JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

"Ameliorative Role of Ascorbic Acid Against *Para*-Nonylphenol Induced Toxicity In Liver of Male *Mus Musculus (P)*"

Anil Binjhade, Manohar Rao Gawande and Vinoy Kumar Shrivastava
Laboratory of Endocrinology, Department of Biosciences,
Barkatullah University, Bhopal India 462026 (M.P.)

Correspondence Author: Prof. Vinoy Kumar Shrivastava,
Laboratory of Endocrinology, Head Department of Bioscience, Dean Faculty of Life Science, Barkatullah University, Bhopal (M.P.) – 462026. E-mail: vinoyks2001@yahoo.com

Present investigation aimed to study, whether *para*-nonylphenol (p-NP) induced, oxidative stress in the liver of male *Mus musculus* and co-administration of ascorbic acid 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 receved normal diet and water adlibitum, group II exposed with (250 mg/kg/0.2ml/day) *para*-nonylphenol orally and Group III exposed with *para*-nonylphenol along with co-administered with ascorbic acid (10ppm) and While, Group IV supplemented with ascorbic acid alone for 30 and 60 days, and hepatic 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 pyeknotic change in hepatocytes after 30 and 60 days. While, the animals supplemented with vitamin-C alongwith *para*-nonylphenol up to 30 and 60 days showed recoveries in hepatic 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, ascorbic acid, enzymes, *Mus musculus*, histopathology, oxidative damage**.**

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 [1]. Nonylphenol accumulates in plants and animals in the aquatic environment [2]. There are probably diverse routes of human exposure; not only via contaminated foods and drinking water, but also via dermal absorption or inhalation [3, 2]. 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 [4] and disrupts the gonad development in neonatal rats [5] .

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" [6]. 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 [7]. Vitamin-C prevents free radical damage in the lungs and may even help to protect the central nervous system from such damage [8] . 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 [7]. Hepatic damage induced by lead has been reported to be neutralized by a combination of ascorbic acid and thiamine [9]. Protective effects of vitamin-C against chlorpyrifos poisoning on haematological and biochemical changes were reported [10]. Vitamin-C plays an important role in pesticide

and chemical toxicity protection especially in the hepatic toxicity as antioxidant agent and prevents the effect of free radicals for vital cells [11, 12,13,14,15].

Thus in this study we have tried to evaluate ameliorative role of vitamin-C against *para*-nonylphenol toxicity in hepatic 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°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 livers were immediately dissected out, cleaned, dried and weighed. Some part of liver were used for histopathological observation [16] and rest part were used for quantifying enzymological parameters *i.e.* GOT and GPT adopting the methodology of [17], ACP and ALP [18] and biochemical parameters *i.e.* Cholesterol [19] and Creatinine [20] (Jaffe's Colorimetric method).

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 [21].

RESULTS:

Body Weight:

The animals treated with *para*-nonylphenol showed decrease in their body weight throughout the experiment, and this decrease was more significant in later part of the experiments *i.e.* 60 days as compared to control groups. While, the recoveries in their body weight were noticed when vitamin-C were given with *para*-nonylphenol to the experimental animals. However, the animals exposed with vitamin-C alone only showed a constant increase in their body weight throughout the experiment (fig. 1).

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

The hepatic enzyme activities *i.e.* ACP, ALP, GOT and GPT were significantly elevated after the *para*-nonylphenol exposures in both duration *i.e.* 30 and 60 days as compared to control groups (figs. 2, 3, 4 & 5). While, the animals supplemented with vitamin-C along with *para*-nonylphenol, these enzyme activity (*i.e.* ACP, ALP, GOT and GPT) were lowered towards normalcy in 30 and 60 days groups in comparison to *para*-nonylphenol exposed groups (figs. 2, 3, 4 and 5). In connection to this, the animals exposed with *para*-nonylphenol also significantly elevated cholesterol and creatinine levels after *i.e.* 30 and 60 days in comparison to control groups (figs. 6 and 7). While, the animal supplemented with vitamin-C with *para*-nonylphenol up to 30 and 60 days, lowered their values (*i.e.*cholesterol and creatinine) and elevated their values (*i.e.*protein) towards to normalcy as compared to *para*-nonylphenol treated groups (figs. 6 & 7). However, the animals treated with vitamin-C showed not much variations in enzyme activities and biochemical contents in comparison to control groups (figs. 2,3,4,5,6 and 7).

Hepatic Histopathology:

The hepatic microphotographs of control *Mus musculus* showed well defined hepatocytes with cytoplasmic granules with prominent and spherical nuclei. The sinusoid separated by hepatic cells and central vein and bile ducts were surrounded by hepatocytes (fig. 8). While, the animals exposed with *para*-nonylphenol up to 30 days showed hyper trophied with reduced nuclei and devoid degenerative and pycknotic nuclear in hepatocyte. A part from this mild congestion of sinusoidal spaces in the centrilobular area were also noticed (fig. 10). Whereas, *para*-nonylphenol exposed up to 60 days hepatic cells showed necrosis characterized by variable proliferative connective tissue with disturbed hepatic architecture with pycknotic nuclei (fig. 11). While, the animals supplemented with vitamin-C along with *para*-nonylphenol up to 30 and 60 days showed recoveries in their cellular structure (figs. 12 & 13). In contrast to this, vitamin-C alone supplemented animals showed normal hepatic architectures like control groups (figs. 9).

DISCUSSION:

The toxicity of any compound depends on many factors, such as the chemical and physical form of the compound, rout of administration, dose and duration of exposure dietary level of the interacting elements, physiological conditions (pregnancy, lactation etc), nutritional status, age and sex of the exposed individuals [22,23,24,25]. Nonylphenol is a xenobiotic compound have estrogenic in nature known as an endocrine disruptor, also enhances the uterine DNA and protein synthesis in immature female rats and fishes [3,26]. The *para*-nonylphenol contaminated diet decreased in body weight in rats and mice fed [27,28,29]. This reduction in body weight may explained by the ability of *para*-nonylphenol to generate free radicals [30] which may lead to DNA breakage, inhibition of protein biosynthesis and gluconeogenesis, lipid peroxidation, disruption of oxidative phosphorylation in mitochondria, inhibition of blood clotting and apoptosis [31] In our study, body weights were significantly decreased after 60 days after *para*-nonylphenol exposures and this decrease was ameliorated by vitamin-C when co-administered with *para*-nonylphenol. This may suggest that *para*-nonylphenol directly or indirectly inhibits protein synthesis and gluconeogenesis within the animals may resulted in decreased in their body weight and this effects were ameliorated by vitamin-C supplementation, because vitamin-C is well known antioxidant and it is having the capacity to reduce oxidative damage caused by toxicants [32,27,33,12,13].

Regarding enzyme activities it is well known that GOT is present in many tissues including liver, heart, muscle, kidney, and brain. It is released into serum during damage of tissue [34,35,36]. GPT also normally found largely in liver and is released into the blood stream as the result of liver injury. High levels of GOT and GPT are usually indicative of liver and kidney damage in animals [37,38,39] and humans [40,41]. 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 [42]. Liver ALP is mobilized most rapidly into blood and its levels in plasma may increase at early periods of liver damage. High ALP level is usually indicative of cholestasis. Cholestasis may also result in a progressive liver disease biliary cirrhosis [43,44]. ALP and ACP are known to be involved in a variety of metabolic activites such as permeability [45], protein synthesis and gonadal maturation [46]. [47] Ram and Satyaneshan, reported that the increase in the activity of ALP or ACP might be due to the necrosis of liver, kidney and other organs. Antioxidants may prevent the harmful effects of free radicals, and suppress the formation of reactive intermediary metabolites [48,49,50,51,52]. In our study a significant increase in enzyme activities especially in hepatic transaminases (GOT, GPT) of male mice after 30 and 60 days of *p*-NP treatment. Increase in GOT, GPT, ACP and ALP revealed that hepatic cellular damage could be correlated with the disturbed enzyme activities. However, the co-administration of vitamin-C along with *para*-nonylphenol neutralizing the enzymatic activities and restored near to the normal level. These results were corroborated with [53], and other authors 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 mammals [33,12,54].

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. Our research work also mentioned significant increases in cholesterol were recorded in animals treated with *para*-nonylphenol for 30 and 60 days respectively. [55] Labana, *et al*., (2001) 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, our investigation showed that these increased levels in cholesterol concentration was decreasing to its normal value after the supplementation of vitamin-C along with *para*-nonylphenol to the experimental animals.

Creatinine serves as the high energy phosphate storehouse in muscle and it is an end product of creatinine metabolism. Creatinine is largely formed in muscle by the irrepressible and non-enzymatic removal of water from creatine phosphate. Elevate values are also observed in certain 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 induced liver dysfunction. 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*.

During present study, the histoarchitecture of liver of control group *Mus musculus* showed normal structure of hepatocytes with prominent nuclei and cytoplasm, while, the liver of *para*-nonylphenol exposed animals showed sever necrotic changes with marked degenerative alterations, cellular disintegration distorted and pycnotic nuclei. *para*-nonylphenol induced many histopathological changes in the liver of rat and the magnitude of such changes was time dependent. Similar results were obtained by various workers characterized by congestion of blood vessels, cytoplasmic vacuolization of the hepatocytes, fat degeneration and leukocyte infiltrations [56,57,58,59,33]. Moreover, liver exposed with *para*-nonylphenol and supplemented with vitamin-C showed amelioration characterized by normal hapatocytes with no degenerative changes suggesting recovery in hepatic cells when compared with *para*-nonylphenol treated animals. Thus these findings revealed that vitamin-C could prevent the hepato cellular damage against the *para*-nonylphenol induced toxicity. Similarly, the ameliorative role of vitamin -C was also observed by [33,12,13,14,15]. 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 for providing all infrastructural facilities for the research work.

References:-

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

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

[3]. Clark, L.B., R.T. Rosen, T.G. Hartman, J.B. Louis, I.H. Suffet, and R.L. Lippincott, (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

[4]. Daidoji T, H. Inoue, S. Kato, and H. Yokota, (2003); Glucuronidation and excretion of nonylphenol in perfused rat liver. *The American Society for Pharmacology and Experimental Therapeutics DMD* 31:993–998,

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

[6]. 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.

[7]. Gaby S.K., and V.N. Singh, (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.

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

[9]. Wang C., J. Liang, C. Zhang, Y. Bi, X. Shi and Q. Shi, (2007): Effect of ascorbic acid and thiamine supplementation at different concentrations on lead toxicity in liver. *Ann. Occup. Hyg*., 51(6): 563-569.

[10]. Ambali S., D. Akanbi and N. Igbokwe, (2007): Evaluation of subchronic chlorpyrifos poisoning on hematological and serum biochemical changes in mice and protective effect of vitamin C. *J Toxicol Sci.* 32(2): 111-20.

[12]. Gautam V. and V. K. Shrivastava, (2011): Protective role of vitamin C against lindane toxicity on the histo-architecture of the epididymis and vas deferens of male mice (Mus musculus), Egyptian Journal of Biology, 13: 30-36.

[13]. Murmu S. and V.K. Shrivastava, (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.

[14]. Naseer M.I., N. Ikram Ullah, H.Y. Lee, E.W. Cheon, J. Chung and M.O. Kim, (2011): Neuroprotective Effect of Vitamin C Against Ptz Induced Apoptotic Neurodegeneration In Adult Rat Brain. *Pak. J. Pharm. Sci.*, 24(3): 263-268.

[15]. Amal I. E. R. and I. E. Fawzy, (2012): Protective effects of ascorbic acid and zinc against cadmium-induced histopathological, histochemical and cytogenetic changes in rats. *Comunicata Scientiae* 3(3): 162-180.

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

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

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

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

[20]. Toro G. and P.G. Ackermann, (1975): Practical clinical chemistry. Boston, Little Brown and Co, 154.

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

[22]. Schalm, O.W., N.C. Jain and E.J., Corroll, (1986): Veternary heamatology, (4thed.). Lee and Fibiger, Philadelphia.

[23]. Haratym-Maj, A., (2002): Hematological alterations after pyrethroids poisoning in mice. *Ann. Agri. Environ. Med*., 9:199-206.

[24]. Khan, A., H.A.M. Faridi, M. Ali, M.Z. Khan, M. Siddique, I. Hussain and M. Ahmad, (2009a): Effects of cypermethrin on clinico-hemato-biochemical and pathological parameters in male Dwarf goats (*Capra hircus*). *Exp. Toxicol. Pathol.*, 61(2): 151- 160.

[25]. Khan M.R., W. Rizvi, G.N. Khan, R.A. Khan, and S. Shaheen, (2009b): Carbon tetrachloride induced nephrotoxicity in rats: Protective role of *Digera muricata*. *J. Ethnopharmacol*. doi:10.1016/j.jep.2008.12.006.

[26]. Chitra, K.C. and P.P. Mathur, (2004): Vitamin E prevents nonylphenol induced oxidative stress in testis of rats. *Indian J Exp Biol.*, 42: 220-3.

[27]. AL-Shinnawy, (2008): Vitamin "C" as ameliorative agent Against thiodicarb toxicated Male albino rats (*rattus norvegicus*) Egypt. *Acad. J. biolog. Sci.*, 1(2): 177- 187

[28]. Kyselova V., J. Peknicova, D. Buckiova and M. Boubelik, (2003): Effects of p-nonylphenol and resveratrol on body and organ weight and *in vivo* fertility of outbred CD-1 mice. *Reprod Biol Endocrinol*, 1: 30.

[29]. Aslam F., A. Khan, M. Z. Khan, S. Sharaf, S. T. Gul and M. K. Saleemi, (2009): Toxico-pathological change induced by p-NP in broiler chicks; their attenuation with vitamin E and selenium. *Exp.Toxicol. Pathol., Doi:* 10.1016/ j.etp., 06.004

[30]. Tyl R.W., C.B. Myers, M.C. Marr and C.N. Castillo, (2006): Three-generation evaluation of dietary para-nonylphenol in CD (Sprague-Dawley) rats. *Toxicol Sci* 92: 295-310, 2006.

[31]. Okai, Y., E.F. Sato, K. Higashi-Okai and M. Inoue, 2004): Enhancing effect of the endocrine disruptor paranonylphenol on the generation of reactive oxygen species in human blood neutrophils. *Environ. Health Prespect.*, 112(5): 553-556.

[32]. Aydogan M. and N. Barlas, (2006): Effect of maternal 4-ter-octylphenol exposure on the reproductive tract of male rat adulthood. *Reprod. Toxicol.* 22: 455-460

[33]. Korkmaz A., M. Aydogan, D. Kolankaya and N. Barlas, (2010): Influence of vitamin-C on bisphenol A, nonylphenol and octylphenol induced oxidative damage in Liver of rats. *Food and Chemical Toxicol*., 48: 2865-2871.

[34]. Reham A. Mohamed, R.S. Ramadan and L.A. Ahmed, (1999): Effect of Substituting Pumpkin Seed Protein Isolate for Casein on Serum Liver Enzymes, Lipid Profile and Antioxidant Enzymes in CCl -intoxicated Rats. *Advances in Biological Research* 3 (1-2): 09-15.

[35]. Jain N., R. Shrivastava, A.K. Raghuwanshi and V.K. Shrivastava, (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

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

[38]. Tiwari A. and V.K. Shrivastava, (2001): Acetaminophen poisoning induced ezymological and biochemical changes in liver and kidney of male mice Mus musculus. *Biochem. Cell Arch*. (1 & 2): 71-76.

[39]. Gawande M.R. and V.K. Shrivastava, (2009): Carbendazim induced histopathological chanes in testis and epididymis and some enzyme activities (ACP, ALP) in testis of male *Rattus rattus*. *Journal of Experimental Zoology.*, 12(1): 153-156.

[40]. Gil F., V. Fiserova-Bergerova and N. H. Altman, (1988): Hepatic protection from chemical injury by isofluorane. *Anesth. Analg.*, 67: 860-867

[41]. Ray D.C. and G.B. Drummond, (1991): Halothane hepatitis. *Br. J. Anaesth*., 67: 84-99.

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

[43]. Murray R.K., D.K. Granner, P.A. Mayes and V.W. Rodwell, (1988): Harper's Biochemistry (A Lange Medical Book), Appleton and Lang (Eds.)., 649—664.

[44]. Burtis C.A. and E.R. Ashwood, (1994): Tietz textbook of clinical chemistry (2nd ed.), WB Saunders Co., *Philedelphia*, U.S.A., pp. 1275-1512,

[45]. Seth J.S., H.B. Tewari and P.P. Sood, (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.

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

[47]. Ram R. and A.G. Sathayanesan, (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.

[47]. Sinisa F.D., C. Gordana, D. Jelena and D. Vukosava, (2008): The influence of vitamin C supplementation on the oxidative status of rat interscapular brown adipose tissue. *Journal of Thermal Biology*, 33(4): 238-243.

[49]. Naziroglu M., (1999): Protective role of intraperitoneally administered vitamin E and selenium in rats anesthetized with enflurane. *Biol. Trace Elem. Res.*, 69: 199-209.

[50]. Olmez D. and A.Z. Karakilcik, (2004): Protective role of $-carotene dietary supplementation on activities of functional liver enzymes and some biochemical parameters in experimental hepatotoxicity of rats. *J. Nutr. Biochem.*, (In press).

[51]. Gautam V. and V.K. Shrivastava, (2006): Dichlorovos induced changes in enzyme activities in testis and adrenal glands of male *mus musculus* (P). *Asian J. of Bio Sci.*, 1(2): 24-25

[52]. Khan A. and V.K. Shrivastava, (2007): Antagonastic effect of Kamdhenu ark (Cow Urine Distillate) and bioenhancing role with zinc in liver of male *Mus musculus*. *International Journal of Cow Science.*, 2(1): 14-18.

[53]. Yanardag R., O. Ozsoy-Sacan, S. Ozdil and S. Bolkent, (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.

[54]. Abdel-Monem U. M., H. Qar and R. A. Attwa, (2012): Detoxification of dietary diazinon by clay, vitamin C and vitamin E in rabbits. *World Applied Sciences Journal* 19 (1): 144-152,

[55]. Labana S., R.C. Bansal and M. Akhtar, (2001): Age related effects of oerganochlorine insecticide lindane on intestinal brush border membrane in rats. *Indian Journal of Biochemostry & Biophysics.*, 38: 249-252.

[56]. Niwa T., Y. Maekawa, M. Fujimoto, K. Kishimoto, Y. Yabusaki, F. Ishibashi, and M. Katagiri (2002): Inhibition of human hepatic cytochrome P450s and steroidogenic CYP17 by nonylphenol. *Biol Pharm Bull.* 25:235–238.

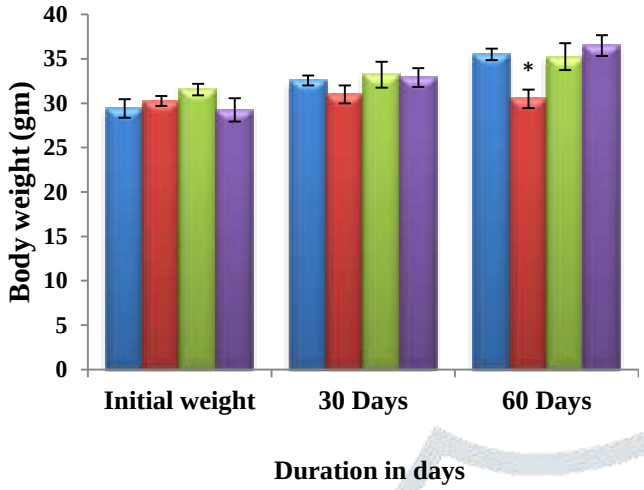
[57]. Durak I., T. Guven, M. Birey, H.S. Ozturk, O. Kurtipek and M. Yel, (1996): Halothane hepatotoxicity and hepatic free radical metabolism in *guiena pigs*: The effects of vitamin E. *Can. J. Anaesth*., 43: 741-748.

[58]. Durak K., O.F. Bilgen, T. Kaleli, P. Tuncel, R. Ozbek and K. Turan, (1996): "Antioxidant effect of *αα*-tocopherol on fracture haematoma in rabbits," *Journal of International Medical Research*., 24(5): 419–424.

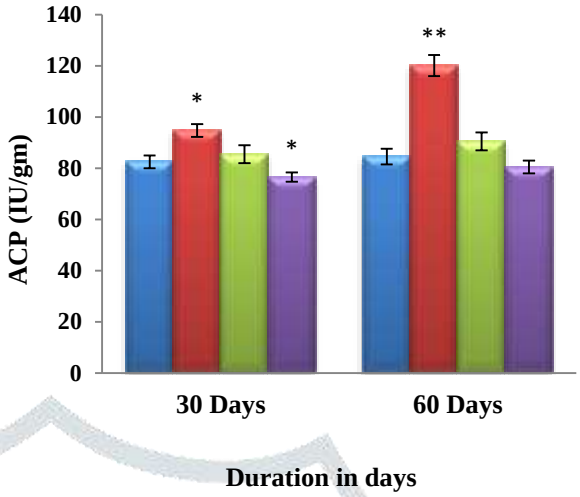
[58]. Trevor Green A., A. Cindy Swain, John P. Van Miller, B and Ronald L. Joiner (2003): Absorption, bioavailability, and metabolism of *para*-nonylphenol in the rat. *Regul Toxicol Pharmacol.* Aug; 38(1):43-51

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

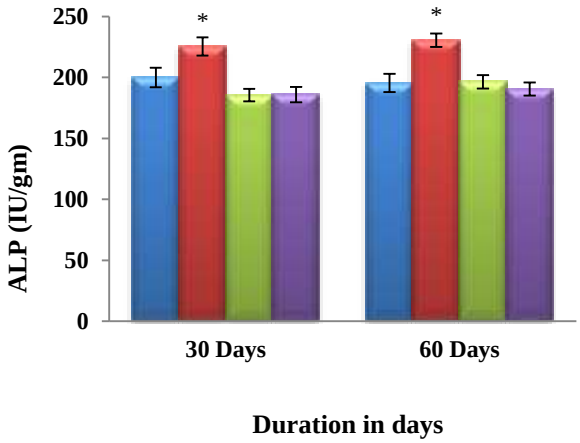
Hist. 1 :- Body weight (gms) of male *Mus musculus* (P)



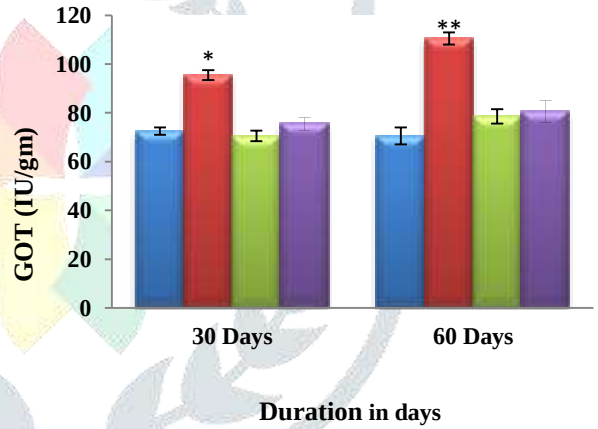
Histo. 2 :- Acid Phosphatase (ACP) (IU/gm) in Liver of male *Mus musculus* (P)



Histo.3:Alkaline Phosphatease (ALP)



Histo.4: Glutamate Oxaloacetate Transaminase (GOT) (IU/gm)



Where:-

Control

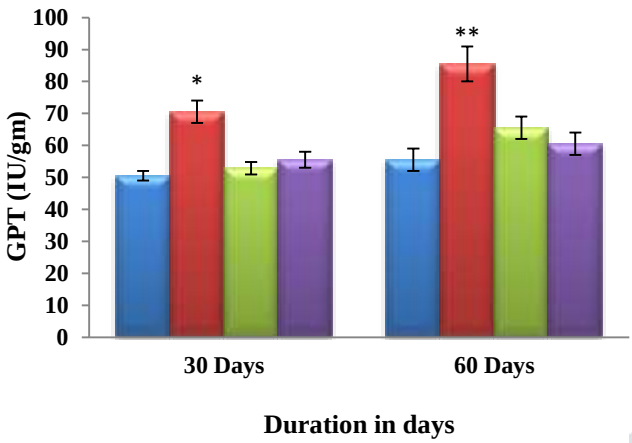
Treated with *para*-Nonylphenol

Vitamin-C supplemented

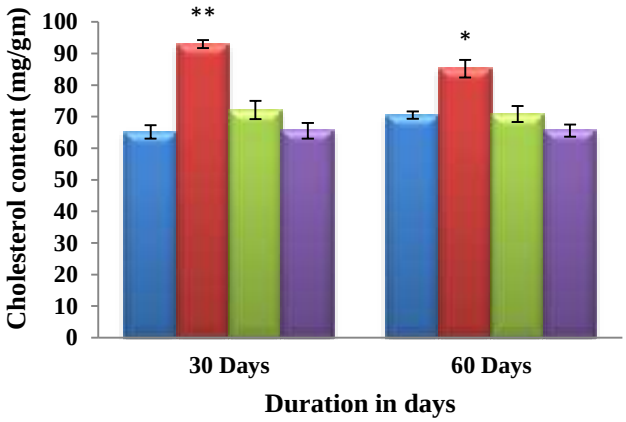
***para*-Nonylphenol with Vitamin-C**

Histogram: Showing changes in Body weight and Liver enzymes of male *Mus musculus* (P) after the exposure of *p*-NP, *p*-NP along with Vitamin-C supplemented, Vitamin-C alone and Control group after 30 and 60 days.

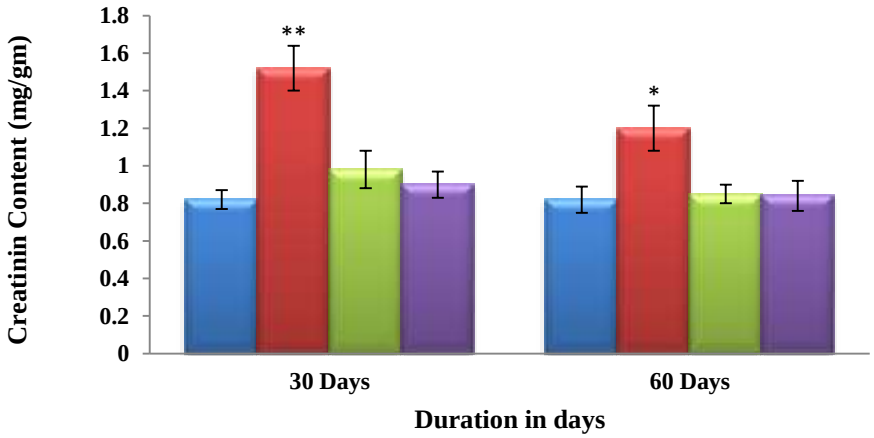
Histo.5: Glutamate Pyruvate Transaminase (GPT) (IU/gm) in Liver of male *Mus musculus* (P)



Histo.6: Cholesterol content (mg/gm) in Liver of male *Mus musculus* (P)



Histo.7: Creatinine content (mg/gm) in Liver of male *Mus musculus* (P)



Where:-

Control

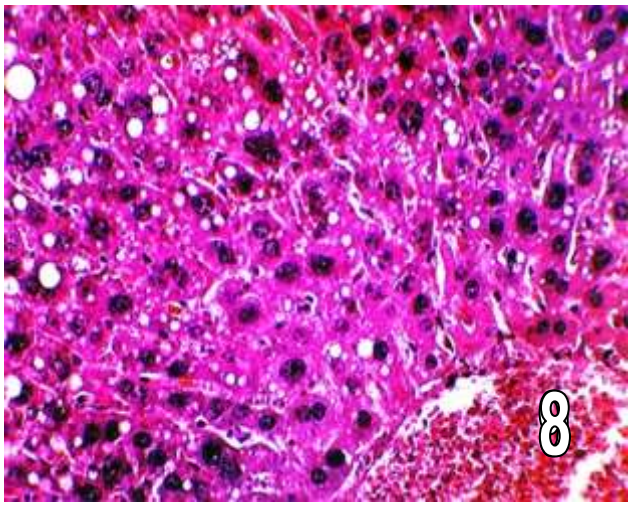
Treated with *para*-Nonylphenol

Vitamin-C supplemented

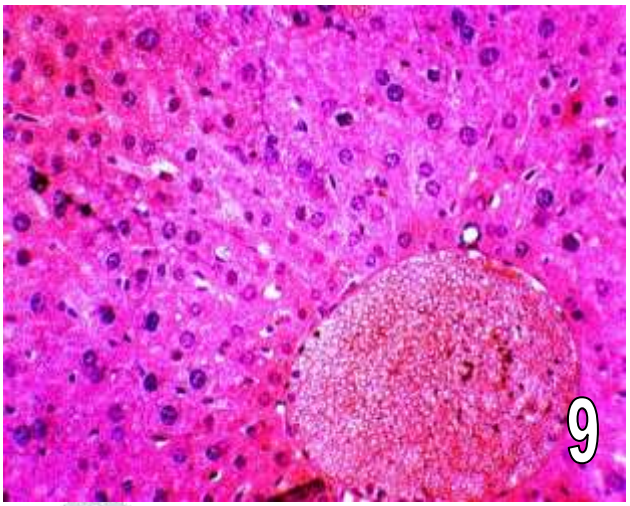
***para*-Nonylphenol with Vitamin-C**

Histogram: Showing changes in Liver enzymes, Cholesterol & Creatinine of male *Mus musculus* (P) after the exposure of *p*-NP, *p*-NP along with Vitamin-C supplemented, Vitamin-C alone and Control group after 30 and 60 days.

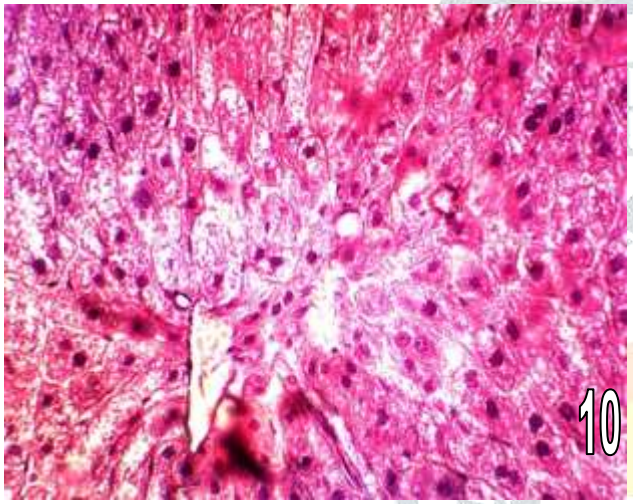
Microphotographs of Liver transverse section (H & E 400x)



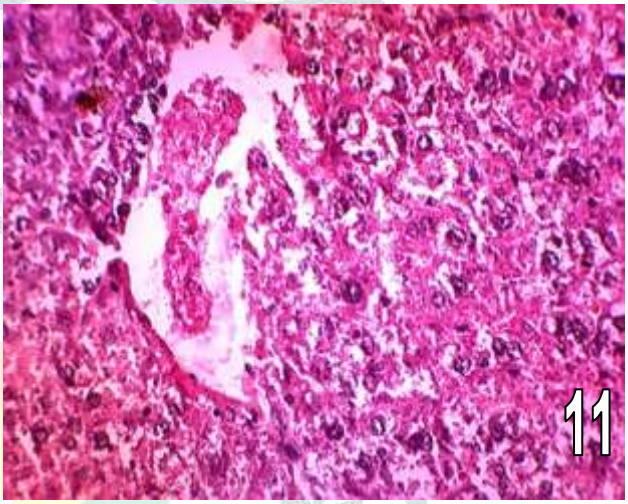
Control



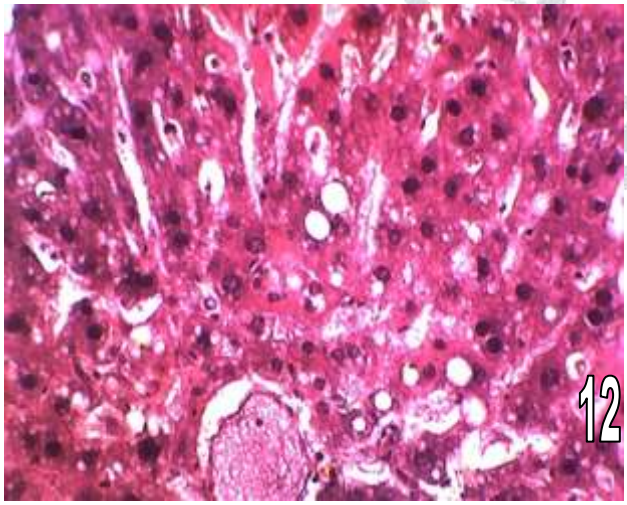
Vitamin-C supplemented



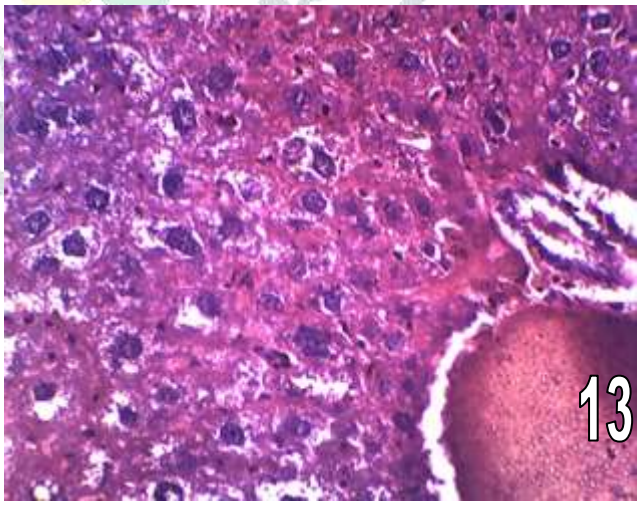
p-NP treated for 30 days



p-NP treated for 60 days



p-NP treated+vit.-C supplemented for 30 day



p-NP treated + vit.-C supplemented for 60days