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Role of Some Micronutrients in Ameliorating the Destructive Effect of Trichloroethylene on Kidney and Testes of Male Rats

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ABSTRACT

Trichloroethylene (TCE) is a widely used volatile compound to which considerable numbers of human are exposed via breathing, through the skin or through drinking water but rarely through food. The main symptoms of exposure are headache, dizziness, and confusion. Beyond the effects to the central nervous system, workplace exposure to TCE has been associated with toxic effects in many organs including kidney and testes.This work aimsto investigate the role of vitamin C and / or zinc against the destructive effect of daily oral TCE intake through biochemical, tissue and DNA studies for kidney and testes during short (3 weeks) and long (15 weeks) terms. Also a hematological study for complete blood count (CBC) has been carried out.The results showed that TCE increases the level of urea creatinine and uric acid and decreases the level of total testosterone significantly. It alsoshowed deformation in tissues and DNA degradation after short and long term treatment with TCE.Administrationof vitamin C and/or zinc improved the disruptedeffect of TCE oral intakein all the investigated parameters either significantly or non-significantly.It couldbe concluded that, both occupational workers and normal people whom may be exposed to TCE can use vitamin C and/ or zinc to compensate theTCE hazardous effect.

Key words: Trichloroethylene TCE/Vitamin C/Zinc/Kidney/testes.

INTRODUCTION

The chemical compound trichloroethylene is a chlorinatedhydrocarboncommonly used as an industrial solvent. It is a clear non-flammable liquid with a sweet smell. The IUPAC name is trichloroethene and the industrial abbreviation is TCE[(1)].Trichloroethylene (TCE) is a major environmental contaminant. Histopathological examinations revealed that TCE causes body toxicity including kidney and testes, moreover it leads to carcinogenicity. However, biochemical mechanism and tissue response to toxic insult are not completely elucidated. It is hypothesized that TCE induces oxidative stress to various rat tissues and alters their metabolic functions[(2)]Workers in degreasing operations have the highest risk of exposure to TCE. People who live near factories that use TCE may be exposed to low levels in the air.The most common exposure mechanisms are via Breathing, Touching or Drinking/Eating. People who use TCE as a solvent (such as typewriter correction fluid or paint remover) may breathe significant amounts of the compound. Since TCE evaporates quickly, people who shower in contaminated water may breathe the vapors.TCE can be absorbed through the skin therefore, people who use the compound without solvent-resistant gloves may be exposed. Also, exposure can occur when people work with contaminated soil or bathe in contaminated water. TCE released onto soil readily enters groundwater. Therefore, people who drink water from wells located near TCE disposal sites may be exposed. Plants grown on contaminated soil do not absorb TCE.

TCBhas been detected at very low levels in many processed foods as a result of its use in equipment-cleaning[1].

The kidneyis one of the target organs for TCE[3]. Renal effects of TCE are generally attributed to its conjugation with GSH and subsequent metabolism within the proximal tubules to generate the primary metabolite, 1,2,-dichlorovinyl-L-cysteine DCVC, which is further metabolized to another reactive intermediate [4]. DCVC was demonstrated to be a potent cytotoxicant in freshly isolated rat cells with production of mitochondrial dysfunction as an early effect[5]. Changes in mitochondrial Ca^{2+} ion homeostasis also appear to be a key step in DCVC-induced renal toxicity[6].Studies of trichloroethylene on male reproductive end points have been done primarily in rodents. It wasreported that trichloroethylene at an oral dose of 1,000 mg/kg/day (5 days/week for 6 weeks) inhibited copulatory behavior in male rats.

A study of male mice exposed to trichloroethylene via inhalation found significantly increased percentages of abnormal sperm at the highest test concentration[7].Leydig cells were hyperplastic. All the tubules had Sertoli cells but were almost devoid of spermatocytes and spermatids. "Fibrines" were present in the tubular lumens. Testicular dehydrogenase and glucose-6-phosphate dehydrogenase were significantly reduced and glutamyltransferase and ß-glucuronidase were significantly increased. The authors concluded that postmeiotic stages of spermatogenesis in rats were susceptible to trichloroethylene-induced insult. They suggested that exposure to trichloroethylene "may cause testicular toxicity, which in turn affects postmeiotic cells of spermatogenesis, Sertoli cells, and Leydig cell functions" [8].

Exposure to TCE causing DNA faulty that leads to activation of apoptosis. Apoptosis, or programmed cell death, is a normal component of the development and health of multicellular organisms. It is a process in which cells play an active role in their own death. Upon receiving specific signals instructing the cells to undergo apoptosis, a number of distinctive changes occur in the cell. Similarlythe degradation of enzymes such as DNasesbegins to cleave the DNA in the nucleus[9]. Diet represents a specific modulator of the individual status as well asmajors sources of exposure to contaminants. Furthermore, micronutrients such as vitamins (A, E, C, and B complex) and minerals (iron, iodine, zinc, selenium…) are essential dietary components required in minute amounts for healthy physiological function. Several toxicological studies indicate that antioxidants such as vitamin A, E and C may have either a direct or indirect endocrine modulating activity. Therefore, exogenous supplementation with micronutrients may reduce the damaging effects of organochemicals on the critical biomolecules of the biological systems[10].

This work aims to study the ameliorating effect of vitamin C and / or zinc on the destruction of kidney's and testes's biochemistry, tissues and DNA after short and long term exposure to TCE.

MATERIALS AND METHODS

Male albino rats (96 rat) weighing about 120-140 g were divided mainly to short term and long term studies (A and B). The group of each study was divided into two subgroups (a and b) which were further subdivided into four groups (1, 2, 3 and 4)in addition to the recovery subgroup in the short term study.

Short term study (A)

a- Control groups

1st group not treated. 2nd group daily orally treated with 50 mg/kg body weight vitamin C for 3 weeks. 3rd group daily orally treated with 220 mg/kg body weight Zn as ZnSO4 for 3 weeks.4th group daily orallytreated with 50 mg/kg vitamin C and 220 mg / kg Zn as ZnSO4 for 3 weeks.

b- TCE intoxicatedgroups

1st group daily orally treated with 750 mg/kg body weight TCE for 3 weeks. 2nd group daily orally treated with 750 mg/kg body weight TCE and 50 mg/kg body weight vitamin C for 3 weeks. 3rd group daily orally treated with 750 mg/kg body weight TCE and 220 mg/kg body weight Zn as ZnSO4 for 3 weeks. 4th group daily orally treated with 750 mg/kg body weight TCE and 50 mg/kg vitamin C and 220 mg / kg Zn as ZnSO4 for 3 weeks. 1st group daily orally treated with 750 mg/kg body weight TCE for 3 weeks. After that the group was left without treatment until the 15th week for recovery (recovery group).

Long term study (B)

It was divided to the same groups except that the duration of TCE treatment was for 15 weeks and the recovery group doesn't involve.The study started at late summer and ended at autumn Initial and final body weights were recorded.Organs weights of both kidney and testes were recorded for each group at the end of both short and long terms treatments.Blood samples were collected for serum creatinine, urea, uric acid and testosterone detection. Part of the blood was collected on EDTA for the hematological study (CBC count).Enzymatic colorimetric method was used for the determination of urea [11]. Creatinine was estimated using kinetic method [12]. Quantitative enzymatic colorimetric method was used for the determination of uric acid in serum [13]. Coat-A-count total testosterone is a solid-phase ^{125}I radioimmunoassay kit designated for the quantitative measurement of total testosterone levels in serum[14].The red blood cells and white blood cells were counted by visual means [15]. Platelet was counted as described in references[16].

Blood hemoglobin content was determined [17].Statistical analysis for mean, standard error, F-test and Duncan test using SPSS program was carried out. Autopsy samples were taken from the kidneys and testes of sacrificed rats from each group and fixed in 10% formal saline solution for forhistopathological examination by the light microscope. A minimum of 10 fields for each organ slide were examined and assigned for severity of changes [18].Nucleic acids DNA of kidney and testes wereextracted[19] andDNA fragments were detected by agarose gel electropharesis[20].The percent of (DNA) degradation of kidney was correlated with urea creatinine and uric acid. Also DNA degradation of testes was correlated with testosterone.

RESULTS AND DISCUSSION

The mean body weight changes in grams during short and long termstudies and mean weights of kidney and testes in grams are presented in table 1.It was obvious that the net body weight increased in thecontrol groups during 3 and 15 weekshigher than those of the corresponding intoxicated groups with TCE. Many authors studied the effect of TCE toxicity via inhalation or drinking water on body and organ weights. A decrease in body weight gain was observed in animals exposed to TCE[7, 21, 22, 23]This decrease may be due to the disturbance effect of TCE on anabolic rate.Also body weight gain was significantly reduced by 12 weeks of exposure, indicating systemic toxicity [8].

The kidney weight of the normal short term groups was in a higher rangethan that of the intoxicated groups. On the other hand, the kidney weight range of the normal long term groups was lower than that of intoxicated groups as shown in Table (1).It was found that significant increase in kidney weights; indicate the sensitivity of kidney as target tissues in TCE-toxicity[2, 23, 24].Also it wasreported thatkidney weight was slightly increased[25].This increase may be contributed to that the kidney tissues try to compensate its function.E U also reported that kidney weights were increased in rats exposed to 400 ppm of trichloroethylene for 8 months. In a long term Inhalation study rats and

mice were exposed to 0, 100, 300, 600 ppm trichloroethylene for 104 and 78 weeks for rats and mice respectively[26].

The testes weight of the short term intoxicated group increasedthan that of the control groups. On the other hand, the testes weight of the long term intoxicated groups decreased than that of the control groups as shown in table 1.It was stated that significant reduction in absolute testicular weight and alteration in testicular enzyme activity took place. This may be due to the damage effect of TCE causing testes atrophy[27, 28, 29].

In the current study the groups of rats intoxicated with TCE and supplemented with vitamin C or zinc showed a slight improvementin body and organs weightwhile the group supplemented with both showed higherimprovement compared with the normal (Table 1).This might be due to the antioxidantion role of vitamin C and zinc ,in addition, the role of vitamin C in regeneration of vitamin E to maintain its role as antioxidant and halt peroxidation of cellular membrane to maintain and repair tissue damage[30].In a long term exposure study of cluster of differentiation CD-1 mice were administered trichloroethylene in drinking-water (1.0, 2.5 or5.0 g L-1) for 4 - 6 months. A significant reduction in body weight (males and females at 5.0 g L-1) and increased kidney weight (males and females at 5.0 g L-1) were observed [31].

Statistical evaluation of the results for short (3 weeks) and long (15 weeks) term studies of creatinine, urea, uric acid and testosterone are shown in Table (2). The findings in the present work showed that administration of TCE for short and long period resulted in a significant rise in serum urea, uric acid and creatinine ($p<0.05$) compared to that of the control group (Table 2). It was reported that TCE administration increased blood urea and serum creatinine indicating toxicity and severe damage to kidney[2]. Biochemical indications of renal damage were observed in rats continuously exposed to 800 ppm trichloroethylene for 12 weeks[26, 29].The treatment of the intoxicated group with each of vitamin C and Zn element and both for short and long terms showed insignificant improvement compared to intoxicated group except in case of the short term groups treated with both vitamin C and Zn element a significant decrease in creatinine and urea level was noted (Table 2).

It is clear that administration of TCE dramatically decrease the serum testosterone level in the intoxicated groups compared to the control groups in both short and long term studies. Treatment with both vitamin C and Zn element shows a significant increase in the testosterone level of this group compared to the intoxicated group in short and long term studies (Table 2).In the present study short and long terms of toxicity have been conducted on the male rats via oral injection of TCE. As the results show a significant ($p<0.05$) decrease in serum testosterone level.Earlier researchers reported that inhalation of TCE may bring about testicular toxic effects, and the results indicated significant reduction in absolute testicular weight, and marker alteration in the testicular enzymes activity associated with spermatogenesis and germ cell maturation[27].

It was stated thatdepletion of testosterone through castration or destruction of the pituitary gland or hypothalamuswere manifested as a resultof exposure to TCE[32]. Also it wasreported that a significant decrease in total epididymal sperm count, sperm motility, specific activities of enzymes Glucose 6-p dehydrogenase (G6pDH) and 17 betahydroxy steroid dehydrogenase (17-beta HSD) with concomitant decrease in serum testosterone concentrations in TCE inhaled rats showing reduced male reproductive efficiency[8]. On the other hand testosterone level in the current study was improved by supplementation with dietary vitamin C and zinc due to the effect of each nutrient in repairing damage.

It wasstated that toxic effects were accompanied by significant elevation of testicular lipid peroxidation, decreased plasma testosterone level and a drop in copper and zinc concentrations in

testes. The administration of ascorbic acid after toxic treatment blunted the increased testicular lipid peroxidation and the decreased plasma testosterone level probably by the protective antioxidants effect and by compensating the loss of copper and zinc from testes[30]. The observed higher levels of serum testosterone in long term group (15 weeks) than the short term group (3 weeks) might be due to seasonal change as observed in an experiment with wild boar. It was found that all steroids showed a clear seasonal pattern with highest concentrations in autumn and early winter and low levels from January to July[33]. These results indicate that the seasonal variation in testicular steroid production was regulated by photoperiod.The observed higher level of serum testosterone may be also an indicative of maturity of the pituitary–gonadal axis in male rats [34].

The TCE effect after short term (3 weeks) of hematological study CBC are shown in Table (3) and that of long term (15 weeks) are shown in Table (4). The results of the present study revealed significant reduction in erythrocytic count, hemoglobin, platelets, hematocrit, MCH, MCV, MCHV and segmented neutrophils.On the other hand there was significant increase in WBC count, lymphocytes, monocytes, basophilseosinophils and staff neutrophils.These changes may be due to the increase in erythrothetic destruction as a result of abnormalities in the environment contaminated with TCE. It was foundthat asignificant increase innumber of typical lymphocytes, and white blood cell count was 10,100mm^3 with 27% eosinophilia and this is due to sensitization to trichloroethylene or more likely to one of its metabolites[35].

The current tissue studies showed histopathological changes in kidney and tests (Fig. 1-6). Focal inflammatory cell infiltration inbetween and surrounding the glomeruli and tubules at the cortex with swelling in the tubula epithelium were observed in kidney(Fig. 1&2). Also it showed focal haemorrage in the corricomedullary portion, and severe congestion in sclerotic blood vessels following TCE administration for both periods. These results are in agreement withreportedan increase in kidney weight,glomerular nephrosis, degeneration, desquamation of tubular epithelium and characteristic amyloid deposition in glomeruli[36].

The data in Fig. 5 revealed that the histopathological examination of kidney of TCE- rats supplemented with vitamin C and /or zinc illustrated moderate atrophy of glomerular interstitial hemorrhage and lymphocytic infiltration. Renal tissues exhibited mild congestion in its blood vessels with mild dilatation in some renal tubules and normal glomerular structure[37].Ascorbic acid (AA) treatment reversed some of the changes in biochemical indices, as well as histopathological alterations induced by toxicity. The findings imply that reactive oxygen species play a causal role in induced renal injury, and that AA exerts reno-protective effects, probably by radical scavenging and antioxidant activities [38].

Histological examination of testicular tissue of TCE- intoxicated rats for short and long periods of treatment (Fig. 3&4)showed degenerated spermatogonial cells in the lumen of the seminiferous tubules with appearance of homogenous eosinophilicalbuminous material inbetween. Also it showed multinumber of sertoli cells in the lumen of the degenerated tubules and showing mitosis in the spermatogonial cells of some seminiferous tubules and azospermia in most seminiferous tubular lumen. It was reported that inhalation of TCE by male rats for 12 and 24 weeks brings about significant reduction in absolute testicular weight, and alters marker testicular enzymes activity associated with spermatogenesis and germ cell maturation, along with marked histopathplogical changes showing depletion in germ cells and spermatogenic arrest[27]. Various organs were affected in the male reproductive system subjected to TCE . Enzyme induction and oxidative metabolism appear to be important in the systemic toxicity and may play a role in the reproductive toxicity of TCE. Oxidative metabolites of TCE are formed in the mouse epididymis resulting in epididymal damage, and at systematically toxic high doses, TCE may adversely affect the maturation of sperm and decreasing sperm motility [39].

This study revealed that the treatment of the TCE- intoxicated animals with vitamin C and/ or zinc induced marked amelioration of pathological lesions induced in testicular tissue (Fig. 6). The recovery group that was administered TCE for short term (3 weeks) then was left longer (for 15 weeks) without TCE administration, induced deleterious effects as oligospermia secondary to germ cells damage combined with testicular atrophy and interstitial fibroblasts cells proliferation. Many investigators reported nearly similar results and attributed their findings to disturbance in endocrine function[40].

Relation between DNA gel electrophoresis density(which indicate DNA degradation) for each organcorrelated with the corresponding biochemical parameters (kidney correlated with urea, creatinine and uric acid and testes correlated to testosterone). These correlations presented in curves with the regression line, equation and correlation coefficient (r) for long term groups in Fig.(7).The results show negative correlation between testes DNA degradation andtestosterone level. On the other hand there was a positive correlation between kidneys DNA degradation and each of the urea, creatinine and uric acid levels. These correlation confirm the biochemical results of both kidney and testes.

The present study also revealed that TCE has slight apoptotic effect on kidney and testicular tissues for short period of treatment, but inducedclear apoptotic effect after long period of treatment. On the other hand vitamin C and / or zinc supplementation for the treated rats with TCE resulted in anti-apoptotic potentials in the prevention of DNA damage in TCE – intoxicated rats (Fig. 8). Kidney tubule meganucleocytosis was reported in the male rats exposed to 300 and 600 ppm trichloroethylene[26, 29].Apoptosis detected by agarose gel electrophoresis [20]which is used to demonstrate the ladder pattern of DNA fragmentation (a hallmark of apoptosis) which is generated by endonucleolytic cleavage of genomic DNA into nucleasomal – size DNA of approximately 180 bp long (monomers) or oligonucleotides, which are multiple of 180 bp (oligomers).Toxic nephrosis was reported in rates(doses 500 and 1000 mg kg-1 bw) and mice (1000 mg kg-1 bw) administered trichloroethylene in corn oil by gavage 5 days per week for 103 weeks[31]. The workers of the Classification and Labelling ofDangerous Substances, on the advice of its specialist experts, considered that trichloroethylene should be regarded as an *in-vivo* mutagen in somatic cells. It is therefore classified as a category 3 mutagen in the EU[26].

The present study revealed that TCE- has slight apoptotic effect on kidney and testicular tissues for short period of treatment, but induced apoptosis after long period of treatment. On the other hand vitamin C and / or zinc supplementation for the treated rats with TCE resulted in antiapoptotic potentials in the prevention of DNA damage in TCE – intoxicated rats. The obtained apoptotic effect of trichloroethylene may be attributed to their cytotoxic consequence[41]. On the similar ground, TCE significantly potentiate the MPP (+) induced cell death associated with observed DNA fragmentation, which is one of the hallmarks of apoptosis. In addition, TCE markedly reduced the efflux of MPP (+), which is an inhibitor of multidrug resistance proteins (MRPs),mimicked the NSAIDs- induced effects, increasing cell toxicity and promoting the accumulation of MPP(+). These results suggest that TCE might cause a significant increase in the intracellular accumulation of MPP (+) via the suppression of reverse transport by the blockade of MRP, resulting in the potentiation of MPP (+) induced cell death[42].

CONCLUSION

Conclusion of that work indicates that receiving TCE orally for short or long periodscauses damagesat the biochemical, histopathological or molecular levels. Treatment with micronutrients as vitamin C and/or zinc improve that damage significantly in most cases.

Table (1): Body weight changes(g) and kidney and testes weights (g) of normal and TCE intoxicated groups after both short and long term studies.

	Normal groups				TCE-intoxicated groups				Recovery Samples were collected after 15 weeks
	Control	Vit. C	Zn	Vit. C+Zn	TCE	TCE + Vit.C	TCE + Zn	TCE + Vit. C+Zn	
	Short term (3 weeks)								
Initial and final body weight	137 ±4.24	135±4.67	144±3.30	140±3.47	138±10.21	123±1.65	140±2.36	135±1.78	151±11.22
	178 ±6.23	184±5.1	189±3.68	185±4.54	174±16.42	165±2.94	181±4.65	178±0.96	189±11.39
Net weight	41	49	45	45	36	42	41	43	38
Kidney weight	1.70 ±0.06	1.72 ±0.14	1.68 ±0.11	1.71 ±0.06	1.62 ±0.06	1.67 ±0.02	1.63 ±0.04	1.68 ±0.10	1.64±0.05
Testes weight	2.34 ±0.09	2.37±0.19	2.38 ±0.11	2.35±0.07	2.61 ±0.13	2.57±0.13	2.52±0.08	2.50±0.126	1.95 ±0.234
Long term (15 weeks)									
Initial and final body weight	130±6.41	131±1.25	137±3.07	126± 6.62	143.20±3.55	135±2.44	142±3.44	125±3.14	-
	246±6.62	281±11.27	288±7.19	278±19.94	201.40±5.39	195±4.51	204±5.65	188±4.24	-
Net weight	116	150	151	152	58	60	62	63	-
kidney weight	1.35 ±0.04	1.44 ±0.06	1.48±0.03	1.39±0.06	2.17±0.24	1.84±0.10	1.53±0.027	1.48±0.08	-
Testes weight	2.75 ±0.17	2.75±0.07	2.79±0.19	2.79±0.12	1.82±0.16	2.32±0.07	2.35±0.21	2.45 ±0.06	-

Table (2): Mean ±SE and Duncan test of renal functions and testosterone of normal and TCE intoxicated groups after both short and long term studies.

Measured Parameters	Normal groups				TCE-intoxicated groups				Recovery Samples were collected after 15 weeks
	Short term 3 weeks								
	Control	Vit. C	Zn	Vit. C+Zn	TCE	TCE + Vit.C	TCE + Zn	TCE + Vit. C+Zn	
Urea (mg/dl)	37.88±2.18^{c}	38.75±1.25^{c}	39.62±1.62^{c}	37.37±2.78^{c}	51.87 ±2.28^{a}	48.63±2.92^{ab}	48.75 ±4.86^{ab}	42.50±3.21^{bc}	45.75 ±5.15^{b}
Uric acid(mg/dl)	3.80±0.09^{b}	3.64±0.10^{b}	3.82±0.36^{b}	3.41 ±0.34b	4.98 ±0.27^{a}	4.48±0.43^{ab}	4.51±0.50^{ab}	4.36 ±0.38^{ab}	3.88±0.20^{b}
Creatinine (mg/dl)	0.59±0.03^{b}	0.52±0.03^{b}	0.56±0.03^{b}	0.57±0.02b	0.74±0.05^{a}	0.74±0.07^{a}	0.62 ±0.04^{ab}	0.60 ±0.01^{b}	0.58±0.02^{b}
T-Testosterone (ng/dl)	129.18± 12.68^{bc}	132.87± 13.09^{bc}	141.91± 6.19^{b}	171.32± 3.08^{a}	78.13± 10.15^{e}	85.55± 5.76^{de}	87.66± 5.76^{cd}	110.29± 9.15^{bc}	267.18± 16.51^{a}
	Long term 15 weeks								
Urea (mg/dl)	42.50 ±1.08^{b}	41.25±4.48^{b}	42.25±2.26^{b}	42.75±4.46^{b}	56.00±3.40^{a}	53.25±1.86^{ab}	49.87 ±4.51^{ab}	48.37 ±2.87^{ab}	-
Uric acid(mg/dl)	3.22±0.10^{b}	3.21 ±0.10^{b}	3.24±0.10^{b}	3.22±0.27^{b}	4.38±0.34^{a}	4.14±0.22^{a}	4.05±0.35^{ab}	3.94±0.20^{ab}	-
Creatinine (mg/dl)	0.53±0.03^{b}	0.51±0.03^{b}	0.53±0.03^{b}	0.51±0.02^{b}	0.73±0.06^{a}	0.67±0.02^{a}	0.62±0.04^{ab}	0.59±0.04^{ab}	-
T –Testosterone (ng/dl)	324.27± 16.42^{a}	338.46± 3.50^{a}	345.33± 9.72^{a}	347.00± 7.90^{a}	164.55± 21.88^{c}	202.44± 12.36^{c}	206.90± 25.48^{c}	253.44± 16.02^{b}	-

Data are means of 6 replicates ± SE. One way F- test of urea, uric acid, creatinine and testosterone are significant ($p<0.05$). Means in the same row have the same letter are not significantly different at 0.05.

Table (3):Mean ±SE and Duncan test of hematological parametersfor normal and TCE intoxicated groups after short term (3 weeks) study.

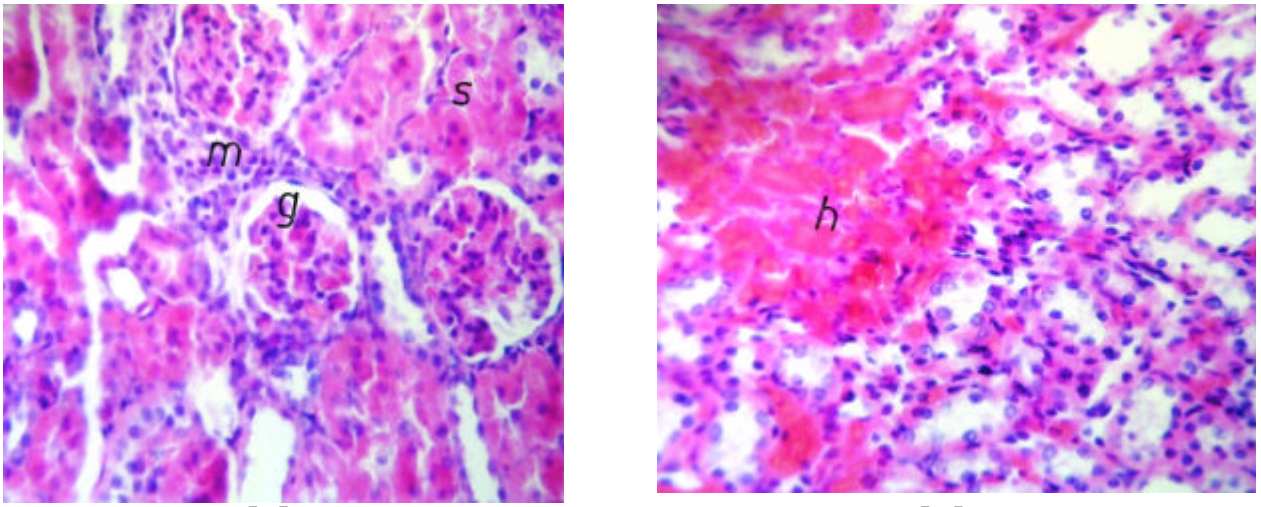
	Normal groups				TCE-intoxicated groups				Recovery Samples Were collected after 15 weeks
	Control	Vit. C	Zn	Vit. C+Zn	TCE	TCE + Vit.C	TCE + Zn	TCE + Vit.C+Zn	
Red blood cell counts($\times 10^6$µl)	5.26 ±0.34^{a}	5.33 ±0.29^{a}	5.55 ±0.29^{a}	5.60±0.26^{a}	3.60 ±0.11^{c}	3.79 ±0.08^{bc}	4.16 ±0.06^{bc}	4.31 ±0.10^{b}	4.93±0.28^{ab}
Hemoglobin(g/dl)	13.04 ±0.11^{a}	13.08 ±0.21^{a}	13.08±0.26^{a}	13.34±0.27^{a}	11.13±0.18^{d}	11.45±0.18^{cd}	11.99±0.22^{bc}	12.25±0.14^{b}	12.51±0.15^{b}
P.C.V %	36.50±0.65^{a}	39.50±0.65^{a}	37.75±0.48^{a}	37.25±0.85^{a}	36.25±2.28^{a}	36.25±0.48^{a}	37.00±0.41^{a}	38.25±0.85^{a}	37.57±1.14^{a}
M.C.V Fl	85.03±0.37^{a}	84.83±0.15^{a}	84.28±0.11^{a}	84.10±0.18^{a}	82.37±0.85^{b}	83.73±0.75b^{b}	83.53±0.19^{b}	85.30±0.16^{a}	84.00±1.08^{a}
M.C.H Pg	28.80±00.15^{a}	29.25±0.12^{a}	29.78±0.63^{a}	29.25±0.12^{a}	27.67±0.23^{c}	28.15±0.17^{ab}	28.18±0.85^{ab}	28.27±0.19^{a}	28.65±0.55^{a}
M.C.H.C %	36.35±0.13^{a}	36..20±0.09^{a}	36.65±0.09^{a}	36.75±0.65^{a}	33.10±0.07^{c}	34.62±0.12^{b}	35.37±0.19^{ab}	35.38±0.23^{ab}	36.40±0.13^{a}
Platelets count ($\times 10^3$µl)	265.00±8.66^{a}	265.60±8.78^{a}	272.50±15.66^{a}	270.00±15.58^{a}	189.13±9.46^{c}	201.25±6.11^{bc}	205.00±8.02^{bc}	225.63±8.83^{b}	262.50±8.81^{a}
White blood cell count($\times 10^3$µl)	4.64 ±0.19^{b}	4.65 ±0.17^{b}	4.59 ±0.16^{b}	4.50 ±0.12^{b}	7.75±0.24^{a}	7.03±0.66^{a}	7.00 ±0.24^{a}	6.10±0.21^{a}	5.40±0.37^{b}
Monocytes %	4.00 ±0.46^{a}	4.00±0.50^{a}	4.00±0.53^{a}	3.88 ±0.44^{a}	5.00±0.46^{a}	4.00±0.57^{a}	5.00±0.46^{a}	5.00±0.42^{a}	3.00±0.19^{a}
Lymphpcytes %	34.00 ±1.78^{c}	33.00±1.70^{c}	32.38±1.55^{c}	32.50±1.50^{c}	53.00±1.56^{a}	44.13 ±1.51^{b}	42.63±1.61^{b}	37.50±2.17^{c}	38.37±2.18^{c}
Basophils %	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}
Eosinophils %	2.00±0.32^{b}	3.00±0.42^{b}	2.00±0.27^{b}	2.00±0.27^{b}	5.00±0.56^{a}	5.00±0.26^{a}	3.00±0.53^{b}	2.50±0.42^{b}	2.00±0.00^{b}
Segmented neutrophils %	57.87±2.32^{a}	58.00±2.04^{a}	59.63±1.63^{a}	59.88±1.69^{a}	35.37±2.06^{c}	43.63 ±1.44^{c}	45.13±0.99^{b}	54.25±1.26^{ab}	53.37±2.36^{a}
Staff neutrophils %	1.00±0.26^{b}	1.00±0.27^{b}	1.00±0.00^{b}	0.75±0.00^{b}	1.88 ±0.22^{a}	2.25±0.31^{a}	2.00±0.00^{a}	1.00±0.19^{b}	1.25±0.31^{b}

Data are means of 6 replicates ± SE, means in the same row have the same letter are not significantly different at 0.05

Table (4): Mean ±SE and Duncan test of hematological parametersfor normal and TCE intoxicated groups after long term (15 weeks) study.

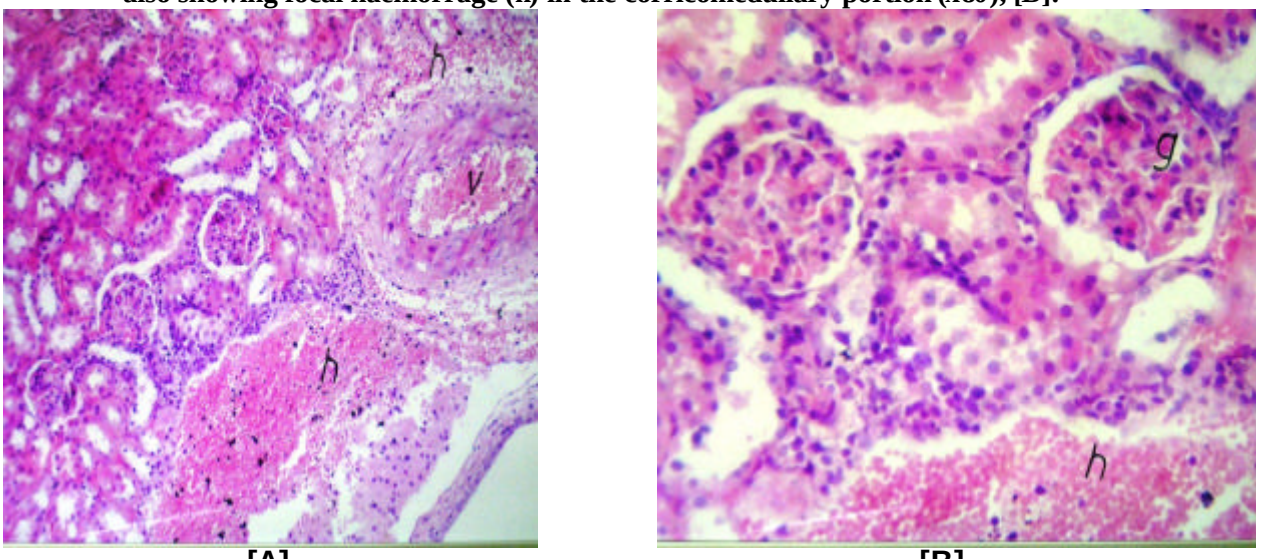
Groups / Variables	Normal groups				TCE-intoxicated groups			
	Control	Vit. C	Zn	Vit. C+Zn	TCE	TCE + Vit.C	TCE + Zn	TCE + Vit. C+Zn
Red blood cell counts ($\times 10^6$µl)	5.35±0.32^{a}	5.35±0.30^{a}	5.65±0.32^{a}	5.63±0.23^{a}	3.47±0.09^{d}	3.71±0.09^{cd}	4.12±0.06^{cd}	4.33±0.14^{bc}
Hemoglobin (g/dl)	13.17±0.09^{a}	13.27±0.20^{a}	13.40±0.29^{a}	13.27±0.15^{a}	10.91±0.09^{d}	11.15±0.09^{cd}	11.55±0.16^{c}	12.32±0.18^{b}
P.C.V %	44.02±1.06^{a}	43.50±1.19^{a}	44.05±1.31^{a}	45.00±1.05^{a}	39.50±1.55^{c}	42.05±1.09^{bc}	43.42±1.53^{ab}	43.10±0.31^{ab}
M.C.V Fl	87.75±1.88^{a}	86.37±0.24^{a}	86.52±1.68^{a}	88.17±2.26^{a}	76.07±3.05^{b}	76.35±1.87^{b}	76.82±2.30^{b}	78.32±1.47^{b}
M.C.H Pg	30.15±0.71^{a}	30.22±0.40^{a}	30.70±0.66^{a}	30.00±0.81^{a}	27.35±0.89^{c}	27.70±0.24^{c}	28.05±1.03^{b}	29.67±0.52^{ab}
M.C.H.C %	36.52±0.18^{a}	36.50±0.17^{a}	36.92±0.13^{a}	36.50±0.17^{a}	30.67±0.22^{c}	32.32±0.16^{bc}	33.70±0.12^{b}	34.60±0.11^{ab}
Platelets count ($\times 10^3$µl)	288.75±10.76^{a}	293.12±9.67^{a}	295.25±7.66^{a}	296.87±4.52^{a}	183.75±9.80^{d}	188.75±9.19^{d}	201.25±7.48^{cd}	220.62±9.93^{c}
White blood cell count($\times 10^3$µl)	5.21±0.29^{d}	5.08±0.35^{d}	5.05±0.38^{d}	5.07±0.39^{d}	14.05±0.74^{a}	13.50±0.65^{ab}	12.16±0.66^{bc}	10.90±0.37^{c}
Monocytes %	2.87±0.39^{c}	3.00±0.53^{c}	3.00±0.42^{c}	2.00±0.26^{c}	5.00±0.46^{a}	4.12±0.44^{ab}	4.00±0.37^{ab}	3.25±0.37^{bc}
Lymphpcytes %	36.00±1.33^{c}	36.13±2.24^{c}	35.63±1.66^{c}	35.50±1.21^{c}	67.00±1.89^{a}	65.38±1.58^{a}	60.75±2.21^{b}	56.38±1.73^{b}
Basophils %	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}	2.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}
Eosinophils %	2.00±0.00^{d}	2.25±0.25^{d}	2.00±0.19^{d}	2.00±0.00^{d}	5.00±0.33^{a}	4.00±0.42^{b}	3.00±0.57^{c}	3.00±0.33^{c}
Segmented neutrophils %	55.87±1.64^{a}	55.62±2.39^{a}	56.37±1.93^{a}	53.50±1.32^{a}	18.75±1.71^{d}	22.00±1.79^{d}	28.25±3.00^{c}	34.13±1.58^{b}
Staff neutrophils %	1.00±0.26^{c}	2.00±0.37^{c}	2.00±0.42^{c}	2.00±0.19^{c}	3.50±0.33^{a}	3.00±0.32^{ab}	3.00±0.37^{ab}	2.25±0.26^{bc}

Data are means of 6 replicates ± SE, means in the same row have the same letter are not significantly different at 0.05



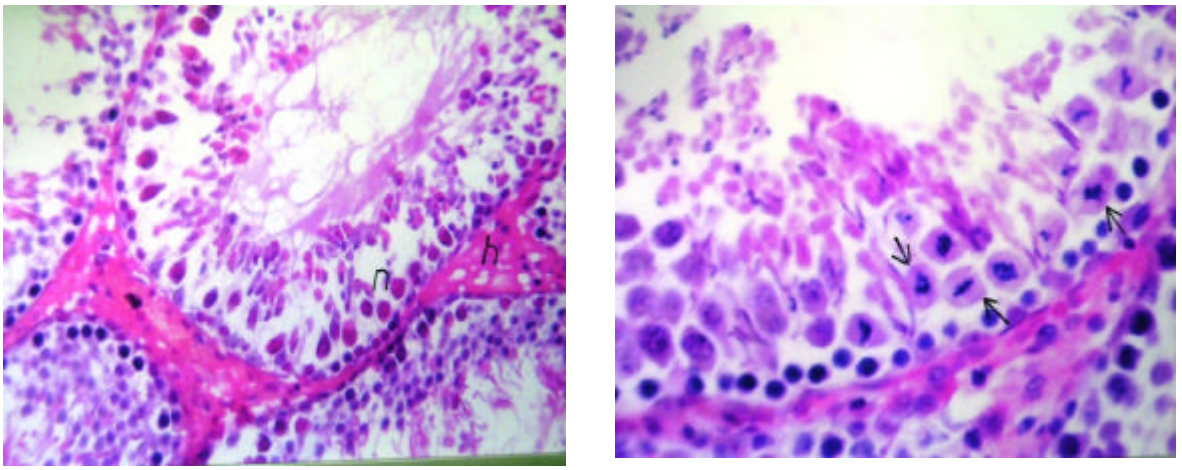
[A] **[B]**

Fig. (1): Histopathological sections (stained with H& E) of the kidney tissue of TCE-intoxicated group for 3 weeks showing focal inflammatory cells infiltration inbetween and surrounding the glomeruli (g) & tubules at the cortex with swelling in the tubula epithelium(s) (x80) [A], also showing focal haemorrage (h) in the corricomedullary portion (x80), [B].



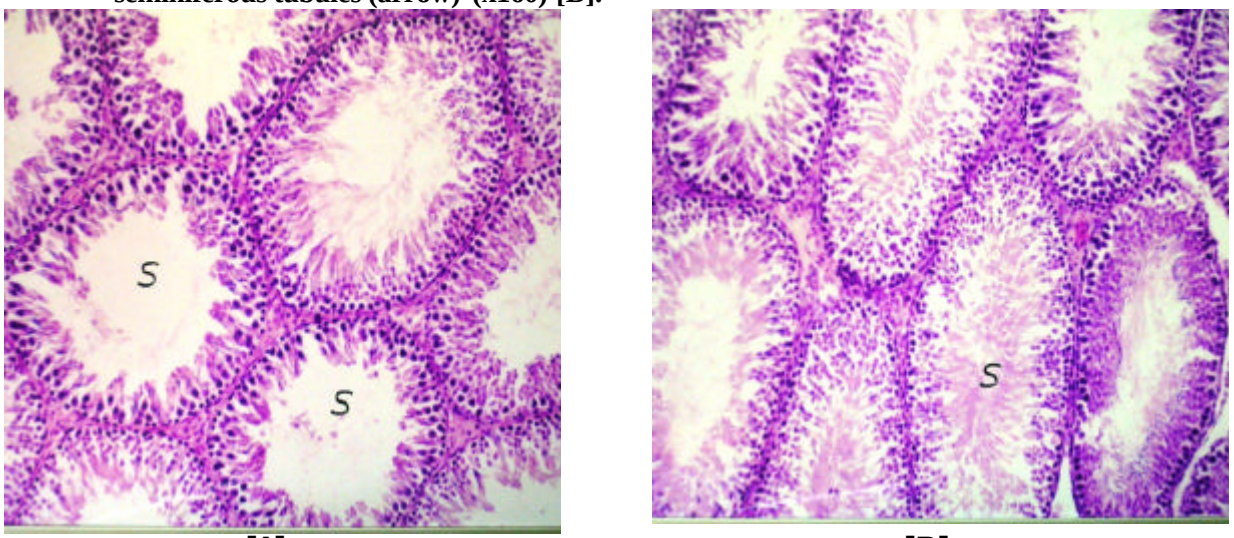
[A] **[B]**

Fig. (2): Histopathological sections (stained with H& E) of the kidney tissue of TCE-intoxicated group for 15 weeks showing severe congestion in sclerotic blood vessels (v) with focal haemorrage (h) (x40) [A], also showing focal inflammatory cells infiltration (g) in between the glomeruli& tubules (x80), [B].



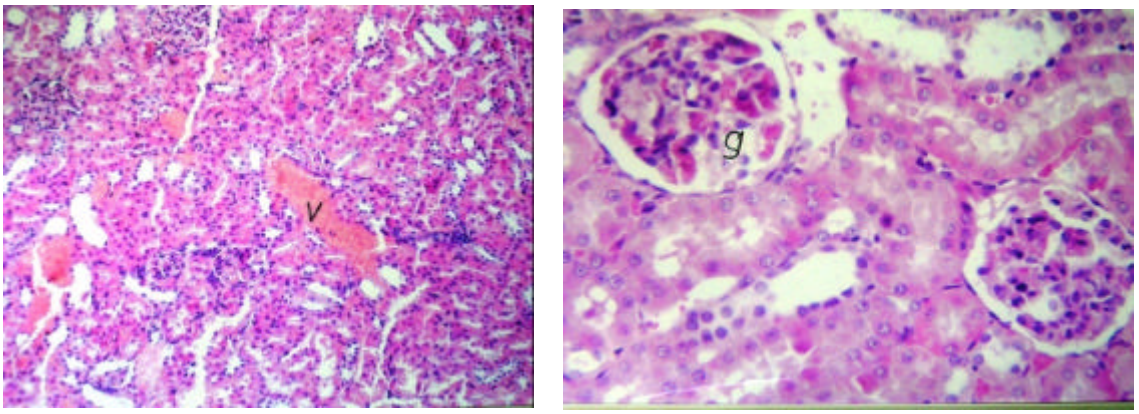
[A] **[B]**

Fig. (3): Histopathological sections (stained with H& E) of the testes tissue of TCE-intoxicated rats for 3 weeks, showing degenerated spermatogonial cells (n) in the lumen of the seminiferous tubules with appearance of homogenous eosinophilicalbuminous material in between (h) (x80)[A], also showing multinumber of sertoli cells in the lumen of the degenerated tubules and showing mitosis in the spermatogonial cells of some seminiferous tubules (arrow) (x160) [B].



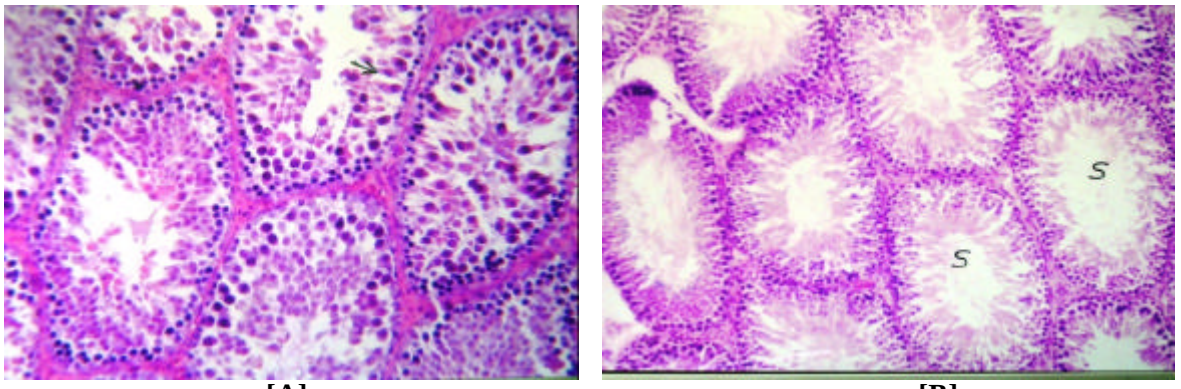
[A] **[B]**

Fig. (4): Histopathological sections (stained with H& E) of the testes tissue of TCE-intoxicated rats for 105 days, showing azospermia in most seminiferous tubular lumen (s) (x40) [A], also showing intact histological structure (s) (x40) [B].



[A] **[B]**

Fig (5): Histopathological section (stained with H& E) of the kidney tissue of TCE- intoxicated rats supplemented with vitamin C& zinc for 3 weeks showing congestion in the cortical blood vessels (v), (x 40) [A] kidney tissue of TCE- intoxicated rats supplemented with vitamin C& zinc for 15 weeks showing hyperemic glomeruli tuft (g), (x80) [B].



[A] **[B]**

Fig (6): Histopathological section (stained with H& E) of the testes tissue of TCE-intoxicated rats supplemented with vitamin C& zinc for 3 weeks showing the sertoli cells as predominant cells in seminiferous tubules(arrow)(x80) [A] testes tissue of TCE-intoxicated rats supplemented with vitamin C& zinc for 15 weeks showing intact histological structure (s)(x40) [B].

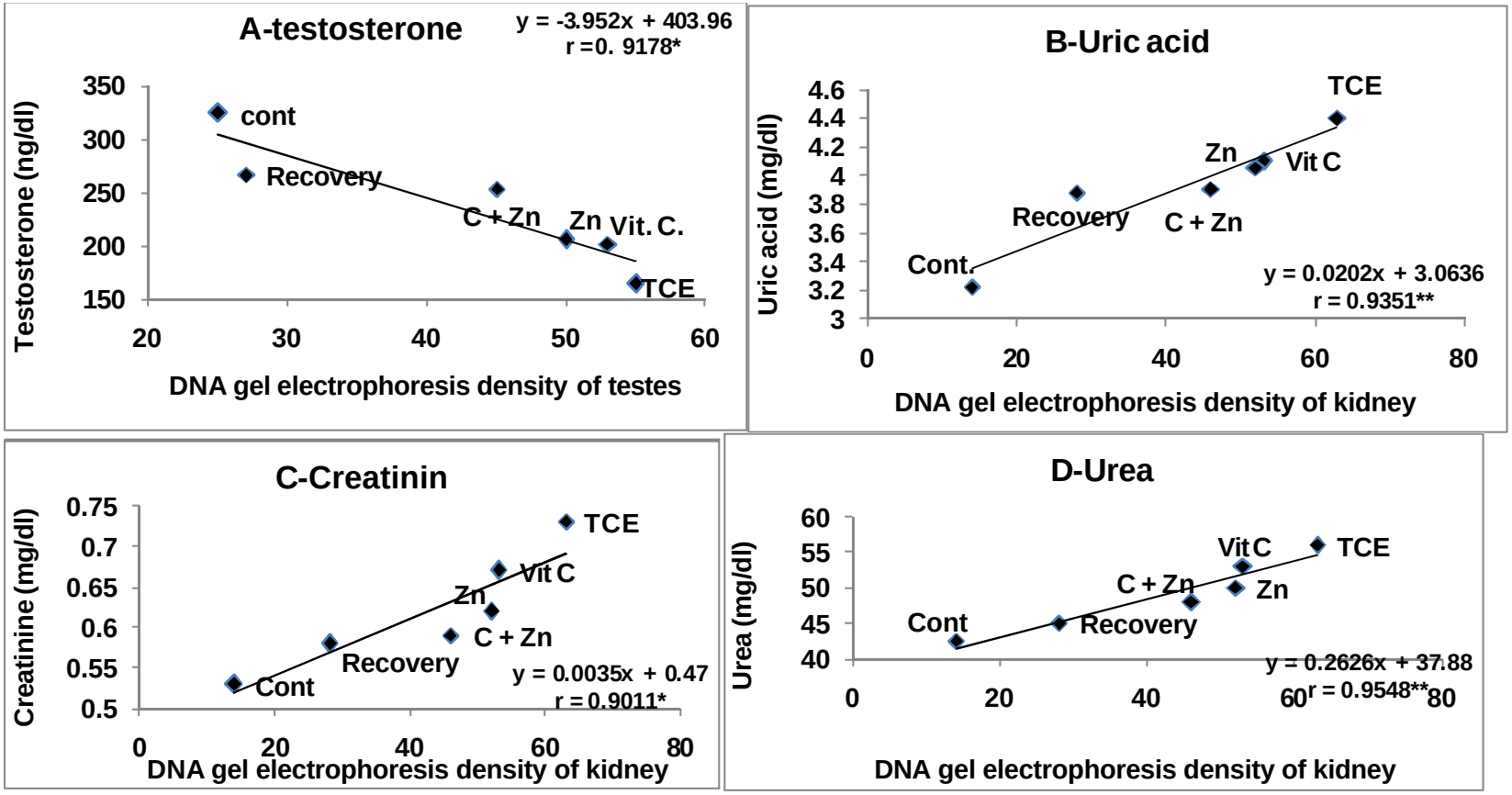
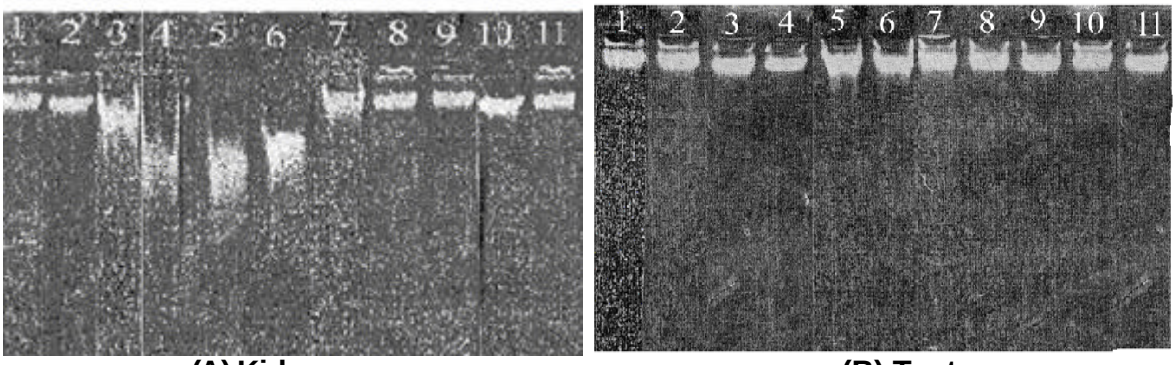


Figure (7): Correlation between DNA gel electrophoresis density of testes with testosterone (A) and of kidney with uric acid (B), creatinine (C) and urea (D) in long term (15 weeks) study groups



(A) Kidney **(B) Testes**

Figure(8):Apoptotic laddering pattern showed by Agarose Gel Electrophoresis of DNA fragments of kidney [A] and testes [B] tissues of normal and TCE- intoxicated rats and rats supplemented with vitamin C and / or zinc for long term.

Lane 1 &2: DNA fragments extracted from the normal rats.
Lane 3 &4: DNA fragments extracted from TCE- intoxicated rats for short term of treatment.
Lane 5 &6:DNA fragments extracted from TCE- intoxicated rats for long term of treatment.
Lane 7: DNA fragments extracted from TCE- intoxicated rats supplemented with vitamin C for long term of treatment.
Lane 8: DNA fragments extracted from TCE- intoxicated rats supplemented with zinc for long term of treatment.
Lane 9& 10: DNA fragments extracted from TCE- intoxicated rats supplemented with vitamin C and zinc for long term of treatment**.**
Lane 11: DNA fragments extracted from the withdrawal rats.

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