"Ameliorative role of Vitamin-C against *para*-nonylphenol induced toxicity in kidney of male *Mus musculus (P)*"

[1]Anil Binjhade and [2]Vinoy Kumar Shrivastava
Laboratory of Endocrinology, Department of Biosciences,
Barkatullah University, Bhopal. India-462026 (M.P.)

In the present study, the *para*-nonylphenol (p-NP) induced oxidative stress in the kidney of male *Mus musculus* and co-administration of vitamin-C can ameliorate any possible oxidative stress. For which 20 male *Mus musculus* (P) were divided in to four groups of 5 each. Group I served as control received normal diet and water adlibitum, group II exposed with (250 mg/kg bw) *para*-nonylphenol orally and Group III exposed with *para*-nonylphenol (250mg/kg bw) along with co-administered with Vitamin-C (10ppm) and While, Group IV supplemented with Vitamin-C alone (10ppm) for 30 and 60 days, and histopathological, enzymological *i.e.* GOT, GPT, ACP and ALP and biochemical parameter *i.e.* cholesterol and creatinine levels were carried out. In histological observations, noted that *para*-nonylphenol induced hypertrophied, degenerative and pyknotic change in renal cells after 30 and 60 days. While, the animals supplemented with vitamin-C alongwith *para*-nonylphenol up to 30 and 60 days showed recoveries in renal cells. In connection to this, the enzyme activities *i.e.* GOT, GPT, ACP and ALP and biochemical parameter *i.e.* cholesterol and creatinine levels were significantly elevated by *para*-nonylphenol in 30 and 60 days treatement, However, there change were become lowered towards normalcy when vitamin-C supplemented alongwith *para*-nonylphenol.

Key words: *para*-Nonylphenol, Vitamin-C, enzymes, *Mus musculus,* histopathology, oxidative damage**.**

Corresponding Author: Prof. Vinoy Kumar Shrivastava,
Laboratory of Endocrinology, Head Department of Bioscience,
Dean faculty of life sciences, Barkatullah University, Bhopal (M.P.) - 462026.
E-mail: vinoyks2001@yahoo.com

INTRODUCTION

para-Nonylphenol is an organic compound of the wider family of alkylphenols and it has xenobiotic properties. It is a product of industrial synthesis form during the alkylation process of phenols particularly in the synthesis of polyethoxylate detergents. It has also been found in polyvinyl chloride (PVC) used in the food processing and packaging industries. Nonylphenol (NP) is widely used as lubricating oil additives, plasticizers and surface-active agents. Apart from this, alkylphenols are also widely used as components in chemically synthesized products such as plastics, detergents, and other formulated products (Nimrod and Benson, 1996). Nonylphenol accumulates in plants and animals in the aquatic environment (Ahel *et al.*,., 1993). There are probably diverse routes of human exposure; not only via contaminated foods and drinking water, but also via dermal absorption or inhalation (Clark *et al.,* 1992; Ahel *et al.,* 1993). It is also known that NP is estrogenic in nature although *p*-nonylphenol is known as an endocrine disruptor. On the other hand, some previous reports have indicated that estrogenic agents cause free-radical mediated effects on various physiologic functions. NP also enhances the uterine DNA and protein synthesis in immature female rats (Lee and Lee, 1996). and disrupts the gonad development in neonatal rats (Nagao *et al.,* 2000).

As we known that vitamin-C (Ascorbic acid) is a water-soluble antioxidant and its role is to neutralize free radicals by donating electrons to free radicals such as hydroxyl and superoxide radicals and quench their reactivity" (Bendich, 1990). Vitamin-C protects the DNA of the cells from the damage caused by free radicals and mutagens. It prevents harmful genetic alteration within cells and protects lymphocytes from mutations to the chromosomes (Gaby and Singh, 1991). Vitamin-C prevents free radical damage in the lungs and may even help to protect the central nervous system from such damage (Kronhausen et al., 1989). Moreover, vitamin-C also protects us by preventing the development of nitrosamines, the cancer-causing chemicals that stem from the nitrates contained in many foods (Gaby and Singh, 1991). The investigations also showed that chronic administration of nonylphenol by animals leads to pathological changes in skin, esophagus, lungs, liver, kidneys and also urogenital tract (Sitting, 1981; Michałowicz and Duda, 2006; Korkmaz *et al.*, 2009a; Derai *et al*., 2013). Vitamin-C is found almost exclusively in foods of plant origin. Kidney is the only food of animal origin considered to be a significant source of vitamin-C. Particularly rich sources of vitamin-C are citrus and soft fruits, with most leafy green vegetables being moderately rich sources.There is a gradual decline in the amount of vitamin-C as food sage (Platt et al., 1963).

The beneficial and protective effect of combined administration of vitamins C and E on renal function (Derai *et al.*, 2013). Specifically, ascorbic acid, the known chelating agent with antioxidant features, was widely reported with the capability of protecting cells from oxidative stress (Ramanathan *et al.*, 2002; Patra and Swarup, 2004; Korkmaz *et al.*, 2009a).
Thus in this study we have tried to evaluate ameliorative role of vitamin-C against *para*-nonylphenol toxicity in renal of male *mus musculus* up to 30 and 60 days by observing hispathological and biochemical parameters *i.e.* ACP, ALP, GOT, GPT, cholesterol and creatinine levels.

MATERIALS AND METHODS:

Experimental Animals: In present investigation 20 sexually matured male *Mus musculus* (Parke's strain) weighing about 30±5 gms were brought from Govt. Veterinary College, Mhow, Dist. Indore (M.P.) and acclimated to laboratory conditions [22±3^{o}C room temperature and light and dark photoperiod (14L: 10D h)] in the animal house of Laboratory of Endocrinology, Bioscience Department, Barkatullah University, Bhopal (M.P.). Hygienic conditions were maintained with rice husk bedding in separate polypropylene cages as per ethical rules. Animals were fed on standard mice feed and tap water *ad libitum*.

Chemicals and Reagents: *para*-nonylphenol (Hi-media Analytical Chemicals) and Vitamin-C (ascorbic acid, analytical grade) were obtained from V.K. Traders, M.P. Nagar, Zone-2, Bhopal (M.P.). As *para*-nonylphenol is not soluble in water, so corn oil were used as a vehicle.

Experimental Design: Total 20 male *Mus musculus* were divided in four groups of five each, the first group received daily dose (0.2ml/day) of vehicle *i.e.* corn oil and fed with balanced diet and water *ad libitum*, served as control. Group second received *para*-nonylphenol (250mg/kg body weight/0.2ml/day) dissolved in corn oil, orally through cannula, while group third, received similar dose of *para*-nonylphenol as group second and supplemented with vitamin-C (10 ppm) through drinking water. The animals of fourth group were supplemented with vitamin-C (10 ppm) alone through drinking water for 30 and 60 days. Five animals from each group were sacrificed by cervical dislocation on day 31st and 61st and kidney were immediately dissected out, cleaned, dried and weighed. Some part of kidney were used for histopathological observation (Ehrlich 1886) and rest part were used for quantifying enzymological parameters *i.e.* GOT and GPT adopting the methodology of Reitman and Frankel, (1957), ACP and ALP (Bergmeyer method, 1963) and biochemical

parameters *i.e.* Cholesterol (Roeschlau *et al.*, 1974), and Creatinine (Jaffe's Colorimetric method by Toro and Ackermann, 1975).

Statistical analysis: All results were expressed as the mean ±S.E.M. from five animals per group. One way analysis of variance (ANOVA) followed by student's 't' test was used to determine the significance of the difference between the groups. Values of $P<0.05$ were considered statistically significant (Fisher and Yates, 1953).

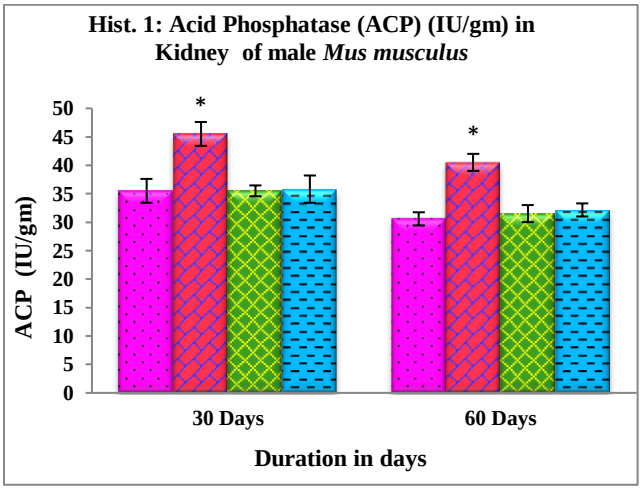
RESULTS:

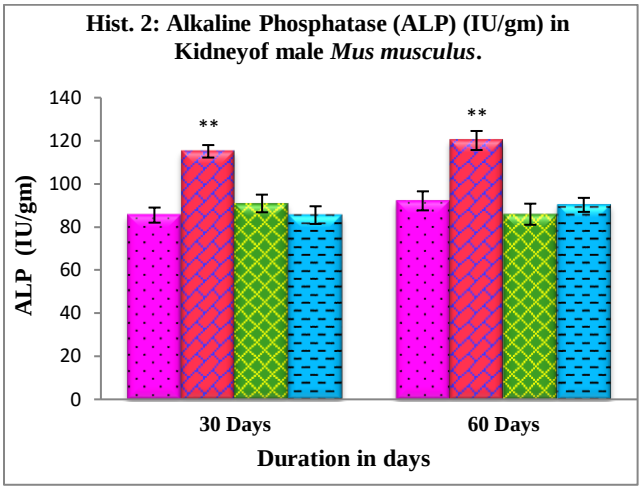
Enzymological activities and biochemical levels in Kidney of male *Mus musculus* (P)

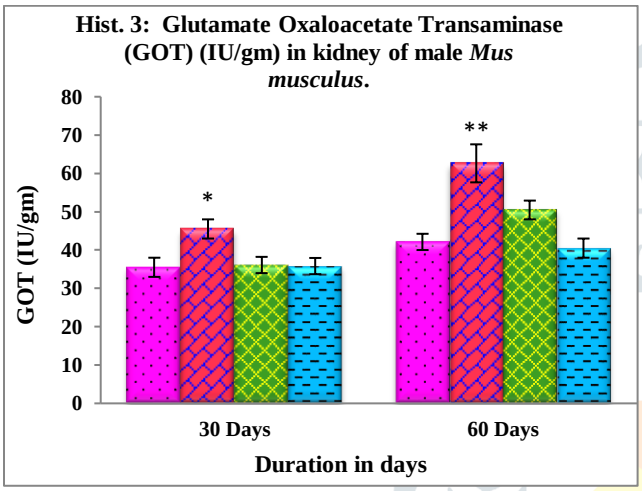
Our result find out from the Histogram 1, 2, 3 & 4 it was noticed that the animals showed a significant increased in their renal enzyme activities (ACP, ALP, GOT and GPT) of *para*-nonylphenol treated groups 30 and 60 days as compare to control group. However, the animals treated with *para*-nonylphenol along with vitamin-C showed ameliorative effects after 30 and 60 days similar to control group in their enzyme activity throughout the experiments. A part form this, the renal cholesterol and creatinine levels of male *Mus musculus* significantly increased in animals exposed with *para*-nonylphenol in all duration groups *i.e.* 30 and 60 days (Hist. 5 & 6). While, the animals supplemented with vitamin-C along with *para*-nonylphenol lowered these levels *i.e.* cholesterol and creatinine towards normal level in all duration *i.e.* 30 and 60 days group in comparison to *para*-nonylphenol treated groups. Whereas, vitamin-C alone supplemented group showed normal values of above mentioned enzymes and biochemical contents in all duration *i.e.* 30 and 60 days (Hist. 1, 2, 3, 4, 5 & 6)

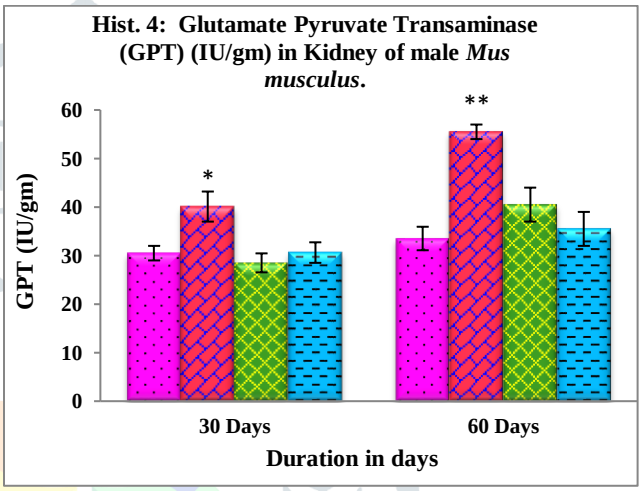
Renal histopathology:

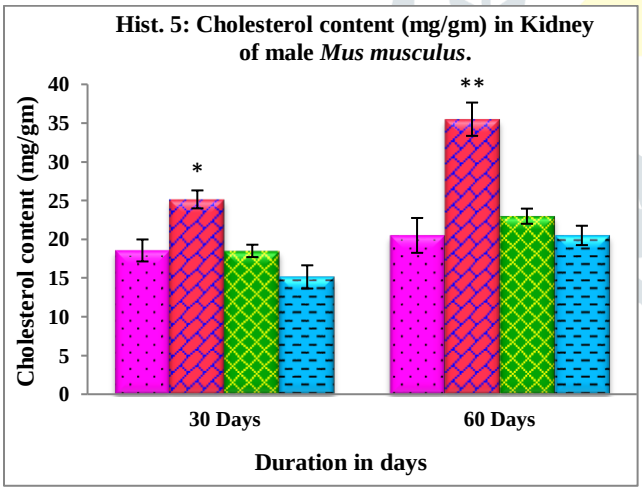
The microphotographs of the kidney of control *Mus musculus* showed proximal convoluted tubule with prominent and spherical nuclei, lumen were filed with brush border of all microvilli of cuboidal epithelial cells, normal glomerulus, Bowmen's capsules, collecting ducts and loop of Henley (figs. 1 & 7). While, the animals exposed with *para*-nonylphenol up to 30 days showed variable changes characterized by in the proximal convoluted tubules (figs. 3 & 9). After 60 days of *para*-nonylphenol treatment showed degenerative changes in proximal convoluted tubule and distal convoluted tubules contained lack of brush border of microvilli, glomerulus and Bowmens capsules (figs. 5 & 11). However, the animals treated with *para*-nonylphenol along with vitamin-C showed recoveries in histoarchitecture of kidney, showed normal cellular structure after 30 day 60 days as compared to *para*-nonylphenol exposed groups (figs. 4, 6, 10 & 12). In connection to this, the animals treated with vitamin-C alone showed normal histoarchitecture of renal tissue (figs. 2 & 8).

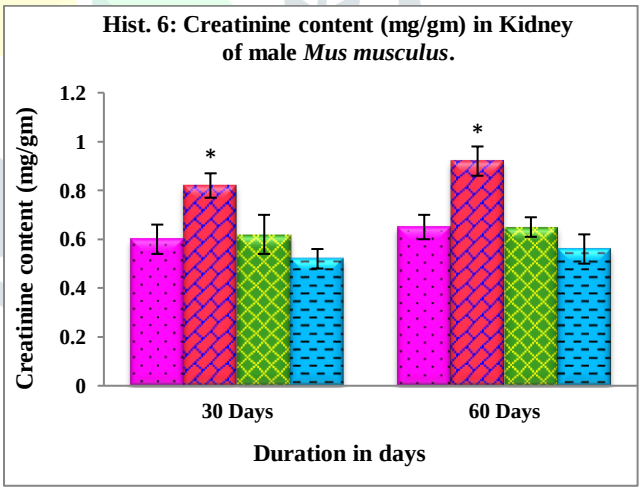










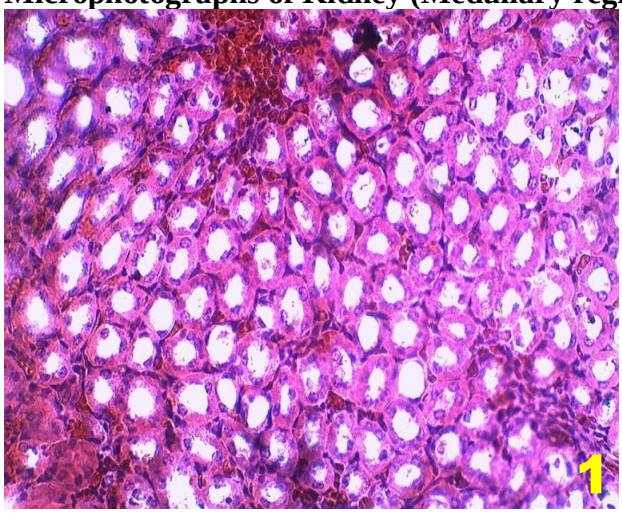


Where:- The experimental groups

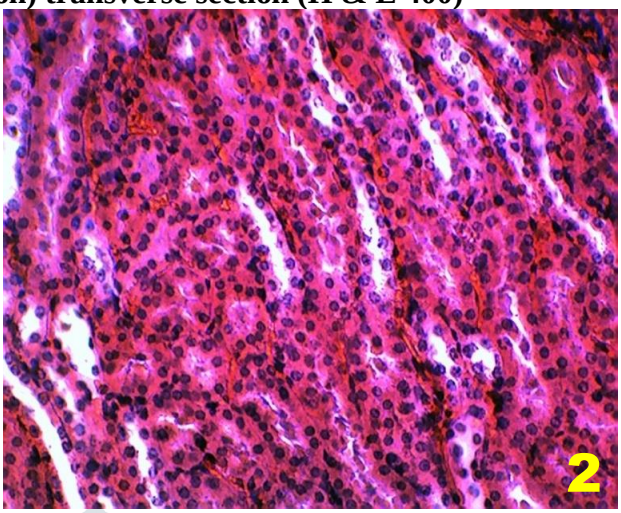
Control. **Treated with *para*-Nonylphenol.** ***para*-Nonylphenol with Vitamin-C.** **Vitamin-C**

± SEM of five animals
* Significant different ($p<0.05$) from the control vs treated by student's 't' test
** More significant different ($p<0.01$) from the control vs treated by student's 't' test
*** Highly significant different ($p<0.001$) from the control vs treated by student's 't' test

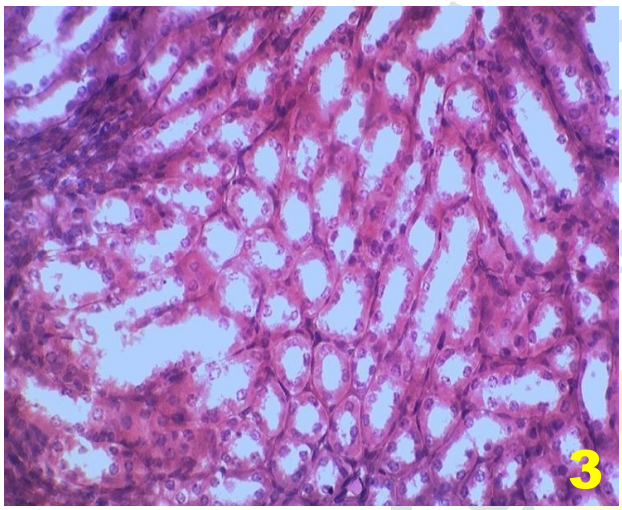
Microphotographs of Kidney (Medullary region) transverse section (H & E 400)



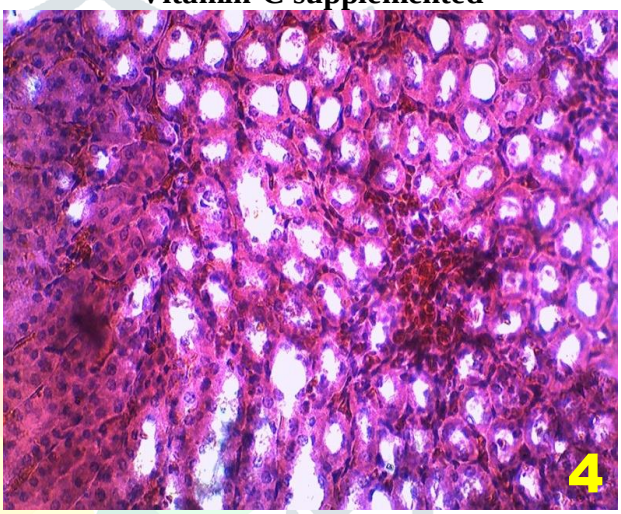
Control



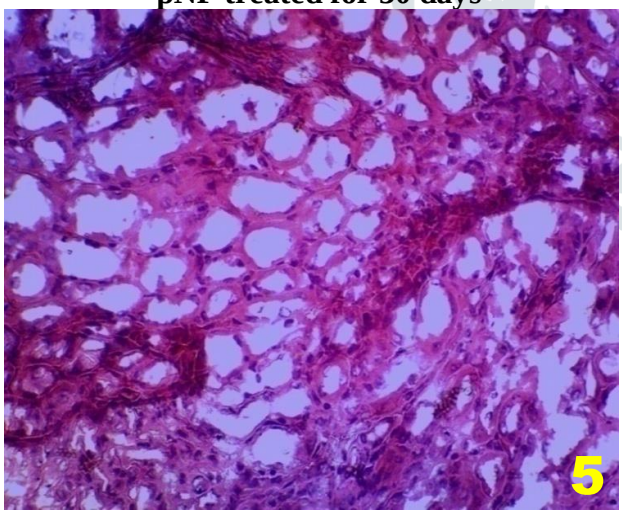
Vitamin-C supplemented



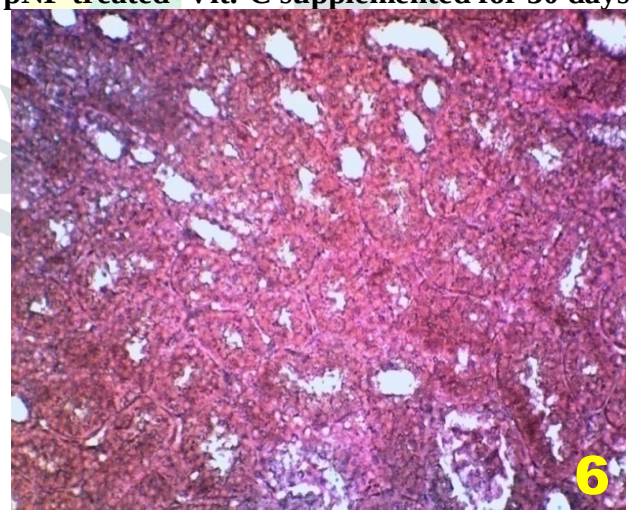
pNP treated for 30 days



pNP treated+Vit.-C supplemented for 30 days

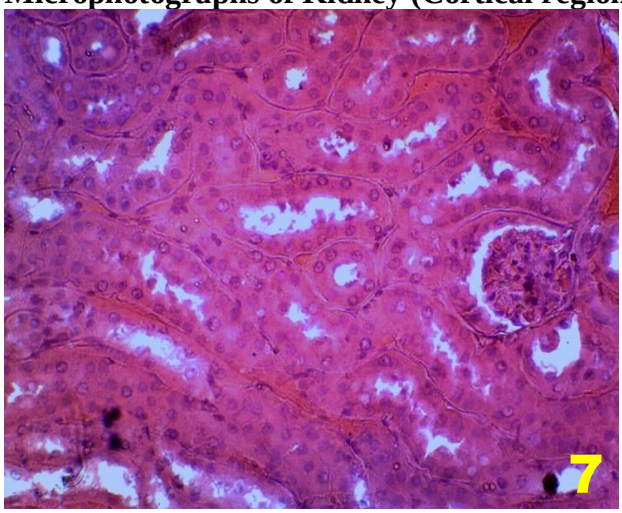


pNP treated for 60 days

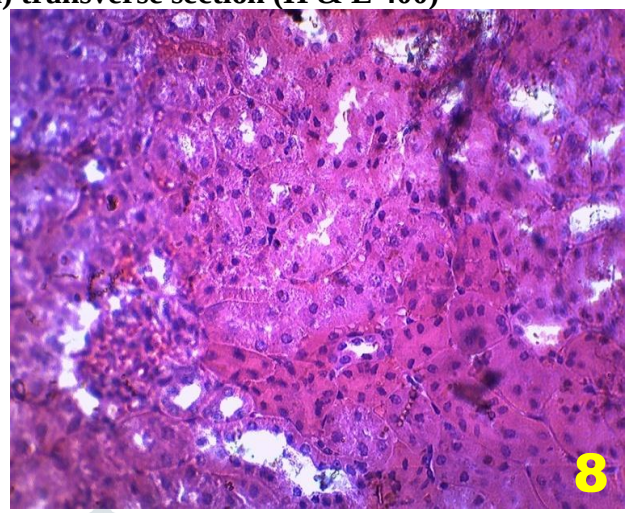


pNP treated+vit.-C supplemented for 60 days

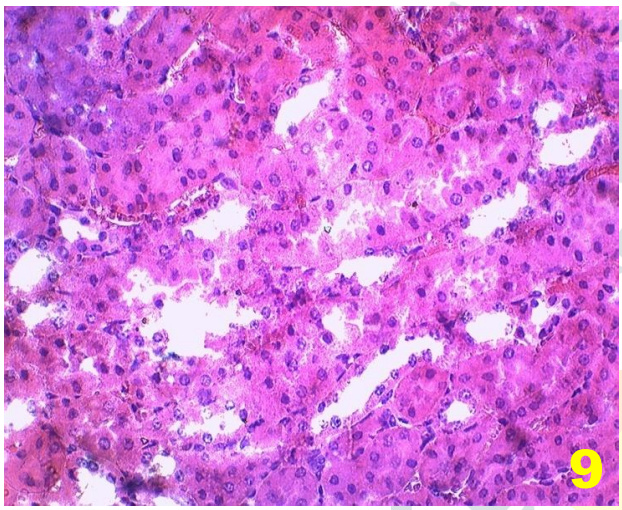
Microphotographs of Kidney (Cortical region) transverse section (H & E 400)



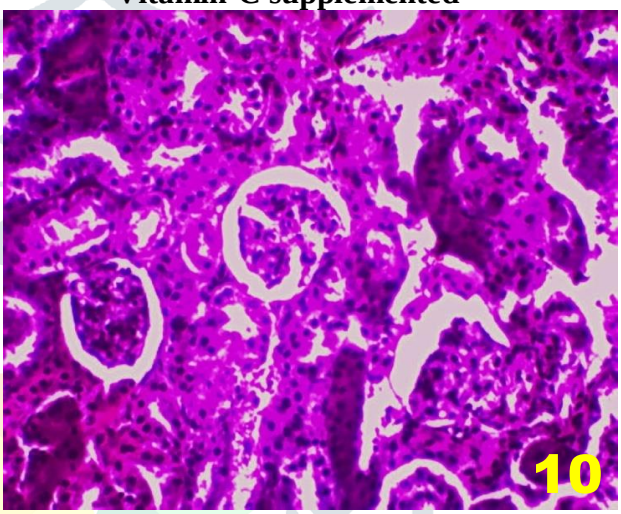
Control



Vitamin-C supplemented



pNP treated for 30 days



pNP treated+Vit.-C supplemented for 30 days

pNP treated for 60 days

pNP treated+vit.-C supplemented for 60 days

DISCUSSION:

Man-made environmental pollutants, especially those with estrogenic activity, have increased public concern regarding their effects on the health of reproductive system, digestive system, excretory system etc. A number of chemicals used in industrial processes, agriculture, and the production of various domestic products enter the body from the environment. Chemicals similar in structure to estrogen or androgen can bind to estrogen receptors (ERs) or androgen receptors (ARs) and interfere with the actions of endogenous steroid hormones. These chemicals are known collectively as endocrine disruptors (EDs). The risk posed by exposure to environmental EDs is very real, especially with respect to reproductive abnormalities during the early phase of life in human and wild animals (Witorsch, 2002; Kimura *et al.,* 2006; Lukacova *et al.,* 2013).

Kidney is large, bean shaped organ, located in the retroperitoneal tissue of the abdominal cavity. Medial border of the kidney is concave and contain a hilum through which the renal vessels and nerves pass and through which the expanded origin of the ureter, called the pelvis, leaves. A section through the kidney shows that these structures intermingle just within the hilum of the kidney, in a space called the renal sinus. The space between these structures filled largely with loose connective tissue and adipose tissue. The cut face of the kidney also shows that the substance of the kidney can be divided into an outer region, the cortex and an inner region, the medulla. A connective tissue capsule covers the outer surface of the kidney (Ross and Reith, 1985).

GOT is present in many tissues including liver, heart, muscle, kidney, and brain. It is released into serum during damage of tissue. GPT normally found largely in liver and is released into the blood stream as the result of liver and kidney injury. Increase of transaminase activity (GOT and GPT) along with decrease in the ALP activity may be the consequences of *para*-nonylphenol induced pathological changes in tissues. The increase in serum GOT and GPT accompanied by decrease of ALP enzyme activity is related to the intensity of cellular damage due to chemical induced cellular alteration varying from simple increase of metabolism to death of cell (Giray *et al.,* 2001; Cunny *et al.,* 2007; Mehranjani *et al.,* 2007; Gawande *et al.,* 2010; Jain *et al.,* 2012; Chouhan *et al.,* 2013). The GOT, GPT and ALP may be released to plasma, and serum levels of these enzymes would increase. High serum levels of GOT and GPT are usually indicative of liver and kidney damage in animals (Knights *et al.,* 1987; Gawande *et al.,* 2010; Jain *et al.,* 2012; Walaa *et al.,* 2015) and humans (Gil *et al.,* 1988; Ray and Drummond, 1991). The results of our investigation also support the reports of another study performed using antioxidants and renal substances (Patra *et al.,* 2001; Korkmaz *et al.,* 2009). ALP and ACP are known to be involved in a variety of metabolic activites such as permeability (Seth *et al.,* 1969), growth and cell differenttion (Dolder *et al.,* 2006), protein synthesis and gonadal maturation (Shaffi *et al.,* 1974) and steroidogenesis (Hayley *et al.,* 2009). Ram and Satyaneshan, (1985); Murmu and Shrivastava, (2011); Jain *et al.,* (2012) and Chouhan *et al.,* (2013) reported that the increase in the activity of ALP or ACP might be due to the necrosis of liver, kidney and other organs. In our work, an increase in ACP, ALP, GOT and GPT activities were noticed in liver and kidney of male mice after 30 and 60 days of *para*-nonylphenol treatment. However, neutralizing the enzymatic activities and restored near to the normal level are observed when the animals were exposed with *para*-nonylphenol with vitamin-C supplementation. These results were corroborated with Yanardag *et al.,* (2007) as he say that the combination of vitamin-C and Vitamin E has a protective role on chemical induced changes in lipid per oxidation, glutathione levels and antioxidant enzyme activities in liver, kidney and intestine tissue and in some serum parameters of rats.

Histopathology: In present investigation the kidneys from the animals exposed with *para*-nonylphenol showed variable changes in the proximal and distal convoluted tubules, contained lack of brush border of tall microvilli and degenerative changes in renal tubules. In accordance with these findings (Cunny *et al.,* 1997; Latendresse *et al.,* 2001; Rochelle *et al.,* 2006; Jain *et al.,* 2012; Walaa *et al.,* 2015) also observed degenerative changes in

kidney of rats/mice after the exposure of chemical toxicants. While, the animals exposed to *para*-nonylphenol and supplemented with vitamin-C showed recovery in renal histoarchitecture characterized with well-defined organized structure of renal tissue and prominent nuclei. It is well documented that vitamin-C have the ability to reduce the production of reactive oxygen species, the inhibition of protein and DNA synthesis caused by *para*-nonylphenol.

In case of cholesterol, they serve as the reservoir of high energy value and stored in the adipose tissue and act as an important component of cell membrane. The increased level of cholesterol may be due to increased synthesis and accumulation of it in the kidney or impaired biliary function and the increased level of triglycerides probably may be due to the cells apoptosis because the triglycerides are the main components of cell membrane (Shivanadappa and Krishnakumari, 1981) Our research work also mentioned significant increases in cholesterol were recorded in animals treated with *para*-nonylphenol for 30 and 60 days respectively. A similar result was obtained by Baker *et al.,* (1978) in rats treated with specific phenols. Mehranjani *et al.,* 2007; Nassr-Allah and Hameid, 2007**;** Nahed and Saad, 2008; Riva *et al.,* 2010 reported that phenolics affected triglycerides and cholesterol in different experimental mammals. Mehranjani *et al.*, (2007); Korkmaz *et al.,* (2009a&b); Walaa *et al.,* (2015) also observed increase in levels of cholesterol and phospholipids; it may be the result of enhanced biosynthesis of these compounds. It is speculated that treating mice with *para*-nonylphenol increased tissue lipogenesis and probably this has been achieved through acceleration at acetyl-Co-A to be the precursor of cholesterol biosynthesis. However, present investigation showed that these increased values in cholesterol concentration was decreasing to its normal value after the supplementation of vitamin-C along with *para*-nonylphenol to the experimental animals throughout the experiment. The value of cholesterol; become normal after supplementation of vitamin-C is may be due to vitamin-C has nutritive anti-oxidation properties, which can neutralized the free radicals generated and react directly with peroxides, and executed an important antioxidant function as generated by *para*-nonylphenol.

Creatinine serves as the high energy phosphate storehouse in muscle and it is a end product of creatinine metabolism. Creatinine is largely formed in muscle by the irrepressible and non-enzymatic removal of water from creatine phosphate. Creatinine in the waste products of protein metabolism that need to excreted by the kidney therefore marked increase in serum creatinine as noticed in this study conform an indication of functional damages of kidney (Garba *et al.,* 2007). Serum creatinine is increased in renal failure. Elevate values are also observed in certain other conditions like congestive heart failure, shock and mechanical obstruction of the urinary tract. The present study revealed significant increase in creatinine levels after exposure of *para*-nonylphenol for 30 and 60 days respectively. Increase in creatinine level may suggest that the *para*-nonylphenol may induce kidney dysfunction. Present study was also supported by Cooper *et al.,* (2006) and Jain *et al.,* (2012) reported a increase in creatinine in *para*-nonylphenol treated male mice. Similar report was recorded by Cunny *et al.,* 1997; Antonia *et al.,* 2005 in rats treated with phenolics. While, supplementation of vitamin-C along with *para*-nonylphenol on experimental animals revealed no changes on creatiñine value as compare to control animals throughout the experiment. Means vitamin- C has some properties to normalize the *para*-nonylphenol effect on *Mus musculus*.

In a study Dogan and Kolankaya, (2005), showed that oral administration of anzar honey which contain large amounts of vitamin-C prevents ethanol induced oxidative damage in the rat stomach also, they demonstrate that vitamin-C exert protective effects against ischemia/reperfusion induced renal injury (Korkmaz and Kolankaya, 2009b). However, there are limited investigations concerning the effect of vitamin-C on endocrine disrupting chemicals-induced oxidative stress in the tissues.

In fine, it is revealed by these finding that vitamin-C is an important antioxidant for the neutralizations of many free radicals and we should include it in our daily diet.

ACKNOWLEDGEMENTS

Authors are very grateful to Ministry of Higher Education, Government of Madhya Pradesh for financial support and head of the Department of Bioscience and Dean Faculty of Life sciences for providing all infrastructural facilities for the research work.

REFERENCES:

Ahel M., McEvoy J. and Giger W., (1993): Bioaccumulation of the lipophilic metabolites of nonionic surfactants in freshwater organisms. *Environ Pollut*., 79: 243–248.

Antonia C.M., Zsuzsanna K., John A., Reidy S.P., Caudill J.E. and Larry L.N. (2005): Urinary Concentrations of Bisphenol A and 4-Nonylphenol in a Human Reference Population. *Environmental Health Perspectives*., 113(4): 25-32.

Baker E.L., Landrigan P.J., Bertozzi P.E., Field P.H., Basteyns B.J., and Skinner HG (1978): Phenol poisoning due to contaminated drinking water. *Arch Environ Health*., 33: 89-94.

Bendich A., (1990): "Antioxidant micronutrients and immune responses". In: Bendich, A. and Chandra, R.K. Micronutrients and immune functions. N.Y. *Academy of Sciences*, N.Y, (eds.): 175.

Bergmeyer H.U. and Bernt E., (1963): In methods of enzymatic analysis, edited by H.U. Bergmeyer. *Academic press, Weimheim, N.Y. and London.*, (eds):pp 837.

Chouhan S., Khan N., Chauhan R., Raghuwanshi R.K. and Shrivastava V.K., (2013): Cyclophosphamide induced changes in certain enzymological (got gpt acp and alp) parameters of adult male *rattus norvegicus*. *IJRRPAS.*, 3(1): 155-163.

Clark L.B., Rosen R.T., Hartman T.G., Louis J.B., Suffet I.H., and Lippincott R.L., (1992): Determination of alkylphenol ethoxylates and their acetic acid derivatives in drinking water by particle beam liquid chromatography/electrospray mass spectrometry. *Int J Environ Anal Chem.*, 47: 167-180

Cooper S., Latendresse J.R., Doerge D.R., Twaddle N.C., Fu X. and Delclos K.B., (2006): Dietary modulation of p-nonylphenol-induced polycystic kidneys in male Sprague–Dawley rats. *Toxicol Sci.*, 91: 631–42.

Cunny H.C., Mayes B.A., Rosica K.A., Trutter J.A. and Van Miller J.P. (1997): Subchronic toxicity (90-day) study with para-nonylphenol in rats. *Regul.Toxicol.Pharmacol*., 26: 172–178.

Derai E.l.h., Kechrid Z. and Bouhafs L. (2013): The beneficial effect of combined administration of vitamins C and E on renal function and selected parameters of antioxidant system in diabetic rats fed zinc-deficient diet. *African Journal of Biotechnology*., Vol. 12(43): 6232-6240

Dogan A. and Kolankaya D. (2005): Protective effects of Anzer honey against ethanol induced vascular permeability in the rat's stomach. *Exp. Toxicol. Pathol*., 57, 173-178..

Ehrilich P. (1886): Fragekasten Zeitschnft Fur Wissenschaflliche Mikroskopic and Fur Mikroskopische Technik., 3: 150.

Fisher R.A. and Yates F. (1953): Statistical tables. London: Oliver and Boyd (eds.).

Gaby S.K., and Singh V.N. (1991): "Vitamin C," – Vitamin intake and health: A Scientific Review, Gaby, S.K., Bendich, A., Singh, V. and Machlin, L. (eds.). Marcel Dedder, N.Y.

Garba S.H., Adelaiye A.B. and Mshelia L.Y. (2007): Histopathological and biochemical changes in the rats kidney following exposure to a pyrethroid based mosquito coil. *Journal of applied sciences research*, 3: 1788-1793.

Gawande M.R., Murali N., Binjhade A. and Shrivastava V.K. (2010): Carbendazim induced Histopathological changes in Adrenal, Thyroid Glands and some Enzyme Activities in Adrenal Gland of *Rattus rattus*. *International Journal of Biological Technology.*, 1(2): 38-43

Giray B., Gurbay A. and Hincal F. (2001): Cypermethrin-induced oxidative stress in rat brain and liver is prevented by vitamin E or allopurinol. *Toxicol Lett*., 118: 139-146.

Hayley M., Scott J., Mason I. and Richard M.S. (2009): Steroidogenesis in the Fetal Testis and Its Susceptibility to Disruption by Exogenous Compounds, *Endocrine Reviews.*, 10: 2009-0016.

Jain N., Shrivastava R., Raghuwanshi A.K. and Shrivastava V.K. (2012): aspirin induced changes in serum ACP, ALP, GOT, GPT, bilirubin and creatinine in corelation with histopathological changes in liver and kidney of female albino rat. *International journal of pharma*., 4(3): 9-12

Kimura N., Kimura T., Suzuki M. and Totsukawa K. (2006): Effect of gestational exposure to Nonylphenol on the development and fertility of mouse offspring, *J. Reprod. Dev*., 52, 789-795

Knights K.M., Gourlay G.K., Hall P.M., Adams J.F. and Cousins M.J. (1987): Halothane hepatitis in an animal model: time course of hepatic damage. *Br. J. Exp. Pathol*., 68: 613—624

Korkmaz A., Aydogan M., Kolankaya D. and Barlas N. (2009b): Vitamin-C co-administration augments bisphenol A, nonylphenol and octylphenol induced oxidative damage in kidney of rats. *Environ. Toxicol*., in press., 10.1002/tox.20556.

Korkmaz, A., Kolankaya D. (2009a): The protective effects of ascorbic acid against renal eschemia-reperfusion injury in male rats. *Renal Fail*., 31: 36-43.

Kronhausen E., Kronhausen P.D. and Harry B. (1989): Formula for Life (eds.). William Morrow and Co., New York.

Lee P.C. and Lee W. (1996): *In vivo* estrogenic action of Nonylphenol in immature rats. *Bull Environ Contam Toxicol.*, 57: 341–348.

Lukacova J., Zuzana K., Eva T., Peter M. and Norbert L. (2013): The toxic effect of 4-nonylphenol on male reproductive system. *(Special issue on BQRMF).*1455-1468.

Mehranjani M.S., Abnosi M.H., Naderi A. and Mahmodi M. (2007): Preventive effects of wheat germ oil on sex hormones, liver enzyme, lipids and proteins in rat serum following treatments with p-nonylphenol. *Journal of Biological Sciences*., 7: 1406-1411.

Michałowicz J., Duda W. (2006): Phenols – Sources and Toxicity; *Polish J. of Environ. Stud. Vol. 16, No. 3 (2007)*,347-362

Murmu S. and Shrivastava V.K. (2011): Protective Action of an Anti-oxidant (Vitamin-C) Against Bisphenol-toxicity in *Cirrhinus mrigala* (Ham.) *Turkish Journal of Fisheries and Aquatic Sciences.*, 11: 25-29.

Nagao T., Saito Y., Usumi K., Nakagomi M., Yoshimura S. and Ono H. (2000): Disruption of the reproductive system and reproductive performance by administration of nonylphenol to newborn rats. *Human Exp Toxicol.*, 19: 284–296.

Nahed S. G. and Amal S. S. (2008): Effect of Environmental Pollution by Phenol on Some Physiological Parameters of *Oreochromisniloticus. Global Veterinaria.*, 2 (6): 312-319.

Nassr-Allah H., Abdel H. (2007): Physiological and Histopathological Alterations Induced by Phenol Exposure in *Oreochromis aureus* Juveniles. *Turkish Journal of Fisheries and Aquatic Sciences.* 7: 131-138.

Nimrod A.C., Benson W.H. (1996): Environmental estrogenic effects of alkylphenol ethoxylates. *Crit Rev Toxicol.,* 26: 335-364.

Patra R., Swarup D., Dwivedi S. (2001): Antioxidant effects of tocopherol, ascorbic acid and L-methionine on lead induced oxidative stress to the liver, kidney and brain in rats. *Toxicology.,* 162: 81–8.

Platt B.S., Eddy T.P. and Pellett P.L. (1963): Food in Hospitals. Anonymous (Eds.). Nuffield Provincial Hospitals Trust and Oxford University Press, London.

Ram R. and Sathayanesan A.G. (1985): Mercuric chloride, cythion and ammonium sulfate induced changes in the brain, liver and ovarian alkaline phosphatase content in the fish *Channa puntactus*. *Environ Ecol.,* 3: 263–268.

Ramanathan K., Balakumar B., Panneerselvam C. (2002): Effects of ascorbic acid and alpha tocopherol on arsenic induced oxidative stress. *Hum Exp Toxicol*., 21: 675–80.

Reitman S. and Frankel D. (1957): A Calorimetric Method for the determination of serum glutamate oxaloacetic and glutamic pyruvic transaminase. *A.M.J. Clin. Pathol.*, 28: 56-63.

Riva C., Porte C., Binelli A. and Alfredo P. (2010): Evaluation of 4-onylphenol in vivo exposure in Dreissena polymorpha:Bioaccumulation, steroid levels and xidative stress. *Comparative Biochemistry and Physiology.*, 152: 175–181.

Rochelle W.T., Christina B., Myers M.C., Marr N.P., Castillo J.C., Seely C.S., Sloan M.M., Veselica R.L. Joiner John P., Van M. and Glenn S.S. (2006): Three-Generation Evaluation of Dietary *para*-Nonylphenol in CD (Sprague-Dawley) Rats. *Toxicol. Sci.*, 92(1): 295–310.

Roeschlau P., Bernt E. and Gruber F. (1974): Clinicle Chemistry. *Clin.Biochem*., 12: 226.

Ross M.H. and Reith E.J. (1985): Histology A Text and Atlas, (ed. 1, Harper International), Harper & Row, Publishers (Eds.), J.B. Lippincott Company New York, ISBN 0-06-350617-3.

Seth J.S., Tewari H.B. and Sood P.P. (1969): On the distribution pattern of alkaline phosphatase activities and their functional significance amongest the spinal ganglion cells of squirrel. *Acta.Neuro.Belga*., 69: 51-57.

Shaffi S.A., Jafri A.K., Khawaja D.K. (1974): Alkaline phosphatase activity in the ovary of the cat fish *Clarius batrachus* (Linn) during maturation. *Curr. Sci.,* 43: 51-52.

Shivanadappa T. and Krishnakumari M.K. (1981): Histochemical and biochemical changes in rats fed dietary benzene hexachloride. *Indian Journal of Experimental Biology.,* 19: 1163-1168.

Sitting M. (1981): Handbook of Toxic and Hazardous Chemicals Park Ridge (eds.), NJ: Noyes Data Corporation, 185.

Toro G. and Ackermann P. (1975): Quantitative determation of creatinine in serum or heparinized plasma or urine by colometric kinetic method. Practical little brown and company boston, 237-238.

Walaa M.S. Ahmed, Walaa A. Moselhy and T.M. Nabil (2015): Bisphenol A Toxicity in Adult Male Rats: Hematological, Biochemical and Histopathological Approach, *Global Veterinaria.*, 14 (2): 228-238.

Witorsch R.J. (2002): Endocrine disruptors: can biological effects and environmental risks be predicted. *Regul Toxicol Pharmacol.*, 36: 118–130.

Yanardag R., Ozsoy-Sacan O., Ozdil S. and Bolkent S. (2007): Combined effects of vitamin C, vitamin E, and sodium selenate supplementation on absolute ethanol-induced injury in various organs of rats. *Int. J. Toxicol*., 26: 513-23.