

The Effect of Oral Administration of Bisphenol A on Tissue and Liver Enzymes, Serum Lipids and Blood Glucose in Rats

Mahnaz Taherianfard*, Sara Abdolmaleki**, Zahra Alimoradi***, Hamidreza Afsoun**** and Mohammad Alimoradi*****

*Associate Professor, Department of Physiology, School of International, Shiraz University, Shiraz, Iran

**Ph.D. Department of Chemistry, Faculty of Science, University of Kurdistan, Sanandaj, Iran

***B.Sc. Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

****M. D, Human Physiology Medical Biology Research Center (MBRC), Kermanshah University of Medical Sciences, Kermanshah, Iran

*****M.Sc. Human Physiology Medical Biology Research Center (MBRC), Kermanshah University of Medical Sciences, Kermanshah, Iran

(Corresponding author: Mohammad Alimoradi)

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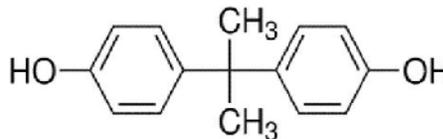
ABSTRACT: Bisphenol A (BPA) is a synthetic compound of carbon with chemical formula $(\text{CH}_3)_2\text{C}(\text{C}_6\text{H}_4\text{OH})_2$. In this study 20 adult male rats were selected and divided into 4 groups of 5 including group I (Control), group II (Shame) and groups III and IV (receiving BPA at doses of 5 and 50 $\mu\text{g}/\text{kg}/\text{day}$ respectively). BPA was given to rats by gavage for 15 days and blood samples were collected. Serum lipids (Triglyceride, Total Cholesterol, HDL and LDL), Serum liver enzymes (AST, ALT and ALP) and serum glucose values were measured. According to the data, using BPA at 2 doses caused a significant reduction ($P < 0.05$) in liver enzymes level and a significant increase in LDL level, but had no effect on lipids and blood sugar. BPA at the low dose caused a significant increase in Triglyceride. Therefore within these doses and during the research (2 weeks), BPA does not cause fatty liver.

Keywords: Bisphenol A, Liver Enzymes, Lipids, Blood Sugar

INTRODUCTION

Xenoestrogens are synthetic estrogens widely used in the industrial compounds and they are different chemically with the natural estrogen made by the body's endocrine system, but their estrogen effects are almost the same on living organisms (Wolstenholme *et al.*, 2010). Xenoestrogens literally composed of the prefix "Xeno" meaning foreign or alien and estrogen which is the importantly reproductive hormone of the female sex (Keri *et al.*, 2007). Other disparate groups with estrogenic effects are phytoestrogens (estrogenic compounds in plants) and mycoestrogens (estrogenic compounds in fungi, including mycotoxin) (Smith *et al.*, 1998). Pervasive presence of the estrogens has created a major concern for the health of individuals and populations. These chemicals accompanied with wastewater of houses or factories enter the water cycle and pollute the plants and aquatic. Researches have proved the presence of Xenoestrogens in the several medical failures. In the last ten years scientific studies have showed the adverse effects of these chemicals on human health (Vidaeff and Ramin, 2005). One of environmental Xenoestrogens is BPA. It is an inexpensive synthetic organic compound with chemical

formula $\text{C}_{15}\text{H}_{16}\text{O}_2$, that was used in the production of polycarbonate plastics and epoxy resins, drink bottles, glasses, contact lenses, furniture, utensils disposable and etc (Hajszan and Leranath, 2010).



Scheme 1. BPA (Kang *et al.*, 2006)

Since 1950, BPA has been widely used in manufacturing plastics polycarbonate, epoxy resin, some dental materials and as a non-polymeric additive in plastics (Wolstenholme *et al.*, 2010). BPA is a compound with similar structure of natural steroids of the body that is replaced with them and destroyed the construction and operation of steroid hormones. Also it may disrupt steroids function in the process of liver (Yasutake *et al.*, 2009; Assy *et al.*, 2008). BPA can interfere in the synthesis and clearance of hormones and also change the hormone receptor gene expression and activity of genes of the target tissues (Maclusky *et al.*, 2005).

Fatty liver is a reversible disease characterized by the accumulation of triglyceride and other fats in liver cells and liver inflammation. In this disease, usually more than 5% of liver weight is fat (Sherlock *et al.*, 2002). Most patients are 40-60 years old people. Fatty liver is more common in women and also in children older than 10 years can be created. This liver dysfunction is chronic and will remain stable for many years (Zelber-Sagi *et al.*, 2006).

Two main types of fatty liver are:

1. Alcoholic fatty liver: This condition may occur in people who consume alcohol. Alcohol increases blood fats level by accumulating of fat in the liver and causes fatty liver disease, but in Iran this problem has other reasons.

2. Non-Alcoholic fatty liver: Nonalcoholic fatty liver is known as a major health problem. In fact, non-alcoholic fatty liver is a chronic liver disease which includes a wide range of clinical symptoms (from asymptomatic to severe inflammation of the liver with fibrosis and cirrhosis sometimes). NAFLD can be diagnosed through increasing the level of liver enzymes (alanine aminotransferase and aspartate aminotransferase), as well as by ultrasound and MRI (Magnetic Resonance Imaging) (Angulo., 2002).

Most effective parameters that causes NAFLD involve: Obesity, hyperglycemia, type 2 diabetes and hyperlipidemia (Pagano *et al.*, 2002). Studies have shown more than 75% of obese people have this disease (Hamaguchi *et al.*, 2005). NAFLD in the northern and southern parts of United States, Australia and New Zealand, the Middle East and Europe has a high prevalence rate from 10 to 24 % (Angulo *et al.*, 2002). Around 20 to 30 % of adults in developed countries have increase in fat accumulation in liver (Bedogni *et al.*, 2004; Bellentani *et al.*, 2000). The half of these people have diabetes and around 80 % of them are obese (Gupte *et al.*, 2004; Del Gaudio *et al.*, 2002). The results of studies in Iran reveal about 2.04-2.09 % of people have NAFLD (Pourshams *et al.*, 2005; Sotoudehmanesh *et al.*, 2006; Sohrabpour *et al.*, 2010). Studies have shown that the incidence of many diseases, including type 2 diabetes, coronary heart diseases, normal growth weakness, cancer particularly in the reproductive organs, memory impairment, IQ neurological are related to the use of BPA (Singleton *et al.*, 2003).

Due to the important effects of this substance on human health and little researches in this area, especially in Iran, the main aim of this study is to evaluate of the effect of oral administration of BPA on Serum lipids (Triglyceride, Total Cholesterol, HDL and LDL), Serum liver enzymes (AST, ALT and ALP) and blood glucose values in rats.

MATERIALS AND METHODS

The recent study is a prospective clinical trial in which 20 male rats of the Sprague-Dawley race, the aged 18 weeks and weighing 220-300 grams were treated by gavage for 15 days. Before the research, all the animals were kept in standard Plexiglas cages in order to adapt to conditions for two weeks. Water and food were freely access to them. First, nasogastric tube which known as esophageal or gavage needle was used. The reason of the use of metallic gavage was that rats could not chew it. Metallic gavage pipe sizes 8, 5 and 5.2 cm was determined based on the distance between the lips of the animal to the last gear. Animals at condition of controlled temperature of $22 \pm 2^\circ\text{C}$, humidity of $60 \pm 5\%$, 12 hours of light and 12 hours of darkness were kept (lamps were on from 6 am to 6 pm). Animals were randomly divided into 4 groups of 5 as follows:

Group I: A control group without the use of any particular matter

Group II: A shame group consist of sesame oil and alcohol (as a solvent for BPA)

Group III: A group receiving daily 5 $\mu\text{g}/\text{kg}/\text{day}$ of BPA for 2 weeks

Group IV: A group receiving daily 50 $\mu\text{g}/\text{kg}/\text{day}$ of BPA for 2 weeks

Appropriate volume at doses 5 and 50 $\mu\text{g}/\text{kg}/\text{day}$ of BPA solution were administered for 2 weeks. In any promise gavage 4 ml drug and solvent were fed. During gavage, no anesthetic or anesthesia was used and all the rats were awake. At the end of the gavage period, immediately blood samples were taken from rats heart, all the animals were killed. The rats did not use any food 6 hours before blood sampling. Blood sampling was done at the amount of 1-1.5 ml. The sample was transferred to a test tube containing EDTA and immediately was frozen at -18 to -20°C . Then the specimens were centrifuged by Rotofix centrifuges at 3000 rpm for 15 minutes and the separated plasma with an average volume between 0.5 to 0.7 ml was collected with a pipette and transferred to microtube. Plasma was placed in Selectra device and the data was measured. This device consists of two parts, in one part solution is poured according to Pars test field pre-built solution and in another part solution of the enzyme or the other measurable lipid is poured. Serum lipid of LDL and HDL are two solutions of pre-built solutions of Pars test field. Hypochlorous acid 9% is used to rinse through plasma fatal. Measured numbers based on a defined wavelength in the device are read in different kits.

Statistical analysis: In this study, data was analyzed by using statistical program (SPSS version 19). For statistical analysis, the statistical test of (One way-ANOVA) and Tukey's test were used.

Also the numbers were considered as Mean ± SEM and the significance level of (P<0.05) in the results section. The values are quantitative variables and are known as dependent or response variables. On the other hand, the two control groups have been considered as the variable, which determine the groups under study. Data are presented as mean ± standard error in the results section.

FINDINGS

According to the findings of this study AST, ALT and ALP in groups III and IV have had significant decrease

(P<0.05) comparing with the group I. In addition, there was no significant difference (P<0.05) between groupie with groups III and IV (Fig. 1).

Also in this study, results showed that triglyceride and LDL cholesterol of the group III have had a significant increase comparing with group I (P<0.05). But group IV compared with the groups I and II had no significant difference (P>0.05); furthermore, the serum total cholesterol, serum HDL and serum glucose in groups III and IV not shown significant different (P>0.05) compared with the groups I and II (Fig. 2 and 3).

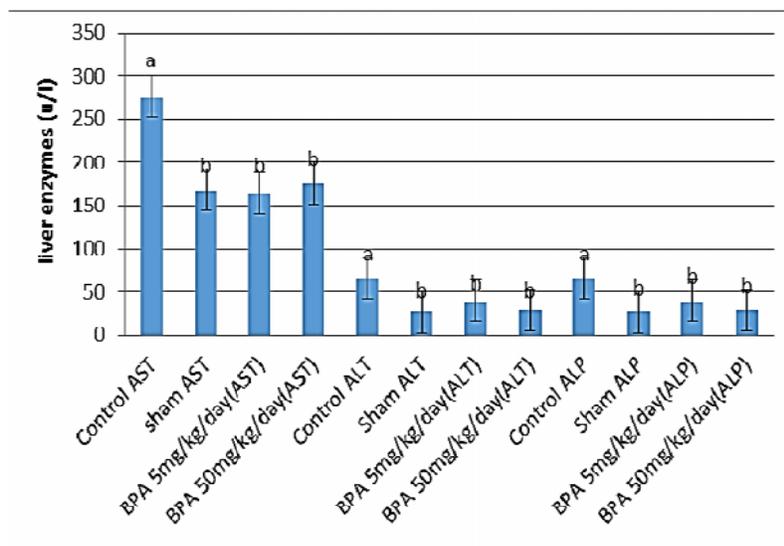


Fig. 1. Effect of oral administration of PBA on liver enzymes ALT, AST and ALP.

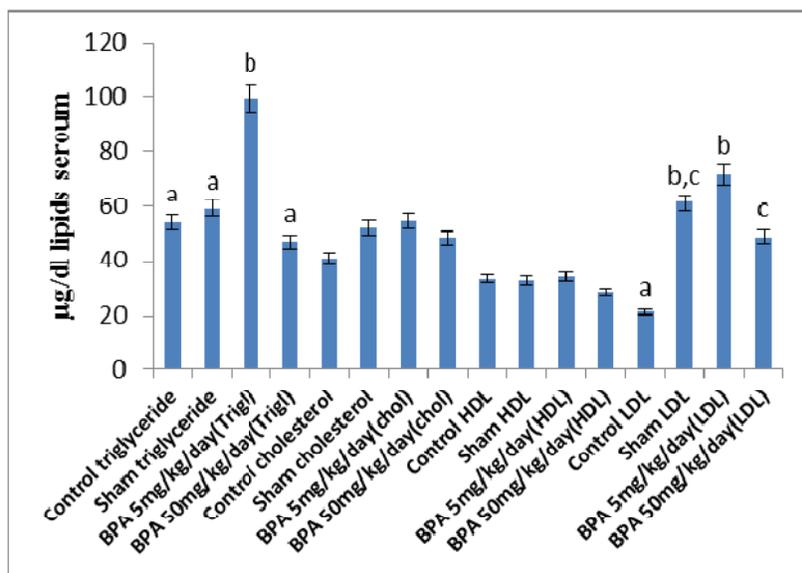


Fig. 2. Effect of oral administration of PBA on triglyceride, total cholesterol, LDL and HDL serum.

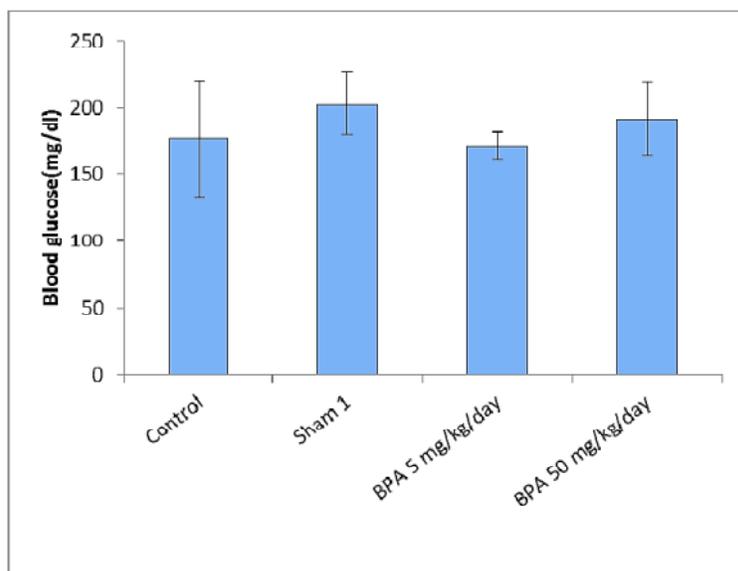


Fig. 3. The effect of oral administration of BPA on serum glucose.

The study of obtained photomicrographs of rats liver proved that groups I and II don't have change in the tissues. Destruct in the cytoplasm and the nucleus of hepatocytes was observed in normal mode (Fig. 4A and

B). BPA administered at dose of 50 $\mu\text{g}/\text{kg}/\text{day}$ increases hepatocyte polymorphism comparing to other groups (Fig. 4D).

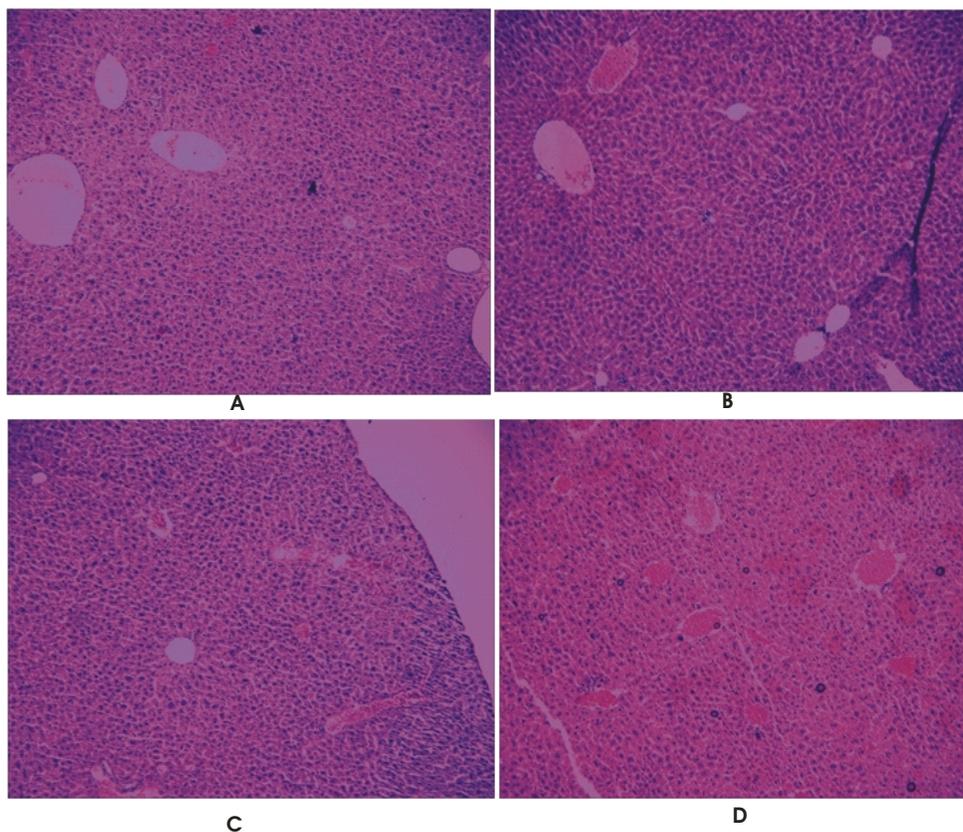


Fig. 4. Photomicrographs of rats liver tissue. A) group I, B) group II, C) group III, D) group IV (H & E, $\times 100$).

DISCUSSION

According to the results of this study, in groups III and IV a significant decrease in amount of liver enzymes AST, ALT and ALP was observed compared with the group I. Also, there was no significant difference between group II with groups III and IV. It seems that properties of sesame oil have reduced the amount of AST enzyme. In a study done by Hassan and colleagues, 4 doses of BPA 50, 10, 1, 1% $\mu\text{g}/\text{kg}/\text{day}$ were fed to the rats for 4 weeks. The group that consumed dose of 50 $\mu\text{g}/\text{kg}/\text{day}$ showed a significant increase in liver enzymes of AST and ALP than the control group (Hassan *et al.*, 2012). In the research done by Sangai and Verma, within 30 days of the consumption of doses of 60 and 120 $\mu\text{g}/\text{kg}/\text{day}$ of BPA a significant increase were observed in liver enzymes AST, ALT and ALP (Sangai *et al.*, 2012). In a research conducted by Mourad and Khadrawy on the rats receiving doses of 25 and 10 $\mu\text{g}/\text{kg}/\text{day}$ of BPA for 6 and 10 weeks, 5 days per week, the results showed the group which received 6 weeks dose of 25 $\mu\text{g}/\text{kg}/\text{day}$ had significantly increase in liver enzymes AST and ALT than the control group. But in the group which consumed the dose 10 $\mu\text{g}/\text{kg}/\text{day}$ for 6 and 10 weeks, there was no significant difference (Mourad *et al.*, 2012). In the research conducted by Helal *et al.*, subcutaneous injection at the amounts of 10 and 30 $\mu\text{g}/\text{kg}/\text{day}$ of BPA after 15 and 30 days significantly increased ($p < 0.01$) the liver enzymes level of ALT, AST and ALP (Helal *et al.*, 2013). It seems that the difference in level of liver enzymes in our study with the previous studies is due to the longer exposure of rats to BPA in the previous studies (4-6 weeks) than the present study (2 weeks) and also the difference in the less amount of BPA prescribed (5 $\mu\text{g}/\text{kg}/\text{day}$) in this study. According to the results of this study, in group III significant increase in triglyceride level not observed compared to groups I and II. In the study done by Kukkonen and Rahamaa, at dose of 50 $\mu\text{g}/\text{kg}/\text{day}$ of BPA for two weeks significant difference in serum triglyceride level was not observed and this confirms the validity of the research (Nieminen *et al.*, 2002). In research conducted by Helal *et al.*, subcutaneous injection at the amounts of 10 and 30 $\mu\text{g}/\text{kg}/\text{day}$ of BPA after 15 and 30 days significantly increased ($p < 0.01$) serum triglyceride level. BPA mimics estrogen activity and can be cytotoxic (Mourad *et al.*, 2012). According to the results of this study, comparing serum total cholesterol in groups III and IV with groups I and II do not show significant difference. In the study conducted by Kukkonen and Rauhamaa, at dose of 50 $\mu\text{g}/\text{kg}/\text{day}$ of BPA for two weeks significant change in serum level of total cholesterol was not observed (Nieminen *et al.*, 2002). According to the research of Helal *et al.*, subcutaneous injection at the doses of 10 and 30 $\mu\text{g}/\text{kg}/\text{day}$ of BPA after 15 and 30 days significantly increased ($p < 0.01$) serum total cholesterol level. BPA

can have different effects on rats in each race (mother and son) (Wyatt, 2011). According to the results of this study, in the groups III and IV significant difference was not observed in amount of serum HDL compared with groups I and II. It seems that the administration at these doses of BPA and the duration of treatment had no effect on serum HDL level. In the study carried out by Kukkonen and Rauhamaa of consumption at dose 50 $\mu\text{g}/\text{kg}/\text{day}$ of BPA for two weeks significant difference in serum level of HDL was not observed (Nieminen *et al.*, 2002). According to the research of Helal *et al.* subcutaneous injection at doses of 10 and 30 $\mu\text{g}/\text{kg}/\text{day}$ of BPA after 15 and 30 days caused a significant decrease ($p < 0.01$) in serum HDL level (Mourad *et al.*, 2012). It seems that the difference in the amount of serum HDL in our study and the research of Helal *et al.* (2013) can be related to administration of BPA (orally or by subcutaneous injection). The research of Gail and colleagues shows the ways administration of BPA orally or subcutaneously are effective on the metabolism and absorption by the body (Prinsa *et al.*, 2011). According to the results of this study, in groups II, III and IV a significant increase was seen in LDL cholesterol level compared to group I. This shows that the consumption of sesame oil and BPA increases LDL cholesterol value. In the study conducted by Kukkonen and Rauhamaa, the consumption at dose 50 $\mu\text{g}/\text{kg}/\text{day}$ of BPA for 2 weeks led to a significant increase in LDL cholesterol (Nieminen *et al.*, 2002). According to the research done by Helal *et al.*, subcutaneous injection at the amounts of 10 and 30 $\mu\text{g}/\text{kg}/\text{day}$ of BPA after 15 and 30 days significantly increased ($p < 0.01$) of LDL cholesterol level (Mourad *et al.*, 2012). According to the research conducted by Wyatt BN in male rats, the consumption of BPA increased adipose tissue but there was not observed significant difference in the female rats (Wyatt, 2011). The results of our study exhibited that serum glucose level don't have significant difference in groups III and IV than groups I and II. It seems using at these doses of BPA and the duration of treatment had no effect on the blood sugar level. According to the study done by Jayashree and colleagues, administration at doses 20 and 200 $\mu\text{g}/\text{kg}/\text{day}$ of BPA for 29 days did not have a positive or negative effect on the value of serum glucose (Jayashree *et al.*, 2013). The natural amount of glucose in rat is 106-278 $\mu\text{g}/\text{dl}$. BPA can change the function of pancreatic beta cells through nonclassical membrane ER (ncmER), in vitro (Ropero *et al.*, 2002). According to the research conducted, using at doses 3.5, 35 and 350 $\mu\text{g}/\text{kg}/\text{day}$ of BPA for 6 weeks significantly decreased blood sugar level in strains of female rats but significant effect in male rats was not observed (Wyatt, 2011). It seems that the difference in the results of the level of serum glucose with the previous studies is due to the rats' longer exposure to BPA in the previous studies (6 weeks) than the present study (2 week).

CONCLUSION

The results suggest that administration at high and low doses of BPA have been significantly decreased the liver enzymes and it has not cause any significant change in serum total cholesterol, serum HDL and serum glucose levels, but has led to the increase of LDL cholesterol level.

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REFERENCES

- Assy, N; Nasser, G; Kamayse, I; Nseir, W; Beniashvili, Z; Djibre, A and Grosovski, M (2008). Soft drink consumption linked with fatty liver in the absence of traditional risk factors. *Can. J. Gastroenterol. Hepatol.*, **22**: 811-816.
- Angulo, P (2002). Nonalcoholic fatty liver disease. *N. Engl. J. Med.* **346**: 1221-1231.
- Bedogni, G and Bellentani, S (2004). Fatty liver: how frequent is it and why. *Ann. Hepatol.* **3**: 63-65.
- Bellentani, S; Saccoccio, G; Masutti, F; Croce, LS; Brandi, G; Sasso, F and Cristanini, G (2000). Prevalence of and risk factors for hepatic steatosis in Northern Italy. *Ann. Intern. Med.*, **132**: 112-117.
- Del Gaudio, A; Boschi, L; Del Gaudio, GA; Mastrangelo, L and Munari, D (2002). Liver damage in obese patients. *Obes. Surg.*, **12**: 802-804.
- Gupte, P; Amarapurkar, D; Agal, S; Baijal, R; Kulshrestha, P; Pramanik, S and et al (2004). Nonalcoholic steatohepatitis in type 2 diabetes mellitus. *Can. J. Gastroenterol. Hepatol.*, **19**: 854-858.
- Hajszan, T and Leranath, C (2010). Bisphenol A interferes with synaptic remodeling. *Front. Neuroendocrinol.*, **31**: 519-530.
- Hamaguchi, M; Kojima, T; Takeda, N; Nakagawa, T; Taniguchi, H; Fujii, K and et al (2005). The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. *Ann. Intern. Med.* **143**: 722-8.
- Hassan, ZK; Elobeid, MA; Virk, P; Omer, SA; El Amin, M; Daghestani, MH and AlOlayan, EM (2012). Bisphenol A Induces Hepatotoxicity through Oxidative Stress in Rat Model. *Oxid. Med. Cell. Longev.* 2012: 1-6.
- Helal, EG; Badawi, MM; Soliman, MG; Abdel-Kawi, NA; Fadel, HA and Abozaid, NM (2013). Physiological and Histopathological studies on Bisphenol-A compound as xenoestrogen in male albino rats. *EJHM.* **50**: 127-136.
- Jayashree, S; Indumathi, D; Akilavalli, N; Sathish, S and Selvaraj, J and Balasubramanian, K (2013). Effect of Bisphenol A on insulin signal transduction and glucose oxidation in liver of adult male albino rat. *Environ. Toxicol. Pharmacol.* **35**: 300-310.
- Keri, RA; Ho, SM; Hunt, PA; Knudsen, KE; Soto, AM and Prins, GS (2007). An Evaluation of Evidence for the Carcinogenic Activity of Bisphenol A. *Reprod. Toxicol.*, **24**: 240-252.
- Mourad, IM and Khadrawy, YA (2012). The sensitivity of liver, kidney and testis of rats to oxidative stress induced by different doses of bisphenol A. *Life.*, 2012; **50**: 19.
- Maclusky, NJ; Hajszan, T and Leranath, C. (2005). The environmental estrogen BPA inhibits Estradiol-induced Hippocampal synaptogenesis. *Environ. Health. Perspect.*, **113**: 675-679.
- Nieminen P (2002). Effects of bisphenol A and phytosterols on the European polecat (*Mustela putorius*) and the field vole (*Microtus agrestis*). University of Helsinki.
- Pagano, G; Pacini, G; Musso, G; Gambino, R; Mecca, F; Depetris, N and et al (2002). Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: further evidence for an etiologic association. *J. Hepatol.* **35**: 367-372.
- Pourshams, A; Malekzadeh, R; Monavvari, A; Akbari, MR; Mohamadkhani, A; Yarahmadi, S (2005). Prevalence and etiology of persistently elevated alanine aminotransferase levels in healthy Iranian blood donors. *Can. J. Gastroenterol. Hepatol.*, **20**: 229-33.
- Prins, GS; Ye, SH; Birch, L; Ho, SM and Kannan, K (2011). Serum bisphenol A pharmacokinetics and prostate neoplastic responses following oral and subcutaneous exposures in neonatal Sprague-Dawley rats. *Reprod. Toxicol.* **31**: 1-9.
- Ropero, AB; Soria, B and Nadal, A (2002). A non classical estrogen membrane receptor triggers rapid differential actions in the endocrine pancreas. *Mol. Endocrinol.*, **16**: 497-505.
- Sangai, NP and Verma, RJ (2012). Quercetin ameliorates Bisphenol A -induced toxicity in mice. *Acta Pol. Pharma.*, **69**: 557-563.
- Sherlock, S and Dooley, J (2002). Diseases of the Liver and biliary System. 11th ed. Oxford: Blackwell Science. 423-433.
- Singleton, DW and Khan, SA.(2003). Xenoestrogen exposure and mechanisms of endocrine disruption. *Front Biosci.* **1**: 110-118.
- Sotoudehmanesh, R; Sotoudeh, M; Ali-Asgari, A; Abedi-Ardakani, B; Tavangar, SM and Khakinejad, A (2006). Silent liver diseases in autopsies from forensic medicine of Tehran. *Arch. Iran. Med.*, **9**: 324-8.
- Sohrabpour, AA; Rezvan, H; Amini-Kafiabad, S; Dayhim, MR; Merat, S and Pourshams, A (2010). Prevalence of Nonalcoholic Steatohepatitis in Iran. A Population based Study. *Middle East J. Dig. Dis.*, **2**: 14-19.

- Smith, DR; Striplin, CD; Geller, AM; Mailman, RB; Drago, J; Lawler, C.P. and Gallagher, M. (1998). Behavioral assessment of mice lacking D1 dopamine Receptors. *Neurosci. Res.*, **86**: 135-146.
- Vidaeff, AC and Ramin, SM. (2006). From concept to practice: the recent history of preterm delivery prevention. Part I: cervical competence. *Am. J. Perinat.*, **23**: 3-13.
- Wolstenholme, JT; Rissman, EF and Connelly, JJ (2010). The Role of Bisphenol A in shaping the Brain, Epigenome and behavior. *Horm. Behav.*, **59**: 296-305.
- Wyatt, BN (2011). The effects of bisphenol-A on adipose tissue development, metabolism, and endocrine function and the role it may play in the development of obesity. Master's Thesis, University of Tennessee.
- Yasutake, K; Nakamuta, M; Shima, Y; Ohyama, A; Masuda, K and Haruta, N (2009). Nutritional investigation of non-obese patients with non-alcoholic fatty liver disease: the significance of dietary cholesterol. *Scand. J.Gastroenterol.*, **44**: 471-477.
- Zelber-Sagi, S; Nitzan-Kaluski, D; Halpern, Z and Oren, R (2006). Prevalence of primary non-alcoholic fatty liver disease in a population-based study and its association with biochemical and anthropometric measures. *Liver Int.* **26**: 856-863.