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RESEARCH ARTICLE

MORPHOLOGICAL, HEMATOLOGICAL and HISTOPATHOLOGICAL EFFECTS of PROPYL PARABEN on ENDOCRINE GLANDS of MALE RATS at PREPUBERTAL PERIOD

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ABSTRACT

Propyl paraben (propyl 4-Hydroxybenzoate) is frequently used in various products due to its physical and chemical properties and cheapness, and its use is increasing day by day due to the development of the industrial industry. This study is conducted on the side effects of propyl paraben on male rats' endocrine glands. Accordingly, each oil control, positive control (3 mg/kg/day flutamide=FLU), negative control (0.4 mg/kg/day testosterone propionate = TP) and 10, 250 and 750 mg/kg/day testosterone propionate + propyl paraben 6 groups were formed, one of which had six rats.

While a decrease was observed in thymus and spleen weights in the 10, 250 and 750 mg/kg/day testosterone propionate + propyl paraben dose groups compared to the fat control group, an increase was observed in the weight of the thyroid tissue in the 250 mg/kg/day testosterone propionate + propyl paraben dose group compared to the positive control and 10 mg/kg/day testosterone propionate + propyl paraben dose groups. It has also been shown with various damages in endocrine organs in histopathological examinations. Therefore, we can say that propyl paraben has a negative effect on the endocrine organs examined.

Keywords: *Propyl paraben, Endocrine glands, Toxicity, Prepubertal male rats*

1. INTRODUCTION

To utilize antimicrobial efficiency of parabens, they are used alone or mixed as a preservative in pharmaceutical products, foods and cosmetic products such as shampoos, lotions, deodorants etc. [1-3]. They are effective in between pH 4.5-7.5 and have several preservative criteria which is ideal such as not causing to fade in colors, being odor free and tasteless and continuing their efficiency by remaining stable in wide temperature interval [4]. Parabens can be taken by gastrointestinal tract, breathed their dust particles or absorbed through the skin. When parabens are taken orally, they are metabolized in the liver and intestines to the main metabolite parahydroxybenzoic acid (PHBA). They are eliminated from body by being conjugated in three

ways which are p-carboxyphenyl sulphate, p-hydroxybenzoyl glucuronide, p-carboxyphenyl glucuronide and they leave body through urine and feces [5].

By the 2000s, packaged and processed food production began to increase and parabens began to be added to the foods produced in this way. Researchers had not yet expressed their concerns about parabens until this period [6,7,8].

Propyl paraben (PP) is frequently used in cosmetic products due to its antimicrobial effect [9,10,11]. In addition, it is considered suitable for use because it is odorless and does not add any color or taste to the product it is added to. When all these features are added to the fact that it is cheap, institutions such as the USA FDA have authorized many institutions for its use. [9,11].

For a chemical to be used in any product, it is an extremely serious task to first determine whether it has toxic effects for both the environment and humans. Each country has different regulations and rules for the use of any chemical [12]. Here, in order to determine the properties of a chemical, researchers have determined evaluation criteria with their studies [13] and it has been seen that propyl paraben is an endocrine disruptor.

There are more than one way to be exposed to a paraben, mainly by ingestion and inhalation, and depending on these exposure pathways, the duration of metabolism and excretion also changes [14,15]. It is then metabolized and conjugated by the enzyme esterase. Parabens can accumulate in the body, and much of this build-up occurs with daily use of them as a comic [16]. Oral parabens, on the other hand, are metabolized by the esterase enzyme and removed from the body through urine and feces [17].

Parabens have estrogenic action, since they can bind to estrogen receptors. This process could affect the hormonal balance of the body. They have the ability to block 17-hydroxysteroid dehydrogenase, an estrogen-inactivating enzyme [18]. Parabens are lipophilic pollutants that have been shown to build up in fatty tissue [19]. Shin et al. [20] established a pharmacokinetic model for PP, which may also be used to examine the pharmacokinetics and toxicokinetics of other parabens. In summary, orally administered PP was swiftly absorbed (less than 2 hours) and totally removed [20].The European Union (EU) and the Association of Southeast Asian Nations (ASEAN) banned five parabens in 2014 and 2015. (isopropylparaben, isobutylparaben, phenylparaben, benzylparaben, and pentylparaben). Studies on propyl paraben, methyl paraben, ethyl paraben and butyl parabens, which are frequently used parabens, have been shown to have toxic effects on mitochondrial activity and DNA [7]. However, no clear relationship has been shown between paraben exposure and cancer risk. Parabens have been proven in animal models to operate as weak xenoestrogens, with activity rising with the length of the alkyl group. In a study with mice, it was observed that the administration of paraben caused some epigenetic differences [21].

It has also been supported by studies that more than one ailment in humans, such as infertility and cancer, is associated with parabens [22]. It has been observed that propyl paraben application causes a decrease in follicle growth [23]. In terms of endocrine disruption, parabens were found to have the ability to bind to estrogen receptors, indicating estrogenic activity [24, 25]. Despite what is known about parabens' toxicological effects on the reproductive system, there is still a lack of research about their harmful effects on endocrine organs and the mechanisms that underpin them.

Therefore, in this work, because of the limited research on PP, it was selected and examined the toxicological effects on endocrine glands of prepubertal male rats. It is aimed that the results obtained will contribute to the limited literature available and be a start to the parts that need to be clarified on this subject.

2. MATERIALS and METHODS

2.1. Chemicals

Hangzhou Dayang Chem. Co., Ltd. supplied testosterone propionate (TP, 97%). Sigma-Aldrich provided flutamide (FLU, 98%) and propyl paraben (PP, 98%). Because androgen ligands are hydrophobic, all test compounds were dissolved in oil.

2.2. Animals and Laboratory Conditions

36 prepubertal male Wistar albino rats, 6 weeks old, weighing 170-210 g, were obtained from the Experimental Animals Production Center of Hacettepe University. Afterwards, the experiment was started with the permission of the ethics committee (2015/86-13) obtained from the Hacettepe University Ethics Committee. All rats were kept in polypropylene cages at 22°C and 50% relative humidity in a room with a cycle of 12 hours of light and 12 hours of dark throughout the experiment. Pellets were given as feed and drinking water in glass bottles.

2.3. Grouping Experimental Animals and Doses

Subcutaneous injections of testosterone propionate (TP) were used. Flutamid (FLU) and PP were given for 10 days through oral gavage with oil. For oral administration, the total dosage amount was 5 ml/kg/bw/day. The Hershberger Bioassay OCSPP Guideline 890.1400 was used to calculate the daily dosages of TP (0.4 mg/kg/day) and FLU (3 mg/kg/day). This test is A Short-term Screening Assay for (Anti) Androgenic Properties it also shows toxicity in endocrinal glands such as liver, kidney and adrenal glands. It has been preferred because it is an optional short-term test that has been used frequently recently [26, 27].

At the age of six weeks, rats were castrated and allowed eight days to recover. After the recovery period, the animals were randomly assigned to one of six groups (n = 6) based on their body weights: an oil control group (5 mg/kg/day oil), a negative control group (0.4 mg/kg/day TP), a positive control group (3 mg/bw/day FLU plus 0.4 mg/kg/day TP), and three propyl paraben dose groups (10, 250, and 750 mg/kg/day plus 0.4 mg/kg/day TP). Food and water consumption, as well as body weights, were noted daily, and the daily dosage was adjusted for body weight. Using sterilized needles, dosages were delivered to the dorsal-scapular region for subcutaneous injection. Subcutaneous and oral deliveries were modified with total dose volumes of 0.5 ml/kg and 5 ml/kg, respectively [28]. The rats were killed within 24 hours of the last injection following 10 days of therapy. The pancreas, adrenal gland, thymus, thyroid, and spleen were all removed and weighed.

2.4. Histopathologic Analysis

After the tissue samples were taken, they were weighed and kept in Bouin's solution for 8 hours. Following this technique, tissue were sliced and stained with Hematoxylin and eosin. An Olympus BX51 light microscope was used to view stained preparations. It was photographed with the Bs200prop software connected to this microscope and all the changes observed for each section were recorded.

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Thyroid slices stained with H&E were assessed for epithelial height and follicular diameter. The outer follicle layers of the thyroid gland were defined as the marginal region and the middle region as the central region. Using a 40X objective lens, morphmetric measurements were taken in the core and marginal areas of the thyroid glands [29,30]. The distance of luminal-basal cell membranes was measured for the height of the follicular epithelium. The size of the epithelium in the thyroid follicles was measured at 12.5 and 7 o'clock and recorded. An average of 20 follicles were counted from each rat thyroid preparation [31,32,33]. The data in the marginal and central regions were evaluated separately and presented as bar graphs.

2.5. Hematological Analysis

White blood cells (WBC), lymphocytes % (lym), monocytes % (mon), red blood cells (RBC), MCV (mean corpuscular volume), hematocrit level (Hct), mean erythrocyte hemoglobin concentration (MCHC), Hemoglobin (Hb) level, platelet count (Plt), mean erythrocyte hemoglobin (MCH) were calculated using a hematology analyzer MELET SCHLOESING MS9-5 (France).

2.6. Statistical Analysis

The data was statistically evaluated using the SPSS IBM-23 statistical software (USA). The variances were homogenous, according to Levene statistics. The analysis of variance (ANOVA) method was utilized. To establish the difference between groups, the Tukey post-hoc test was utilized. The mean and standard deviation of all data were reported (SD). Statistical significance was determined at $p \leq 0.05$ (n=6).

3. RESULTS

3.1. Body and Organ Weight Results

Organ weight results were given in Figure 1. There was a statistically significant decrease in thymus weights in the negative control and positive control groups and in all TP + PP application groups compared to the oil control group. In addition, a significant decrease in thymus weights was observed in the 10 and 250 mg/kg/day of TP + PP dose groups compared to the negative control group. The spleen weights of the 750 mg/kg/day TP + PP dose group decreased significantly when compared to the oil control, positive control, negative control, and 10 mg/kg/day TP + PP dose groups.. In addition, a statistically significant decrease was observed in spleen weight in the 250 mg/kg/day of TP + PP dose group compared to the oil control and negative control groups. The positive control group had a significant decrease in thyroid weight when compared to the negative control and 250 mg/kg/day TP + PP dose groups. When compared to the negative control and the 250 mg/kg/day TP + PP dose group, the 10 mg/kg/day TP + PP dose group showed a significant decrease in thyroid weight. When TP + PP doses of 10, 250, and 750 mg/kg/day were compared to the negative control dose group, there was a significant decrease in adrenal weight. When compared to the oil control and positive control dose groups, the 10 mg/kg/day of TP + PP dose group showed a substantial decrease in adrenal weight. There was no statistically significant difference in pancreatic weights between the treatment and control groups. The data were not graphed since there was no statistically significant difference in pancreatic weights.

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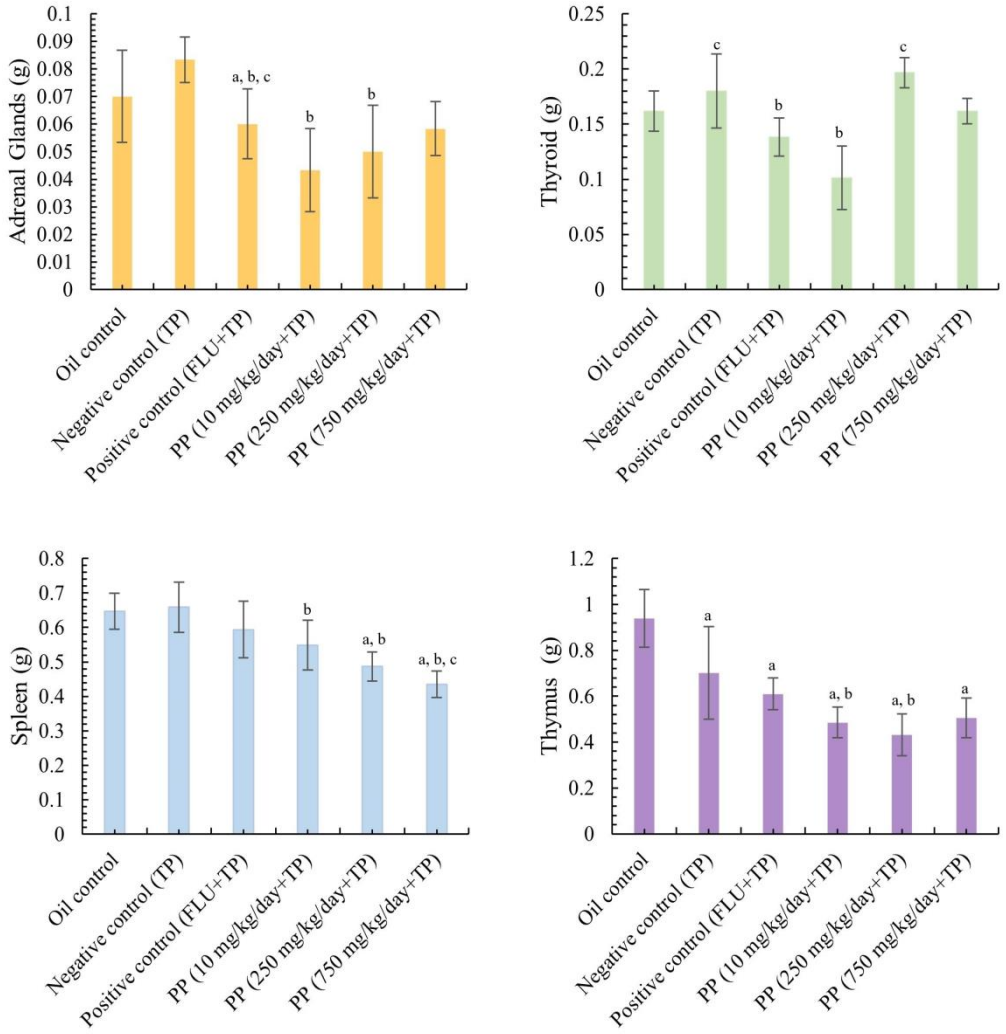

Figure 1. Absolute organ weights of controls and TP+PP dose groups. Values are given as mean ± SD. [a] Significant difference from oil control, [b] Significant difference from negative control (TP) and [c] Significant difference from positive control (FLU+TP), $p<0.05$ (Significance level $p\leq0.05$).

3.2. Hematologic Analysis

Hematologic analysis results of rats belonging to application groups are shown in Table 1. In the 250 mg/kg/day of TP + PP group, a significant decrease was observed in lym %, mon % and plt values in general compared to the oil control and negative control groups. In addition, a significant decrease was observed in RBC and Hct values in comparison with the negative control group. WBC values were increased compared to the oil control and negative control groups.

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Table 1. Results of hematologic analysis of male rats in the control and exposure to propyl paraben at dose of 10, 250, and 750 mg/kg/day.

Values are given mean ± SD for six animals in each group. "n" number of rats. [a] Significant difference from oil control,

Parameters	Oil Control	Negative Control (TP) 0.4 mg/kg/day	Positive Control (Flutamid+TP) 3 mg/kg/day	TP+PP 10 mg/kg/day	TP+PP 250mg/kg/day	TP+PP 750mg/kg/day
n	6	6	6	6	6	6
WBC	3.72 ± 0.81	3.20 ± 0.81	4.34 ± 0.49	4.00 ± 0.76	**5.85 ± 1.30** [a,b]	3.28 ± 1.38
Lym %	80.58 ± 7.28	85.08 ± 5.38	**68.20 ± 4.91** [a,b]	**3.10 ± 0.75** [a,b]	**3.98 ± 0.69** [a,b]	**7.12 ± 7.43** [a,b]
Mon %	5.88 ± 0.36	6.57 ± 0.67	**4.58 ± 0.57** [a,b]	**0.18 ± 0.04** [a,b]	**0.22 ± 0.04** [a,b]	**0.27 ± 0.15** [a,b]
RBC	**6.49 ± 0.39** [b]	**10.66 ± 0.43** [a]	**9.11 ± 2.15** [a]	**7.06 ± 0.31** [b]	**7.38 ± 0.51** [b]	**7.01 ± 0.45** [b]
MCV	43.32 ± 5.58	34.75 ± 3.19	39.22 ± 6.51	56.62 ± 2.43	54.20 ± 1.11	135.55±199.61
Hct	**38.12 ± 2.63** [b]	**49.60 ± 3.71** [a]	**25.95 ± 3.94** [a,b]	**40.50 ± 3.53** [b]	**39.4 ± 3.33** [b]	**37.22 ± 3.07** [b]
MCHC	**18.43 ± 0.94** [b]	**39.90 ± 2.88** [a]	**31.20 ± 1.36** [a,b]	**35.58 ± 0.35** [a,b]	**37.00 ± 1.57** [a]	**35.08 ± 4.19** [a,b]
Hb	13.47 ± 0.83	14.62 ± 1.08	**10.22 ± 2.31** [a,b]	14.62 ± 1.29	14.25 ± 0.40	13.97 ± 1.17
Plt	**4900.33±954.6**[b]	**9335.67±257.40**[a]	**4248.17±200.53**[b]	**963.17±234.33**[a,b]	**1129.17±72.70**[a,b]	**885.83±124.94**[a,b]
MCH	**35.70 ± 1.57** [b]	**17.95 ± 0.83** [a]	**11.82 ± 1.15** [a]	**20.28 ± 0.79** [a,b]	**19.78 ± 1.12** [a]	**19.83 ± 0.69** [a,b]

[b] Significant difference from negative control (TP) (Significance level p≤0.05).

A significant decrease was observed in the lym %, mon % and plt values in the 10 mg/kg/day of testosterone propionate + propyl paraben group compared to the oil control and negative control groups. In addition, a significant decrease was observed in RBC and Hct values compared to the negative control group. There was an increase in MCHC values compared to the oil control group and a decrease compared to the negative control group. A significant decrease was observed in the lym %, mon % hct and hb values in the positive control group compared to the oil control and negative control groups. Plt values were found to be significantly lower in the positive control group than in the negative control group. In comparison to the oil control group, there was an increase in MCHC levels. When compared to the oil control group, the RBC, Hct, MCHC, and Plt values in the negative control group increased significantly. MCH levels were found to be significantly lower in the oil control group.

3.3. Histopathologic Analysis

The histopathological examination results of the tissues are shown in Figure 2 and 3. Degeneration was demonstrated in the adrenal tissue in the negative control group. Congestion and edema were found in 250 and 750 mg/kg/day TP+PP dose groups. Cell melting was observed in the positive control group and 750 mg/kg/day TP+PP dose group. In thyroid tissue, colloidal degeneration was shown in negative and positive control group, also all TP+PP group. Follicular degeneration was shown in 250 mg/kg/day TP+PP group. In thymus tissue, congestion, edema and cell degeneration were demonstrated in the negative control group.

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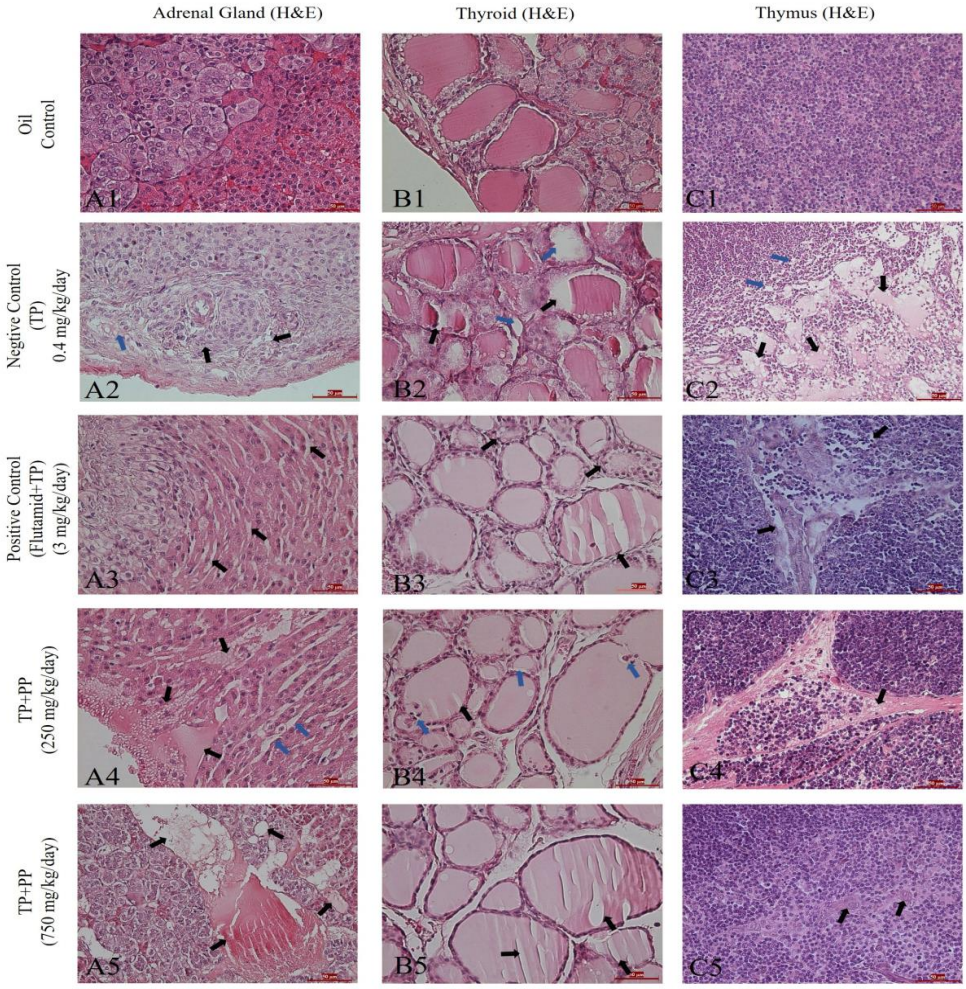

Figure 2. Histological images of the controls and TP+PP dose groups of the adrenal, thyroid and thymus glands with H&E stain. Histological images of the adrenal gland in oil control group (A1), degeneration (black arrow) and edema (blue arrow) in negative control group (A2), cell melting (black arrow) in positive control group (A3), congestion, edema (black arrow) and cell melting (blue arrow) in 250 mg/kg/day TP+PP group (A4), congestion and edema (black arrow) in 750 mg/kg/day TP+PP group (A5). Histological images of the thyroid in oil control group (B1), colloid degeneration (black arrow) in negative control group (B2), colloid degeneration in positive control group (B3), follicle degeneration (black arrow) and colloid degeneration (blue arrow) in 250 mg/kg/day TP+PP group (B4), colloid degeneration (black arrow) in 750 mg/kg/day TP+PP group (B5). Histological images of the thymus in oil control group (C1), congestion and edema (black arrow), cell degeneration (blue arrow) in negative control group (C2), degeneration (black arrow) in positive control group (C3), degeneration (black arrow) in 250 mg/kg/day TP+PP group (C4), megakaryocyte (black arrow) in 750 mg/kg/day TP+PP group (C5). 50µm bar, 400X magnification, H&E: Hematoxylin and eosin.

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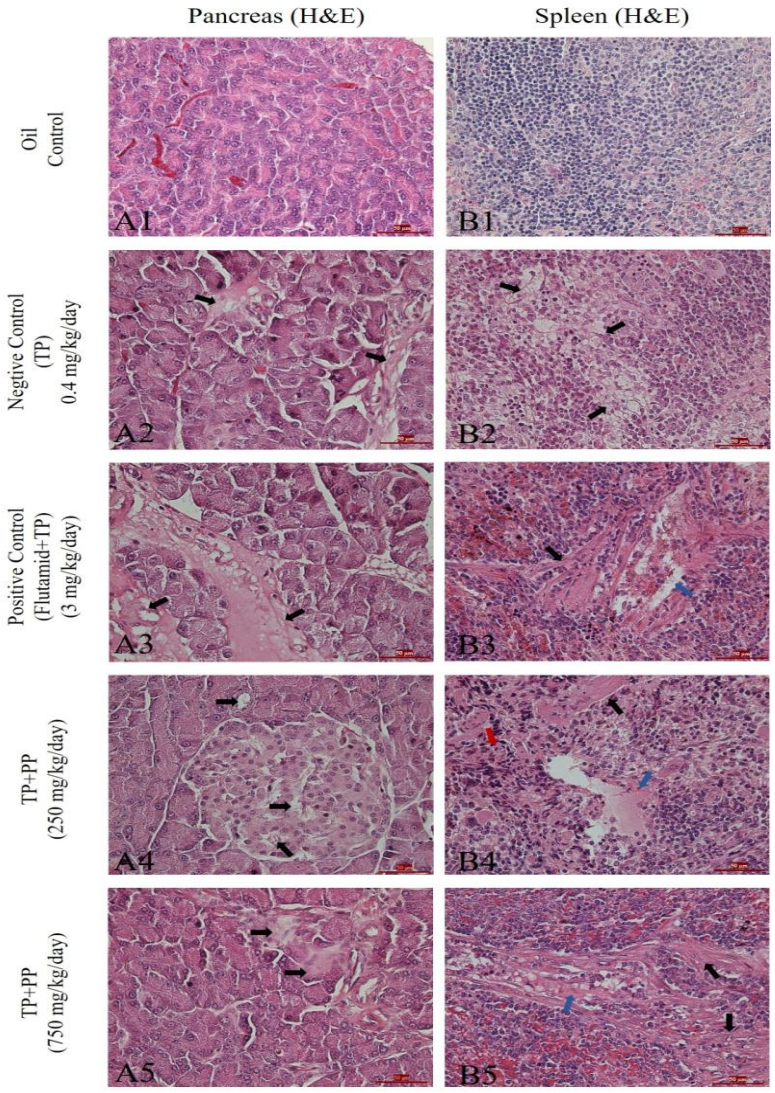

Figure 3. Histological images of the controls and TP+PP dose groups of the pancreas and spleen with H&E stain. Histological images of the pancreas in oil control group (A1), congestion and edema (black arrow) in negative control group (A2), congestion and edema (black arrow) in positive control group (A3), cellular degeneration (black arrow) in 250 mg/kg/day TP+PP group (A4), cellular degeneration (black arrow) in 750 mg/kg/day TP+PP group (A5). Histological images of the spleen in oil control group (B1), edema (black arrow) in negative control group (B2), connective tissue formation (black arrow), edema (blue arrow) in positive control group (B3), connective tissue formation (black arrow), edema (blue arrow), cell degeneration (red arrow) in 250 mg/kg/day TP+PP group (B4), connective tissue formation (black arrow), edema (blue arrow) in 750 mg/kg/day TP+PP group (B5). 50µm bar, 400X magnification, H&E: Hematoxylin and eosin.

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Degeneration was observed in the positive control and 250 mg/kg/day TP+PP group. Megakaryocytes were also observed in the 750 mg/kg/day TP+PP group. Congestion and edema in pancreatic tissue were seen in negative and positive control groups. In addition, cell degeneration was found in 250 and 750 mg/kg/day TP+PP dose groups. In the spleen tissue, edema was shown in the negative and positive control groups. Cell degeneration was shown in the 250 mg/kg/day TP+PP dose groups. Connective tissue formation was also observed in the positive control and 250-750 mg/kg/day TP+PP dose groups.

Figure 4 shows the findings of the thyroid gland morphological measurements. In comparison to the oil control group, follicular epithelial heights increased in both the marginal and central areas. In the negative and positive control groups, as well as the 250 mg/kg/day TP+PP dosage group, these increases were statistically significant. In comparison to the oil control group, there was no statistically significant difference in follicular diameter in both the marginal and central areas.

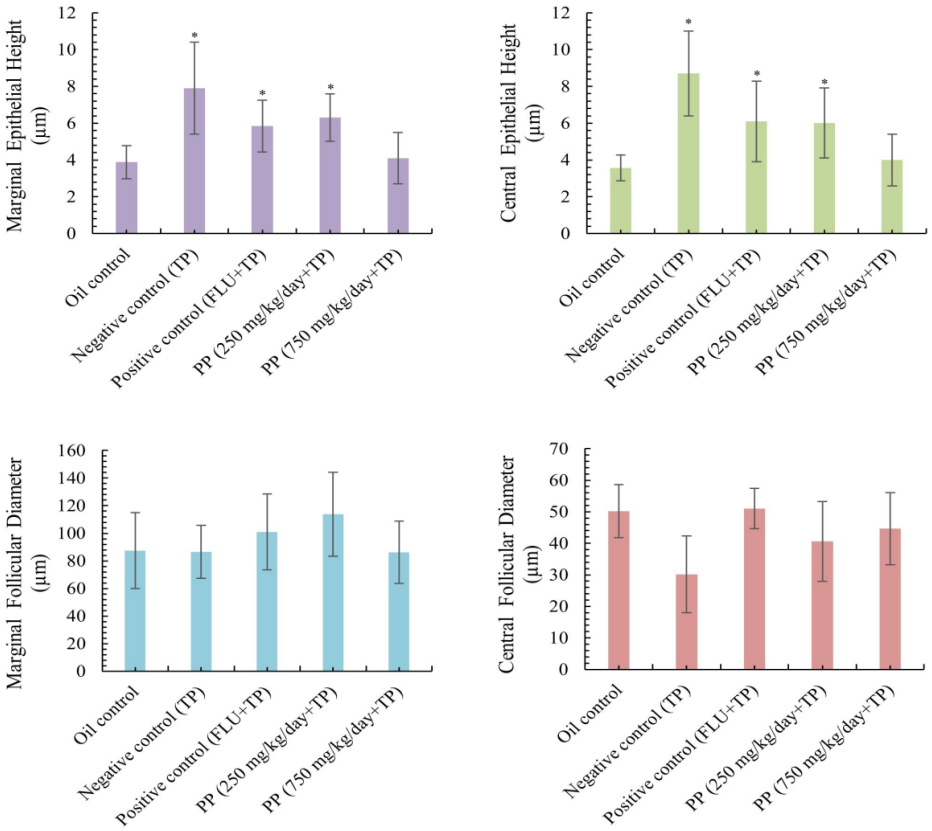

Figure 4. Thyroid gland morphometric measurement results. Values are given as mean ± SD. *statistically different from oil control group. (Significance level p≤0.05).

4. DISCUSSION

The use of parabens as preservatives can generally be made individually or in combination, and it is mostly found in cosmetic products in this way [34]. According to the US Food and Drug Administration's Voluntary Cosmetic Registry Program (VCRP) it has been shown that the most common paraben found in cosmetic products is methyl paraben [35].

It is known that propyl paraben dissolves in water and has a high concentration power [11]. It biodegrades rapidly under aerobic conditions and has a low to moderate bioaccumulation potential [36]. It has an estrogenic activity but is known to be lower compared to other chemicals [37]. They were considered harmless due to their low activity [38], but their occurrence in body fluids and cancerous tissues suggests that these chemicals may be more harmful than they appear [40,41]. PP was found in human urine at 75.3 g/L and below 0.27 g/L in cord blood plasma samples [42]. PP has been proven in animal studies to increase cell proliferation in the rat forestomach [43,44]. In another study with hamsters, it was shown that propyl paraben causes toxic effects on the urinary bladder [44]. It may also affect the viability of human sperm [45]. Furthermore, other studies have found that PP treatment reduced testosterone secretion in rats and mice [46]. As a result, inadvertent and ongoing exposure to this preservative has negative consequences for human and environmental health.

The harmful impact of parabens grows as the chain length in their chemical structure increases. When exposed on a regular basis, BP has the most damaging impact of any paraben. Despite studies demonstrating this reproductive toxicity with parabens, insufficient evidence has been found to determine whether it is related to estrogenic events. Recent research, notably PP [35], have generally shown that parabens have lower chronic toxicity than previous studies based on acceptable laboratory methods.

Recently it was found that parabens disrupt hormones function through to interfere with normal hormone functions on various levels, an effect that is linked to increased risk of the different diseases linked with the different hormones, such as obesity, cancer, allergic diseases [47,48]. As proof of this, it has been said that paraben has the ability to accumulate in body fluids such as blood. Nowak and his colleagues in 2018 say further studies are necessary to find out how parabens affect human health in their review [49].

In our investigation, the 10 mg/kg/day TP+PP dosing group had a substantial decrease in thyroid weight when compared to the negative control. Furthermore, the weights of the adrenal gland decreased in the 10, 250, and 750 mg/kg/day TP+PP dosing groups. Vo et al. (2010) discovered that female rats treated with several parabens (including butyl-, ethyl-, methyl-, propyl-, isopropyl-, and isobutyl-paraben) had substantial alterations in body and organ weights (adrenal glands, liver, ovaries, thyroid glands, and kidneys) [50]. According to studies, parabens can interact with thyroid hormone and sex hormone, altering the endocrine system by interfering with the previously stated hormones [51].

There is currently a scarcity of scientific evidence linking paraben exposure to particular harmful consequences on the thyroid hormone system. In pre-pubertal female rats, oral treatment of 250 mg PP mg/kg/day resulted in a 50% drop in blood T4 levels [50]. Despite this, no histological changes on the thyroid glands of the treated mice were observed, according to the authors. A study

was conducted by Meeker et al. on whether there is a relation between the presence of propyl paraben and methyl paraben in the urine and the level of thyroid hormone, and no correlation was found [52]. In addition, low maternal free T4 levels have been linked to propyl paraben, while high maternal T4 levels have been linked to methyl paraben [53].

Two studies have been conducted on parabens and their results have been different. Accordingly, no correlation was found between cortisol level and paraben concentration when looking at the blood of mothers and newborns [54], but a decrease in cortisol levels was seen in the fluids taken from the children of mothers associated with butyl paraben. Congestion, edema, degeneration, and cell melting were detected in the adrenal gland in the negative-positive controls and the 250-750 mg/kg/day TP+PP dosage groups. The effect of parabens on the decrease in adrenal gland hormones may be attributed to this harm, according to recent studies.

Degeneration of the thymus gland was seen in the 250 mg/kg/day TP+PP dosage group in our investigation. In addition, anomaly was observed in the pancreatic tissue. In a study with Vero cells, an increase in oxidative stress was observed when these cells were exposed to propyl paraben, and accordingly DNA damage was detected [55]. It was also found that the amount of toxicity was greater in combination exposure. This damage could have been caused by propyl paraben-induced oxidative stress.

There was an increase in DNA damage and oxidative stress after exposure to propyl paraben [56]. According to another study, it has been shown to be toxic in human lymphocyte cells and it was thought that its continuous use may increase this toxicity [57]. Identical results were reported in our study, which were similar to the detrimental effect of propyl paraben on blood cells seen in earlier studies. The number of lymphocytes and monocytes in the 10 and 250 mg/kg/day TP+PP dosage groups decreased dramatically.

In both animals and humans, parabens can affect the endocrine system and cause oxidative stress [58]. Poor pregnancy and fetal deterioration and long-term deterioration in health have been attributed to parabens [59]. As a result of the fact that the number of platelets produced in the bone marrow is not at a sufficient level, the number of platelets in the body decreases. When the spongy structure of the bone marrow is damaged, it cannot produce enough blood cells. Butyl paraben has been shown to have toxic effects on osteogenic and chondrogenic development, and it has been proven to do so by activating the peroxisome proliferator or glucocorticoid receptor [60]. Similar to the effect of butyl paraben, a remarkable decrease was observed in the amount of platelets at 10 and 250 mg/kg/day of TP+PP dose groups in our study. Parabens can cause oxidative damage by causing the formation of glutathione (GSH) [61]. If an individual's MCH value is lower than the specified reference range, it can be said that the person has a problem with the production of red blood cells or the amount of iron in the body. In spleen, in 250 and 750 mg/kg/day of testosterone propionate+propylparaben dose groups, there were congestion and connective tissue formation. The damage to the spleen as a result of the oxidative stress caused by propyl paraben, similar to other parabens, may have resulted in a decrease in the amount of MCH. Meanwhile, data from other pregnant cohorts show that urine paraben can influence circulating inflammation markers such as IL-6, IL-10, CRP, and TNF- α [62].

In this study, PP causes morphometric changes in the thyroid glands. The positive and negative control groups, as well as the 250 mg/kg/day TP+PP dosage group, all showed a substantial

increase in marginal and central thyroid epithelial height in comparison with the oil control. Studies have shown that endocrine disruptors and environmental contaminants increase the height of the thyroid gland follicular epithelium [63]. It is stated that the height of the follicular epithelium is associated with active follicles in the thyroid gland. These studies and our study suggest that substances classified as endocrine disruptors cause follicle hyper stimulation [64,65].

5. CONCLUSION

The studies discussed above have raised concerns about the reproductive system and hormonal changes in the general population of parabens, leading to a concentration of research in this area. Restrictions have been put on the use of parabens, trying to reduce the use of the most. In addition to these, studies should be carried out for new substances that can be used as an alternative to parabens. Paraben exposure has not been totally eliminated. On this issue, more research is required.

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APPENDİX A

T.C.
HACETTEPE ÜNİVERSİTESİ
Hayvan Deneyleri Yerel Etik Kurulu

Sayı : 52338575-152

HAYVAN DENEYLERİ YEREL ETİK KURUL KARARI

TOPLANTI TARİHİ	:	05.11.2015 (PERŞEMBE)
TOPLANTI SAYISI	:	2015/08
DOSYA KAYIT NUMARASI	:	2015/86
KARAR NUMARASI	:	2015/86 – 13
ARAŞTIRMA YÜRÜTÜCÜSÜ		Prof.Dr. Nurhayat BARLAS
HAYVAN DENEYLERİNDEN SORUMLU ARAŞTIRMACI		Ecem ÖZDEMİR
YARDIMCI ARAŞTIRMACILAR	:	Arş.Gör. Gözde KARABULUT
ONAYLANAN HAYVAN TÜRÜ ve SAYISI	:	42 adet wistar albino sıçan

Üniversitemiz Fen Fakültesi Biyoloji Bölümü öğretim üyelerinden Prof.Dr. Nurhayat BARLAS'ın araştırma yürütücüsü olduğu 2015/86 kayıt numaralı ***"Propil Parabenin Olgunlaşmamış Erkek Sıçanlarda Antiandrojenik Etkilerinin Araştırılması"*** isimli çalışma Hayvan Deneyleri Yerel Etik Kurulu Yönergesi'ne göre uygun bulunarak oy birliği ile onaylanmasına karar verilmiştir.

Sorumlu araştırmacı deneylere başlangıç tarihini Etik Kurula bildirmekle yükümlüdür

Prof. Dr. Sema ÇALIŞ
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