrophages.

Keywords | Vitamin E, Vitamin C, Sodium nitrate, Antioxidant, Nephroprotective, Immunomodulatory, Histopathology, Kidney, Mice, Biochemical indices

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INTRODUCTION

Sodium nitrate is used in several health and industrial related activities such as meats and seafood preservation and as color-fixative (Filipec and Janči, 2023). In addition, sodium nitrate has been utilized as an intestinal relaxant, bronchial dilator, vasodilator, and antidote for cyanide poisoning in both human and veterinary medicine (Kamel *et al*., 2023).

Due to the extensive use of nitrates as agricultural fertilizers, which can reach humans and animals through many routes, the problem of nitrate has received a lot of attention in recent years (Mande *et al*., 2012; El-Sawi *et al*., 2024). An essential component of the nitrogen cycle in the environment, nitrate is a naturally occurring type of nitrogen. Fertilizers, decomposing vegetation, manure, and other organic leftovers combine to make it. Air, soil, water, and vegetables all contain nitrate, which the human body naturally produces (Rajini *et al*., 2022).

In reality, it has been observed that some crops, including maize, guinea corn, carrots, potatoes, sunflower, pumpkins, and cabbage, can collect significant amounts of nitrates even when fertilizer is applied at the standard rate of 150 kg/ha (Awodi *et al*., 2005; Yarube, 2011). $NaNO_3$ is also utilized as a food additive, mostly as a preservative and antibacterial (Shee *et al*., 2010). Vegetables and drinking water may contain higher quantities of nitrate than in the past due to the increased use of synthetic nitrogen fertilizers and livestock manure in intensive agriculture (Lin *et al*., 2023).

Vitamin E -sodium selenite is a naturalistic constituent of cellular bilayer unite membrane pay vital role in preservation of the integrity of cell membrane (Acharya *et al*., 2004). Under cellular and molecular levels, vitamin E -sodium selenite act by one of the following mechanisms: The first mechanism depends on antioxidant activity of the vitamin which protect cellular membrane and proteins from oxidative stress of ROS (Abdelhalim *et al*., 2020; Mandil *et al*., 2023), another mechanism depends on the ability of this vitamin for interaction and regulation of specific enzymes that have direct effect on cellular membranes as well as lipid (Zingg *et al*., 2013).

Vitamin C consisting of two compounds have the ability to be changed in form: L- ascorbic acid (potent reducing agent) and L dehydroascorbic acid (potent oxidizing agent). Vitamin C is an extremely important co-factor contributes in numerous biochemical activities by donation or reduction for electrons. Vitamin C is found in lemon, fruits and vegetables (Doseděl *et al*., 2021; Dresen *et al*., 2023).

The novelty of current study based on the use of vitamins E and C which are popularly used as a protectant from sodium nitrate. The present experimental study aims to determine nephroprotective and immunomodulatory activity of vitamins C and E -sodium selenite in experimental sodium nitrate intoxication of mice. Effect of urea and creatinine concentration on scavenging activity of monocytes and macrophages in vitamin E-sodium selenite treated group.

MATERIALS AND METHODS

Experimental design

A total of thirty adult male mice of gross weight 25-30gm were kept in a good vented cage, standard feeds and drinking water in 20-25°C, with half day light and allowed to adapt with environment for 7 days before experiment which extended for fourteen days.

Acute toxicity testing for sodium nitrate by using a single non-lethal dose procedure was applied for 14 days according to the following plan (Parasuraman, 2011).

Mice were randomly divided into 6 categories(5/each): T1 administered vitamin E -sodium selenite (0.5 ml/L), T2: vitamin C (0.5 gm/L); T3: sodium nitrate 0.5gm/L; T4: Vitamin E -sodium selenite (0.5 ml/L) + sodium nitrate (70 mg/kg); T5: vitamin C 0.5gm/ L - + sodium nitrate (70 mg/kg) and T6: distal water for 14 days .

The given dose for vitamins include, (0.5ml/1L DW) for Vitamin E -sodium selenite; (0.5gm/1L DW) for Vitamin C. Sodium nitrate prepared by dissolving 0.5 gm on 1L DW and given via gavage needle in a dose (70mg/kg) (Hameed *et al*., 2020).

Observation of signs in exposed mice

Classical sings for nitrate administration were observed such as slight weakness and dyspnea, cyanotic mucous membranes.

Mortality ratio index

No mortality was reported in all groups.

Collection of samples

At the end of the day 14th each mouse was euthanized by cervical dislocation procedure for blood collection into falcon tube (without anticoagulant) and centrifuged (1500 rpm, 10 min) Serum was separated and stored at -20 °C until analyzed (Al-Ezzy, 2016; Al-Ezzy *et al*., 2016; Hameed *et al*., 2020).

Postmortem examination

Upon gross examination for internal organs including kidneys, no gross pathological changes were observed.

Detection of blood urea

Urea was detected by Enzymatic colorimetric method (Berthelot modified method) according to biomegreb-Tunis (Hammed, 2015).

Detection of serum creatinine

Creatinine was detected by Kinetic test without deproteinization method according to biomegreb-Tunis (Hammed, 2015).

Nitro blue tetrazolium reduction test

All animals inoculated intraperitoneally with hanks balanced salt solution. The injected solution containing peritoneal monocytes/macrophage cells withdrawn via sterile syringe gage 25. Then the withdrawn cells was added to nitro blue tetrazolium solution in test tube and incubated for 25 minute at 37°C. Further processing was done according to (Hameed *et al*., 2020).

Histopathological study

Both Kidneys were removed form euthanized mice and preserved in Buffered formalin 10%. Paraffin blocks were prepared with two days and sections were obtain after cutting with rotary microtome in four micrometers (Jameel *et al*., 2014; Humadi *et al*., 2021; Sultan *et al*., 2023).

Data analysis

Data were expressed as (mean±SD) (Al-Ezzy, 2016; Al-Ezzy *et al*., 2020). ANOVA test of Vassar Stats online program was used for analysis (Al-Ezzy, 2015; Hameed and Al-Ezzy, 2019). SPSS used for correlation between variables with significant level (P<0.05) (Al-Ezzy, 2016). The least significant differences (LSD) was used to identify significant differences (Al-Ezzy, 2015, 2016).

Table 1: Descriptive statistics of urea (mg/dl) according to experimental groups.

Groups	Urea (mg/dl)		
	Min.	Max.	Mean± SD
Vitamin E -sodium selenite	30	38	33.75 ± 3.24037
Vitamin C	38	51	42.75 ± 5.31171
sodium nitrate	45	50	48.00 ± 2.00000
vitamin E+ sodium nitrate	20	25	22.50 ± 1.92725
vitamin C+ sodium nitrate	26	28	27.13± 0.83452
Control	25	27	26.00 ± 0.75593

RESULTS AND DISCUSSION

Table 1 and Figure 1, revealed urea level increased obviously among T3 (48.00 ± 2.00000 mg/dl) and T2 (42.75 ± 5.31171 mg/dl) and seriously decreased in T4, (22.50 ± 1.92725 mg/dl). Table 2 and Figure 1, revealed creatinine level increased obviously among T3 (0.8500± 0.09258mg/dl) followed by T2 (0.8000± 0.07559 mg/dl). Table 3, revealed statistical difference in urea level among T2 and T6 (P value=.002), T3 and T6 (P value=0.000), T1 and T3(P value<0.0001), T2 and T3(P value= 0.003089), T4 and T3(P value <0.0001), T5 and T3 (P value<0.0001). As indicated in Table 4, Statistical difference in creatinine among T2 and T6 (P value= 0.031), T1 and T3(P value<0.0001), T4 and T3(P value<0.0001), T5 and T3(P value<0.0001) were described.

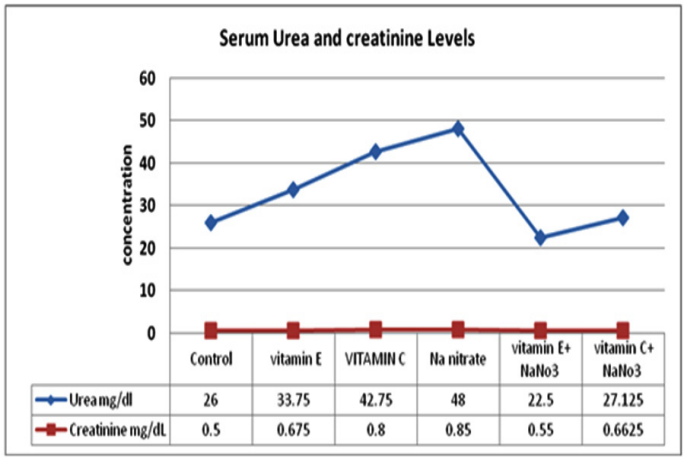

Figure 1: Levels of serum urea and creatinine (mg/dl) according to experimental groups.

Table 2: Descriptive statistics of serum creatinine (mg/dl) according to experimental groups.

Groups	Creatinine (mg/dl)		
	Min.	Max.	Mean± SD
Vitamin E -sodium selenite	0.60	0.70	0.6750± 0.04629
Vitamin C	0.70	0.90	0.8000± 0.07559
sodium nitrate	0.70	0.90	0.8500± 0.09258
vitamin E+ sodium nitrate	0.50	0.60	0.5500± 0.05345
vitamin C+ sodium nitrate	0.60	0.70	0.6625± 0.05175
Control	0.40	0.60	0.5000± 0.07559

As illustrated in Figure 2, A: Normal control mice show renal corpuscle consists of glomerulus and a two-layered glomerular Bowman's capsule that encloses glomerulus; the proximal convoluted tubules and distal convoluted tubules. B: Histopathological Changes of mouse kidney for sodium nitrate treated group: In the cortex, there is a sever congestion of renal blood vessels, perivascular cuffing, and infiltration of inflammatory cells in the dilated blood vessels. Extensive distension of Bowman's space with sever congestion of glomerular capillaries and extensive cellular swelling of proximal and distal convoluted tubules with vacuolar degeneration surrounding proximal and distal convoluted tubules and tubule necrosis with dilation and vacuolar degeneration of proximal and distal convoluted tubules.

Table 3: Differences in serum urea (mg/dl) among experimental groups.

Groups	Parameter	ANOVA compared with control group		ANOVA compared with sodium nitrate group	
	Urea(mg/dl)	F	P value	F	P value
Vitamin E -sodium selenite	Between Groups	2.604	.168	153.23	<.0001
Vitamin C	Between Groups	28.359	.002	11.66	0.003089
sodium nitrate	Between Groups	67.500	.000		
vitamin E+ Sodium nitrate	Between Groups	.100	.907	813.5	<.0001
vitamin C+ Sodium nitrate	Between Groups	.208	.819	884.08	<.0001

Table 4: Differences in serum creatinine (mg/dl) among experimental groups.

Groups	Parameter	ANOVA compared with control group		ANOVA compared with Sodium nitrate group	
	Creatinine (mg/dl)	F	P value	F	P value
Vitamin E -sodium selenite	Between Groups	1.250	.363	24.77	<.0001
Vitamin C	Between Groups	7.500	.031	1.69	0.210002
sodium nitrate	Between Groups	1.250	.363		
vitamin E+ sodium nitrate	Between Groups	2.500	.177	65.33	<.0001
vitamin C+ sodium nitrate	Between Groups	.179	.842	27	<.0001

Table 5: Correlation between blood urea concentration and nitro blue tetrazolium activity.

Blood urea concentration according to group(mg /dl)		Nitro blue tetrazolium activity according to treated group				
		vitamin E-sodium selenite treated group	Vitamin C treated group	Sodium nitrate treated group	vitamin E-sodium selenite + Sodium nitrate treated group	vitamin C+ Sodium nitrate treated group
Control	R	-0.143	0.028	-0.016	-0.133	0.012
	P value	0.452	0.882	0.933	0.482	0.951
Vitamin E -sodium selenite	R	-0.031	0.126	-0.044	-0.048	0.094
	P value	0.870	0.508	0.818	0.799	0.622
Vitamin C treated group	R	-0.094	0.069	-0.027	-0.096	0.046
	P value	0.620	0.718	0.885	0.613	0.809
Sodium nitrate treated group	R	-0.142	-0.002	-0.006	-0.128	-0.011
	P value	0.453	0.991	0.976	0.500	0.952
Vitamin E -sodium selenite +Sodium nitrate treated group	R	-0.101	-0.144	0.044	-0.068	-0.117
	P value	0.595	0.447	0.817	0.721	0.537
Vitamin C + Sodium nitrate treated group	R	0.210	0.002	0.196	0.278	0.095
	P value	0.265	0.993	0.300	0.137	0.616

Section C revealed histopathological changes of mouse kidney for (vitamin E -sodium selenite received group): In the cortex, there is a congestion of renal blood vessels, perivascular cuffing, and infiltration of inflammatory cells in the dilated blood vessels. Distension of Bowman's space. Congestion of glomerular capillaries, proximal and distal convoluted tubules showed cellular swelling. While in section D: Histopathological Changes of mouse kidney for (vitamin C treated group): In the cortex, there is a congestion of renal blood vessels, perivascular cuffing, and infiltration of inflammatory cells in the dilated blood vessels. Congestion of glomerular capillaries, proximal and distal convoluted tubules showed cellular swelling. Section E revealed histopathological changes of mouse kidney for sodium nitrate and vitamin E treated group: In the cortex, there is a sever congestion of renal blood vessels, perivascular cuffing, and infiltration of inflammatory cells in the dilated blood vessels. Extensive distension of Bowman's space and sever congestion of glomerular capillaries, extensive cellular swelling of proximal and distal convoluted tubules. Section F revealed histopathological changes of mouse kidney for sodium nitrate and vitamin c treated group: In the cortex, there is a sever congestion of renal blood vessels, perivascular cuffing, and infiltration of inflammatory cells in the dilated blood vessels. Extensive distension of Bowman's space and sever congestion of glomerular capillaries, extensive cellular swelling of proximal and distal convoluted tubules

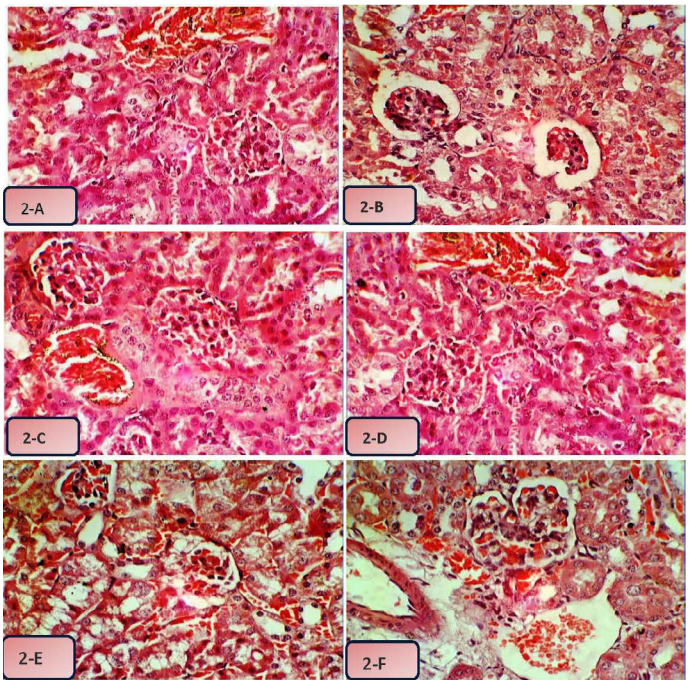

Figure 2: A: Normal control mice show renal corpuscle consists of glomerulus and a two-layered glomerular Bowman's capsule that encloses glomerulus; the proximal convoluted tubules and distal convoluted tubules. B: Histopathological Changes of mouse kidney for sodium nitrate treated group: In the cortex, there is a sever congestion of renal blood vessels, perivascular cuffing, and infiltration of inflammatory cells in the dilated blood vessels. Extensive distension of Bowman's space with sever congestion of glomerular capillaries and extensive cellular swelling of proximal and distal convoluted tubules with vacuolar degeneration surrounding proximal and distal convoluted tubules and tubule necrosis with dilation and vacuolar degeneration of proximal and distal convoluted tubules. C: Histopathological Changes of mouse kidney for (Vitamin E -sodium selenite received group): In the cortex, there is a congestion of renal blood vessels, perivascular cuffing, and infiltration of inflammatory cells in the dilated blood vessels. Distension of Bowman's space. Congestion of glomerular capillaries, proximal and distal convoluted tubules showed cellular swelling. D: Histopathological Changes of mouse kidney for (Vitamin C treated group): In the cortex, there is a congestion of renal blood vessels, perivascular cuffing, and infiltration of inflammatory cells in the dilated blood vessels. Congestion of glomerular capillaries, proximal and distal convoluted tubules showed cellular swelling. E: Histopathological Changes of mouse kidney for sodium nitrate and vitamin E treated group: In the cortex, there is a sever congestion of renal blood vessels, perivascular cuffing, and infiltration of inflammatory cells in the dilated blood vessels. Extensive distension of Bowman's space with atrophic changes and sever congestion of glomerular capillaries, extensive cellular swelling of proximal and distal convoluted tubules. F: Histopathological Changes of mouse kidney for sodium nitrate and vitamin C treated group: In the cortex, there is a sever congestion of renal blood vessels, perivascular cuffing, and infiltration of inflammatory cells in the dilated blood vessels. Distension of Bowman's space and sever congestion of glomerular capillaries, extensive cellular swelling of proximal and distal convoluted tubules with cellular debris.

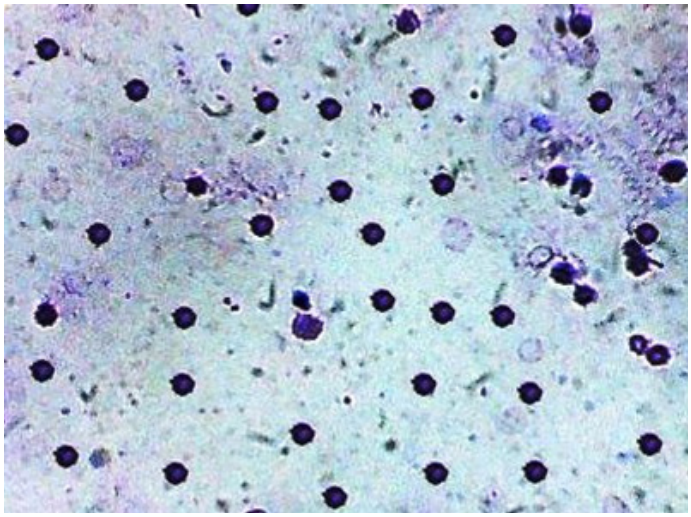

Figure 3: Positive deep blue color indicates intracytoplasmic formazan formation by peritoneal monocytes-macrophages. Negative cells appear as pale shadow with intact cellular membrane.

Figure 3, reveals the activity of nitro blue tetrazolium of peritoneal monocytes-macrophages. They have positive intracytoplasmic formazan in deep blue color. Negative formazan forming cells appear as pale shadow with intact cellular membrane. Table 5, reveals the absence of correlation between blood urea concentration and Nitro blue tetrazolium activity in all groups. Table 6, reveals the absence of Correlation between blood creatinine concentration and Nitro blue tetrazolium activity in all groups.

Urea level increased obviously among sodium nitrate treated group which come in line with (El-Wakf *et al*., 2008), recorded significant increase in urea and creatinine levels in male rats received $NaNo_3$ with water for 120 successive days at doses 21.7 and 47.4 mg $NaNo_3$/kg/day. The level of urea seriously decreased in T4, which come in line with (Hirneth and Classen, 1984; Shehata, 2005). This may be due to the antioxidant activity of vitamin C, which has an inhibitory effect on the conversion of nitrate to nitrite and nitric oxide, since it is known that nitrite is eight fold more toxic than nitrate (Hirneth and Classen, 1984; Bassetti *et al*., 2018).

Creatinine level increased obviously among T3 followed by T2 which indicate a status of acute kidney injury was developed and increase in formation and production

Table 6: Correlation between blood creatinine concentration and nitro blue tetrazolium activity.

Blood creatinine concentration according to group (mg /dl)		Nitro blue tetrazolium activity according to group				
		Vitamin E-sodium selenite treated group	Vitamin C treated group	Sodium nitrate treated group	Vitamin E-sodium selenite + Sodium nitrate treated group	Vitamin C+ Sodium nitrate treated group
Control	R	-0.075	-0.153	0.048	-0.043	-0.123
	P value	0.694	0.418	0.800	0.822	0.519
Vitamin E -sodium selenite treated group	R	-0.109	0.022	-0.012	-0.102	0.009
	P value	0.566	0.910	0.949	0.592	0.962
Vitamin C treated group	R	-0.034	0.091	-0.032	-0.045	0.067
	P value	0.859	0.633	0.866	0.812	0.724
Sodium nitrate treated group	R	-0.109	0.022	-0.012	-0.102	0.009
	P value	0.566	0.910	0.949	0.592	0.962
Vitamin E -sodium selenite + Sodium nitrate treated group	R	0.048	-0.130	0.046	0.065	-0.096
	P value	0.799	0.494	0.809	0.734	0.614
Vitamin C + Sodium nitrate treated group	R	0.055	-0.064	-0.046	0.026	-0.080
	P value	0.774	0.735	0.809	0.890	0.674

of reactive oxygen species by mitochondria of renal tubules and hence leads to tubular damage and affecting glomerular filtration rate entirely. Renal tubules are particularly vulnerable to oxidative stress and damage, since mitochondria are one of the main sites within the cell for manufacturing of free radicals via the respiratory chain and NADPH oxidases (Eirin *et al*., 2016; Gyurászová *et al*., 2019). This comes in line with (El-Wakf *et al*., 2008), reported significant increase in urea and creatinine in male rats provided sodium nitrate in the drinking water for 4 months at estimated doses of 21.7 and 47.4 mg sodium nitrate/kg/day.

Considerable differences in urea level were reported between T6 and T2, T3. Considerable differences were reported between T3 and T1, T2, T4, T5. Considerable difference in creatinine was reported between T2 and T6; T1 and T3, T4 and T3, T5 and T3. This come in line with that reported by (Ghlissi *et al*., 2015) that vitamin E as documented antioxidants act mainly as radical scavenger which is essential inhibitor of membrane lipid peroxidation. As vitamin E is a lipid soluble agent which facilitate crossing of cell membranes and exerts its effect both on cells and membranes which explain the ameliorative effects of sodium nitrate on tubular damage which reflected by significant reduction in the level of the of urea and creatinine. On the opposite hand, vitamin C, which exerts powerful antioxidant properties on the hydrophobic compartments, can scavenge chain initiation by removing aqueous radicals.

As vitamin C have the ability to give away a hydrogen atom and lead to formation a proportionally a stable ascorbyl free radical which play a role in oxidation of proteins, lipids or even cellular DNA (Verma *et al*., 2007; Rouhier *et al*., 2008). Vitamin C act as effective scavenger against oxygen and nitrogen oxide species, (O_2^-, H_2O_2, OH^-, and O_2). This repertoire has a great role in protection against any damage induced by free radicals. On the other hand, vitamin C synergies the antioxidant property of vitamin E by reducing tocopheroxyl radical which leads to prevent any free radical induced damage to membranes and other vital the cellular components (Pehlivan, 2017).

Histopathological changes of mouse kidney for vitamin E -sodium selenite treated group and vitamin c revealed that in the cortex, renal blood vessels were congested with perivascular cuffing and infiltration of inflammatory cells in the dilated blood vessels and distension of Bowman's space. Congestion of glomerular capillaries, proximal and distal convoluted tubules showed cellular swelling .All these changes indicate a significant increase in immunological response of renal tissue. These results come in line with that reported by (Liu *et al*., 2015), specified that vitamin E has the capacity to afford protection to kidney tissue from lipid peroxidation and free oxygen radicals, and this action regulates its therapeutic action against acute kidney injury in experimental models. While histopathological changes of mouse kidney for sodium nitrate treated group, (T3) revealed sever congestion of renal blood vessels in the cortex, perivascular cuffing, and infiltration of inflammatory cells in the dilated blood vessels. Extensive distension of Bowman's space and sever congestion of glomerular capillaries, extensive cellular swelling of proximal and distal convoluted tubules with degenerative changes.

All these changes clearly indicate the stimulation of the immune response in dose dependent manner of vitamin

E, which stimulated the humoral immune response by increasing the pool of inflammatory cells, as well as the expansion in the area of the renal tubules and the collection of cells and fluids in a remarkable manner. All these histopathological changes confirm the role of vitamins (E and C) in protecting the renal tissue in comparison with the tissue changes in the group that was given sodium nitrate, there was severe pooling of inflammatory fluids and sever congestion of renal blood vessels in the cortex, perivascular cuffing, and infiltration of inflammatory cells in the dilated blood vessels. Extensive distension of Bowman's space and severity of glomerular capillaries, extensive cellular swelling of proximal and distal convoluted tubules, which confirms that damage to the renal tubules and thus normal glomerular filtration of kidney function is affected (Ghlissi *et al*., 2015).

Different physiological strategies were developed to get rid the excess reactive oxygen species and maintain them on normal physiological levels (Schieber and Chandel, 2014; Halliwell, 2023). The use of an extra elements of naturalistic antioxidants such as selenium and vitamin E aids in elimination of oxidative impairment induced by free radicals (Cemek *et al*., 2010; Akbari *et al*., 2022). On the other hand, Selenium required for construction of selenoproteins which is the core of antioxidant enzyme glutathione peroxidase structure and function, Selenium eventually promote the activity of the enzymes (Ognjanović *et al*., 2008, Angelone *et al*., 2024). Vitamin E as potent antioxidant which is soluble in lipid, considered as vital element in different metabolic activities by protection from lipid peroxidation of cellular membranes and vital compartments. On the other hand vitamin E play fundamental role in anti-inflammatory reactions by suppression of platelet from accumulation, and boosting of immune response (Al-Attar, 2011; Sharma, 2024).

α-tocopherol form of vitamin E has active antioxidant potency due to their ability to cross the cellular plasma membrane greater than other tocopherols, hence they has greater biopotency (Pekİner, 2003; Shastak *et al*., 2023). The biopotency and efficacy of antioxidant activity were not affected by high efficacy of cellular uptake of α-tocopherol alone but also affected by temperature, substrate types, presence of appropriate solvent, presence of prooxidants, presence of synergistic compounds (Budilarto and Kamal-Eldin, 2015; Barouh *et al*., 2022). As α-tocopherol form of vitamin E is integrated within the cellular membrane this will leads to several physiological benefits which leads to protection of sub cellular structures such as, first, Inhibition for polyunsaturated fatty acid destruction by reactive oxygen species (Kanarovskii *et al*., 2018). Second benefit, anchoring of vitamin E with lipid bilayer of biological membranes leads to reduction of membrane permeability and increase its stability (Raederstorff *et al*., 2015; Trela-Makowej *et al*., 2022). The third one, formation of complex due to anchoring of vitamin E with lipid bilayer of biological membranes leads to stabilization of membrane-bound phospholipases (Marquardt *et al*., 2013; Kearns *et al*., 2023). The last one, Formation of complex due to anchoring of vitamin E with lipid bilayer of biological membranes leads to stabilization and protection of the polypeptide chains of cellular intrinsic proteins to counteract the modification results from free fatty acids (Pekİner, 2003; Lopez *et al*., 2014).

Previous researches revealed that dietary selenium and vitamin E intake promotes glutathione peroxidase levels and hence sufficiently reduce the level of reactive oxygen species and protect tissue from extreme damage (Abdel Samie *et al*., 2018).

The present experiment reveals no correlation between blood urea and creatinine concentration and phagocytic function of monocyte -macrophage in the form of nitro blue tetrazolium activity and formazan formation in all groups. As tubular tissue were riches with macrophages, as well as mitochondria as a source of ROS, current results indicate that the level of tubular damage does not reach to the end stage renal disease and the glomeruli at this time have the ability to work and tolerate the active damage of ROS induced by sodium nitrate in dose and time dependent manner (Podkowińska and Formanowicz, 2020).

CONCLUSIONS AND RECOMMENDATIONS

Vitamin E -sodium selenite and vitamin C have important role in enhancement of functional activity and clearance of urea and creatinine. Sodium nitrate cause significant increase in urea (48.00 ± 2.00000 mg/dl) and creatinine(0.8500± 0.09258) levels addition of vitamin E to sodium nitrate significantly reduce urea (22.50 ± 1.92725 mg/dl) and creatinine concentration (0.5500± 0.05345 mg/dl) addition of itamin C to sodium nitrate cause significant amelioration in the level of urea (27.13±0.83452 mg/dl) and creatinine (0.6625±0.05175mg/dl). Vitamin E -sodium selenite and vitamin C have important role as potent antioxidant in amelioration of histopathological effects of sodium nitrate on renal tissue. Concentrations of blood urea and creatinine have no effect on potent scavenging activity of monocytes and macrophages.

Further studies were recommended for evaluation of the effect of vitamin E -sodium selenite and vitamin C on behavioral effects of sodium nitrate, postmortem changes and gross pathological changes on experimental animals.

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NOVELTY STATEMENT

The novelty of current study based on the use of vitamins E and C which are popularly used as a protectant from sodium nitrate.

AUTHOR'S CONTRIBUTION

All authors are equally contributed in planning, writing a draft and final manuscript, experimental design and laboratory work, statistical analysis.

Conflict of interest

The authors have declared no conflict of interest.

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