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Protective Effect of Vitamins C and E against Nitrocellulose Thinner Induced Nephrotoxicity in Albino Wistar Rats

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Abstract

Effect of vitamins C (vitamin C) and E (vitamin E) on nitrocellulose thinner (NCT)-induced nephrotoxicity in male rats was assessed. Six groups of six rats each, were orally administered 0.5 ml distilled water, 0.5 ml soybean oil, 40.0 mg NCT/kg bwt, 40.0 mg NCT/kg bwt+200 mg vitamin C/kg bwt, 40.0 mg NCT/kg bwt+200 IU vitamin E/kg bwt, and 40.0 mg NCT/kg bwt+200 IU vitamin E+200 mg vitamin C/kg bwt, respectively, for 30 days. The animals were sacrificed, 24 hours after last experimental treatments, blood and kidney tissues were collected for analyses of indicators of nephrotoxicity using standard methods. The results showed a significant ($p<0.05$) increase in serum creatinine (sCr), urea, uric acid, K^+, HCO_3^- and Cl^- and decrease in serum Na^+ levels, as well as severe renal histological changes in rats exposed to NCT only, compared to the values obtained for the control groups receiving only distilled water and soybeans oil, respectively. No significant ($p>0.05$) difference was recorded for these parameters in rats receiving soybean oil, compared with control rats receiving distilled water. These results indicated that exposure to NCT induced nephrotoxicity in rats. It was also observed that administration of vitamin C and vitamin E, in combination and singly, to rats exposed to NCT produced relatively normal renal histological status, and levels of the assayed serum nephrotoxicity indicators within the control range; suggesting vitamin C and vitamin E to be potent in preventing NCT-induced nephrotoxicity in rats. However, comparative percentage decreases (CPD) in sCr, uric acid, K^+ and HCO_3^- indicated that combined vitamin C and vitamin E administration produced a higher protective potency than single administration; and that vitamin C produced a higher potency than vitamin E against NCT-induced nephrotoxicity.

Keywords: Nitrocellulose-thinner; Nephrotoxicity; Vitamins C and E; Nephroprotection

Introduction

The kidneys are known to play a vital role in the excretion of most hydrophilic metabolic wastes from the body. The renal tissues are therefore the major tissue responsible for the clearance of wastes products of metabolic activities from the body. The functional state of the renal tissues may therefore be assessed by the rate at which the tissues clear or sequester these metabolic wastes from the blood. Abnormality or dysfunction of the renal tissues usually results in the accumulation of these metabolic wastes in the blood, due to the failure of the renal tissues to effectively clear the metabolic wastes from circulation. Hence, the concentrations of most of these wastes substances tend to build or increase in the blood, to the levels that are above the normal range. In routine clinical and research investigations, the plasma or serum concentrations of some wastes metabolites and electrolytes are commonly determined to assess the functional integrity of the renal tissues. Among the waste metabolites that are commonly considered in renal function assessment include creatinine, urea, blood urea nitrogen and uric acid, while sodium, potassium, chloride, bicarbonate, magnesium, and sometimes calcium ions are among the electrolytes that are often used to assess the renal functions [1-3].

The functional state of the renal tissues may be adversely affected by several factors, including chemical insults. Chemical insults to the renal tissues may arise from the exposure to toxic chemical agents, or their metabolites. These toxic substances can interact with the macromolecular constituents of the renal tissues, and produce a compromise in the renal functions. Our previous studies revealed that nitrocellulose thinner is one of the chemical agents that can cause an adverse effect on the functional state of the renal tissues [4,5]. Nitrocellulose thinner (NCT) is an industrial solvents commonly used in furniture, paints and automobile spray-painting industries. It contains such organic chemical agents, as ethylbenzene or toluene and butyl acetate. These chemical substances are known to constitute chemical pollutants in different environments. WHO [6] reported that these chemical pollutants are detectable in household and workplace air. Hence, exposure to chemical pollutants from NCT in indoor and outdoor environments may be common. Individuals may be exposed to this solvent by direct inhalation of the volatile constituents, or ingestion of foods and drinks contaminated by the solvents during use. Particularly, occupational exposures to mixtures of toluene, ethylbenzene and butyl acetate have been reported in painting or lacquering workplaces [7-9].

Exposure to NCT, and related organic solvents has been reported to induce haematotoxicity, hepatotoxicity and nephrotoxicity in humans and experimental animals [4,8-13]. The presence of chemical constituents of nitrocellulose thinner, like other xenobiotics, may activate some drug metabolizing enzymes to transform these chemical substances into various metabolites in the body [14]. These metabolites, which may be very reactive, in the course of their renal excretion are likely to interact with the renal tissues to express some toxic effects [15,16]. These interactions may produce cellular injury, resulting in tissue damage. Damage to the renal tissues is likely to result in the overall compromise of the functional status of the kidneys.

Exposures to certain reactive or toxic metabolites have been reported to produce varying degrees of renal dysfunction in humans and experimental animals [3,17,18].

Most toxicity effect(s) associated with exposure to chemical agents and their metabolites are known to be indication of tissue, or tissue components-reactive metabolite species interactions in the body. The presence of antioxidants has been reported to provide protective mechanism against different toxicity effects associated with the reactive species generated by chemical substances into the body tissues [19]. While some antioxidants are endogenously generated within the body, others (including antioxidant vitamins) may be provided as micronutrients in the diets. According to the report, such antioxidants vitamins as ascorbate (vitamin C) and α-tocopherol (vitamin E) have been demonstrated to provide protection against chemicals induced oxidative stress in different tissues via several mechanisms [20-22].

According to Carr et al. [19], the effectiveness of ascorbate in mitigating the pathophysiological processes of oxidative stress tends to be higher than that of α-tocopherol. The reported increased protective effectiveness of ascorbate against oxidative stress may likely be attributed to its effectiveness in scavenging a wide range of reactive oxygen and nitrogen species, as well as its ability to regenerate α-tocopherol, and possibly tetrahydrobiopterin, from its radical species. Also, ascorbate is known to act as a co-antioxidant and possibly prevent the pro-oxidant activity of α-tocopherol [19,23-26]. However, our earlier study showed that vitamin E is relatively more effective than vitamin C in providing protective measures against gasoline vapour-induced hepatotoxicity in rats [27]. It is therefore possible that co-administration of vitamin C and E may be more effective in protecting the tissues against chemicals induced oxidative stress processes, than singular administration. On the basis of the reported contradicting protective effectiveness of vitamins C and E against chemicals-induced toxicities, this study assessed the protective effectiveness of co-administration and singular administration of vitamins C and E against nitrocellulose thinner induced nephrotoxicity in rats.

Materials and Methods

Animal handling and treatment

Thirty six apparently normal matured male albino Wistar rats (180 to 200 g), obtained from Biochemistry Department Experimental Research Animal House of the University of Calabar, Calabar, Nigeria, were used in this study. They were fed with standard laboratory diet and allowed free access to tap water ad libitum. The work was carried out under 12 hours light/dark cycle illumination and prevailing tropical room temperature.

Preliminary acute toxicity studies in mice, gave LD 50 of 16.0 ml/kg (i.e., 160.2 mg/kg, by weight) body weight of nitrocellulose thinner (solubilized in Grand pure soya beans oil). Hence, 4.0 ml/kg (i.e., 40 mg/kg, by weight) body weight concentrations (25% of LD50) were used in this study.

The animals were distributed into six groups, with six rats each, as highlighted below:

Group 1: Comprised of six rats receiving 0.5 ml of distilled water only for thirty (30) days

Group 2: Comprised of six rats receiving 0.5 ml soya beans oil only for thirty (30) days

Group 3: Comprised of six rats receiving 40.0 mg/kg body weight of nitrocellulose thinner (NCT) for thirty (30) days.

Group 4: Comprised of six rats receiving 40.0 mg/kg body weight of NCT + 200 mg/kg body weight of vitamin C for thirty (30) days

Group 5: Comprised of six rats receiving 40.0 mg/kg body weight of NCT + 200 IU/kg body weight of vitamin E for thirty (30) days

Group 6: Comprised of six rats receiving 40.0 mg/kg body weight of NCT + 200 IU and 200mg/kg body weight of vitamins E and C, respectively, for thirty (30) days

The choice of the dosage of the vitamins was based on our previous report that daily administration of vitamins C (200 mg/kg body weight) C and E (200IU/kg body weight) produced protective effect against gasoline-induced hepatoprototoxicity in rats [27]. The vitamins were administered to the rats, one hour after NCT administration, and all the administrations were carried out once daily, six days per week, throughout the experimental period. The animals were sacrificed, 24 hours after the 30th day of experimental period. All animal experiments were carried out according to the Guidelines of Institution's (University of Calabar, Nigeria) Animal Research Ethics Committee, with reference to the Guide for the Care and Use of Laboratory Animals [28].

Collection and preparation of tissues for analyses

Blood and kidneys were collected for analyses. Blood samples were collected by cardiac puncture, under chloroform vapor anaesthesia, 24 hours after termination of experimental treatments, into sterile plain sample bottles. The blood samples were allowed to clot and centrifuged with Table-top centrifuge (MSE model, England) at 3000 rpm for 10 minutes to obtain the serum, which was used for the biochemical assays. The kidneys collected were dissected out carefully, blotted free of blood, sliced and immersed in 10% phosphate buffer formalin and in 2.5% phosphate buffer glutaraldehyde (pH 7.4) for histological analysis.

Biochemical analyses

The concentrations of creatinine, urea, uric acid and electrolytes (including Na^+, K^+, $HCO3^-$ and Cl^-) in the serum were determined using referenced standard methods [29-32]. Reagent kits obtained from Biosystems Laboratories (S. A. Costa Brava, Barcelonia, Spain) and Randox Laboratories (United Kingdom) were used in the study. All the reagent kits were of analytical grade.

Histological analysis

The slices of kidney tissues were fixed in 10% formosaline for 24 hr, after which they were dehydrated with 100% ethanol solution and then embedded in paraffin. They were thereafter sectioned at 7 μm thickness, and stained with haematoxylin and eosin (HE) for histological examination. Images of kidney sections processed by HE were examined under light microscope for morphological changes.

Statistical analysis

Results were presented as mean ± S.E.M. The data generated from the study were statistically analysed using one-way analysis of variance (ANOVA) with SPSS (version 17.0) and Microsoft Excel programmes. Student "t" test was also used for pair-wise comparison, and differences were considered significant at $p<0.05$.

Results

The results of this study are presented in figures 1-4 and slides 1a-f. The results showed that serum creatinine, urea, uric acid, K+, HCO3- and Cl- levels were significantly ($p<0.05$) increased, while the serum Na+ level decreased significantly in rat model, following exposure to NCT, when compared with the control (Figure 1-3). However, there was no significant ($p<0.05$) difference in the serum creatinine (1.5 ± 0.5 mmol/l), urea (36.3 ± 4.1 mmol/l), uric acid (2.3 ± 0.8 mmol/l), Na + (142.8 ± 7.1 mEq/l), K+ (1.5 ± 0.6 mEq/l), HCO3- (13.0 ± 2.1 mEq/l) and Cl- 81.1 ± 3.8 mEq/l) levels of rats receiving soybeans oil only, compared, respectively, to the levels (1.5 ± 0.5 mmol/l, 36.9 ± 3.8 mmol/l, 2.4 ± 0.6 mmol/l, 143.6 ± 6.2 mEq/l, 1.6 ± 0.3 mEq/l, 12.8 ± 2.6 mEq/l and 80.7 ± 4.6 mEq/l, respectively) recorded for the control rats receiving distilled water only. Also, administration of vitamins C and E, either singularly or in combination, to rats exposed to NCT produced a significant ($p<0.05$) decrease in serum creatinine, urea, uric acid, K+, $HCO3^-$ and Cl- levels, and increase in serum Na+ level to levels within the control range, compared respectively to the levels obtained for rats exposed to NCT only (Figure 1-3). The levels of these serum indices recorded for rats exposed to NCT and treated with vitamins C and E were not significantly ($p<0.05$) different from the levels recorded for rats in the control. This observation implies that administration of vitamins C and E to rats exposed to NCT maintained serum creatinine, urea, uric acid, Na+, K+, HCO3- and Cl- levels within the control range.

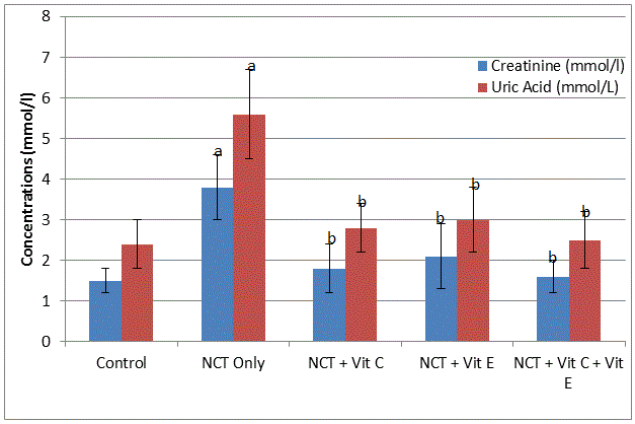


Figure 1: Effect of vitamins C (Vitamin C) and E (Vitamin E) on serum creatinine and uric acid concentrations of rats exposed to Nitrocellulose thinner (NCT). a=$p<0.05$ compared with control; b=$p<0.05$ compared with NCT only.

Moreover, it was observed from the results of this study that administration of vitamin C only to rats exposed to NCT produced a significant ($p<0.05$) increase in comparative percentage decrease in serum creatinine and K+ concentrations, compared respectively to the comparative percentage decrease recorded for rats exposed to NCT and administered vitamin E only (Figure 4). However, the comparative percentage decrease in serum creatinine, uric acid, K+ and HCO3- concentration recorded for rats exposed to NCT and treated with vitamins C and E, in combination, was significantly ($p<0.05$) higher, compared respectively to the comparative percentage decrease in serum creatinine, uric acid, K+ and HCO3- concentration recorded for rats exposed to NCT and treated singularly with vitamins C and E (Figure 4). The results obtained from this study therefore suggested that vitamin C is more potent than vitamin E in protecting the renal tissues against NCT induced toxicity on one hand, and that on the other hand, combined administration of vitamins C and E produced a higher protective potency against NCT induced renal tissues toxicity than administration of vitamins C and E singly in rats.

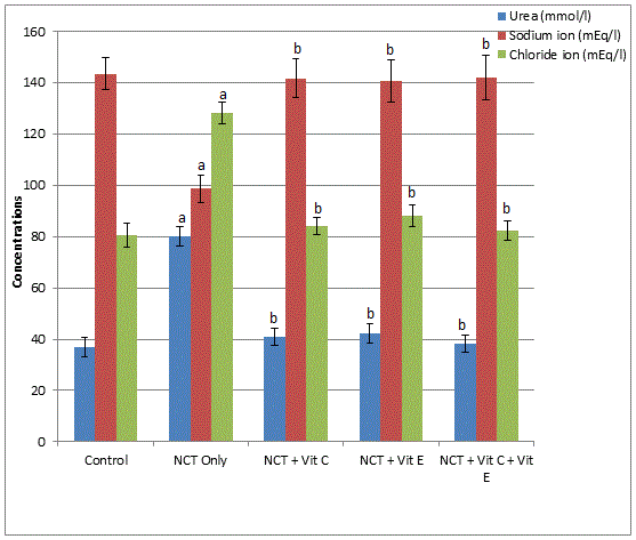


Figure 2: Effect of vitamins C (Vitamin C) and E (Vitamin E) on serum urea, sodium and chloride ions concentrations of rats exposed to Nitrocellulose thinner (NCT). a=$p<0.05$ compared with control; b=$p<0.05$ compared with NCT only.

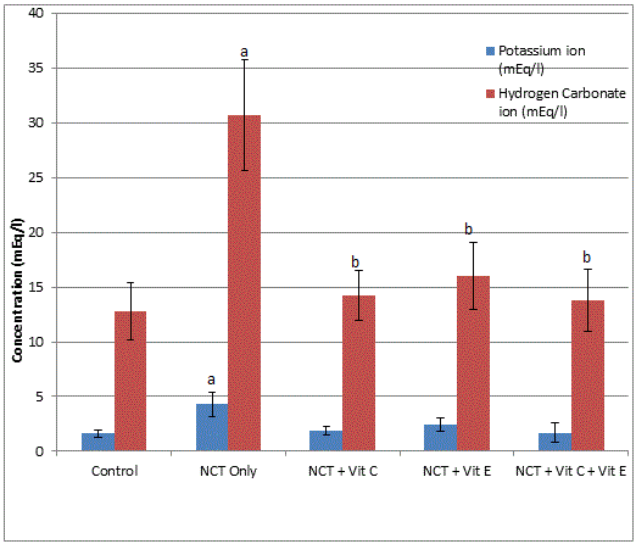


Figure 3: Effect of vitamins C (Vitamin C) and E (Vitamin E) on serum potassium and hydrogen carbonate ions level of rats exposed to Nitrocellulose thinner (NCT). a=$p<0.05$ compared with control; b=$p<0.05$ compared with NCT only.

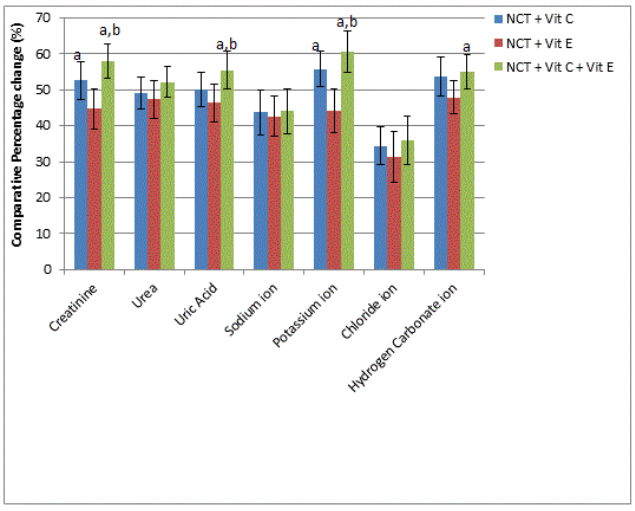


Figure 4: Comparative percentage effect of vitamins C (Vitamin C) and E (Vitamin E) on some serum metabolites and electrolytes of renal function assessments in rats exposed to Nitrocellulose thinner (NCT). a=p<0.05 compared with NCT+Vitamin E; b=p<0.05 compared.

Slide 1c shows the various negative changes in renal histological structure following exposure to nitrocellulose thinner, compared to the relatively normal histological structures observed for the control groups (Slides 1b and c), and the groups exposed to nitrocellulose thinner and treated with vitamins C and E (Slides 1 d-f). These results show that exposure to nitrocellulose thinner caused adverse changes in renal tissues histological pattern, such as necrosis of glomeruli and tubules, atrophic glomeruli, glomerular capsule and tubules dilatation. And that administration of vitamins C and E to rats exposed to nitrocellulose thinner-prevented the occurrence of these nitrocellulose thinner induced adverse changes in renal tissues histological integrity in male rats (Slides 1 d-f).

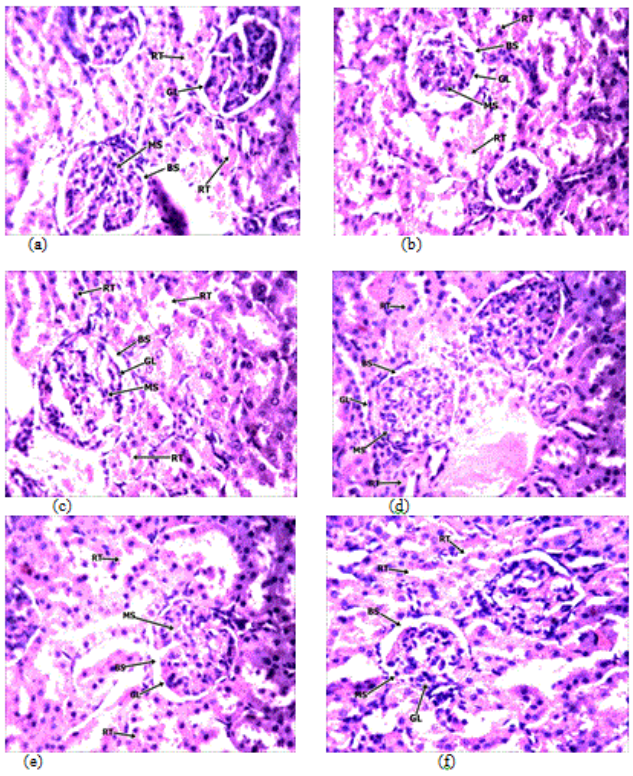
Discussion

It is generally known that exposure to some chemical solvents, including nitrocellulose thinner, may cause severe organ tissue toxicities. Particularly, previous studies reported that exposure to nitrocellulose thinner is capable of exerting deleterious effects on the renal tissues, hence nephrotoxicity [4,5,33]. In our earlier studies, exposure to nitrocellulose thinner was observed to cause elevation of serum creatinine, urea, BUN, uric acid, K^+, and renal tissue malondialdehyde (MDA), as well as decreased serum protein, Na^+, Ca_2^+, HCO_3^-, Cl^- levels and renal tissue superoxide dismutase (SOD) activity in rats [4,5]. Also, a significant distortion in the architectural integrity of the ultrastructural profile of the renal tissues was also observed to be associated with exposure to nitrocellulose thinner in these previous studies. Similar increase in serum creatinine, urea, uric acid, Na^+, K^+, HCO_3^- and Cl^- levels was observed to be associated with exposure to nitrocellulose thinner in this present study. Also, marked distortions in the architectural integrity of the ultrastructural status of the renal tissues following exposure to nitrocellulose thinner were also recorded in this study. Elevation in serum creatinine, uric acid, urea and blood urea nitrogen has been reported to be strongly associated with the development of renal disease, hence renal dysfunction or failure [10,34,35]. The observations made from this present study in correlation with our earlier reports [5,27] therefore indicated that exposure to nitrocellulose thinner is among the risk factors for nephrotoxicity, with the possibility of producing renal failure.

Literature reports that some of these chemical substances produce renal toxicity via the generation of **free radicals** and other reactive species in the course of their metabolism [36-38]. The results of this study therefore suggest that nitrocellulose thinner contain some chemical substances with nephrotoxic potentials. It is generally known that when the level of the reactive species, or pro-oxidants, generated in the course of chemical agents' metabolism in the tissues overwhelms the endogenous protective antioxidants, they tend to interact with the tissues' macromolecular components. The interaction of these reactive species, generated from chemical agents' metabolism, with the renal tissues is likely to result in the nephrotic damage and necrosis [39]. It may therefore be assumed that the reactive metabolites of the nitrocellulose thinner's constituents might have interacted with the renal tissues to induce the nephrotoxicity reported in this present work and our previous studies [4,5,13]. The observation made from the results of this study therefore supports of our earlier reports that NCT induced nephrotoxicity in experimental animals [4,5,13]. However, administration of antioxidants supplements have been reported to provide some degree of protection against the chemical agents generated reactive species tissue challenges. Vitamins C and E have been reported to be among the vitamin supplements with effective antioxidant properties in the various biological systems [20-22].

Vitamin C has been reported to mediate its antioxidant effect by scavenging free reactive oxygen specie [40-44]. This indicates that Vitamin C may inhibit the chain reactions of chemical agents-generated free radicals or scavenged the reactive free radicals before reaching their tissue targets; while Vitamin E is reported to act by breaking the antioxidant chain that prevents reactive oxygen species from interacting with membrane macromolecules to produce cell membrane damage [45]. Also, Factor et al. [46], demonstrated that vitamin E can directly reduce ROS production by interfering in the union between the membrane and the NADPH oxidase complex. Also administration of vitamin E, have been reported to play a vital role in the prevention of lipid peroxidation, to protect the tissues against chemical injury [47,48]. In this study, the levels of metabolites and electrolytes assayed were observed to fall within the control range following administration of vitamins C and E to rats exposed to nitrocellulose thinner. Also, relatively normal histological integrity of the renal tissues was observed in rats exposed to nitrocellulose thinner and treated with vitamins C and E. The results of this present study therefore showed that vitamins C and E supplements are capable of providing protection against nitrocellulose induced nephrotoxicity in rats.

In agreement with the report of Car et al. [19], the results of this present study showed that vitamin C is relatively more potent than vitamin E in protecting the renal tissues against NCT induced renal toxicity in rats. However, it was observed that the protective effectiveness of combined administration of vitamins C and E, as a combined therapy, was higher than that of single therapy administration; and that the protection potency of vitamin E was more than that of vitamin C. The specific mechanism(s) of the higher protective potency of the combined, than single, administration of vitamins C and E against nitrocellulose thinner induced nephrotoxicity is a subject for further investigation.



Slide 1: Sections of renal tissues of, (a) control rats receiving distilled water only, (b) rats receiving soya beans oil only, (c) rats receiving 40.0mg NCT/kg bwt, (d) rats receiving 40.0 mg NCT/kg bwt+200 mg Vitamin C/kg bwt, (e) rats receiving 40.0mg NCT/kg bwt+200IU Vitamin E/kg bwt and (f) rats receiving 40.0mg NCT/kg bwt+200IU Vitamin E/kg bwt+200mg Vitamin C/kg bwt. Slides 1a and b show normal kidney tissues showing prominent glomeruli (GL) and renal tubules (RT). The glomeruli are surrounded by clear bowman's space (BS) and a cellular mesangium (MS) consisting of deeply stained basophilic oval to round mesangial cells. The renal tubules are closely packed with empty lumen, lined by cuboidal epithelial cells with regular cellular outline. Also, the intervening interstitium is scanty and contains normal blood vessels. Slide 1c shows atrophic glomeruli and some swollen glomeruli with loss of bowman space. Their mesangial cells are scanty with areas of fibrosis. The renal tubules are closely packed and lined by swollen epithelial cells with some tubules showing detachment of epithelial cells from their basement membrane. Also, the intervening interstitium is scanty and contains congested blood vessels and thick wall arterioles. Slide 1d, e and f show prominent glomeruli and renal tubules. The glomeruli are surrounded by clear bowman's space and a cellular mesangium. The renal tubules are closely packed with empty lumen, lined by cuboidal epithelial cells having regular cellular outline and moderate eosinophilic cytoplasm and prominent nuclei with nucleoli. Also, the intervening interstitium is scanty and contains blood vessels.

Based on the results obtained from this study, it may be concluded that coadministration of vitamins C and E is more potent than single administration, in providing protection against nitrocellulose thinner-induced nephrotoxicity in male albino Wistar rat model. Also, it is very likely that the results obtained from this study may be applicable to humans since most biochemical and physiological activities in rats correlate those in human. However, more investigations are needed to verify this assertion.

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