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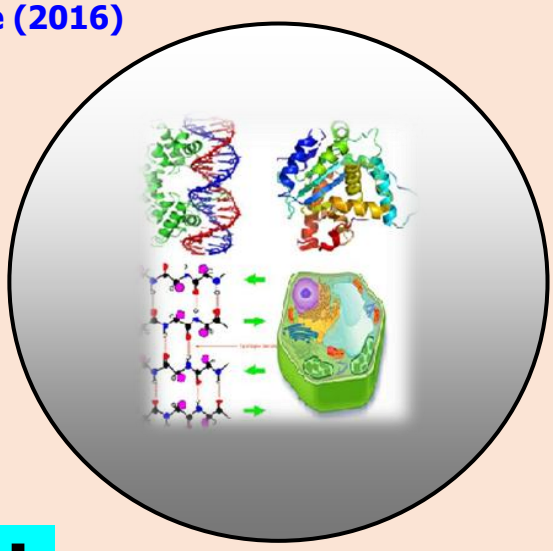

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RESEARCH PAPER

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Biocompability of Apricot or/and Rutin on the Di-2-(Ethylhexyl) Phtalates Induced Rat Testis

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ABSTRACT

Phthalates are carcinogens may cause foetal death, malformation, teratogenicity in particular, testicular damage and toxic effects on the reproductive system in laboratory animals. It is reported that disorders during development of testicles and other elements of the male reproductive system particularly cause more damages than the damages in adults. We have aimed to investigate the protective and inhibitory effect of apricot and rutin on damaged testis tissues by Di-2-(Ethylhexyl) Phtalates (DEHP) in rats. Total 42 rats were used for this experiment. DEHP was used in order to creating testicular damage. Rats were feed with either apricot or rutin to evaluate antitoxic effects. After 28 days of feeding period animals were sacrified and testis tissues taken. Oxidative stress parameters such as Malondialdehyde (MDA), Nitric Oxide (NO), Glutathione (GSH), were studied to observe the effects of apricot and rutin. Histological examination of testis tissues carried out by hematoxylin and eosin stain.We observed that apricot and rutin administered groups had lower MDA and NO levels than DEHP administered groups. There was no statistically difference between groups in terms of GSH levels. Histological analysis showed that apricot and rutin administered groups has less damage than DEHP administered groups.We suggest that feeding with natural food can be preventing testicular toxicity of DEHP by reducing oxidative stress.
Key Words: DEHP, Testis, Rutin, Apricot and Oxidative damage.

INTRODUCTION

Di-2-(Ethylhexyl) Phtalates (DEHP) is used for various purposes including wallpapers, building materials used for floor coverings, automobiles and vehicle seats, synthetic curtains, clothes, such as raincoats and rain boots, food-packaging materials, tether, a variety of toys, pacifiers and bottle teats.(Zarean, Keikha et al. 2016). It is determined that phthalates are animal carcinogens and may cause foetal death, malformation, teratogenicity and in particular, testicular damage and toxic

effects on the reproductive system in laboratory animals(Mariana, Feiteiro et al. 2016). It is reported that phthalates adversely affect development of the male reproductive system in the event of prenatal exposure. However, its toxicity mechanism could not be clarified yet (Wang, Chen et al. 2012). DEHP considered being an endocrine disrupting chemical and it is known that especially children are more sensitive to this effect (Ejaredar, Nyanza et al. 2015). It is reported that disorders during development of testicles, penis and other elements of the male reproductive system particularly cause much more damages than the damages in adults (Motohashi, Wempe et al. 2016).

Reactive oxygen species (ROS) are produced by living organisms as a result of regular cellular metabolism. At low to moderate concentrations, they function in physiological cell processes, but at high concentrations, they cause to unwanted alteration on major cell components, such as lipids, proteins, and DNA as well. When the balance between oxidant/antioxidant goes in favor of oxidants this condition is termed as "oxidative stress." Oxidative stress leads to many pathological conditions including cancer, neurological disorders, atherosclerosis, hypertension, ischemia/perfusion, diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, and asthma. Highly organized organisms have enzymatic and non enzymatic antioxidants as an integrated antioxidant systems which are usually efficient in blocking detrimental effects of ROS.(Birben, Sahiner et al. 2012)

In vivo and *in vitro* test systems revealed that apricot has a high antioxidant activity and this antioxidant activity comes from high carotenoid and flavonoid content(Imrak, Kuden et al. 2017).Apricot is rich in flavonoids such as rutin and catechin (Mitani, Horinishi et al. 2013). Rutin, also known as vitamin P, has a high radical scavenging and antioxidant activity and it has antitumoral, antithrombotic, anti-inflammatory and anti-allergic effects. It also plays protective roles against atherosclerosis and coronary heart disease (Saklani, Gupta et al. 2016). Many studies showed that flavonoids possess an antioxidant effect substantially contribute to prevent adverse effects of phthalate toxicity in rats (Ge, Han et al. 2015, Abd-Ellah, Aly et al. 2016).

In line with these studies and information, we have aimed to investigate the protective and inhibitory effect of apricot and rutin on DEHP toxicity.

MATERIAL AND METHODS

Wistar albino-type male rats used for this study were procured from Inonu University, the Reproduction and Research Centre for Experimental Animals. Codes of the Experimental Animal Ethics Committee of Inonu University were observed during the study. 18-week male rats weighing 205±13 gram were housed in standard housing cages until the day of experiment. Their drinking water was changed daily and standard cage cleaning was performed during the study. Rats were housed in ventilated, 12-hour light and 12-hour dark-lit rooms in a room temperature between 24°C and 27°C. Total 42 rats were used for this experiment and experimental groups arranged as above.

Control Group (n=7): The control group was fed with standard pellet feed.

DEHP Group (n=7): The rats in this group were administered 500 mg/kg/day of DEHP by gavage.

DEHP + Apricot Group (n=7): DEHP 500 mg/kg/day were administered to the rats by gavage for 28 days, and the same group was fed with 20% apricot mixed feed.

DEHP + Rutin Group (n=7): The rats in this group were administered DEHP 500 mg/kg/day and rutin 25 mg/kg/day by gavage.

Apricot Group (n=7) was fed with 20% apricot mixed feed.

Rutin Group (n=7): The rats in this group were administered rutin 25 mg/kg/day by gavage.

Preparation for Analysis of Testis Tissues

Rats were sacrificed under general anaesthesia after 28 days. A piece of testis tissue received from the rats was fixed and stored in 10% of formaldehyde for pathological examination. Remaining pieces of the tissue wrapped with aluminium foils and placed in plastic bags. Then, tissue samples were kept in a freezer at -70°C until the day biochemical and histological tests performed.

Tissue Homogenization and Buffers

Approximately 200 mg. testis tissue was homogenized in 2 ml. of Tris-HCl buffer (pH: 7.0) at 16000 rev/min. After homogenization, tubes were centrifuged for 10 minutes at 4000 rpm in a refrigerated centrifuge. Supernatans aliquate into separate eppedorf tubes and stored into freezer until the working day.

Glutatione (GSH) Measurement

The total sulfhydryl groups content was determined as descripted previously(Karabulut, Gul et al. 2010). Briefly prepared a mixture containing 500 µL of the homogenate, 500 µL of 0.3 M Na_2HPO_4, and 500 µL of 0.04% Ellman reagent (DTNB). Absorbance was measured at 412 nm with a spectrophotometer.

Malondialdehyde (MDA) Analysis

MDA, the most important indicator of lipid peroxidation, was analysed as descripted previously (Simsek, Gurocak et al. 2012). The basic principle of the analysis is based on creating by MDA of a pink-colour chromogen by reacting when heated in an acid environment with thiobarbituric acid. Intensity of the pink colour is proportional to the concentration of MDA in the sample.

Nitric oxide (NO) Measurement

NO measurement of testicular tissues was analysed as descripted before (12). Total nitrite (nitrite + nitrate) was measured by spectrophotometry at 545 nm (Pharmacia LKB Ultraspec Plus; Biochrom Ltd, Cambridge, UK) after conversion of nitrate to nitrite by copperized cadmium granules. A standard curve was established with a set of serial dilutions of sodium nitrite. Results were expressed as micromole per liter.

Histological evaluation

Paraffin blocks were cut in 5-mm-thick sections, and the sections were stained with hematoxylin and eosin (H&E) and examined under the light microscope. The histological evaluation was done in a blind, randomly numbered fashion. Six animals and six sections were evaluated for each animal. A four-level grading scale similar to that of Cosentino et al. was used to quantify histological injury (Cosentino, Nishida et al. 1986, Whitney 2012).

Statistical Analysis

Data were evaluated using the SPSS 20 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.) statistical software package. Mean ± standard deviation and standard error values were used as the variables. In addition, homogeneity of the variances, one of the prerequisites of parametric tests, was checked by "Levana" test. Normality assumption was checked by “Shapiro-Wilk” test.The Kruskal-Wallis test and the Bonferroni-Dunn test, one of multiple comparison tests, were used for comparison of three or more than three groups when the comparison by the One Way Variance Analysis and the Tukey HSD test, one of multiple comparison tests, failed. The level of statistical significance was considered as$p < 0.05$ and $p < 0.01$.

RESULTS

Results of oxidative stress parameters belong to experimental groups given in Table 1. The highest GSH level was found in the apricot administered group and the lowest GSH level in the DEHP group, although no statistically significant difference was found between average scores of the groups in terms of the GSH measurement. NO was at the lowest level in the apricot administered group, while it was at the highest level in the DEHP administered group. We observed that apricot reduced the increased NO induced by DEHP in the DEHP and apricot administered group. Again, the level of NO in the rutin and DEHP administered group reduced compared to the group administered only DEHP. The highest MDA levels were observed in the DEHP group. MDA levels reduced in the DEHP + Apricot administered group and the lowest MDA levels was found in the Apricot administered group which was close to the Control group. Interestingly, MDA levels of DEHP + Apricot group was lower comparing to the DEHP-Rutin group.

Histological Results

To assess the effect of DHEP on rat testicular tissue pathology, the testicular tissues from each group were analyzed by H&E staining. Figure 1 represents the histological findings used for injury scoring (injury score of 0–4, respectively). The mean histological injury scores were significantly higher in DHEP-treated groups compared to control and other groups (Figure 1 G and H, Figure 2). The control rats showed normal seminiferous tubule morphology (Figure 1 A and B). Seminiferous tubules, germ cells, Sertoli, and Leydig cells appear complete, without infiltration and haemorrhagic signs. However, the testicular tissues from DHEP treated groups displayed severe degenerative changes in the seminiferous epithelium. Light microscopy revealed that DHEP exposure resulted in interstitial space dilatation, germ cell loss, oedema, Leydig cell proliferation and thicker myoid cell layer in the testicular tubule of rats (Figure 1G–H, Figure 2). Rutin and Apricot groups displayed lower to moderate disruption of the seminiferous epithelium (Figure 1I-L). The antioxidant effect of Rutin and Apricot on the DEHP exposed testes is also observed. No significant difference was detected between Rutin and Apricot groups (Figure 2). However, injury scores in Rutin and Apricottestis groups were significantly reduced compared the injury scores obtained in DEHP exposed groups.

DISCUSSION

Phthalates, or phthalate esters, are phthalic acid esters and added to plastics usually to increase flexibility. They are used to transform hard plastic into polyvinyl chloride flexible plastic (Sampson and de Korte 2011). In our study only DEHP administered group has the highest NO concentration and this elevation was lowered by whether apricot or rutin given groups. Abdel-Khawi et al found that DEHP administration leads to increase in expression of inducible nitric oxide synthase (iNOS) gene in testis tissue (Abdel-Kawi, Hashem et al. 2016). Rutin and apricot might play a role to suppressing this effect of DEHP.

MDA is the well-known indicator of lipid peroxidation and DEHP administered group has the highest MDA level in our study. This elevation of MDA levels due to DEHP administration has been reported by other researchers(Abd El-Fattah, Fahim et al. 2016, Ma, Zhou et al. 2017, She, Jiang et al. 2017). But MDA levels were lowered in the DHEP + Rutin and DEHP + Apricot groups. Surprisingly, DEHP + Apricot group had lower MDA levels than DEHP + Rutin group. This result showed that intake of whole food may have more beneficial effects in point of oxidative stress.

Detailed morphological examinations of testicular lesions induced by phthalates in pubertal and adult rats have shown that sertoli cells are also one of the first targets of phthalates. Incidents like loss of germ cells or entry by germ cells into seminiferous tubular lumen earlier indicate that phthalates have specific effects on the spermatogenic step (Wang, Wei et al. 2004, Xu, Huang et al. 2013). Our study has the parallel results in terms of histological changes.

Age of the animal is a critical factor for testicular toxicity of phthalates. Although reproductive toxicity in young adult rats is seen at high doses, testicular lesions occur with relatively shorter administrations. It is reported that toxicity occurs at lower doses in pubertal animals compared to adult animals and that testicular toxicity and severe toxicity testicular lesions are observed as well as tubular atrophy (Park, Habeebu et al. 2002).

Although observance of these disorders, also called ''phthalate syndrome", in human increases concerns about contact with phthalates, it is seen that no conclusive evidence is available or data are insufficient. Based on human studies, it is observed that the number of studies which associate phthalate contact with male reproductive system disorders has been increasing rapidly in recent years (Zhao, Ao et al. 2012, Erkekoglu, Giray et al. 2014, Ge, Han et al. 2015, Gao, Xu et al. 2017). Although data on biomarkers that can be used to determine contact with phthalates are insufficient, the original value of our study is important in terms of investigating antioxidant effects by directly administering a natural and conventional food to the chemical carcinogenesis mechanism created by phthalates.

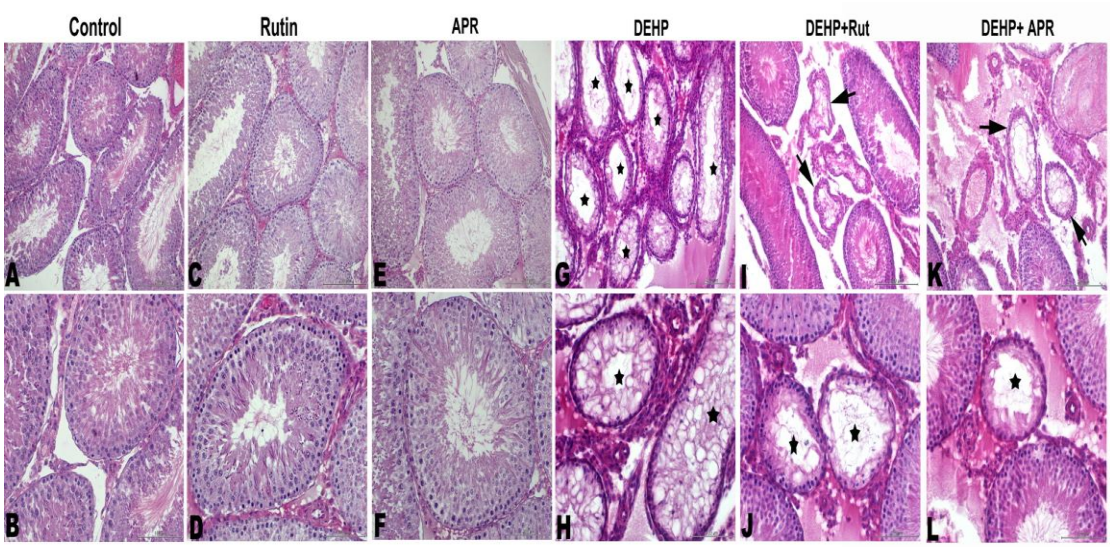

Figure 1. Histological findings used for injury scoring in rat testicular tissues.

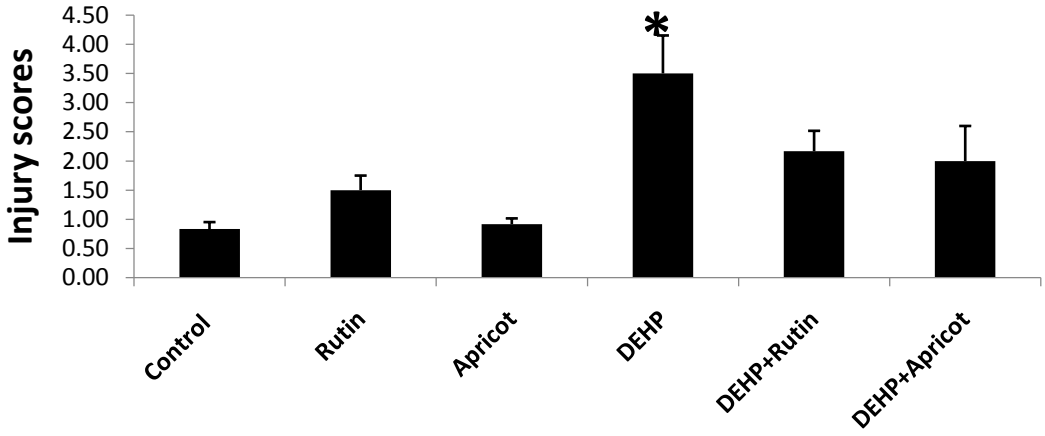

Figure 2. Histologic injury scores after exposure of DEHP, Rutin and Apricot groups. The highest injury score is observed in DEHP exposed testes. This score is found to be statistically significant than other groups (*p<0, 05). The antioxidant effect of Rutin and Apricot on the DEHP exposed testes is also significantly different than DEHP group.

Table 1. Comperation of oxidative stress markers of DEHP administered group and other groups. Different letters in the same column indicate statistically difference between groups (p<0.05).

Groups	GSH		NO		MDA	
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Control	7	1.912 ± 0.069	7	4.443 ± 0.367	7	18.480 ± 0.990^{b}
Apricot	7	1.928 ± 0.064	7	3.686 ± 0.121^{b}	7	17.662 ± 1.523^{b}
Rutin	5	1.828 ± 0.020	5	4.140 ± 0.960^{b}	5	19.665 ± 0.376^{b}
DEHP	6	1.805 ± 0.090	6	5.109 ± 0.612^{a}	6	22.880 ± 1.332^{a}
DEHP + Apricot	6	1.872 ± 0.095	6	3.895 ± 0.847^{b}	6	18.621 ± 5.029^{b}
DEHP + Rutin	7	1.846 ± 0.066	7	4.076 ± 0.261^{b}	7	20.649 ± 0.827^{b}

As a result, the use of apricot, a vitamin and flavonoid, in a study which have not been studied with these combinations previously brings a different perspective to the study. Namely, antioxidant effects were observed in apricot-administered groups comparing to rutin-administered groups in comparison to the food and antioxidant drug extract given in this study. This study will shed significant light on future studies that will be carried out with different doses and combinations and open a new aspect.

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