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Research Article

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The Influence of the Myricetin on the Liver, Kidney, Spleen and Some Endocrine Glands of Male Rats at Prepubertal Period

Myricetinin Prepubertal Dönemde Erkek Sıçanların Karaciğer, Böbrek, Dalak ve Bazı Endokrin Bezler Üzerine Etkisi

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ABSTRACT

In this study, the effects of myricetin exposure to rats from postnatal day (PND) 23 to 53 at various doses were investigated. The male rats were divided into five groups and each group consisted of six animals. Group of rats were treated with myricetin 25 and 50 mg/kg/day in a suspension of corn oil. Positive control males were received gavage orally with 17α-ethinyl estradiol 0.7 and 7 µg /kg /body weight day and control males were received corn oil. End of the study, weights of liver, kidney, spleen, pancreas, thymus and adrenal gland were measured. Organ/body weight ratios were calculated and tissue sections were examined histologically. In liver, the TUNEL method was applied and evaluated. In results, absolute liver weights were decreased statistically in 0.7 and 7 µg/kg/day etinil estradiol and 50 mg/kg/day myricetin treatment groups, compared with the oil control group. Histopathological examination of the liver, kidney and spleen revealed significantly increased frequency of congestion, cell degeneration and mononuclear cell infiltration when compared with the control group. Also myricetin dose groups, the apoptotic cells were increased. This study demonstrated that orally gavages myricetin caused adverse effects on male liver, kidney, spleen and endocrine glands, during peripubertal period to pubertal period.

Key Words

Phytoestrogen, myricetin, endocrine glands, prepubertal male rat, liver, kidney.

ÖZ

Bu çalışmada, doğum sonrası (PND) 23'ten 53'e güne kadar çeşitli dozlarda myrisetine maruz kalmanın etkileri incelenmiştir. Erkek sıçanlar her grup altı hayvandan oluşan beş gruba ayrıldı. Sıçanlar, mısır yağı süspansiyonu içinde 25 ve 50 mg/kg/gün dozlarımda myricetin ile muamele edildi. Pozitif kontrol grubuna oral olarak 0.7 ve 7 µg/kg/vücut ağırlığı gün dozlarında 17α-etinil estradiol ve normal kontrol grubuna mide tüpü ile mısır yağı verildi. Çalışmanın sonunda karaciğer, böbrek, dalak, pankreas, timus ve adrenal bez ağırlıkları ölçüldü. Organ/ vücut ağırlık oranları hesaplandı ve doku kesitleri histolojik olarak incelendi. Karaciğerde TUNEL metodu uygulandı ve değerlendirildi. Sonuç olarak, mutlak karaciğer ağırlıkları, yağ kontrol grubuna kıyasla 0.7 ve 7 µg/kg/gün etinil estradiol ve 50 mg/kg/gün myrisetin uygulama gruplarında istatistiksel olarak azaldı. Karaciğer, böbrek ve dalağın histopatolojik incelemesinde, kontrol grubuyla karşılaştırıldığında, konjesyon, hücre dejenerasyonu ve mononükleer hücre infiltrasyonu sıklığında anlamlı artış olduğu görüldü. Ayrıca myrisetin doz gruplarında, apoptotik hücreler arttı. Bu çalışma, peripubertal dönemden pubertal döneme ağızdan mide tüpü ile verilen mirisetinin erkek sıçanların karaciğer, böbrek, dalak ve endokrin bezleri üzerinde olumsuz etkilere neden olduğunu göstermiştir.

Anahtar Kelimeler

Fitoöstrojen, myrisetin, endokrin bezler, prepubertal erkek sıçan, karaciğer, böbrek.

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INTRODUCTION

In recent years, exposure to the chemical compounds, which are end up with reproductive system defects, is a common worldwide concern. It was reported that some compounds known as the endocrine disruptors have estrogenic and androgenic effects caused male reproductive tract disorders such as tumor formations and decreased sperm count and function, disrupted spermatogenesis [1]. Exposure to these endocrine disruptors like phthalates, bisphenol A, S and F with food packaging, pharmaceutics, pesticides, solvents in cleaning products, detergents and personal care products. Besides the synthetic endocrine disruptors, there are natural estrogens as called as phytoestrogens which are found naturally in fruits and vegetables, and take in to the body with this way all day long [2]. Chemical structure of phytoestrogens is similar to endogenous estrogen, estradiol and acting like an estrogen with binding to the estrogen receptors of body tissues such as gonads, reproductive tract, placenta, mammary gland, central nervous system, bones etc. Hence, they may cause alterations on endogenous sex hormones concentration and sex differentiation and increase the risk of reproductive system tumors or developmental disorders especially during fetal development [3].

With the increase in human consumption of phytoestrogens, studies that address the consequences of phytoestrogens exposure are required. There is a lot of evidence, that consumption of some of these phytoestrogens could be an additive efficient tool to prevent and to treat several dysfunctions and diseases related to aging, mental processes, metabolism, cardiovascular diseases and reproduction cancers, menopausal symptoms, osteoporosis, atherosclerosis and stroke, and neurodegeneration [4-7]. Zhang et al., demonstrated both in vitro and in vivo evidence that combination of myricetin with radiotherapy can enhance tumor radiosensitivity of pulmonary carcinoma A549 and H1299 cells, and myricetin could be a potential radiosensitizer for lung cancer therapy [8].

Phytoestrogens are considered as polyphenolic antioxidants and they have four major classes; isoflavones, flavonoids, coumestans and lignans [9]. Flavonoids are the most common group of polyphenolic compounds in the human diet because of their relatively low toxicity and widely distributed in plants. Foods with a high flavonoid content include parsley, onions, berries, black tea and green tea, bananas, all citrus fruits, Ginkgo biloba, red wine, sea-buckthorns, wine and dark chocolate [10]. Myricetin was able to scavenge the hydroxyl free radicals, restrict H_2O_2-induced DNA strand breakage in human lymphocytes, protect against ROS production in red blood cells [11], active against ROS related oxidative stress in human LDL and human vascular endothelial cells [12]. Beside the strong anti-oxidant effect myricetin has a wide range of activities such as antiphotoaging, anti-inflamatory, anti-cancer properties, anti-platelet aggregation and antihypertensive, immunomodulatory, anti-allergic and analgesic [13-15]. Also, there is some researches on myricetin has focused on the protective effects of myricetin as an anti-tau effect against neurodegenerative diseases like Parkinson's and Alzheimer's diseases which can develop with defection of tau proteins. The compound also has an antigenotoxic and hepatoprotective effects. Furthermore, it has antidiabetic potential for non-insulin-dependent diabetes and antiobesity activities by stimulating the uptake of glucose independent of insulin receptors. Addition of antibacterial and antiviral roles, low concentrations of myricetin was strong inhibitor of reverse transcriptase from Rauscher murine leukemia virus (RLV) and Human Immunodeficiency Virus (HIV) [15].

Leaving aside the possible beneficial protective effects of myricetin, when it is taken at high amounts, it can cause adverse effects on endocrine system. In vitro studies with MCF7 cells show that the estrogenic activity of myricetin which might be associated with breast tumors, especially in postmenopausal women. Also, it has shown in the utherotrophic assay, myricetin has increased relative uterus weights and uterine heights [16]. This product is purchased in tablets as dietary supplement due to its benefits on human health. However, there are not enough comprehensive and long term in vivo animal and human experiments about adverse effects of these supplements. In the present study, we hypothesized that exposure to myricetin in the EDSTAC male pubertal protocol [18] would alter the adverse effects on liver, kidney and endocrine glands, during peripubertal period to pubertal period.

METHODS

Chemicals

17β-ethinyl estradiol (%98) and myricetin (%96) were purchased from Sigma-Aldrich. Chemicals were dissolved in corn oil as a vehicle.

Animals

The animal experiment was approved under number 2010/4-4 by the Animal Experimentations Local Ethics Board of Hacettepe University (Hacettepe, Turkey). Twenty one day male Wistar albino rats were purchased from the Experimental Animals Production Center, Hacettepe University in Ankara, Turkey. All rats were housed in polycarbonate cages with stainless steel covers in an air-conditioned room (12 h light – 12 h dark cycle with a temperature 21±4°C and a relative humidity of %50±5). Rats were weighed weekly and their food intake (g wk-1) was determined. During the experimental period they were provided, ad libitum, with phytoestrogen-free pellet food, and tap water. The diet contains uncooked chicken egg, pasta, table salt, bone soup, corn oil and wood shaving. Food and water comsuming of animals were measured daily.

Doses and Administration of Chemicals

All treatments were administered daily by oral gavage from postnatal day (PND) 23 through 53. Body weights were recorded daily and the dose administered each day and it was adjusted for body weight. The dosing solution was prepared by mixing the compound with corn oil to the desired concentration of 25 or 50 mg/kg. Doses were selected on the basis of consumption of daily flavonol intake in literature and the earlier study which examined estrogenic effects of myricetin at juvenile/peripubertal on female rats [16]. Male rats were divided into five groups and each group consisted of six animals. Group of rats were treated with myricetin 25 and 50 mg /kg/day in a suspension of corn oil. Positive control males were gavaged orally with 17β-ethinyl estradiol 0.7 and 7 µg/kg body weight/day and control males received corn oil only.

Organ Relative Weights and Histopathology

The animals were sacrificed with cervical dislocation on PND 53. The liver, kidney, spleen, pancreas, adrenal gland and thymus were weighed and the relative weights were calculated than fixed in 10% formalin for 24 h before transferring to 70% ethanol until later processing in paraffin. The tissues were embedded in 55oC paraffin, cut at 4 µm thickness, stained with Harris haematoxylin and eosin and then examined under a Leica light microscope (Germany) for histopathological evaluation and photographed with imagine program Pixera Pro 150ES (Pixera Corporation, Santa Clara, California, USA).

Histomorphometric Measurement

In kidney tissue, histomorphometric measurement of glomeruli was carried out in all groups. At least a hundred glomeruli for each group were selected; the shortest and the longest diameters for each glomerulus were measured using the Bs200prop program in an Olympus BX51 system light microscope. The glomerular volume was calculated using the following formula: 4 π(d(G)/2)3/3, where d(G) represented the arithmetic average of the longest and shortest diameters [18].

Immunohistochemical Staining

Cells undergoing programmed cell death were assessed using terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) method. Preparations on APS-coated glass slides were dehydrated in xylene, 100%, 95%, 80%, 70% ethanol and rinsed with phosphate buffer (PBS). Then they were incubated with 20 mg ml^{-1} proteinase K solution in 10 mM Tris/HCl, pH 7.4-8 (Roche) in a humidified chamber for 10 min at 37°C and rinsed in PBS, incubated with a blocking solution (3% H_2O_2 solution in methanol). Then sections were rinsed twice with PBS, the area around selected sections was dried and loaded with TdT-terminal deoxynucleotydyl transferase, 50 ml per section. Slides were rinsed twice in PBS and dried. Then 50 ml of horseradish peroxidase was added per sample and sections were incubated in a humidified chamber for 30 min at 37°C. Samples were rinsed in PBS three times and 50 ml of diaminobenzidine (0.05% DAB) per section was added. Preparations were incubated for 10 min at room temperature, rinsed with PBS, dehydrated with alcohol and xylene and closed with DePeX. The apoptotic cells of liver sections were counted by randomly selected five areas for each slide.

Statistical Analysis

All data except histopathologic evaluation (i.e., initial body weight [PND 23], body and organ weights at necropsy) were analyzed by Analysis of Variance (ANOVA). The Tukey HSD test was used to determine the differences between at which groups for post hoc test. For histological analysis, the animals were sacrificed and the samples were obtained. Later, results were analyzed by ANOVA for homogeneity. Also, Fisher exact test was used for determine the differences between groups for histologic analysis.

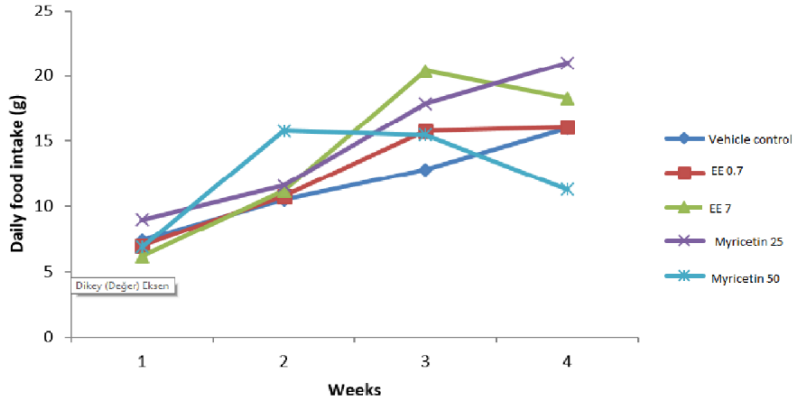

Figure 1. Mean of daily food intakes of rats in the control and treatment groups.

RESULTS

Food-water intake and organ weight

There is no significant difference between the food and water intake of control and treatment groups as seen in Figure 1 and Figure 2. Absolute and relative organ weights of control and treatment groups are given in Table 1. The absolute liver weights were decreased statistically in 0.7 µg/kg/day, 7 µg/kg /day EE and 50 mg/kg/day myricetin treatment groups, compared with the oil control group. Also, relative liver weight was decreased in 50 mg/kg/day myricetin group than control group. Absolute kidney weight was increased in 25 mg/kg/day of myricetin dose group. In spleen, absolute weight was decreased in 50 mg/kg/day myricetin group compared to the 7 µg/kg/day of EE dose group. Thymic absolute weights of rats fed 25 mg/kg/day myricetin showed a significant ($P < 0.05$) increase compared to that in the 0.7 and 7 µg/kg/day EE group.

Histologic Assays

The incidence of exposure-related histopathologic lesions of male rats in the control and treatment groups are given in Table 2 and Figure 3. The oil control group showed regular morphology in all tissues. Histopathological examination of the liver, kidney and spleen revealed significantly increased frequency of congestion when compared with the control group. 25 and 50 mg/kg/day of myricetin dose group showed statistically significant increase frequency of congestion and degeneration in liver compared with the control group.

Also, in kidney tissue, there were significant increase in tubular degeneration and congestion for 25 and 50 mg/kg/day dose group of myricetin. Increased megakaryocytes and fibrosiz were observed in 25 and 50 mg/kg/day of application dose groups in spleen tissue and it is found statistically significant when compared to the control group. In adrenal and thymus, congestion was observed in 25 and 50 mg/kg/day of myricetin dose groups and it was significant. Also, in pancreas, Langerhans islets, cell degeneration was observed statistically significant in 25 and 50 mg/kg/day of myricetin dose groups compared to the control group.

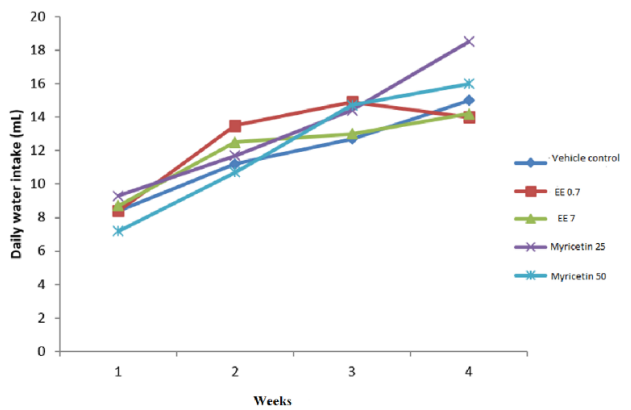

Figure 2. Mean of daily water intakes of rats in the control and treatment groups.

Table 1. Absolute and relative organ weights of rats in the control and treatment groups.

	Oil Control (1 mL)	Ethinyl estradiol (µg/kg/day)		Myricetin (mg/kg/day)	
		0.7 µg/kg/day	7 µg/kg/day	25 mg/kg/day	50 mg/kg/day
Liver					
Absolute (g)	5.394±0.463	3.914±0.284[a]	3.808±0.665[a]	5.347±0.458[b,c]	3.791±0.165[a,d]
Relative (mg/g)	45.15±2.01	41.07±3.54	41.45±1.64	42.69±1.81	35.78±2.17[a,b,c]
Kidney					
Absolute (g)	1.196±0.206	0.991±0.06	0.900±0.119	1.338±0.263b.c	0.950±0.045[d]
Relative (mg/g)	9.943±0.861	10.39±0.743	9.939±1.421	10.81±2.648	8.961±0.44
Adrenal gland					
Absolute (g)	0.0262±0.0017	0.032±0.002	0.0427±0.015[a]	0.0361±0.008	0.0275±0.002[c]
Relative (mg/g)	0.22±0.021	0.335±0.039	0.501±0.29[a]	0.286±0.053	0.26±0.04
Spleen					
Absolute (g)	0.752±0.042	0.685±0.008	0.608±0.124	0.658±0.056	0.653±0.097[c]
Relative (mg/g)	1.956±0.249	2.180±0.108	1.831±0.444	2.094±0.162	2.003±0.151
Thymus					
Absolute (g)	0.0941±0.013	0.0874±0.006	0.0782±0.021	0.1140±0.006[b,c]	0.0935±0.01
Relative (mg/g)	0.789±0.105	0.915±0.068	0.859±0.213	0.915±0.086	0.884±0.12
Pancreas					
Absolute (g)	0.684±0.115	0.675±0.123	0.747±0.136	0.658±0.138	0.655±0.105
Relative (mg/g)	1.771±0.310	2.144±0.368	2.238±0.483	2.028±0.430	2.040±0.445

Values are expressed as mean±SD; N: 6.
[a] Different from oil control.
[b] Different from 0.7 µg/kg/day of ethinyl estradiol.
[c] Different from 7 µg/kg/day of ethinyl estradiol.

Histologic Assays

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Histomorphometric Measurement of Kidney Tissue

The results of histomorphometric analysis of kidney were given in Table 3. According to the results of the analysis of groups, glomerular histomorphometry revealed no significant differences between the control and treatment groups with respect to the analyzed glomerular parameters.

Immunohistochemical Assays

Histologic photomicrographs of liver tissue which was stained with TUNEL method are shown in Figure 4. Besides to this, in 25 and 50 mg/kg/day of myricetin dose groups, the apoptotic cells were increased.

Table 2. Incidence of exposure-related histopathologic lesions of male rats in the control and treatment groups.

	Oil Control (1 mL)	Ethinyl estradiol (µg/kg/day)		Myricetin (mg/kg/day)	
		0.7 µg/kg/day	7 µg/kg/day	25 mg/kg/day	50 mg/kg/day
Liver					
Cellular degeneration	0/6*	1/6*	3/6*	4/6*	4/6*
Congestion	0/6*	5/6*	4/6*	6/6*	4/6*
Mononuclear cell infiltration	1/6*	2/6*	3/6*	6/6*	5/6*
Kidney					
Mononuclear cell infiltration	2/6*	4/6*	3/6*	6/6*	5/6*
Tubular degeneration	1/6*	2/6*	3/6*	5/6*	6/6*
Congestion	0/6*	1/6*	2/6*	6/6*	4/6*
Spleen					
Congestion	0/6*	1/6*	3/6*	5/6*	6/6*
Fibrosis	0/6*	1/6*	3/6*	5/6*	6/6*
Megakaryocyte cell	0/6*	1/6*	3/6*	6/6*	6/6*
Pancreas					
Langerhans degeneration	0/6*	2/6*	3/6*	4/6*	6/6*
Relative (mg/g)	1.956±0.249	2.180±0.108	1.831±0.444	2.094±0.162	2.003±0.151
Adrenal gland					
Congestion	0/6*	1/6*	2/6*	3/6*	5/6*
Cellular degeneration	0/6*	2/6*	3/6*	5/6*	6/6*
Thymus					
Congestion	0/6*	0/6*	4/6*	5/6*	6/6*

Table 3. Histomorphometric measurement of glomeruli in the treatment and control groups.

Groups	Dose	Short diameter (µm)	Long diameter (µm)	Glomerular diameter (µm)	Glomerular volume x 10^6 (μm^3)
Oil control	1 mL	125.610±18.920	165.610±22.567	148.584±15.566	1.641±0.529
Ethinyl estradiol	0.7 µg/kg/day	132.210±20.200	163.321±21.765	146.550±14.234	1.701±0.432
Ethinyl estradiol	7 µg/kg/day	131.122±21.090	162.567±19.789	147.345±14.778	1.699±0.788
Myricetin	25 mg/kg/day	124.334±19.566	162.444±20.779	141.234±23.675	1.590±1.463
Myricetin	50 mg/kg/day	129.677±20.334	168.989±21.556	143.545±24.888	1.700±0.299

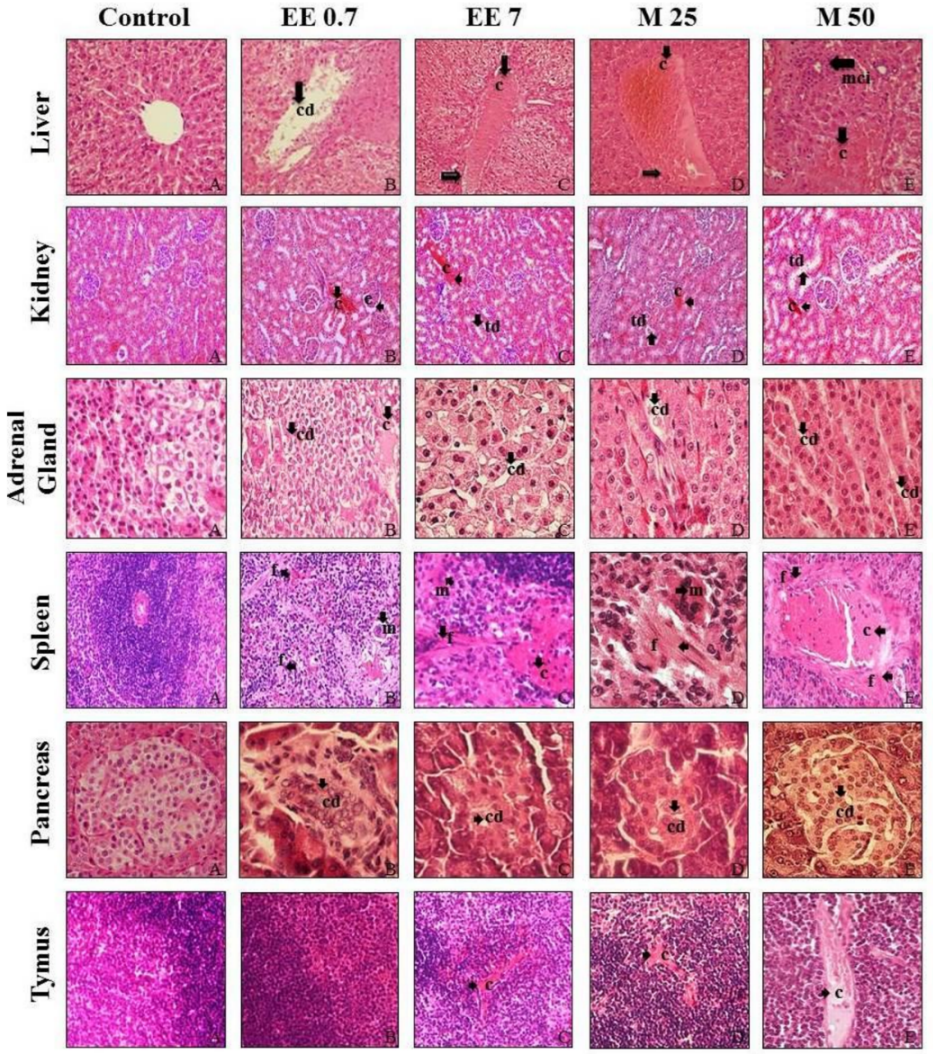

Figure 3. A: Control group, B: 0.7 µg/kg ethinyl estradiol treatment group, C: 7 µg/kg ethinyl estradiol treatment group, D: 25 mg/kg myricetin treatment group, E: 50 mg/kg myricetin treatment group. Histopathological markers: congestion (c), cell degeneration (cd), fibrosis (f), megakaryocyte cell (m), mononuclear cell infiltration (mci), tubular degeneration (td), enlargement of glomerular capsule (e) (All of them stained with contrast to H&E, X200).

DISCUSSION

In the last ten years, according to animal studies some phytoestrogens act as endocrine-disrupting and they have a negative effect on the endocrine system [19]. On the other hand, some epidemiological and experimental studies show that consumption of foods rich in phytoestrogens provide the beneficial health effects in human. A number of factors like age at exposure, the structure of the compound and dose content can cause adverse or beneficial effects [20].

Especially, because of the antioxidant effects of myricetin has an overdose of consumption taken as a food supplement and in the future due to this consumption, several disorders can reveal and also there is not any in vivo or in vitro study about that yet. A purpose of this study is to determine the effects of myricetin on liver, kidney, spleen and some endocrine glands in male prepubertal rats during the transition period to the puberty. Myricetin was given to weaning male rats by oral gavage from postnatal day (PND) 23 through 53 for 30

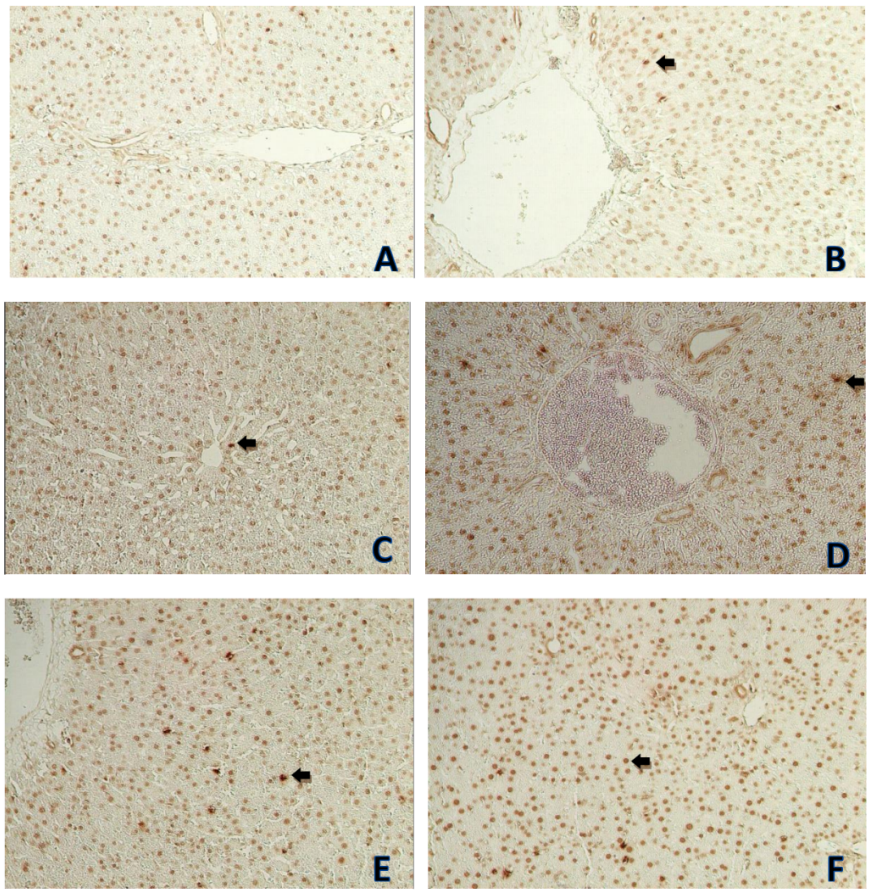

Figure 4. Photomicrographs showing liver tissues of control and treatment groups. A is from control group. B is 0.7 µg/kg day of EE, C is 7 µg/kg day of EE and D is 25 mg/kg day of myricetin, E is 50 mg kg^{-1} day of myricetin. Black arrows shows apoptotic cells in liver (X200).

days. Myricetin was solved in corn oil which was a non-toxic and clean solvent before gavage. Animals were provided with phytoestrogen free food to confirm the effects of myricetin in prepubertal rat development. To determine the applied dose amounts, daily average flavonol intake doses with food and in vivo studies which demonstrate amount of myricetin's effective dose were considered. According to USDA flavonoid database, U.S. population daily flavonoid intake was estimated 189.7 mg and aproximately 12.9 mg of this value was daily flavonol intake [20]. Depending on literature daily flavonol intake database and in consideration of consuming food reinforcement of tablets, myricetin doses which were applied to male rats were determined 25 and 50 mg /kg/day.

Food and water intake of animals were daily recorded and statistically analyzed. Trent et al. reported the male rats fed the phytoestrogen diet significantly consumed more food and water unless gained less weight than male rats fed the phytoestrogen-free diet [21]. In our study, the average food and water consumption of groups are compared but there was no significantly difference between the groups. Organ weights were analyzed statistically between the groups. In our study, there were important significant differences between groups for liver weight. In 0.7 and 7 µg/kg/ day ethinyl estradiol dose groups and in 50 mg/kg/day of myricetin dose group, the liver weight decreased but 25 mg/kg/day of myricetin dose group liver weight was increased. Santhell et al. investigated the antilipidemic effect of myricetin and they found that myricetin caused the decrease in hepatic triglyceride, cholesterol and hepatic lipid levels [22]. In our study, these decrease may be caused from the effect of lipid storage regulation of myricetin. Food intake of high concentrations of flavo-

noids leads to the proteolysis in the intestine, blocking glucose uptake and decreased mineral absorption [23]. The current study clearly demonstrates that myricetin has adverse effects on the liver and kidney, as indicated by increased frequency of lesions in myricetin-treated group.

The affinity of phytoestrogens for estrogen receptors results in effects on a large number of estrogen-regulated systems, including the cardiovascular, metabolic, skeletal, reproductive, and central nervous systems. In this study, we saw a lot of histopathological changes in examined tissues. Especially in all high doses of phytoestrogens, congestion and degeneration had significant increase. In histomorphometry of kidney, there were increases and decreases about all parameters compared to the oil control group but they didn't significant statistically. In spite of the statistically significant tubular degeneration of kidney in dose groups, the histomorphometry wasn't significant. Most studies have evaluated the effects of soy-derived compounds on adiposity. For example, Long-Evans rats or ovariectomized (OVX) ddY mice fed with a soy-rich diet and they have lost weight and they had less adipose deposition than those fed on a soy-free diet [24].

In histopathological analysis, liver, kidney, spleen, pancreas, thymus and adrenal gland were investigated. Liver is the main responsible organ for metabolism of myricetin [25]. In liver, congestion and cellular degeneration were increased in 7 µg/ kg/day of ethinyl estradiol, 25 and 50 mg /kg/day of myricetin dose groups, compared to the oil control. In Tunnel method, we observed that apoptotic cells were increased in the 25 and 50 mg/kg/day of myricetin. This situation may be sourced from the apoptotic effects of depending of the estrogenic effects of myricetin. Studies have shown that, in rats which were fed with phytoestrogens from the prenatal life to adulthood, in germ cells, apoptosis was increased [26]. Myricetin acts as an inhibitor of the anti-apoptatic signalling pathways in pancreatic, cervical, and lung cancer cell lines [27]. Thusly, the reason for the increase in apoptosis of liver may be due to the inhibitor role of myricetin.

In pancreas, in 25 and 50 mg /kg/day of myricetin dose groups, langerhans islets degeneration was increased. Potential mechanisms by which dietary soy may improve glucose metabolism have been recently proposed. Dietary soy increases insulin sensitivity by increasing glucose uptake in skeletal muscles [28]. Studies show that the thymus plays a critical role in immune system development and function. In 25 and 50 mg/kg/day of myricetin dose groups, the congestion was increased.

Flavonoids can affect a wide range of functions which are in molecular, cellular and in tissue levels. When these compounds are consumed in high amounts, the risk and hazard that may occur are not fully known yet. To recommend the widespread use of these compounds, more reliable research should be provided [19]. The lack of compatible results across all species is surprising, but it depends on differences of soy foods/isoflavone supplements ingested, route of administration, exposure time and phytoestrogen metabolism so they may well result from a combination of these factors. There are many explanations for the inconsistent results from these studies. Although experiments of phytoestrogen interventions are countless, most experiments include only small numbers of subjects, are short in time and poor in quality. Interventions will be investigate vary in type (food additives, dietary manipulation and packaged supplements) as well as in concentration and balance of active ingredients. Absorption of phytoestrogens is also different between individuals and affected by other factors such as antibiotic use [29].

Finally, myricetin consumption of the male rats which were on the way from peripubertal period to pubertal period had negative effects on liver, kidney, spleen and some endocrine glands. Otherwise, to make a definitive conclusion on apoptotic activity in the liver and to elucidate the process and role of the Fas system as well as other molecular pathways in myricetin-induced apoptosis, further studies are warranted.

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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