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The effect of sodium benzoate as a preservative on the reproductive system of male rats

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ABSTRACT

In order to investigate the effect of sodium benzoate dosage on reproductive efficiency in mature male rats, the current study was conducted at the Faculty of Science, Qadisiyah University for the period from 15\10\2016 to 15\4\2017. Sixty adult male rats (aged 56 days and weighted 138±8.8 g) have been used in the present study. The rats have been divided randomly into three equal groups (20 rats to each group). The first group included the control (C) group and was injected with physiological saline solution daily. The second group was injected with sodium benzoate at a concentration of (50 mg\kg). The third group was injected with sodium benzoate at a concentration of (100 mg\kg). Each group was divided into two subgroups, each containing 10 rats, depending on the duration of the dosage, 2-3 weeks. After 24 hours on the last day of the experiment, animals were sacrificed, and testicular samples were taken for histopathological study. The result of the histological study on the testis showed a significant decrease in the composition of the sperm in the two groups (T1, T2) where the spermatozoa showed a small number of primary and secondary sperm cells as well as a decrease in the number of sperm and Leydig cells. This decrease increases with increasing concentration and duration compared to the control group. It is concluded from the results of the current experiment that the dosage of animals with sodium benzoate at a concentration of (50,100 mg\kg) body weight has a clear effect on the reproductive efficiency of male rats.

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INTRODUCTION

One of the great advances in human history was the ability to conserve food, nd with the beginning of the twentieth century and the qualitative and evolutionary leaps at all levels, One of the areas in which the development of technology has been the production and manufacture of food, which led to the abundance of food and increase circulation and transport not only within the product country, but exceeded the geographical area and across the border, which in turn led to the impossibility of preparing and packing and storage and transport of many food without the addition of materials maintained by of damage (Saad *et al.,* 2005).

Many methods were used to conserve food. Initially, food preservation techniques started from freezing and drying and then developed into preservatives, which were subsequently increased food species. As a result, the food needed to consume a lot of time instead of moving from place to place. To get fresh food, there must be a way to save it until it is used.

In the past, food was produced and consumed locally, but at present, it is produced in a place and treated elsewhere and then distributed elsewhere. This means that the period between production

and consumption is longer and it becomes necessary to conserve food to prevent damage and prevent undesirable changes in taste and color (Wroblewska, 2009). With the increase in food and fast food production, the use of preservatives has become important in food technologies (Saad *et al.,* 2005).

The availability and consumption of healthy food that equips the body with its basic needs is necessary for humans, Food and its active ingredients have a significant effect on human health, and food safety is not a new concept in the modern world, it is deeply rooted in the history of human civilization, Food damage has been a common problem throughout history, For the activity of organisms or enzymatic activity during food preservation (Jay *et al.,* 2005; Turkoglu, 2007).

About one-third of the population in developed countries suffering from food-borne diseases and more than 250 microbiological, chemical or physical agents responsible for these diseases were estimated in 2011. The centre for diseases control in America estimated about 128,000 people hospitalised of foodborne illnesses and three Thousand dies every year (Makwana *et al.,* 2014).

In our day the food factory forms about 75% of the diet of Western societies (Zengin *et al.,* 2001). Sodium benzoate is one of the industrial additives which is widely used in the food industry and is known as a safe substance (GRAS). It is widely used as a preservative in various products such as pickles, vinegar, jams, juices and other materials such as shampoo and medicine (Yazar *et al.,* 2001).

It is also used to disturb the cycle of urea and prevents inflammation **(**Pahan, 2001). Its use is permitted in oral medications and cosmetics (Batshaw *et al.,* 2001).

Sodium benzoate has an antimicrobial effect such as fungi and some bacteria and its use as an ideal preservative in products that are naturally acidic, especially food and beverages with PH<4.5 (Stanojevic *et al.,* 2009).

Although sodium benzoate is classified as a safe preservative, damage preservatives industrial was noted to him in the research prior (Yolmeh *et al.,* 2014).

Some research has shown that small amounts of benzene can be composed of benzoic acid with vitamin C and gasoline is a carcinogen that breaks down DNA-deficient RNA in the mitochondria (Chang and Ku, 1993). Sodium benzoate is also linked to cancer, as mixing it with vitamin C in soft drinks leads to the formation of benzene (Kitano *et al.,* 2001).

Short –term exposure to benzoates can cause irritation in the skin, eyes and bronchi, but prolonged and continuous contact may cause large skin sensitisation (Lucio *et al.,* 2009).

MATERIALS AND METHODS

Experimental animals: Sixty-five days old adult male Wistar rats (average weight: 138+-8.8g), were breed at the animal house of the College of Veterinary medicine, Al- Qadisiyah University**.** For the period between 15/10/2016 and 15/4/2017.

Experimental protocol: Sixty adult male rats aged 56 days with a mean weight of 138±8.8 g were randomly divided into three groups. Each group included 20 rats. The first group represented the control group and was treated with saline physiological solution. The second group represented the treatment group T1, which was injected with sodium benzoate at a concentration of 50 mg/kg. The third group T2 which was injected with sodium benzoate at a concentration of 100 mg/kg. Each group was then divided into three equal secondary groups according to the duration of the dosage: two weeks and three weeks. Each secondary group included 10 rats. After the last administration of (sodium benzoate), the animals were sacrificed after general anaesthesia by a combination of Xylazine and Ketamine (10mg and 90mg/kg, i.p. respectively). After scarification testes, tissue will be removed for histological examination.

Study of sperm parameters: After the eradication of the epididymis, the tail of the epididymis is isolated and placed in a clean, cold and warm watch bottle placed on a hot plate at 37 ° C to avoid shock to the sperm as a result of cold and contain 5 ml of physiological saline solution and then cut by scalpel blade into very small pieces to release the sperm in it to determine the motility, vitality and sperm concentration (Lucio et al, 2009). The following tests were performed:

Motility of sperm: The percentage of sperm was calculated by Levin and his group (1992) (Sohrabi *et al.,* 2008). By taking a drop of the epidermal seed mixture after mixing it thoroughly with a Pasteur pipette and placed on a glass slide (dry and warm) and covered with the lid of the slice and counting at least 200 sperm per slice using the zoom force 40x and then the percentage of moving sperm was extracted.

The vitality of the sperm: To distinguish the living sperm from the dead, we follow the Bambe method (1998). By taking a drop of the semen mixture and placed near the end of the warm glass slice and then add a drop of Eucin-Ncrosin dye, mix the mixture for 30 seconds and prepare a swab to dry in the air. The 40x magnification shows the

dead heads of the sperm dyed reddish pink, while the heads of the sperm that were alive when mixing with this color were not written and then at least 200 sperm were counted along the swab or slide.

The concentration of sperm: The concentration of sperm was calculated using Lucio (2009) method (Oishi, 2001). The haemocytometer was used for the total count of white and red blood cells. It was installed on the optical microscope and covered with the lid of the counting slice. After this, 10 µL of the diluted sample was taken by a pipette and then slowly inserted under the cover on the sides of the counting slice, leave for about 5-10 minutes for the sperm to settle on the slide and then count at x400 in the five special squares after red blood cells. The sperm concentration was calculated by multiplying the sperm count calculated in five squares in the multiplier factor (106).

Calculation of normal sperm: In order to calculate the normal sperm, the Axiner method and its group were adopted (1999). Two samples were applied for each sample, and the same method was used to calculate the live sperm. After testing the microscope at 40x force, the sperm, Sperm from the total of both surveys and then calculated the percentage according to the following equation:

$$\text{Percentage of normal sperm} = \frac{\text{Number of normal sperm}}{\text{Total number of sperm calculated (normal and non-normal)}} *100$$

Histological study

Preparation of histological sections: The histological sections of the test are preserved in formalin10% were prepared for all animal groups to study them and to identify the effect of treatment with sodium benzoate levels. The method Luna (1968) was followed in the preparation of histological sections (Oishi, 2002).

RESULTS

Changes in the epididymis

Effect of benzoate on the number of sperm: The results of the study showed a significant decrease (P <0.05) in all groups when compared with the control group. The results of the T2 and T1 groups and the control group for two weeks were (56.60 ± 0.045, 48.8 ± 0.8, 42.40 ± 1.45, P <0.05) between the two groups. And T1, T2 and control group for three weeks (58.60 ± 0.43, 44.60 ± 1.28, 36.60 ± 0.45) respectively, with significant difference (P <0.05) between the two groups as shown in the table (1).

Effect of benzoate on sperm movement: The results showed a significant decrease (P <0.05) in the mobile sperm ratio of the T1 and T2 groups compared to the control group. The decrease in T1, T2 and control group for two weeks was 85.6 ± 0.2, 76.6 ± 0.6, 57.9 ± 2.06% There was a significant difference (P <0.05) between the two groups. For three weeks, the results were in T1, T2 and control group (85.8 ± 0.1, 65.6 ± 0.2, 47.6 ± 2.14) The two groups as in Table (1).

Effect of benzoate on sperm vitality: The results showed a significant decrease (P <0.05) in the percentage of sperm vitality in the two groups compared to the control group. The results for T1 and T2 and control group were two weeks (85.9 ± 0.4, 74 ± 1.3, 60 ± 2.3) respectively, between the two groups. The three-week results for T1, T2 and control group were 86.5 ± 0.2, 66.8 ± 2.5, 51.8 ± 0.74% respectively, with significant differences between the groups as in Table (1).

Effect of benzoate on the percentage of normal sperm: The study showed a significant decrease (P <0.05) in the two groups compared with the control group. The results for T1 and T2 and control group for two weeks were 76 ± 0.3, 66.8 ± 0.8, 53.8 ± 1.21% There was a significant difference between the two groups, and the results for the three-week period for T1 and T2 groups and control group (75.1 ± 0.2.56 ± 0.3,43.6 ± 1.3) respectively with significant differences (P<0.05) as in Table (1).

- The results represent the mean ± standard error
- The different small letters indicate significant differences (P <0.05) between the periods for each group
- The different large characters indicate significant differences (P <0.05) among the three groups for each period
- C: control group
- T1: Treatment group with 50 mg/kg body weight in sodium benzoate
- T2: Treatment group with 100 mg/kg body weight in sodium benzoate

Result of histopathological study

In comparison with testes section obtained from control male rats (figure.1 a, b), sodium benzoate treated (50 and 100) groups revealed a clear eruption in the spermatozoa appears with intermediate numbers of primary and secondary sperm cells, while other normal germs appear and their abdomen is full of sperm. Leydig cells appear in average numbers. (figure 2 a,b), whereas those obtained with the proliferation of primary and secondary sperm cells and medium numbers of Leydig cells in the interstitial testicular tissue. Slight congestion in the interstitial tissue with thrombus. Sections obtained from group T2 was a concentration of

Table 1: The effect of treatment with sodium levels on sperm parameters in male, white rats

Groups / parameters	C control		Treatment (T1) (50 mg/kg)		Treatment (T2) (100 mg/kg)	
	2week	3week	2week	3week	2week	3week
Count*(106 /ml)	56.60±0.45 Aa	58.60±0.43 Aa	48.8±0.8 Ba	44.60±1.28 C b	42.40±1.45 Ca	36.60±0.45 D b
Motility	85.6±0.2 Aa	85.8± 0.1 Aa	76.6± 0.6 Ba	65.6± 0.2 C b	57.9± 2.06 Ca	47.6± 2.14 D b
Viability	85.4±0.4 Aa	86.5 ±0.2 Aa	74 ±1.3 Ba	66.8±2.5 C b	60±2.3 Ca	51.8±0.74 D b
Morphology	76±0.3 Aa	75.1± 0.2 Ab	66.8± 0.8 Ba	56±///0.3 C b	53.8± 1.21 Ca	43.6 ± 1.3 D b

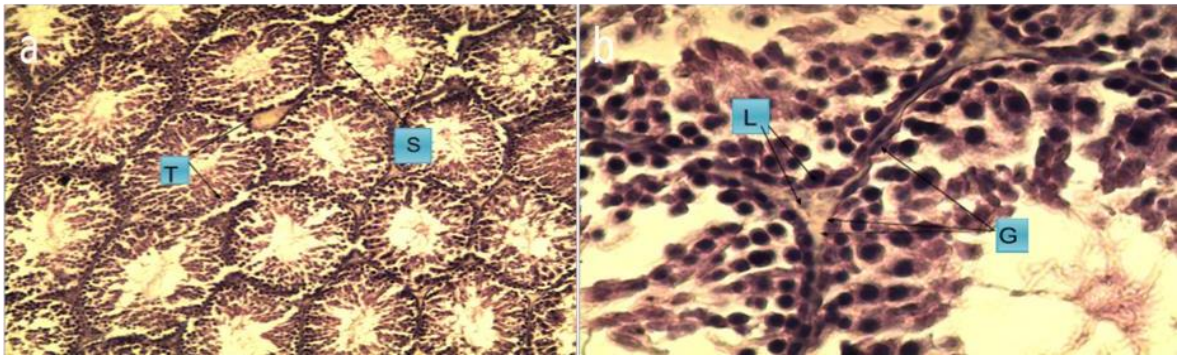

Figure 1: a) The spermatozoa appear enlarged, round and full of tusks (T). A clear proliferation of primary and secondary sperm cells with abundant spermatozoa (S) (4X H & E); b) The multiplication of sperm profiles (G) and primary and secondary sperm cells with Leydig cells in the 40X H & E (L) interstitial tissue

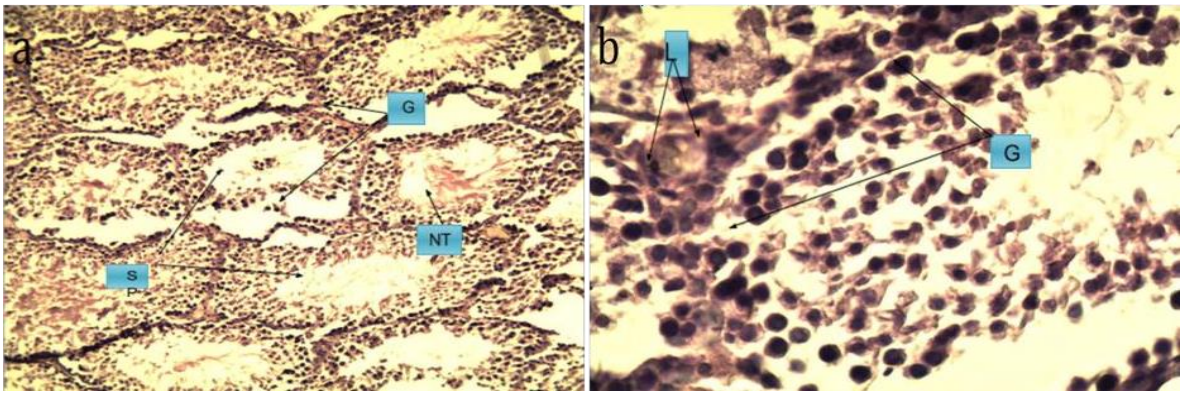

Figure 2: a) Inhibition of sperm formation in some spermatozoa (SP) where there is a clear gap in spermatozoa (G) with moderate numbers of primary and secondary sperm cells, while other germs appear normal and their abdomen is full of sperm (NT). (10X H & E); b) A clear gap in spermatozoa (G) with intermediate numbers of primary and secondary sperm cells and medium numbers of Leydig cells in the testicular interleaved L (40X H & E)

100 mg/kg of sodium benzoate for two weeks showed a simple eruption occurs in the spermatozoa (Fig. 4 a,b) with atrophy in some seminal tissues. The spermatozoa appear to have a large lining with very few sperms inside. The primary and secondary sperm cells appear in small numbers, while LIDC cells appear in small numbers. Group T2-3W was a concentration of 100 mg/kg of sodium benzoate for three weeks the spermatozoa contain a wide lining with very little sperm (Fig. 5 a, b). It is also observed in the sperm follicles with few numbers of primary and secondary sperm cells, Leydig cells appear in very few numbers in the interstitial testicular tissue, blood vessels of the interstitial tissue.

DISCUSSION

Effect of sodium benzoate in some sperm parameters

The experimentation of the experimental animals with sodium benzoate concentration resulted in a clear reduction in the parameters of the sperm under study. The results showed that the morbidity

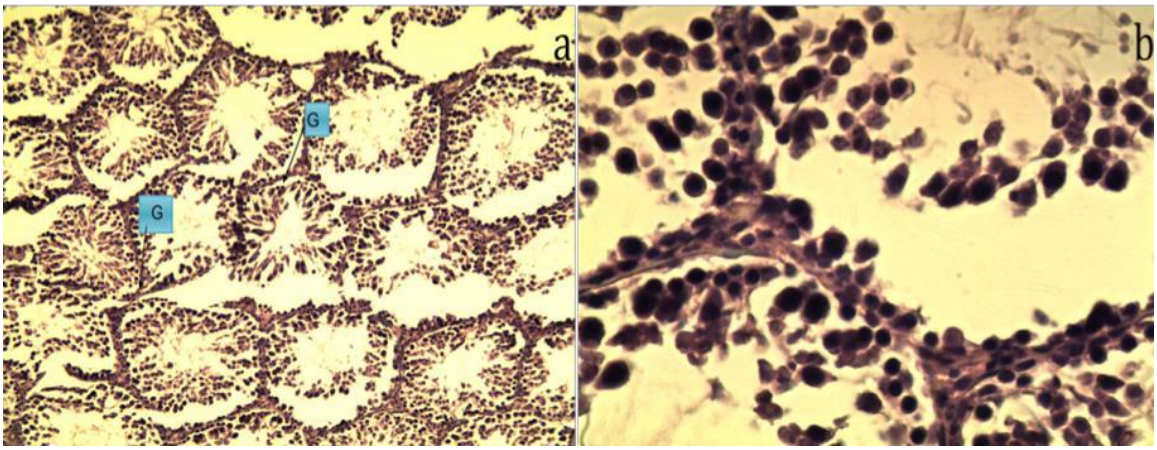

Figure 3: a) We observe a simple burst in spermatozoa (G) with a proliferation of primary and secondary sperm cells and medium numbers of Leydig cells in the interstitial testicular tissue; b) We observe a clear gap in the spermatozoa (G) with a number of primary and secondary sperm cells and medium numbers of LIDC cells in the testicular interstitial tissue (L). (40X H&E)

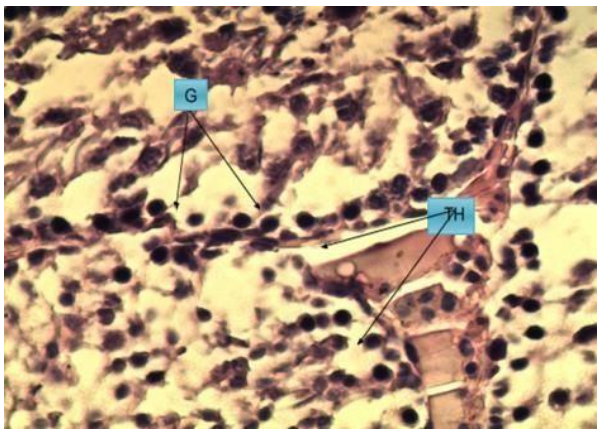

Figure 4: We observe a simple eruption in spermatozoa (G) with atrophy in some spermatozoa (A) and spermatozoa showing a broad lining with very few sperms inside it (M). (10X H&E)

decreased in both groups for both periods compared with the control group.

Concentration of sperm

One of the most important parameters that can reflect the function of the testicle is the natural concentration of sperm in the tail of the epididymis and give a clear assessment of sperm and can be attributed to low concentration of sperm to decrease the concentration of lipid hormone testicular and hormone stimulant as noted Sohrabi and his group (2008) significant decrease in the hormone lipid testicular in mice treated with sodium levels[24]. Because these two hormones are directly responsible for the initiation and persistence of sperm production, the levels of these two hormones have a direct effect on the concentration of sperm. The testicular lipid hormone stimulates the sperm parasites to divide into the bacterial epithlial layer of the spermatozoa. This is therefore essential in the process of semen emergence and is involved with stimulating follicle hormone Spermiogenesis, this is in line with 23 reported that the absence of testicular lipid hormone in rats resulted in a decrease in the concentration of sperm and a decrease in the diameter of the tubules carrying the sperm and preparation of sperm droplets.

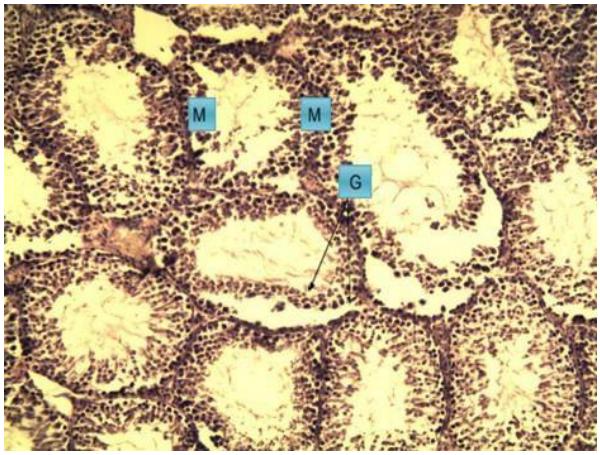

Figure 5: The spermatozoa contain a full lining with a very little sperm (M) and a burst of spermatozoa (G) with few numbers of primary and secondary sperm cells (10X H & E)

Exposure to preservatives, such as paraffin, benzoic acid (a preservative of food derived from benzoic acid), adversely affects the function of the male reproductive system and affects the secretion of the adipose hormone. And its maturity requires a combination of hormones together, such as prolactin hormones and growth hormone, as well as hormones that secrete from the frontal lobe of the pituitary gland, LH, FSH. This is explained by the moral decline in the concentration of sperm in both the two groups during the duration of the dosage (Kumar *et al.,* 2008).

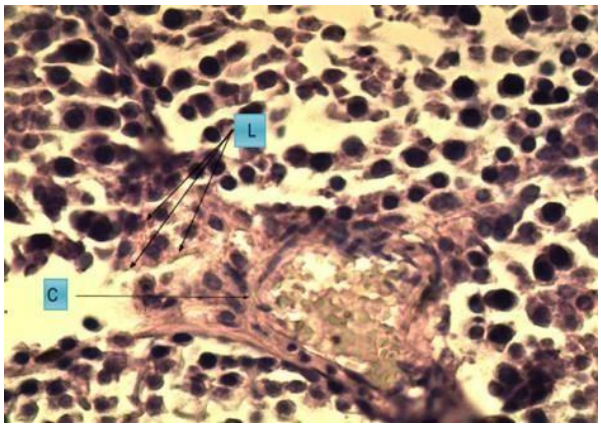

Figure 6: Note that LIDC cells appear in very few numbers in the interstitial tissue of L (L) also shows clear congestion of the blood vessels of the interstitial tissue (C) (40X H & E)

Percentage of sperm movement: The sperm movement is one of the essential features of semen in assessing the possibility of fertility. The active move is necessary for healthy sperm to enable it to penetrate cervical mucus and migration through the female genital canal to fertilize the egg. The weak and non-motile offspring can perform their task even if their number increases. The function of the epididymis (the protection of sperm and the increase in fertility) is under the influence of DHT Dihydrotestosterone, which is the effective form of testicular lipid, which is the primary androgen in the epidermal tissue. The low level of lipid hormone due to exposure to benzoate leads to a decrease in the effectiveness of the epididymis On fertilization and movement as they pass through the epididymis (Shu, 2010). The oxidative stress resulting from treatment with sodium levels can inhibit hypothalamic function, which reduces the production of Gonadotropins from the pituitary gland and thus inhibits the secretion of adipose lipid hormone. This explains Decreased sperm movement due to decreased efficacy of the epididymis

Percentage of distorted and levelled sperm: The results showed a significant decrease in the percentage of normal sperm compared with the control group. This may be due to oxidative stress, which causes high lipid peroxidation, which leads to abnormalities in the sperm. Shu (2010) states that benzoic acid and sodium benzoate stimulate lipid peroxidation. High lipid peroxidation is a clear sign of stress and oxidative damage, a reference to the abnormal regulation of antioxidants (Carocho, M. and Ferreira, 2013). Since benzoic acid and sodium benzoate are oxidants that lead to the formation of free radicals as a result of self-oxidation and these roots can destroy large particles, cells and tissues as testicular tissue and semen contain sources for the production of free radicals, (Which are present in the case of genital infection). These free radicals are powerful oxidizing agents that interact with different molecules and cellular compounds and cause an increase in mutated and dead sperm. Free radicals can also cause DNA damage to sperm during sperm differentiation as they affect the mechanism of normal sperm formation. (El-kannishy *et al.,* 2011) Suggests that the increase in the percentage of dead and deformed sperm may be due to the pathological changes that occurred in the living cells of the bacterium. These cells produce substances necessary to increase sperm efficiency, sperm protection and vitality. (Heise *et al.,* 2006).

Effect of sodium benzoate on changes in testicular tissue

The dosage of the animals in the Sodium levels had a negative effect on the histological characteristics of the test group T1 and T2 for the two and three weeks of the study. This effect was reflected on Leydig cells, Sperm and sperm germs may be attributed to the effect of sodium benzoate on thyroid hormones Studies have shown that sodium benzoate affects the thyroid hormone (TSH), which is the effect on thyroid hormones, where there is a significant decrease in oral treatment with sodium benzoate (Craft *et al.,* 2012).

Studies have shown that thyroid hormones affect Leydig cells and their development after birth. Thyroid hormones induce real stimulation and differentiation of Leydig cells, stimulating them to produce progesterone and estradiol, as well as the testicular lipid hormone, as well as the increase in LH receptors on these cells these hormones act on the differentiation of peroxisome cytoplasmic organelles, stimulating STAR mRNA production as well as acute steroidogenic protein (STAR) production in Leydig cells. These compounds are associated with the process in which cholesterol is transferred to energy houses as well as receptors of thyroid hormones On the surface of Leydig cells and other cells. Studies have also shown that thyroid hormones have a strong effect on Sertoli cells through the regulatory role of one of the proteins produced by Sertoli cells, the anti-mularian hormone, which plays an important role in the development of testicular tissue (Sanocka and Kurpisz, 2004).

The development of the cellular link in the testicular tissue is affected by thyroid hormones .40When this complex link of the testicular tissue, which is composed of the overlapping of the Sertoli cells and the sperm cells on one side and the Sertoli cells and the cells around the tubes, for differentiation (El-Kannishy *et al.,* 2001). The growth of testicular tissue and the formation of sperm and the process of full growth and composition in the epididymis depends on the androgens, especially the hormone

lipid testicular, whose receptors are scattered in the bacterial epithelium that encircles the tuberous tubules (Shivaraj *et al.,* 2011).

REFERENCES

Abed, Salwan Ali, 2017. An occurrence of Anatidae in Sawa Lake: A Ramsar Wetland Site in Southern Iraq, Journal of Advanced Zoology, J. Adv. Zool. 38 (1) 43-51.

Al-Grawi, E.D.C., and G.R.L. Al-Awsi. 2018. "Expression of CDKN2A (P16/Ink4a) among Colorectal Cancer Patients: A Cohort Study." Journal of Pharmaceutical Sciences and Research 10 (5).

Batshaw, M.L.; MacArthur, R.B. and Tuchman, M. (2001). Alternative pathway therapy for urea cycle disorders: twenty years later.J. Pediatr. 138(1):46-54.

Blanco-Munoz, J.; Morales, M. M.; Lacasana, M.; Aguilar, G. C.; Bassol.

Carocho, M. and Ferreira, I. C. (2013). A review on antioxidants, prooxidants and related controversy: Natural, synthetic compound, screening and analysis methodologies and future perspectives.Food Chem Toxicol.51:15-25.

Chang P and Ku K, 1993. Studies on benzene formation in beverages. Journal of Food and Drug Analysis, 1, 385–393

Craft, B. D.; Kerrihard, A. L.; Amarowicz, R. and Pegg, R. B. (2012). Phenol-based antioxidants and the in vitro methods used for their assessment comprehend.Rev.Food Sci. Food Saf.11(2): 148-173.

El-Kannishy, S. M.; El-Baz, R. M.; Abd El-Gawad, S. S.; MArzook, H. F.; Hassan, S. A. and Metwali, A. A. (2011). Sperm nuclear deoxyribonucleic acid denaturation in diazinon/diazoxon sprayer men. J. of American Sci., Vol. 7(6):470-475.

Falah Mahdi Ali, Ahmed Ali Obaid, 2018. Pros-Tate Vaporization by Diode Laser For Patients With Benign Prostatic Enlargement. Asian Journal of Pharmaceutical and Clin- ical Research, Vol 11 Issue 12 December 2018 Page: 523-525. DOI: 10.22159/ajpcr. 2018. v11i12.29572.

Ganong, W. F. (2005). Review of Medical Physiology .22ed. Langg medical book\Mc Graw Hill. The University of California. San Francisco.

Heise, K.; Puntarulo, S.; Nikinmaa, M.; Abele, D. and Portner, H. O. (2006). Oxidative stress during stressful heat exposure and recovery in the North Sea eelpout Zoarces viviparus L. J. Exp.Biol.209(2):353-363.

Ibraheen, Lujain Hussein, and Salwan Ali Abed. 2017. "Accumulation Detection of Some Heavy Metals in Some Types of Fruits in the Local Market of Al-Diwaniyah City, Iraq." Rasayan Journal of Chemistry 10 (2) 339–43. doi:10.7324/ RJC. 2017.1021641.

Jay, JM.; Loessner, MJ.and Golden, DA. (2005). Modern food microbiology. New York: Springer Science.

Kitano, K.; Fukukawa, T.; Ohtsuji, Y.; Masuda, T.; Yamaguchi, H. (2002). "Mutagenicity and DNA-damaging activity caused by decomposed products of potassium sorbate reacting with ascorbic acid in the presence of Fe salt", Food and Chemical Toxicology, vol. 40, iss. 11, pp. 1589-1594.

Kumar V.; Kural, M. R.; Pereira, B. M. J. and Roy, P. (2008). Spearmint induced hypothalamic oxidative stress and testicular anti-androgenicity in male rats – altered levels of gene expression, enzymes and hormones. Food Chem.Toxicol.46(12):3563-3570.

Lateef, Ghaidaa Raheem; AL-Thahab, Azhar Omaran; Chalap AL- Grawi, Eqbal Dohan. The linkage between H. Pylori Infection and TNF-α polymorphism in The Pregnant Women. International Journal of Research in Pharmaceutical Sciences, [S.l.], v. 9, n. SPL1, Apr. 2018. ISSN 0975-7538. Available at: <https://www.pharmascope.org/index.php/ijrps/article/view/1298>. Date accessed: 27 July 2018. doi: https://doi.org/10.26452/ijrps.v9iSPL1.1298.

Li, Y.; Sheng, J.; Sha, J. and Han, X. D. (2008). The toxic effects of microcystin-LR on the reproductive system of male rats in vivo and in vitro, Reprod. Toxicol.26(3-4):239-245.

Lucio, R.A.; Tlachi, J.L.; Lopez, A.A.; Zempoalteca, R.; Velázquez-Moctezuma, J. (2009). Analysis of the parameters of the ejaculate in the laboratory Wistar rat: technical description. Vet Méx; 40:405–15.

Makwana, S.; Choudhary, R.; Dogra, N.; Kohli, P.and Haddock, J. (2014). Nanoencapsulation and immobilization of cinnamaldehyde for developing antimicrobial food packaging material. LWT-Food Sci Technol. 57(2):470-6

Oishi, S. (2001). Effect of butyl paraben on the male reproductive system in rats.Toxicol.Ind.Health.17(1):31-39.

Oishi, S. (2002). Effect of butyl paraben on the male reproductive system in mice. Arch.Toxicol.76(7):423-429.

Pahan, K. (2011). Immunomodulation of experimental allergic encephalomyelitis by cinnamon

metabolite sodium benzoate. Immunopharmacol. Immunotoxicol.33(4):586-593.

S. and Cebrian, M. E. (2010). Exposure to organophosphate pesticides and male hormone prole in horticulturist of the state of Morelos, Mexico. Human reproduction., Vol. 25(7): 1787–1795.

Saad, B.; Bari, M.F.; Saleh, M.I.; Ahmad, K.and Talib, M.K. (2005). Simultaneous determination of preservatives (benzoic acid, sorbic acid, methylparaben and propylparaben) in foodstuffs using high-performance liquid chromatography.J. Chromatogr.A. 1073(1-2):393-397.

Sanocka, D. and Kurpisz, M. (2004). Reactive oxygen species and sperm cells. Repord.Biol. Endocrinol.2:12-25.

Shamran, A. R, Shaker, Z. H, Al-Awsi, G. R. L, Khamis, A. S, Tolaifeh, Z. A. and Jameel, Z. I, 2018. Rapdpcr is a Good DNA Fingerprinting Technique to Detect Phylogenetic Relationships among Staphylococcus Aureus Isolated from Different Sources in Hilla City, Iraq. Biochemical and Cellular Achieves. Vol. 18, Supplement 1, pp. 1157-1161.

Shivaraj, D.; David, M. and Ravi, K. B. (2011). Spermatotoxicity evaluation of deltamethrin and chlorpyriphos etc. by oral gavage in Wister rats. Int. J. Pharm. Bio. Sci., Vol. 2(4): 261-268.

Shu, L. (2010). Effect of benzoic acid, sodium benzoate, potassium sorbate and ethyl hydroxyl benzoate, sodium fluoride alone on rat liver lipid peroxidation. CNKI J.

Sohrabi, D.; Alipour, M. and Gholami M.R. (2008). The effect of sodium benzoate on testicular tissue, Gonadotropins and thyroid hormones level in adult (Balb/C) mice. KAUMS Journal. 12(3):7-11.

Stanojevic, D.; Comic, L.; Stefanovic, O. and Solujic-Sukdolak, S. (2009). Antimicrobial effects of sodium benzoate, sodium nitrite and potassium sorbate and their synergistic action in vitro. Bulg J Agric Sci J.15(4):307-11.

Turkoglu, S. (2007). Genotoxicity of five food preservatives tested on root tips of Allium cepa L. Mutat Res. 626(1-2):4-14.

Wroblewska, B. (2009). Influence of Food Additives and Contaminants (Nickel and Chromium) on Hypersensitivity and other adverse health reactions-A Review.pol. J. Food Nutr.Sci.59(4):287-294.

Yazar, K.; Johnsson, S.; Lind, ML.; Boman, A.; Lidén, C. (2011). Preservatives and fragrances in selected consumer-available cosmetics and detergents. Contact Dermatitis, 64: 265-272.

Yolmeh, M.; Najafi, MBH.; Farhoosh, R.and Salehi, F. (2014). Modelling of antibacterial activity of annatto dye on Escherichia coli in mayonnaise. Food Biosci. 8:8-13.

Zengin, N.; Yuzbasioglu, D.; Unal, F.; Yilmaz, S.and Aksoy, H. (2011). The evaluation of the genotoxicity of two food preservatives: sodium benzoate and potassium benzoate. Food Chem Toxicol. 49(4):9-763.