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## Preventing Effects of Wheat Germ Oil on Sex Hormones, Liver Enzymes, Lipids and Proteins in Rat Serum Following Treatment with p-Nonylphenol

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**Abstract:** The aim of this study is to investigate the preventing effect of wheat germ oil as a rich source of vitamin E on the serum biochemical factors in the rats exposed to para-nonylphenol. Four groups (n = 6) of male Wistar rats was orally given para-nonylphenol (200 mg kg<sup>-1</sup>day<sup>-1</sup>) for 70 days. After treatment the blood samples were obtained and LH, FSH, testosterone, progesterone and estrogen, transaminases, phosphatases, LDH, triglyceride, cholesterol, HDL-cholesterol and protein analysis were carried out. Para-nonylphenol caused a significant increase in LH level where other hormones remained unchanged. In addition a significant reduction was found in AST, ALT and LDH level while alkaline phosphatase increased significantly following treatment with para-nonylphenol. There was no change in the level of lipids and proteins in this study with respect to para-nonylphenol treatment. Co-administration of wheat germ oil with para-nonylphenol compensated the imbalance of the LH, AST, ALT and LDH caused by para-nonylphenol to the control level. However treatment of the rats with only wheat germ oil caused a significant reduction in the level of the hormones except for progesterone. Administration of wheat germ oil in any case caused a reduction in triglyceride level. Co-administration of wheat germ oil with para-nonylphenol eliminated the effect of para-nonylphenol on LH hormone as well as enzymes. It seems that daily used of wheat germ oil may have some benefits to the para-nonylphenol exposure, however more clinical studies are needed to find more information.

**Key words:** p-nonylphenol, wheat germ oil, sex hormones, liver enzymes, proteins, lipids

### INTRODUCTION

There has been an increasing public concern for man-made chemicals in the environment that affect reproductive health by disrupting normal endocrine function in humans and animal Kingdom. Among these chemicals, those with estrogenic activity [endocrine disruptive compounds (EDCs)] are notable (Kimmberg *et al.*, 2000). EDCs interfere with the action of the endocrine system through diverse mechanisms, such as, receptor-mediated enzyme inhibition (Naoki *et al.*, 2006). Alkylphenols, including para-nonylphenol (p-NP) as EDCs, are the final biodegradation products of alkylphenol polyethoxylates, which widely used in detergents, paints, pesticides, cosmetics as well as other industries (White *et al.*, 1994). In biological system, p-NP influences the hypothalamus pituitary function and changes the level of LH (Furuta *et al.*, 2006), FSH (Han *et al.*, 2004; Masutomi, 2003) and testosterone (Ying *et al.*, 2006a), which affects the weight of the reproductive organs and delays spermatogenesis (Lee, 1998; De Jager *et al.*, 1999, 2001). It is proposed that in addition to estrogenic effects (Routledge and Sumpter,

1996, 1997), the reactive oxygen species produced by p-NP also affect the biochemical factors (Sridhar *et al.*, 2004; Chitra and Mathur, 2004). Since antioxidants like vitamin E, eliminate the oxidative stress caused by p-nonylphenol (Chitra and Mathur, 2004). This study was organized to investigate the preventing effects of Wheat Germ Oil (WGO), as a rich source of vitamin E, on the level of serum biochemical factors including sex hormones, liver enzymes, lipids and protein profiles driven by p-NP.

### MATERIALS AND METHODS

**Animals:** Twenty four male adult Wistar rats weighing 250±20 g obtained from pasture institute, Iran, were maintained under standard laboratory conditions in the animal house of Arak University (12 h light and 12 h dark and 20±2°C) with free access to food. The rats were randomly divided in 4 groups (n = 6) including control, p-NP (p-nonylphenol), p-NP + WGO (p-nonylphenol + wheat germ oil) and WGO (wheat germ oil).

**Oral treatment of p-nonylphenol:** p-NP was purchased from ACROS ORGANICS Company (New Jersey, USA).

Using Sesame oil as a carrier, all the groups were given calculated amount ( $0.4 \text{ mL kg}^{-1}$  body weight). p-NP group was treated with p-NP ( $200 \text{ mg kg}^{-1} \text{ day}^{-1}$ ) and p-NP + WGO group was treated with p-NP ( $200 \text{ mg kg}^{-1} \text{ day}^{-1}$ ) and WGO ( $170 \text{ mg kg}^{-1} \text{ day}^{-1}$ ). Control and wheat germ oil groups were given only sesame oil and wheat germ oil, respectively.

**Serum sample collection:** Blood samples were collected from the heart under ether anesthesia. Heparinized plasma was separated using centrifuge (3000 rpm) and stored at  $-80^\circ\text{C}$  to carry out further biochemical-assays.

**Hormones (LH, FSH, estrogen, progesterone and testosterone) assay:** Plasma Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH), estrogen, progesterone and testosterone concentration were measured by enzyme linked immunosorbent assay (ELIZA) using ELIZA reader staffax-303 (Awareness Comp. USA). LH and FSH concentration were measured as described in the instructions provided with the kits (RADIM S.P.A. Roma, Italy), where the testosterone, progesterone and estrogen concentration were measured as described in the instruction provided by kits (DRG Diagnostics GmbH, Germany) in which endogenous hormones of the rats plasma competes with a hormone horseradish peroxidase conjugate for binding to the coated antibody. The absorption of the test samples were read at 450 nm with the help of (COBAS-MIRA ROCHE DIAGNOSTIC INSTRUMENT) automated reader.

**Enzymes (Alkaline phosphatase, acid phosphatase, lactate dehydrogenase and Transaminases) assay:** Serum Aspartate Transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), acid phosphatase (AcP), lactate dehydrogenase (LDH) were measured as described in the instructions provided with the kits (parsazmon co. reagents Boheringer Manheim, Germany) and the absorption of the test samples were read at 340 nm with the help of (COBAS-MIRA ROCHE DIAGNOSTIC INSTRUMENT) automated reader.

**Total protein and albumin concentration assay:** Serum total proteins and albumin concentration were determined using biuret and bromocresol green methods, respectively as described in the instructions provided with the kits (parsazmon co. reagents, Boheringer Manheim, Germany). The absorption of the test samples for total protein and albumin were read at 546 and 540 nm, respectively with the help of (COBAS-MIRA ROCHE DIAGNOSTIC INSTRUMENT) automated reader. Globulins level in the test samples were determine by subtracting albumin values from total protein values.

**Lipid profile assay:** Triglyceride (TG), cholesterol (Chol) and HDL-cholesterol (HDL-Chol) concentration were determined using enzymatic methods GPO-PAP and CHOD-PAP as described in the instructions provided with the kit (Parsazmon Co. Reagents Boheringer Manheim, Germany). The absorption of the test samples were read at 550 nm for TG and 546 nm for Chol and HDL-Chol with the help of (COBAS-MIRA ROCHE DIAGNOSTIC INSTRUMENT) automated reader.

**Serum electrophoresis:** Polyacrylamide gel electrophoresis (PAGE): Nondenaturing Condition (native-PAGE) was carried out with 7% separating and 5% stacking gels. The gel thickness was 0.75 mm and a constant current of 40 mA (gel size  $20 \times 20 \text{ cm}$ ) was applied. Coomassie brilliant blue was used to stain the electrophoresis gels.

**Statistical analysis:** The data was statistically analyzed using one way ANOVA by Tukey test and mean differences was considered statistically significant where  $p < 0.05$ .

## RESULTS

**Sex hormones:** Treatment with p-NP caused a significant elevation in LH but the level of other hormones remained constant when compared to control group. In the case of co-administration of WGO along with p-NP, the level of LH hormone reduced to the normal level, but a significant reduction observed in the level of testosterone and progesterone while FSH and estrogen remained unchanged. A significant reduction of LH, FSH, testosterone and estrogen were observed following administration of WGO alone while the level of progesterone was remained unchanged when compared to the control group (Table 1).

**Serum enzymes:** A significant reduction in AST, ALT and LDH level was observed following administration of p-NP, Where the level of ALP increased significantly but no change was detected in the level of AcP. Administration of WGO along with p-NP compensated the decreasing effect of the p-NP on transaminases level as well as LDH to the level of control group, but in case of ALP a significant elevation was seen. In WGO treated rats except the significant reduction in AST level, all the other enzymes remained the same as in control group (Table 2).

**Serum lipids:** Triglycerides, cholesterol, HDL-cholesterol level was the same in p-NP group when compared to the control ones. Co-administration of WGO with p-NP

**Table 1:** Comparing between Luteinizing Hormone (LH) mIU mL<sup>-1</sup>, Follicle Stimulating Hormone (FSH) mIU mL<sup>-1</sup>, testosterone ng mL<sup>-1</sup>, estrogen pg mL<sup>-1</sup> and Progesterone ng mL<sup>-1</sup> in different groups of rats, 70 days after nonylphenol (200 mg kg<sup>-1</sup> b. wt. day<sup>-1</sup>) and wheat germ oil (170 mg kg<sup>-1</sup> body weight day<sup>-1</sup>) treatment

Group of rats	LH	FSH	Testosterone	Estrogen	Progesterone
Control	1.3±0.3 <sup>a</sup>	1.5±0.3 <sup>a</sup>	3.6±0.3 <sup>a</sup>	5.7±1.1 <sup>a</sup>	2.9±0.3 <sup>a</sup>
p-NP	1.7±0.2 <sup>b,c</sup>	1.5±0.2 <sup>a</sup>	4.4±1.2 <sup>a</sup>	4.6±0.8 <sup>bc</sup>	3.0±0.3 <sup>a</sup>
p-NP+WGO	1.4±0.2 <sup>ac</sup>	1.7±0.14 <sup>a</sup>	2.2±0.10 <sup>b</sup>	5.6±0.5 <sup>ad</sup>	1.7±0.7 <sup>b</sup>
WGO	1.0±0.14 <sup>bd</sup>	1.1±0.1 <sup>b</sup>	2.1±0.2 <sup>b</sup>	4.4±0.6 <sup>bcd</sup>	3.5±0.8 <sup>a</sup>

Values are mean±SD. Means with the same letter(s) code do not differ significantly from each other (ANOVA, Tukey test, p>0.05)

**Table 2:** Comparing enzymes (IU L<sup>-1</sup>); aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and acid phosphatase [AcP (U L<sup>-1</sup>)] in different groups of rats, 70 days after p-NP (200 mg kg<sup>-1</sup> day<sup>-1</sup>) and WGO (170 mg kg<sup>-1</sup> day<sup>-1</sup>) treatment

Group of rats	AST	ALT	LDH	ALP	AcP
Control	95.2±7 <sup>a</sup>	55.5±8.2 <sup>a</sup>	325.3±31.7 <sup>a</sup>	401.3±54.1 <sup>a</sup>	24.4±3.6 <sup>a</sup>
p-NP	84.3±6 <sup>b</sup>	40.3±4.3 <sup>b</sup>	248.8±35.7 <sup>b</sup>	524.8±91.4 <sup>b</sup>	23.2±1.5 <sup>a</sup>
p-NP + WGO	88.2±11.4 <sup>ab</sup>	45.7±7.9 <sup>ab</sup>	272.5±45.4 <sup>ab</sup>	535.0±30.8 <sup>b</sup>	24.0±3.6 <sup>a</sup>
WGO	82.2±9.4 <sup>b</sup>	46.0±6.9 <sup>ab</sup>	331.0±43.7 <sup>a</sup>	357.3±59.4 <sup>a</sup>	20.8±2.1 <sup>a</sup>

Values are mean±SD. Means with the same letter code do not differ significantly from each other (ANOVA, Tukey test, p>0.05)

**Table 3:** Comparing, triglyceride (TG, mg dL<sup>-1</sup>), cholesterol (Chol, mg dL<sup>-1</sup>), high density lipoprotein-cholesterol (HDL-Chol, mg dL<sup>-1</sup>) and cholesterol/high density lipoprotein-cholesterol ratio (Chol/HDL-Chol ratio) in different groups of rats, 70 days after p-NP (200 mg kg<sup>-1</sup> day<sup>-1</sup>) and WGO (170 mg kg<sup>-1</sup> day<sup>-1</sup>) treatment

Group of rats	TG	Chol	HDL-Chol	Chol/HDL-Chol ratio
Control	140.50±2.2 <sup>a</sup>	67.33±9.6 <sup>a</sup>	41.17±8.4 <sup>a</sup>	1.2±0.05 <sup>a</sup>
p-NP	143.00±23.7 <sup>a</sup>	73.33±3.5 <sup>ab</sup>	47.33±10.3 <sup>a</sup>	1.4±0.12 <sup>a</sup>
p-NP + WGO	80.70±7.5 <sup>b</sup>	77.33±11.9 <sup>ab</sup>	68.33±8 <sup>b</sup>	0.91±0.05 <sup>b</sup>
WGO	96.33±10.9 <sup>b</sup>	56.83±6.4 <sup>ac</sup>	40.17±4.2 <sup>a</sup>	1.10±0.04 <sup>ab</sup>

Values are mean±SD. Means with the same letter(s) code do not differ significantly from each other (ANOVA, Tukey test, p>0.05)

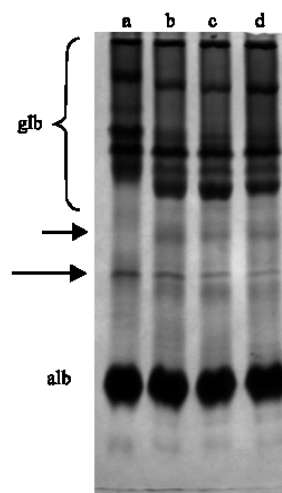
**Table 4:** Comparing Total Proteins (g dL<sup>-1</sup>), Albumin (alb) and Globulin (glb) in different groups of rats, 70 days after p-NP (200 mg kg<sup>-1</sup> day<sup>-1</sup>) and WGO (170 mg kg<sup>-1</sup> day<sup>-1</sup>) treatment

Group of rats	Total proteins	Alb	Glb
Control	7.1±0.9 <sup>a</sup>	3.7±0.5 <sup>a</sup>	3.4±0.9 <sup>a</sup>
p-NP	6.9±0.4 <sup>ab</sup>	3.5±0.1 <sup>a</sup>	3.4±0.4 <sup>b</sup>
p-NP + WGO	6.9±0.4 <sup>ab</sup>	3.6±0.2 <sup>a</sup>	3.3±0.8 <sup>a</sup>
WGO	7.5±0.6 <sup>ac</sup>	3.7±0.1 <sup>a</sup>	3.8±0.5 <sup>a</sup>

Values are mean±SD. Means with the same letter(s) code do not differ significantly from each other (ANOVA, Tukey test, p>0.05)

caused a significant reduction of triglyceride and an elevation of HDL-cholesterol as compared to control and p-NP groups, while cholesterol level remained unchanged. A significant decreased of triglycerides level was found due to administration of WGO alone as compare to control and p-NP groups, while cholesterol level reduced significantly in comparison to the p-NP and p-NP + WGO groups. A reduction in HDL-cholesterol was also seen when compared to p-NP + WGO rats (Table 3).

**Serum proteins:** The total circulating proteins significantly increased in WGO group compared to both p-NP + WGO and p-NP groups but it was the same in the remaining groups. The amount of albumin (alb) and globulin (glb) did not change in any of the groups (Table 4). The electrophoresis profile of the serum proteins showed no variation in the intensity of the albumin (alb) or globulin (glb) region (Fig. 1). Though, the same amount of protein (300 µg) have been loaded on the electrophoresis gel, still at the position of the Arrows which are labeled by numbers, the bands showed change in the intensity. The protein band (arrow 2) in p-NP group



**Fig. 1:** Native electrophoresis of rats serum: (a) nonylphenol, (b) nonylphenol + wheat germ oil, (c) wheat germ oil and (d) control. 7% polyacrylamide gel, 300 µg of the samples has been loaded. Gel was stained by Coomassie blue

showed increase in intensity but arrow 1 indicates faintness in intensity in p-NP group as compare to other groups (Fig. 1).

## DISCUSSION

Para-nonylphchol is thought to imitate endogenous hormones and inhibit their actions, thus could induce reproductive abnormalities (Naoki *et al.*, 2006). In the present study as the results showed treatment with p-NP caused increase LH level in the rats serum. Elevation of LH in the serum of rats following administration of high dose of p-NP (125-250 mg kg<sup>-1</sup> day<sup>-1</sup>) has been reported by the other investigators (De Jager *et al.*, 2001; Han *et al.*, 2004) while low dose of p-NP causes LH suppression (Nagao *et al.*, 2001; Furuta *et al.*, 2006). So it can be concluded that the secretion of LH by anterior pituitary gland depend on the p-NP dosage. Furuta *et al.* (2006) showed that p-NP treatment does not affect the level of FSH, as we found in present study. There are other studies with different results of increasing (Han *et al.*, 2004) or decreasing (Masutomi *et al.*, 2003) of FSH level, it is clear that the effect of p-NP on this hormones has a complex mechanisms as the controversial result conforms. In addition we also found that the p-NP did not influence the testosterone level, this finding is in agreement with the other studies which indicate that p-NP has no effect on the testosterone level in the F1 and F2 rat offspring (Laurenzana *et al.*, 2002a) and adult rats serum following treatment with p-NP (Willoughby *et al.*, 2005). However, some investigators believe the level of testosterone reduces following exposure to high concentration of p-NP (Gong and Han, 2006) but in the present study we conclude that the elevation of LH hormone may stimulate this more testosterone synthesis (18% in our study) by binding to a surface receptor of leydig cells (Ying *et al.*, 2006b). Results of this study confirm the hormonal imbalance due to p-NP exposure may be a reason for reproductive abnormalities and delayed spermatogenesis reported by other investigators (Lee, 1998; De Jager *et al.*, 1999, 2001).

Administration of WGO along with p-NP caused a significant reduction of LH to the control concentration level, although this result is an important finding of present study, but significant reduction of two important hormones (estrogen and progesterone) is also negative considerable point. As the result showed treatment with WGO alone caused a significant reduction in level of the hormones except progesterone in comparison with control ones. This may be due to the fact that WGO in addition to vitamin E (Paramich *et al.*, 2000; Todd *et al.*, 1937),

contains the other plant materials such as phytosterols (Lewis *et al.*, 2003; Nakari, 2005) which have estrogenic activity and can alter cholesterol metabolism (Ostlund *et al.*, 2002) or inhibit the enzymes (Laurenzana *et al.*, 2002b) involved in the synthesis of steroid hormones, which may affect the mechanisms of the hormone secretion. But we have to consider that the WGO has performed two different roles in the control of the hormones level, compensating effect when administrated along with p-NP and negative reducing effect as our results showed. Meanwhile more biochemical investigations are needed to understand these two deferent effects.

ALT, AST and LDH are the enzymes involve in the metabolism of amino acids and pyruvate. Transaminases which play an important role in inter conversion of keto acids to amino acids and in the liver function (Voet and Voet, 1995), thus any imbalance in their activity will be harmful. In the present study, p-NP treatment caused significant reduction of AST, ALT and LDH but elevation of ALP enzyme level in the serum which may be found in a large number of disorders. Previous investigations indicate that the p-NP causes elevation of alkaline phosphatase activity in the cell culture (Wober *et al.*, 2002; Kanno *et al.*, 2004) which is in agreement with present data. Glutamic and aspartic acids are two amino acids which play a main role in transferring ammonia to the urea cycle, thus imbalance of enzymes involved in the metabolism of this amino acids (AST and ALT) could be harmful. Although an increase in the activity of ALT is a remarkable indication of liver damage, but in chronic condition the level of these enzymes may remain unchanged or reduce in comparisons to the normal level. Decrease in the level of these enzymes as we found may be a sign of the liver complication, as Nagao *et al.* (2001) reported histopathologic changes in the liver following administration of p-NP in rats. This may be due to accumulation of p-NP in the liver and bile (Smith and Hill, 2004; Daidoji *et al.*, 2003). Accumulation of chemical compounds like p-NP in the liver lead to the toxicity of the organ and consequently can change the level of enzymes and also p-NP accumulation in bile may cause obstruction of biliary system which might be the reason of ALP elevation. Administration of WGO along with p-NP compensate the decreasing effect of p-NP on AST, ALT and LDH enzymes to the normal level, which may be due to the antioxidant effect of vitamin E (Bansal *et al.*, 2005) content of the WGO. Treatment of rats with WGO alone did not affect the activity of ALT, LDH, ALP and AcP enzymes.

We observed no significant changes in lipids value (triglycerides, cholesterol, HDL-cholesterol and cholesterol/HDL-Chol. ratio) with respect to p-NP treatment. Since p-NP did not affect steroid hormones and in the other hand steroid hormones have a profound effect on lipid metabolism (Ishibashi *et al.*, 2004), so present data for lipid values could be expected. Administration of WGO alone or along with p-NP could reduce significantly the level of triglycerides when compared to the control value. several studies have demonstrated that monounsaturated fatty acid reduce serum triglyceride level (Jenkins *et al.*, 1999; Salmeron *et al.*, 1997; Mensink and Katan, 1987), in addition, WGO has a number of other nutritional and health benefits factors like high content of vitamin E and phytosterol (Jonnala *et al.*, 2005) which may be the reason of its lowering effect on triglyceride. Thus the reducing effect of WGO on triglyceride level is a positive finding of this study.

Finally there were no significant changes in the total protein, albumin and globulin level in p-NP treated rats. However there are many reports to indicate a low level production of specific polypeptide or protein like interleukin-4, IgE (Lee *et al.*, 2003) and vitellogenin (Valerio and Marin, 2005) following treatment with p-NP which do not influence the total proteins in the serum. But as native-PAGE of the serum proteins in the p-NP and p-NP + WGO groups showed intensity of the two bands in the gel electrophoresis profile has changed. These two proteins can be of vehicle type, but the intensity of albumin as well as globulins have shown no variation. Thus we recommend more detail analysis to identify these proteins.

As present data showed, we conclude that although the compensatory effect of WGO was seen in this investigation, but alone administration of WGO imbalanced the enzymes and hormones under investigation. Thus we do not recommend consumption of this plant material as a food ingredient. We suggest more clinical investigations, but it may be used as herbal medicine where ever it is required.

## REFERENCES

- Bansal, A.K., M. Bansal, G. Soni and D. Bhatnagar, 2005. Protective role of vitamin E pre-treatment on N-nitrosodiethylamine induced oxidative stress in rat liver. *Chem. Biol. Interact* 20; 156: 101-111.
- Chitra, K.C. and P.P. Mathur, 2004. Vitamin E prevents nonylphenol-induced oxidative stress in testis of rats. *Indian J. Exp. Biol.*, 42: 220-223.
- Daidoji, T., H. Inoue, S. Kato and H. Yokota, 2003. Glucuronidation and excretion of nonylphenol in perfused rat liver. *Drug Metab. Dispos.*, 31: 993-998.
- De Jager, C., M.S. Bormman, S. Wandrag and G. Van der Horst, 1999. I. Effect of p-nonylphenol, an environmental toxicant with oestrogenic properties, on fertility potential in adult male rats. *Andrologia*, 31: 99-106.
- De Jager, C., M.S. Bormman, S. Wandrag and V.W. Sharp, 2001. The lethal dose and potential reproductive effects of p-nonylphenol in rats: A preliminary study. *Arch. Androl.*, 46: 183-187.
- Furuta, M., T. Funabashi, M. Kawauchi, T.J. Nakamura, D. Mitsushima and F. Kimura, 2006. Effects of p-nonylphenol on the anterior pituitary functions in adult ovariectomized rats. *Neuroendocrinology*, 84: 14-20.
- Gong, Y. and X.D. Han, 2006. Effect of nonylphenol on steroidogenesis of rat leydig cells. *J. Environ. Sci. Health B.*, 41: 705-715.
- Han, X.D., Z.G. Tu, Y. Gong, S.N. Shen, X.Y. Wang, L.N. Kang, Y.Y. Hou and J.X. Chen, 2004. The toxic effects of nonylphenol on the reproductive system of male rats. *Reprod. Toxicol.*, 19: 215-221.
- Ishibashi, H., K. Tachibana, M. Tsuchimoto, K. Soyano, N. Tatarazako, N. Matsumura, Y. Tomiyasu, N. Taminaga and K. Arizono, 2004. Effects of nonylphenol and phytoestrogen-enriched diet on plasma vitellogenin, steroid hormone, hepatic cytochrome P450 1A and glutathione-s-transferase values in goldfish (*Carassius auratus*). *Comp. Med.*, 54: 54-62.
- Jenkins, D.J.A., C.W.C. Kendall, V. Vuksan, L.S.A. Augustin, C.R.D. Mehling, T.R.D. Parker, E. Vidgen, B. Lee, D. Fauldner, H. Seyler, R. Josse, L.A. Leiter, P.W. Connelly and V. Fulgoni, 1999. Effect of wheat bran on serum lipids: Influence of particle size and wheat protein. *J. Am. College Nutr.*, 18: 159-165.
- Jonnala, R.S., N.T. Dunford and S. Irmak, 2005. Policosanol, tocopherol and phytosterol composition of wheat extract. IFT Annual Meeting, July 15-20, New Orleans Louisiana, 54G-1.
- Kanno, S., S. Hirano and F. Kayama, 2004. Effect of phytoestrogens and environmental estrogens on osteoblastic differentiation in MC3T3-E1 cells. *Toxicol.*, 196: 137-145.
- Kimberg, K., B. Korsgaard, P. Bjerregaard and A. Jespersen, 2000. Effects of nonylphenol and 17 $\beta$ -estradiol on vitellogenin synthesis and testis morphology in male platyfish *Xiphophorus maculatus*. *J. Exp. Biol.*, 203: 171-181.

- Laurenzana, E.M., G. Balasubramanian, C. Weis, B. Blaydes, R.R. Newbold and K.B. Delclos, 2002a. Effect of nonylphenol on serum testosterone levels and testicular steroidogenic enzyme activity in neonatal, pubertal and adult rats. *Chem. Biol. Interact.*, 139: 23-41.
- Laurenzana, E.M., C.C. Weis, C.W. Bryant, R. Newbold and K.B. Delclos, 2002b. Effect of dietary administration of genistein, nonylphenol or ethinyl estradiol on hepatic testosterone metabolism, cytochrome P-450 enzymes and estrogen receptor alpha expression. *Food Chem. Toxicol.*, 40: 53-63.
- Lee, P.C., 1998. Disruption of male reproductive tract development by administration of the xenoestrogen, nonylphenol, to male newborn rats. *Endocrine*, 9: 105-111.
- Lee, M.H., S.W. Chung, B.Y. Kang, J. Park, C.H. Lee, S.Y. Hwang and T.S. Kim, 2003. Enhanced interleukin-4 production in CD4<sup>+</sup> T cells and elevated immunoglobulin E levels in antigen-primed mice by bisphenol A and nonylphenol, endocrine disruptors: Involvement of nuclear factor-AT and Ca<sup>2+</sup>. *Immunology*, 109: 76-86.
- Lewis, R.W., N. Brooks, G.M. Milburn, A. Soames, S. Stone, M. Hall and J. Ashby, 2003. The effects of the phytoestrogen genistein on the postnatal development of the rat. *Toxicol. Sci.*, 71: 74-83.
- Masutomi, N., M. Shibutani, H. Takagi, C. Uneyama, K.Y. Lee and M. Hirose, 2003. Alteration of pituitary hormone-immunoreactive cell populations in rat offspring after maternal dietary exposure to endocrine-active chemicals. *Arch. Toxicol.*, 78: 232-240.
- Mensink, R.P. and M.B. Katan, 1987. Effect of monounsaturated fatty acids versus complex carbohydrates on high-density lipoproteins in health men and women. *Lancet*, 1: 122-125.
- Nagao, T.K., H.W. Marumo, S. Yoshimura and H. Ono, 2001. Reproductive effects of nonylphenol in rats after gavage administration: A two-generation study. *Reprod. Toxicol.*, 15: 293-315.
- Nakari, T., 2005. Estrogenicity of phytosterols evaluated *in vitro* and *in vivo*. *Environ. Sci.*, 12: 87-97.
- Naoki, K., K. Takashi, S. Mikiko and T. Kiyoshi, 2006. Effect of gestational exposure to nonylphenol on the development and fertility of mouse offspring. *J. Reprod. Dev.*, 52: 789-795.
- Ostlund, R.E., S.B. Racette, A. Okeke and W.F. Stenson, 2002. Phytosterols that are naturally present in commercial corn oil significantly reduce cholesterol absorption in humans. *Am. J. Clin. Nutr.*, 75: 1000-1004.
- Paranich, V.A., O.I. Cherevko, N.A. Frolova and A.V. Paramich, 2000. The effect of wheat germ oil on the antioxidant system of animals. *Lik Sprava*, 2: 40-44.
- Routledge, E.J. and J.P. Sumpter, 1996. Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. *Environ. Toxicol. Chem.*, 15: 241-248.
- Routledge, E.J. and J.P. Sumpter, 1997. Structural features of alkylphenolic chemicals associated with estrogenic activity. *J. Biol. Chem.*, 272: 3280-3288.
- Salmeron, J., A. Ascherio, E.B. Rimm, G.A. Colditz, A.L. Wing and W.C. Willett, 1997. Dietary fiber, glycemic load and risk of NIDDM in men. *Diabetes Care*, 20: 545-550.
- Smith, M.D. and E.M. Hill, 2004. Uptake and metabolism of technical nonylphenol and its brominated analogs in the roach (*Rutilus rutilus*). *Aqua. Toxicol.*, 69: 359-369.
- Sridhar, M., P. Venkataraman, S. Dhanammal, A. Arunkumar, M.M. Arul, N. Srinivasan and J. Arunakaran, 2004. Impact of polychlorinated bisphenyl (Aroclor 1254) and vitamin C on antioxidant system of rat ventra-prostate. *Asian J. Androl.*, 6: 19-22.
- Todd, A.R., F. Bergel and T. Spence Work, 1937. Studies on vitamin E: The isolation of  $\beta$ -tocopherol from wheat germ oil. *Biochem. J.*, 31: 2257-2263.
- Valerio, M. and M.G. Marin, 2005. Can 4-nonylphenol induce vitellogenin-like proteins in the clam tapes philippinarum? *Environ. Res.*, 97: 43-49.
- Voet, D. and J.G. Voet, 1995. *Amino Acid Metabolism*. In: *Biochemistry*. 2nd Edn., John Wiley and Sons, Inc., New York, pp: 727-776.
- White, R., S. Jobling, S.A. Hoare, J.P. Sumpter and M.G. Parker, 1994. Environmentally persistent alkylphenolic compounds are estrogenic. *Endocrinology*, 135: 175-182.
- Willoughby, K.N., A.J. Sarkar, N.I. Boyadjieva and D.K. Sarkar, 2005. Neonatally administered tert-octylphenol affects onset of puberty and reproductive development in female rats. *Endocrine*, 26: 161-168.
- Wober, J., I. Weisswange and G. Vollmer, 2002. Stimulation of alkaline phosphatase activity in Ishikawa cells induced by various phytoestrogens and synthetic estrogens. *J. Steroid Biochem. Mol. Biol.*, 83: 227-233.
- Ying, F., Y. Gong and X.D. Han, 2006a. Effect of nonylphenol on testosterone secretion of rat leydig cells *in vitro*. *Zhonghua Nan Ke Xue*, 12: 300-322.
- Ying, F., Y. Gong and X.D. Han, 2006b. Effect of nonylphenol on steroidogenesis of rat Leydig cells. *Environ. Sci. Health B.*, 41: 705-715.