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The Effect of Chemical Substances on Endocrine Disorder and Related With Morbidity and Mortality of Sperms in Albino Rats

Rasha Hamid Ayub
Department of Biology, College of Education, University of Samarra, Iraq

Wurood Mohammed Mutar
Department of Biology, College of Education, University of Samarra, Iraq

Qays Assi Ahmed
Department of Biology, College of Education for pure Sciences, University of Kirkuk, Iraq

***Abstract*---**Parabens are a group of alkyl esters of p-hydroxybenzoic acid that is commonly added to personal care products, cosmetics, pharmaceuticals, beverages, and food processing as an antimicrobial preservative. Butylparaben has been reported to cause sperm poisoning. To identify a previous marker of the toxicity of environmental substances or food additives, this study determined whether Butylparaben had an effect on the external appearance of sperm. Where several concentrations of 400mg/kg, 300mg/kg, and 200mg/kg were used daily for 20 days and dissolved in corn oil. It was found that it had an effect, as treatment with this substance led to a reduction in the weight of the testes and epididymis in the adult male rats used understudy. The results also showed a decrease in the number of sperms and their vitality compared to the control group, and the higher concentration used in the study had the greatest effect as compared to the other study groups and control, where the abnormalities in the sperm were represented by the twisting and breaking off the tail with the separation of the head in some sperms and the deformation of the head and the tail agglutination.

***Keywords*---**butylparaben, chemicals, endocrine-disrupting, morbidity, sperm abnormalities.

***Corresponding author**: Ayub, R. H.; Email: rashahamid@uosamarra.edu.iq*
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Introduction

Over the past few decades, endocrine-disrupting chemicals (EDCs) have become a major public health concern. EDC is defined as “an exogenous chemical or mixture of chemicals that interferes with any aspect of the action of the hormone. Humans are at great risk through exposure to endocrine-disrupting chemicals, because these compounds are ubiquitous in the environment (Gore et al., 2015). Absorption of EDCs may occur via various routes including the oral route (ingestion of contaminated drinking water and food), skin contact, inhalation, an intravenous route through drugs, placenta, and breast milk transfer (Kabir et al., 2015). The main mechanism of action of EDCs involves mimicking endogenous hormones and their binding to their receptors based on which they may act as agonists or antagonists to alter the cell signaling pathways regulated by the hormone. Endocrine-disrupting chemicals have different hormonal activities, For example, estrogenic and antiandrogenic properties and thyroid properties. They may also affect different types of nuclear receptors, such as peroxisome proliferator-activated receptors (PPARs) found in reproductive tissues. Chemicals may directly or indirectly disrupt endocrine gland synthesis and affect steroid synthesis (Sweeney et al., 2015).

Through many other pathways observed by in vitro and in vivo studies, endocrine-disrupting chemicals may also affect the male and female reproductive systems. and development of breast gland and breast cancer, prostate cancer, reproductive neuroendocrine systems, thyroid, metabolism and obesity, and cardiovascular disease, Parabens is chemical substance that causes disorder in the function of endocrine gland because it used as preservatives material in most aspects of life in a lot of quantities. Regardless of race, social, economic, or geographical background, studies have shown their presence in developing countries, especially countries where exposure to these substances is still unknown in terms of quantities (Hajizadeh et al., 2020). Parabens are a collective name that expresses alkyl or aryl esters (chain length ranges from methyl to n-butyl, isobutyl, or benzyl) para hydroxybenzoic acid (Abril et al., 2021). Interest in paraben compounds has increased because they are found in Mother' milk, serum, placenta, semen, and adipose tissue (Park et al., 2019; Song et al., 2020). e of the paraben compounds is Butylparaben, where studies have shown that Butylparaben disrupts spermatogenesis by inhibiting androgen production and reducing the number and function of Sertoli cells (Santiago et al., 2020).

The importance of this study on the male reproductive system is a result of the decline in human fertility around the world, and this gradual loss of fertility is coupled with a prominent reason, which is the increase in the body's load of environmental toxins. Scientific evidence has provided convincing information, according to studies and research, that chemicals in the air, water, food, and cosmetic products negatively affect the reproductive capacity of living organisms in several ways. The aim of the present investigation was demonstrated the effect of Butylparaben on the fertility of reproductive system by showed the disorder occur on the sperm.

Materials and Method

Study design

In this experiment, 28 male Sprague dawelu white laboratory rats were obtained from the animal house of the College of Veterinary Medicine at University Tikrit, Iraq. The weights of the animals ranged from 180-195 grams and their ages ranged from 9-11 weeks. The animals were given a week to Adaptation for different environmental condition. The animals were divided into 4 groups, each group contain 7 male rats as following:

- Group1 was the control group and was given a dose of corn oil, group2 was dosed with Butylparaben (Sigma) daily for 20 days. At a concentration of 400 mg/kg/day that was dissolved in corn oil for all concentrations, group3 dosed at a concentration of 300 mg/kg/day, group4 dosed at a concentration of 200 mg/kg/day. After the end of the experiment, all animals were sacrificed by anesthetizing with dimethyl ether, testes, and epididymis were collected for sperm isolation and sperm analysis was evaluated as described.

Quantitative and qualitative calculations

Weight: The animals were weighed on the last day of the experiment using a sensitive digital scale, and the results were recorded for each of the testes and epididymis weights of the males after the end of the study period.

Analysis of sperm characteristics

The epididymis was isolated from the testes and cut into very small parts in a physiological solution to obtain sperms and then calculate the number and vitality of sperms.

Sperm count

The calculation was done by a hemocytometer, mashing the epididymis Caudalie with a sharp scalpel in a dish containing 9.8 ml of 10% formalin buffer solution. Two drops of eosin dye were added to it and a drop of the solution was taken and placed on the counting slide and left for five minutes for the sperms to settle on the counting slide in 5 medium squares, four on the sides and one in the middle. Using a light microscope with a magnification of 40X, the sperm was calculated using the following equation:

$$\text{Total sperm number} = (N/80) \times 400 \times 1000 \times 10 \times 10$$

Sperm viability

The sperm vitality and normal morphology were estimated by preparing glass slides, by mashing the caudal epididymis in 2 ml of 0.9% physiological solution. A drop was taken and placed on the glass slide. A similar amount of eosin-necrosin dye was placed, then mixed them well and spread on the slide to make a light

smear for observation. Sperm shape and vitality by light microscope The dead sperm appeared dyed with the head with eosin dye, while the necrocin served as a floor for the glass slide, while the live sperm did not take the dye, then it was calculated for each smear of at least 200 sperm, and the percentage of sperm vitality was calculated according to the following equation:

live sperm percentage=number of live sperm/total sperm count × 100

Sperm abnormality percentage

The prepared slide was used to count the live and dead sperms to calculate the percentage of deformed sperms, and it was examined under 40X magnification The morphological abnormalities were divided into defects in the head and tail. Central segment sperm abnormalities were included as part of the tail sperm assessment. The percentages of spermatozoa with normal and abnormal shapes were calculated. According to the following formula:

Distorted sperm percentage= distorted sperm count\ total sperm count×100

Statistical analysis

The differences between the groups were analyzed statistically by using the SPSS v.22 software. Data has been represented as ±SD. Statistical significance of data was calculated by the variance (one-way ANOVA) analysis plus tuky check post hoc. $P<0.01$ has been significant.

Result

According to the current study, the results in Table (1) showed the difference between the results of the concentrations used, which are 400, 300, and 200 mg/kg/day, respectively, where the study showed that the higher concentration of Butylparaben, which is 400 mg/kg/day, had the greatest effect. On testis weight and epididymis weight compared with the control group. The study also showed the change in the number of sperms that were calculated, where the number of sperms extracted from the epididymis decreased significantly ($P \leq 0.01$) and the largest effect was also due to the concentration of 400 mg/kg/day and gradually the percentage increased in the remaining concentrations compared with the control group.

Table 1
Showed the differences among weight of testis, epididymis , number of sperms, normal and vital percentage of sperm, concentrations of butylparaben, as compared to the control group

Parameters	control	400 mg/kg/day	300 mg/kg/day	200 mg/kg/day
Testicle weight, g	1.7^{a}	1.2^{cd}	1.4^{c}	1.5^{ab}
Epididymis weight g	0.8^{a}	0.34^{bc}	0.44^{b}	0.56^{ab}
Number of sperm	91^{a}	35^{d}	52^{c}	76^{b}

x 106 / mm 3				
natural%	93[a]	37[d]	56[bc]	69[b]
vitality%	79[a]	32[d]	50[bc]	66[b]

Different letters refers to significant differences (p≤ 0.01) among study parameters, while the same letters that mean no any significant differences.

Evaluation of sperm morphology

The percentages of normal and abnormal sperm are shown in Table (1). Morphological analysis of semen samples showed a significantly low percentage of normal-shaped sperms in all experimental groups (P ≤ 0.01). A significant increase (P ≤ 0.01) in the incidence of abnormal-headed sperms with separation of the sperm head and tail twisting was detected in the second group with a concentration of 400 mg/kg/day. Moreover, the number of sperm head defects was significantly less as the abnormalities included tail-to-tail and mid-segmental asymmetry in the third group with a concentration of 300 mg/kg/day, While in the fourth group with a concentration of 200 mg/kg/day, it showed the appearance of coiled-tail sperms with head abnormalities. Representative micrographs of sperm morphology are shown.

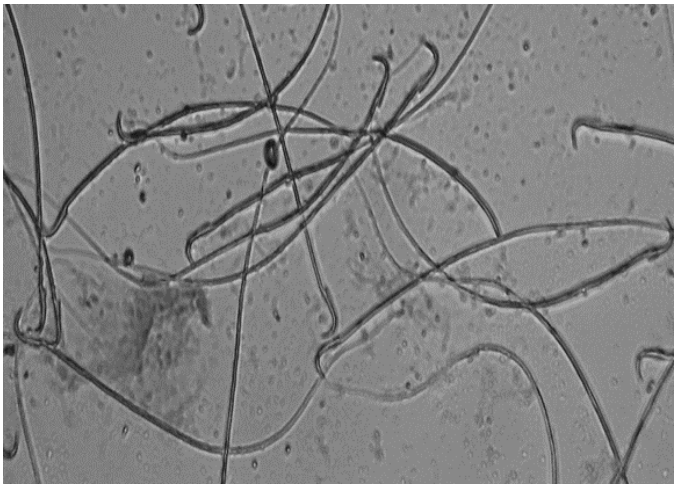

Figure 3. Showing the abnormal shape of tail clumped together and the head was deformed at concentration 300 mg/kg/day (40X)

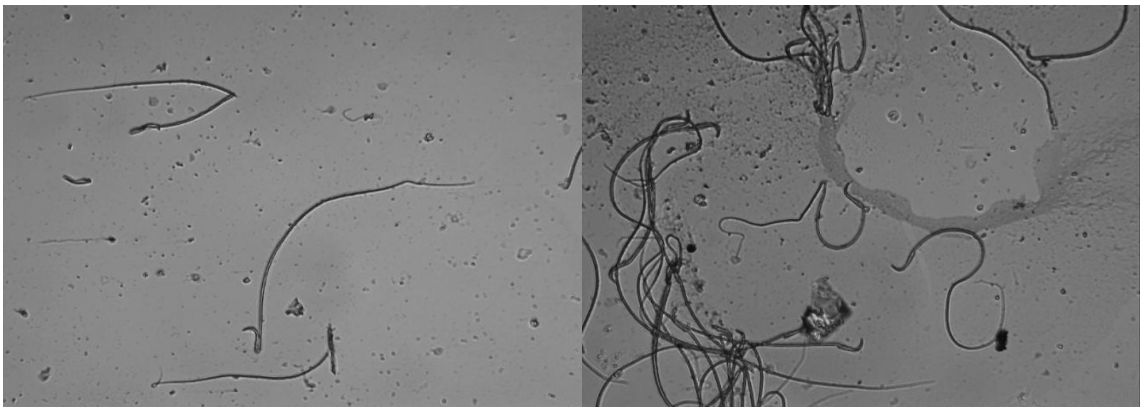

Figure 4. Showing the abnormal shape of midsection fracture, head deformity, and tail fracture at 200 mg/kg/day (40X)

Discussion

In this study, a significant decrease in testis weight, epididymis weight, decrease in sperm concentration and motility, and increase in dead and abnormal sperms of rats treated with butylparaben was documented, and this is identical to the study done by (Zhang et al., 2014). This is due to the use of butylparaben, which is a preservative compound that works to disrupt the work of the endocrine glands. This is because parabens can bind to androgen receptors (AR) and have anti-androgenic activity. Parabens can disrupt the secretion of hypothalamic, pituitary, and peripheral hormones. At the intracellular level, it can interfere with nuclear receptors, transmembrane receptors, intracellular signaling pathways, and modulate gene expression (Yesumanipreethi et al., 2021), Testicular dysfunction and dysfunction are often associated with increased oxidative stress. Hubbar et al. 2020 study agreed that butylparaben had an effect on reproduction in terms of reduced testicular weight, decreased sperm production, and decreased testosterone. Parabens can form ROS that can reduce sperm vitality (Samarasinghe et al., 2018). The change in sperm parameters can be attributed to the direct effect on testicular tissue resulting in reproductive impairment such as decreased sperm count, motility, and morphology.

Adverse effects produce a decrease in circulating androgen caused by butylparaben, which acts to impede androgen signaling, as it has been shown that steroidogenesis can be affected by environmental compounds. Several in vitro and in vivo studies have reported that parabens have estrogenic activity, Gonadotropin feedback systems including FSH/LH are believed to be regulated by xenoestrogen exposure, particularly during Sertori cell development, Which leads to a decrease in the production of testes and sperms (Nishihama et al., 2017). A disturbance in the function of sperm may occur due to parabens interfering with the energy of mitochondria and leading to its disruption by inhibiting the production of ATP, which impairs sperm motility. that the steroidogenic acute regulatory protein (StAR) receptor and the benzodiazepine receptor (Bzrp) play a role during cholesterol transport to the mitochondrion and represent molecular targets of butylparaben that thus interfere with steroidogenesis. And the abnormalities in the sperm that were observed in this study are due to the

imbalance that occurred in the endocrine glands, which led to the interference and disruption of the work of these glands, thus an imbalance occurred in the sex hormones and affected the Sertoli cells and Leydig cells. Butylparaben may also be a suitable vaginal contraceptive due to its ability to inhibit acrosin (an) an enzyme that helps sperm penetrate the egg during fertilization, likely working by damaging the sperm membrane. Sperm DNA damage is likely to be caused by oxidative stress, which can also damage the sperm membrane. Oxidative stress inhibits steroidal enzymes and damages the tissues of the testicles. The sperm membrane and its integrity are damaged, resulting in abnormal structure, loss of motility, and eventually death of the sperm (Kehinde et al .,2018).

Conclusion

This study showed that the use of butylparaben led to a decrease in the number of sperms and an increase in the percentage of disorder sperms with the lack of normal ones and their impact on the weight of the testicle and the weight of the epididymis. existing in the environment to the occurrence of sterility in the future and affect the offspring produced.

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