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Research Article/Özgün Araştırma

Adverse effects of high-dose paracetamol on thyroid gland of female rats

Yüksek dozda parasetamolün dişi sıçanların tiroid bezi üzerine olumsuz etkileri

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Abstract

Aim: The aim of this study is to determine whether paracetamol, an analgesic whose mechanism of action is not yet fully known but used unconsciously, causes toxicity on the thyroid gland.

Materials and Methods: A total of 25 female Wistar albino rats divided into five groups as Control (C), Paracetamol 7 days (P7), Paracetamol 14 days (P14), Paracetamol 21 days (P21) and Paracetamol 28 days (P28). The Paracetamol groups were given 750 mg/kg/day paracetamol via oral gavage administration until the day they were sacrificed. Routine histological procedures were applied to the removed thyroid glands. Thyroid tissue sections were evaluated morphometrically and histopathologically.

Results: Cytoplasmic vacuolization and deterioration in follicle and colloid structures were detected in follicular epithelial cells in thyroid tissue sections of groups given paracetamol. The mean of follicle diameter measurement of the P7 group was significantly decreased compared to the control group (p<0.05). In all paracetamol groups, the mean follicular epithelium height increased significantly compared to the control group (p<0.05).

Conclusion: These results show that high doses of paracetamol cause toxic effects on the thyroid gland depending on the duration of use.

Keywords: Paracetamol (acetaminophen); Thyroid; Histopathology.

Öz

Amaç: Bu çalışmanın amacı, etki mekanizması henüz tam olarak bilinmeyen ancak bilinçsizce kullanılan bir analjezik olan parasetamolün tiroid bezi üzerinde toksisiteye neden olup olmadığını belirlemektir.

Gereç ve Yöntem: Toplam 25 adet dişi Wistar albino rat kontrol (K), Parasetamol 7 gün (P7), parasetamol 14 gün (P14), Parasetamol 21 gün (P21) ve parasetamol 28 gün (P28) olarak 5 gruba ayrıldı. Parasetamol gruplarındaki sıçanlara sakrifiye edilecekleri güne kadar gavaj ile 750 mg/kg/gün parasetamol verildi. Çıkarılan tiroid bezlerine rutin histolojik prosedürler uygulandı. Tiroid dokusu kesitleri morfometrik ve histopatolojik olarak değerlendirildi.

Bulgular: Parasetamol verilen grupların tiroid dokusu kesitlerinde folikül epitel hücrelerinde sitoplazmik vakuolizasyon, folikül ve kolloid yapılarında bozulma saptandı. P7 grubuna ait folikül çap ölçümü ortalaması kontrol grubuna kıyasla anlamlı azaldı (p<0,05). Tüm parasetamol gruplarında folikül epitel yüksekliği ortalaması kontrol grubuna kıyasla anlamlı olarak arttı (p<0.05).

Sonuç: Bu sonuçlar, yüksek dozda parasetamolün kullanım süresine bağlı olarak tiroid bezi üzerinde toksik etkilere neden olduğunu göstermektedir.

Anahtar Kelimeler: Parasetamol (asetaminofen); Tiroid; Histopatoloji.

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Introduction

Paracetamol is currently one of the most widely used drugs for analgesic and antipyretic purposes.[1] It is considered to be one of the safest analgesic/antipyretic drugs in medical use, particularly in special groups such as children, elderly, and pregnant women and today, thousands of preparations throughout the world contain paracetamol. In Turkey, it is present in more than 300 pharmaceutical preparations as of 2015. It is used unconsciously as it is a cheap and easily accessible drug. Paracetamol intoxication is the most encountered one among intentional overdoses.[2]

Although paracetamol is widely used and over a hundred years have passed since its synthesis, its mechanism of action is still not fully understood. A small part of paracetamol is known to be excreted unchanged, but a large part is known to be excreted by undergoing biotransformation to a great extent in the liver and to a certain extent in the kidneys. Paracetamol is excreted as sulfate and glucuronide conjugation and some of it is converted to n acetyl-β-benzoquinone imine (NAPQI), a reactive metabolite, which is formed during the metabolism of paracetamol in the cytochrome P450 system in the liver.[3,4]

However, many studies have shown that high dose or frequent use of paracetamol lead to damage to various tissues, particularly in liver and kidney.[5-8] Furthermore, its toxicity potential has not yet been fully established. Therefore, there are many details that need to be investigated about paracetamol toxicity. There is a limited number of studies investigating the effects of paracetamol on thyroid gland in the literature. The aim of this study was to investigate the effects of high doses of paracetamol used for different duration on thyroid gland and to raise awareness regarding the adverse effects of unconscious use of analgesics on health.

Materials and Methods

Study design

Before starting this study, approval was obtained from Local Ethics Committee for Animal Experiments. A total of 25 adult female Wistar albino rats were used in the study. All animals were kept in a sterile experimental animal unit (55–60°C humidity and 19–22°C) and maintained under a 12:12 hours light/dark cycle. Animals in all groups were allowed to use ad-libitum unlimited feed and tap water. The animals were divided into five groups, each consisting of an equal number of animals: Control (C), Paracetamol 7 days (P7), Paracetamol 14 days (P14), Paracetamol 21 days (P21) and Paracetamol 28 days (P28). Control group was not subject to any procedure. Paracetamol groups were given 750 mg/kg per day via oral gavage administration until the day they were sacrificed.[9] Animal experiments were performed in accord with the National Guidelines for the Use and Care of Laboratory Animals.

Histochemical analyses

On the designated days, all rats were perfused and thyroid glands were removed. The removed tissues were fixed in 10% neutral buffered formalin solution and then examined under the light microscope. After routine histological tissue procedure, tissue samples were embedded in paraffin for sectioning. Then, sections were stained with hematoxylin-eosin (H-E) to measure the intrafollicular diameter and to assess epithelial height with periodic acid Schiff (PAS) to evaluate the amount of intrafollicular colloid in the sections obtained.

Morphometric analyses

In the sections taken for morphometric assessment of the thyroid gland, 10 regions were randomly determined at 4x magnification under the light microscope. Then, five follicles were randomly selected at 10x magnification from each of the identified regions, and their diameters were measured and recorded. Epithelial heights of follicles, the diameter of which was measured at 10x magnification, were measured at 40x magnification. Measurements were made at five different points in a follicle and averages were taken to calculate the height of the follicle epithelium. Photographs of the measured areas were taken using Leica DM 100 light microscope. For a single rat,

diameters of 50 follicles were measured using Leica image transfer apparatus.

Scoring was performed considering the degree of staining in PAS-positive thyroid follicles to evaluate the PAS staining intensity. Staining intensities were divided into four categories: 0 (negative staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining)

Statistical analysis

Statistical analysis was performed using SPSS version 23.0 software. Data were expressed as mean±standard deviation. Differences between groups were determined with normality and homogeneity tests and were evaluated using one-way ANOVA and Tukey tests. A *p* value of <0.05 was considered statistically significant.

Results

Histological findings

Hematoxylin-eosin staining results

When the thyroid sections of the control group were examined, the general structure of the gland was seen to consist of units called follicles (Figure 1). The thyroid follicle lumen was full of colloid and the lumen was covered with single-layer epithelial cells.

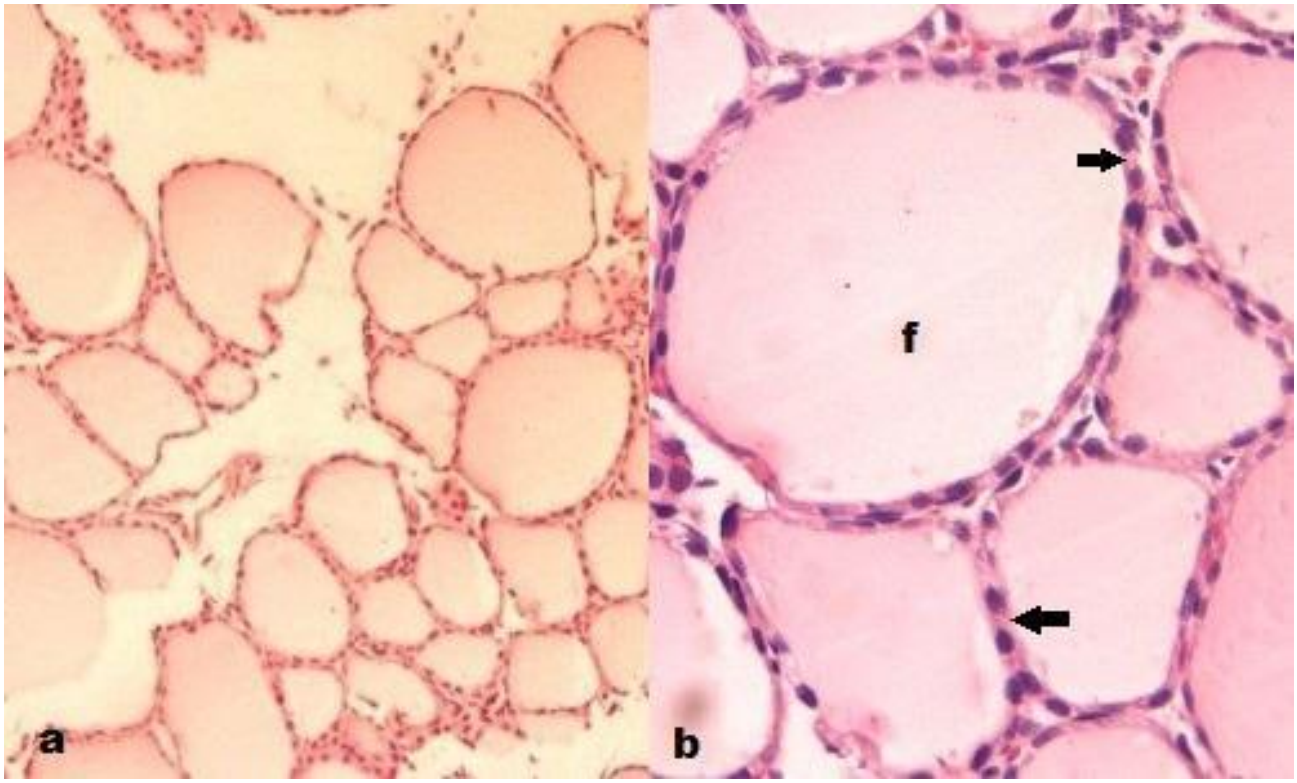

Figure 1. a, b. Control group, thyroid follicles (x100, x400), f; follicle, arrow; follicle epithelium, H-E.

There was a cytoplasmic vacuolization showing significant cellular swelling in some regions of the thyroid follicle epithelium belonging to paracetamol groups. Besides the degenerative changes observed in epithelial cells, there were also deterioration in follicle epithelium continuity in some areas, epithelial cells or cell debris spilled into the follicle lumen, follicular-colloidal degeneration, and interfollicular haemorrhage (Figure 2).

Periodic acid Schiff staining results

PAS staining was performed to evaluate the colloid in the follicle. The PAS staining intensity of the intra-follicular colloid was weaker in the paracetamol groups compared to the control group (Figure 3, Figure 4). Statistical evaluation of scores determined according to staining intensity showed that staining difference was statistically significant ($p<0.05$).

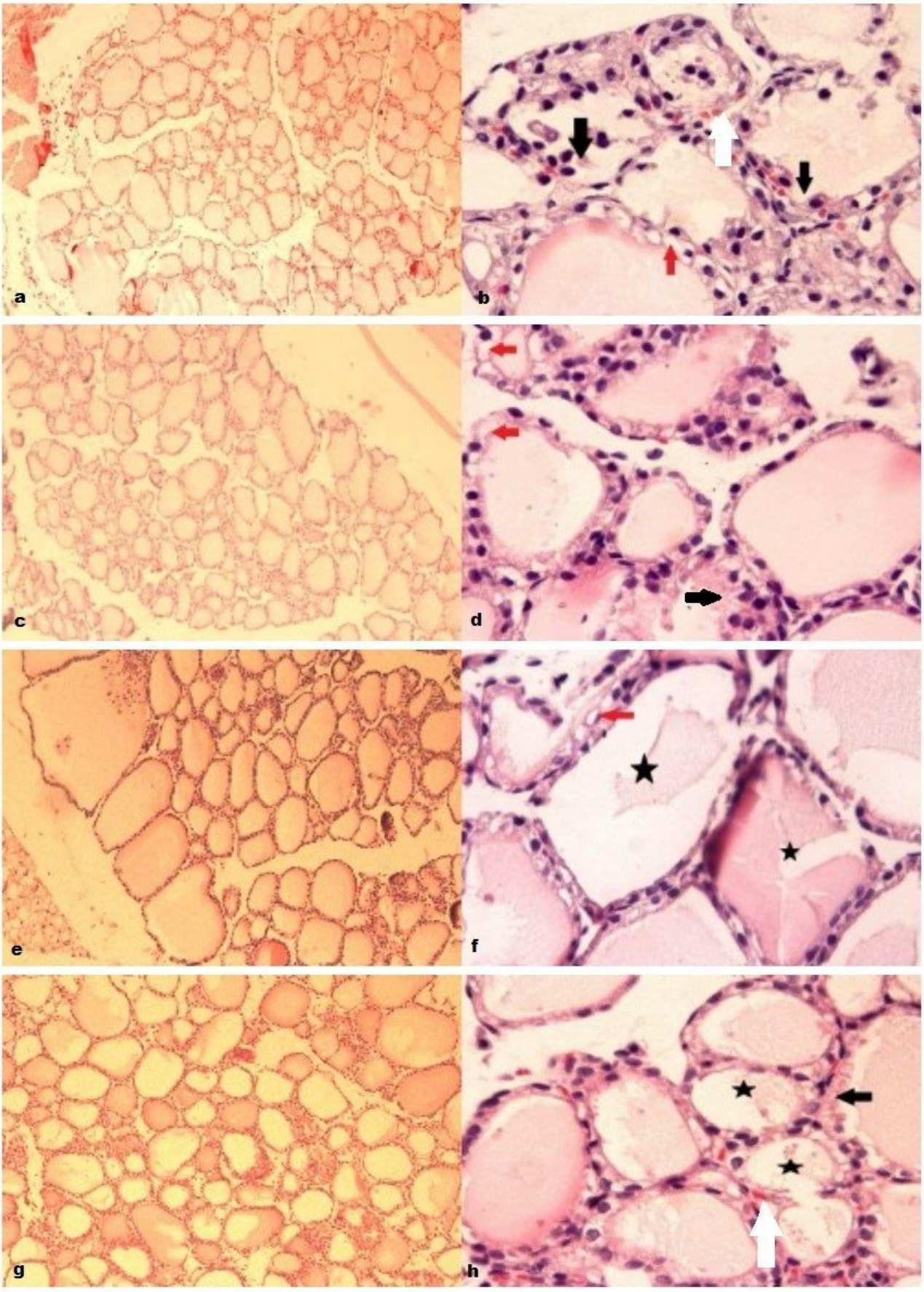

Figure 2. Paracetamol groups, thyroid gland section. a,b. Group P7 (x100, x400), c,d. Group P14 (x100, x400), e,f. Group P21 (x100, x400), g,h. Group P28 (x100, x400), red arrow; cytoplasmic vacuolization, black arrow; epithelium shedding and degeneration, white arrow; interfollicular haemorrhage, *; colloidal degeneration, H-E

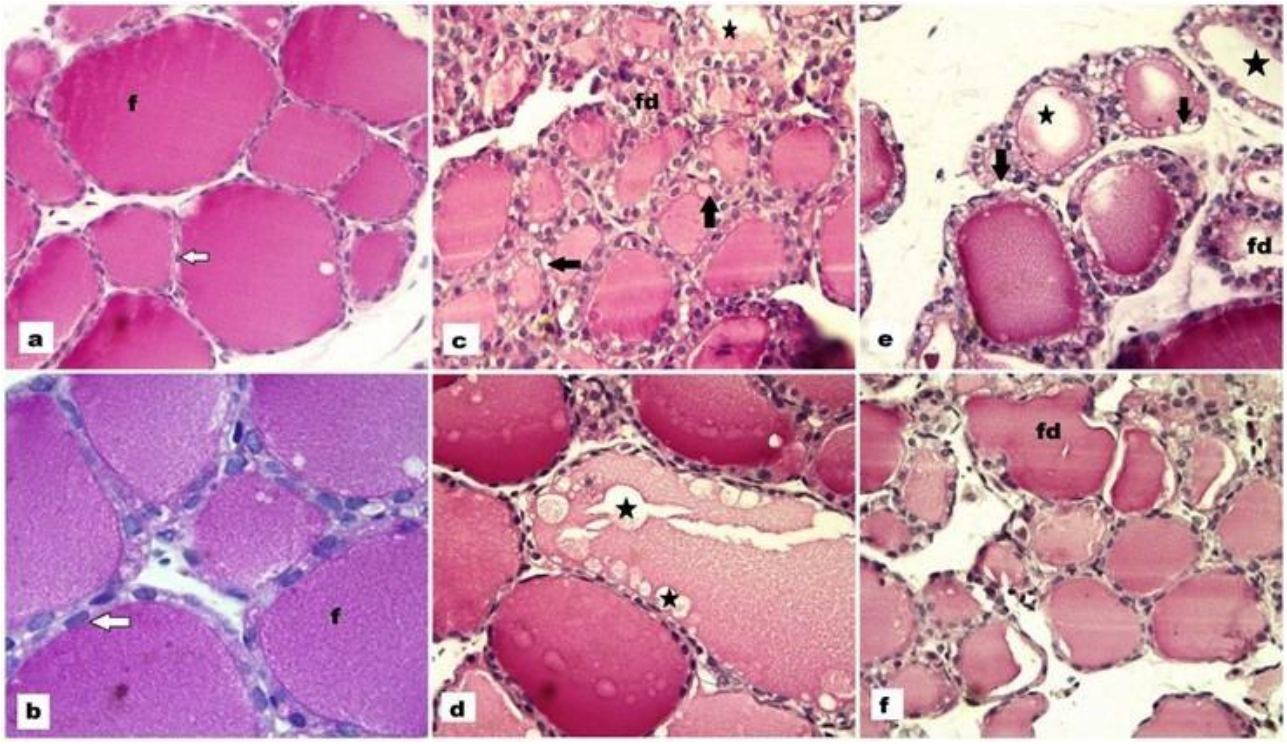

Figure 3. a,b,c, Control group, thyroid gland. (x200, x400, x1000), f; follicle, *; follicle epithelium, PAS.

Morphometric results

Bodyweight results

The bodyweights of the animals in all groups were evaluated both before and after the experiment (Table 1). There was a significant increase in the bodyweight of animals in the control group as a result of normal development ($p<0.05$). On the other hand, there was a decrease in the bodyweights of the animals in paracetamol groups. These decreases were found to be statistically significant in P7, P21 and P28 groups ($p<0.05$), but it was not statistically significant in the P14 group ($p>0.05$) (Table 2).

Morphometric results of the thyroid gland

Evaluation of the data according to the results of one-way ANOVA

A significant difference was observed between the control group and P7, P14, P21, and P28 groups in terms of follicle epithelial height ($p<0.05$) (Table 3). When the staining intensity of colloid was evaluated, it was found to be weaker in the paracetamol groups than the control group and the results were statistically significant ($p<0.05$).

Evaluation of follicle diameter measurement according to post hoc Tukey test results

The histological appearance of follicular diameters were seen to be normal in the control group. The mean follicle diameter of the tissue samples belonging to the control group was measured to be 56.63±5.14. The mean follicle diameter of the tissue samples belonging to the P7 group was measured to be 44.51±4.39. The follicular diameter was found to be smaller in the P7 group compared to the control group, and P14, P21, and P28 groups. Statistical comparison of these groups showed that there was a significant difference between P7 and control groups in terms of mean follicle diameter. However, although there was no significant difference between P7 and other groups (P14, P21, P28) in terms of follicle diameter, there was a decrease in follicle diameter in the P7 group ($p>0.05$) (Table 1).

Evaluation of follicle epithelial height according to Post hoc Tukey test results

The mean follicle epithelial height of the thyroid tissue samples belonging to the control group was 2.19±0.20 (Table 1).

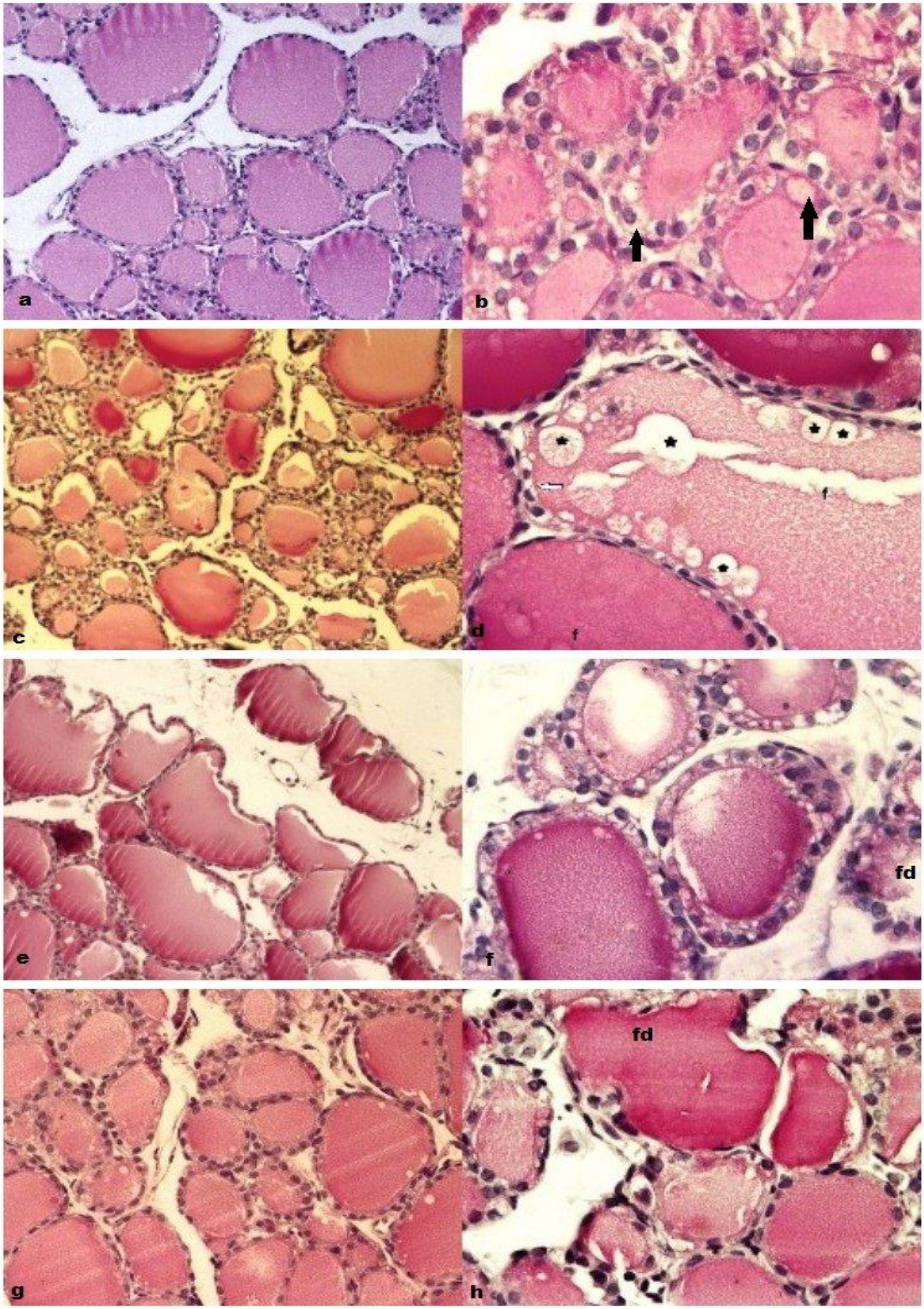

Figure 4. Paracetamol groups thyroid gland section a,b. Group P7 (x200, x400), c,d. Group P14 (x200, x400), e,f. Group P21 (x200, x400), g,h. Group P28 (x200, x400), black arrow; vacuolization in the epithelium,*; colloidal vacuolization and degeneration, fd; follicular degeneration, PAS.

Comparison of the paracetamol groups and control group showed that there was a significant increase in the mean follicle epithelial height in the paracetamol groups (p<0.05).

Table 1. Morphometric results (The groups are different in follicle epithelial heights: P7 v C, p<0.01**; P14 v C, p=0.01* ; P21 v C, p<0.01** ; P28 v C, p<0.05*; in follicle diameter: P7 v C, p<0.05*).

Morphometric results					
	N	Follicle Diameter (±SD)	Follicle Epithelial Height (±SD)	Body Weight (g) Pre-experimental	Body Weight (g) Post-experimental
P7	5	44.51±4.39	3.20± 0.36	235.4	226.4
P14	5	48.80±9.94	2.82 ±0.25	236.6	233.6
P21	5	48.50±1.75	2.99±0.27	261.6	244.6
P28	5	49.88±5.36	2.80±0.14	238.2	227.2
CONTROL	5	56.63 ± 5.14	2.19 ± 0.20	226.4	232

Table 2. Comparison of body weights measured before and after the experiment according to Wilcoxon T test results (statistical significance level was accepted as ** p<0.01, * p<0.05).

Test Statistics[a]					
	p7 post-experiment - p7 pre-experiment	p14 post-experiment - - p14 pre-experiment	p21 post-experiment - p21 pre-experiment	p28 post-experiment - p28 pre-experiment	control post-experiment - control pre-experiment
Z	-2.032	-1.095	-2.023	-2.032	-2.032
Asymp. Sig. (2-tailed) p	.042*****	.273	.043*	.042*	.042*

Table 3. Results of one-way analysis of variance in comparison of groups (statistical significance level was accepted as ** p<0.01, * p<0.05).

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Follicle Diameter	Intergroup	386.036	4	96.509	2.733	.058
	Ingroup	706.209	20	35.310		
	Total	1092.246	24			
Follicle epithelial height	Intergroup	2.814	4	.703	10.309	.000**
	Ingroup	1.365	20	.068		
	Total	4.179	24			
PAS + staining intensity	Intergroup	8.960	4	2.240	10.182	.000**
	Ingroup	4.400	20	.220		
	Total	13.360	24			

Discussion

Paracetamol (acetaminophen) is one of the most commonly used analgesic and antipyretic drugs throughout the world. Its use has been reported to increase considerably in recent studies[1]. The widespread use of a drug can cause intoxication and toxicity. Many studies have shown that high dose or frequent use of paracetamol lead to damage to various tissues, particularly in liver and kidney.[10-12] This study investigated the effects of high doses of paracetamol on the thyroid gland depending on time.

In this study, the mean follicle diameters in thyroid glands of rats in all groups were measured. According to the data obtained, the follicle diameters were smaller in all paracetamol groups than in the control group, however, only the mean follicle diameter in the P7 group was significantly smaller than the other groups. Another important finding in the study was that the follicle epithelium height was higher in all paracetamol groups than in the control group. The highest increase in epithelial height was in the P7 group. In a study by İbrahim[13] investigating the effects of 500 mg/kg paracetamol on the thyroid gland, paracetamol at this dose has

been reported to cause an increase in follicle cell height and a decrease in thyroid hormones and mean follicle diameter. The results of the present study are compatible with this study.

In other studies, paracetamol treatment has been reported to cause cyclic adenosine monophosphate (cAMP) inhibition, leading to a significant reduction in thyroid hormones. The decrease in the thyroid-stimulating hormone (TSH) levels is known to inhibit the stimulating effect of TSH on follicular cells in the thyroid gland. In this study, the increase in the length of follicular cells and the decrease in thyroid diameter in paracetamol-treated groups can be explained by this mechanism. In other words, it can be said that high doses of paracetamol cause a decrease in TSH level and this decrease inhibits the stimulating role of TSH on the follicular cell in the thyroid gland, eventually leading to an increase in the length of follicular cells and a decrease in thyroid diameter in the paracetamol-treated group.

Studies on the effects of paracetamol on the thyroid gland are limited, but many studies have shown that different agents such as medications[14,15], stress[16] heavy metals[17] and pesticides[18] have structural and functional effects on the thyroid gland. In the present study, colloidal degeneration of thyroid glands, epithelial cell deposition in the lumen, deformity of follicles, disturbances in the colloidal area, and follicle epithelium spills were observed in the thyroid glands of paracetamol groups.

We also evaluated the PAS staining intensity of colloid. Staining intensity was found to be weaker in the paracetamol groups than the control group and the results were statistically significant. It can be said that the amount of thyroglobulin in the structure of the glycoprotein in the colloid decreases in paracetamol groups. In a study by Gerard et al. involving old mice, active follicles have been reported to have a round core cubic or cylindrical epithelium whereas hypofunctional follicles are surrounded by squamous epithelial cells. They have shown that the lumen is filled with dark dense colloid in hypofunctional follicles whereas the lumen is composed of colloid that are more clearly stained in the active follicles.[19]

One of the remarkable findings of the present study is that the bodyweight of animals in the control group showed a significant increase as a result of the normal development process, but there was a decrease in body weight in the paracetamol groups. This is one of the clinical signs of hyperthyroidism. Hyperthyroidism is a catabolic condition associated with increased energy expenditure[20,21] increased lipolysis[22,23] and increased protein turnover.[24,25] These metabolic effects lead to loss of body weight due to a decrease in both fat stores and lean body mass.[26]

As a result of the findings of the present study, paracetamol can be said to cause hyperactivity in the thyroid gland due to the decrease in body weight of female rats, an increase in the height of follicle epithelial cells and weaker staining of colloid in paracetamol groups.

Conclusion

These results of this study indicate that high doses of paracetamol can cause to toxic effects along with degeneration on thyroid gland depending on the duration of use but more detailed stereological and biochemical studies must be performed. The results presented in the literature are of great importance in terms of gaining awareness regarding the use of paracetamol that is widely used at the present time as it is an easy-to-access and cheap drug. Therefore, the society should be informed about the possible side effects of paracetamol to avoid over-the-counter use of paracetamol.

Furthermore, the results will contribute to the scientific literature on the effect of paracetamol on the thyroid gland and will be used as data in the studies to be performed in this regard. There is a need for further studies on the toxicity potential of paracetamol, which has not been fully understood yet.

Ethics Committee Aproval

The study was approved by the local ethics committee for animal experiments of Ondokuz Mayıs University (2014/23).

Author Contributions

The authors contributed equally to the study.

Conflict of Interest

The authors declared no conflict of interest.

Financial Disclosure

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Statements

This study International Congress on 2nd International Congress of Forensic Toxicology, 26-30 May 2016, at Ankara/TURKEY has been presented as an poster presentation.

Peer-review

Externally peer-reviewed.

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