

Effects Study of Some Common Food Additives on Histological and Functional Thyroid Gland in Young Male Albino Rats

SARMAD A. ABBAS¹, HUSSEIN A. AL-HAMADAWI¹

¹Department Biology, College of Education, University of Al-Qadisiyah, Iraq.
Corresponding author: Hussein A. Al-hamadawi

ABSTRACT

The current study was conducted in the animal house / Department of biology / Al- University of Qadisiyah from November to February This is for the purpose of studying the effect of some food additives on the histological structure and function of the thyroid gland, as well as some oxidative indicators.

The current study included 32 young male albino rats randomly distributed into four equal groups The control dosed 1 ml of normal drinking water during the 30-day trial period, the first treatment group (T1) Tetrazine was dosed at a concentration of 25 mg/kg body weight for the duration of the 30-day trial period, The second treatment group (T2) received a chocolate brown dye at a concentration of 200 mg/kg body weight for the duration of the 30-day trial, The third treatment group (T3) dosed the preservative sodium benzoate at a concentration of 50 mg/kg body weight for the duration of the 30-day trial.

The results of the statistical analysis showed a significant decrease in the concentration of Thyroxine T4, Thyroid Stimulating Hormone(TSH), enzyme concentration level Superoxide dismutases)SOD(, and Significant increase in the level of concentration of malondialdehyde (MDA) In the first group) T1(and the second group (T2) Compared with the control group. The results also showed a significant increase in (MDA) A significant decrease in the level of (SOD) in a group (T3) Compared with the control group, there was no significant difference in the concentration of T4 & TSH in a group (T3) compared to the control group. The histological study also showed that pathological histological changes occurred in (T1) (T2))T3) Represented by changing the diameter of the thyroid gland's follicles and colloids Where there are many small irregular thyroid follicles, as well as bubbles in the colloidal substance And the occurrence of hyperplasia and an increase in the thickness of the lining layer of the vesicles.

We conclude from the current study that eating food additives causes some negative on the histological structure and function of the thyroid gland.

Keywords: Thyroid gland ,Tartrazine, Chocolate Brown HT E155, Benzoate sodium.

INTRODUCTION

After the great development in the field of food industry and marketing and the increase in consumption It became necessary to add a lot It has substances such as preservatives, dyes and flavors that increase its nutritional value and taste as well Attracting the consumer to it and preserving it for long periods, and processed foods have become an important part of human nutrition (AL-Shinnawy, 2009) . Food additives are any substance that is added to food and works to change any of its qualities, and it includes all substances Which are not from the natural ingredients of food and are added to it and used for the purpose of preserving food or improving its shape, color or taste (Martins et al., 2019).

The increase in the use of food additives in many products such as cheese, dairy, fish, preserved meat products, ice cream, juices, jams, sweets, etc. Disagreement grows over its effects and risks as the US Centers for Preventive Disease Control records 67 million foodborne diseases killed 5,000 people in one year This required setting strict conditions by the US Food and Drug Administration Food and Drug Administration (FDA) For approval of food additives and manufactured ingredients (Mister & Hatcock,2012) .

Al-Harthy et al (,216) mentioned Excessive use of food additives can expose the consumer to potential danger especially in foods and beverages that are eaten randomly and in groups Food additives are classified into food dyes, which are substances that are added to food, drink, medicine, and cosmetics to make them more attractive, reach the natural color, or simulate the original color and maintain the consistency of the food product(Bawazir, 2016; :silva,2022) The global output of dye is estimated to be more From 800,000 tons per year (Palmieri et al,2005) .

The dyes are of natural or synthetic sources, The most common type of dye is a dye (Carmoisine) (E122) As a cochineal-red synthetic azo dye, it is widely used in the pharmaceutical, cosmetic, toothpaste, textile and food industries (Sarvestani & Doroudi ,2022). The tartrazine dye (E102) It is a common synthetic azo dye that imparts a lemon yellow color and is widely used in the manufacture of many food products such as sweets, ice cream, jams and soft drinks, as well as in pharmaceuticals and cosmetics.

(Floriano, et al,2018; Mason & Chanforan, 2015) ,Chocolate brown HT (155) dye .It is a bis-azo dye It has a reddish-brown color, odorless, and is widely used for coloring various types of foods (Neshe et al,2016) Some studies have indicated that the use of these dyes for long periods causes some diseases such as indigestion anemia, growth retardation, neuropathy in the brain, allergic diseases, asthma, skin rashes, diseases of the liver, kidneys and spleen, as well as cancerous diseases(Kamel & El-lethey,2011; Elbanna et al., 2017; Amin& Al-Shehri, 2018; Al-Shinnawy& El-Kattan, 2013).

On the other hand, preservatives are substances that prevent oxidation and extend the useful life of goods to enable their distribution in many parts of the world. (Franco et al,2019) Preservatives are divided into natural Preservative Such as salt, sugar, organic acids such as lactic acid, acetic acid, spices and their oil, honey, and other substances Synthetic Preservative Such as Sodium acetate)SA(Sodium benzoate (SB) potassium sorbate (PS) & butyl parabens (BP) Which are widely used in food and pharmacy industries) Gupta,2021) . Consuming preserved food may cause some disorders and pathological changes because it has been eaten for long periods since childhood, and this has been proven by studies and scientific research It was found that excessive use of sodium chloride As a preservative, it increases blood pressure, Kidney Failure, Heart attack (Abdulmumeen, et al., 2012).

The thyroid gland is part of the body's endocrine system. It is the largest specialized organ of the endocrine glands in the human body They are necessary for the normal growth of the body in infancy and childhood. The thyroid gland is composed of follicles lined with epithelial cells filled with a colloidal sticky substance It is made up of Thyroglobulin protein Which is associated with iodine, which is the only source of thyroid hormones and manufactures two types of hormones T3 ,T4 (Nilsson and Fagman, 2017).

The thyroid gland is the main responsible for the metabolic process in the body of the organism and plays an essential role in regulating the basic metabolic rate and stimulating physical and psychological growth In addition to its vital role in calcium metabolism, it is distinguished from the rest of the glands by the ability of its cells to absorb iodine and to produce and store its

hormones in the gland itself and secretes them when needed as the thyroid gland secretes its hormones directly into the bloodstream as one of the endocrine glands (Ravichand et al., 2005) the biosynthesis and secretion of thyroid hormones are controlled by (negative feedback mechanism) For the axis of the hypothalamus - pituitary gland - thyroid gland secretion is stimulated by a hormone (TSH)

MATERIALS AND WORKING METHODS

Experimental animals: This study was conducted in the animal house of the Department of Biology / Faculty of Education/ Al-Qadisiyah University used 32 young male albino rats purchased from the animal house of the College of Science / Al-Qadisiyah University, their weights ranged between (90-110) grams The animals were placed in plastic cages of dimensions 50 x 35 x 15 cm, with an average of eight animals per cage, in a room of 3 x 4 m. All animals were exposed to the same conditions of temperature 20-25°C regulated by air conditioner As for the average illumination, it was 12 hours of light and 12 hours of darkness. The animals were given concentrated feed and free water, then randomly distributed and left for two weeks to acclimatize, after which the animals were weighed to determine the appropriate dose. It was divided into four groups, eight animals per group.

Experiment design: The animals were divided into four groups, as follows:

- 1 The control group (C) dosed 1 ml of normal drinking water for the duration of the 30-day experiment.
- 2 The first treatment group (T1): dosed 1 ml of Tartrazine dye at a concentration of 25 mg / kg of body weight and the diet throughout the 30-day trial period.
- 3 The second treatment group (T2): 1 ml of Chocolate Brown HT E155 tincture dosed at a concentration of 200 mg / kg of body weight and the diet throughout the 30-day trial period.
- 4 The third treatment group (T3): dosed 1 ml of the preservative sodium benzoate at a concentration of 50 mg / kg of body weight and the diet throughout the 30-day trial period.

Animal Sacrifice: After 24 hours after the last dose, the rats were anesthetized using the substance (ketamin and xylaine) Partially and then withdraw blood by medical syringes of 5 ml from the heart directly while the animals are anesthetized by Heart Puncture The blood was placed in plastic tubes, then placed in a centrifuge for 15 minutes at a speed (3000 r/min) to obtain blood serum and micropipette. The serum was withdrawn and kept in labeled Eppendorf tubes and placed at a temperature of (-20) °C until the tests were performed. Then the animals were dissected and the thyroid gland was removed, then placed in Petri dishes containing physiological solution and cleaned of excreta, after which part of it was placed in 10% formalin until preparation tissue sections.

Study of some biochemical parameters

1 Determination of T4 concentration. Using a ready-made test kit, a measurement was made T4 using ELISA technology Using a kit (KIT) manufactured by sunlong Biotech Chinese and then read absorbance O.D. at 450 nm using Microtiter Well Reader.

2 Determination of (TSH) concentration Using the ready-made test kit, a measurement was made (TSH) using ELISA technology, (TSH) Hormone concentration is measured, The assay depends on the amount of interaction between the antibodies in the reagent enzyme and hormone antigens (TSH) In the serum in KIT and the manufacturer of the company sunlong Biotech Chinese Then the absorbance was read O.D. at 450 nm using Microtiter Well Reader

3 **determination of superoxide dismutase(SOD) in Blood:** Depending on the method of researchers (Spitz & Oberley, 1989) Hormone concentration (SOD) was estimated rat serum samples. The absorbance value is estimated at 560 nm.

4 **determination of Malondialdehyde (MDA) in Blood:** Using the researchers' method (Spitz & Oberley, 1989) Hormone concentration (MDA) was estimated rat serum samples. The absorbance value is estimated at 532 nm.

Histological study: The tissue sections were prepared according to the method approved by (Bancroft et al, 2008) The samples were washed with tap water and the fixative was removed for an hour.

Histological examinations: The diameter of the thyroid follicles and the thickness of the epithelium lining them were measured using the scale Ocular micrometer power of 40 x 10 sections were examined and then the general average was calculated to extract the average diameter of the thyroid vesicles and the thickness of their lining layer for each group.

Statistical Analysis: The results of the statistical analysis were subjected in order to find out the significant differences between the control group and the treatments using the test (F) These significant differences were determined at the level of probability 5% (Al-Rawi & KhalafAllah 2000)

RESULTS AND DISCUSSION

The results of the statistical analysis in Table (1) showed a significant decrease

($P < 0.05$) The level of thyroxine hormone T4 concentration in the first treatment group (T1) They were compared with the control, and these results were in agreement with a study conducted with (Shakoor et al, 2022) The reason for this decrease in T4 concentration was attributed to the role of tartrazine dye in dramatically increasing the formation of free radicals This leads to oxidative stress, which leads to damage to body tissues, including the thyroid gland The result did not agree with another study (Elekima et al, 2017) On albino rats to study the effect of tartrazine dye on thyroid hormones, it was attributed to no significant change in T4 concentration. The reason is due to the dose of the dye used in the study The results of the current study also did not agree with another study (Abdel-Aziz et al, 2019) Where there was a significant increase in the concentration of T4 hormone, it is possible that the reason for the increase in the level of T4 hormone is the increase in the level of TSH enzyme. It also showed a significant decrease ($P < 0.05$) In the level of T4 hormone concentration in the second group (T2) when compared with the control group and the third treatment group (T3) his study agreed with another study (Kurebayashi et al, 1988) The young male mice were dosed with a dye called Rose Bengal For a period of two weeks and after the end of the trial period, the decrease in the concentration of T4 hormone is attributed to the presence of free radicals at high levels in the body tissues, which leads to tissue damage, including thyroid tissue, or this decrease in T4 concentration may be due to a decrease in the level of (TSH) stimulating hormone that stimulates T4 secretion, which was proven by the current study As for the results of the third treatment group (T3) Orally dosed with the preservative sodium benzoate did not make any significant difference in ($P < 0.05$) When compared with the control group and it agreed with the study) Helal et al, 2019) Where it was found that there was no change in the concentration of T4 in young male white rats when they were given the preservative sodium benzoate and monosodium glutamate. The reason for the lack of significant change is attributed to the antagonistic action of sodium benzoate and sodium monoglutamate, as the benzoate works to reduce the level of T4 hormone concentration, while sodium monoglutamate increases the concentration of T4 hormone. On the other hand, a study concluded (Sabr & ebrahim, 2015) To find out the effect of sodium benzoate on white male rats, which were dosed for a period of two weeks from body weight, and when conducting blood serum analysis of rats, it was noted that there was no significant decrease in the level of T4 hormone concentration.

In addition, the results showed a significant decrease ($P < 0.05$) in the concentration level hormone (TSH) in the table (1) In the first treatment group (T1) compared to control he results of this decrease in the level of TSH hormone concentration came with a study conducted by (Elekima et al, 2017; Abdel-Aziz et al, 2019; Shakoor et al, 2022) To find out the effect of tartrazine dye on the thyroid gland in albino rats, they found a significant decrease in the level of luteinizing hormone (TSH) It has been

suggested that the reason for this decrease is due to the occurrence of oxidative stress and the presence of free radicals during the biotransformation, or due to damage to the tissue of the thyroid follicles, or due to the daily dose of tetrazine dye. The results of the blood test of the second group(2) of rats also showed Treatment with chocolate brown, a significant decrease ($P<0.05$) In the level of hormone concentration (TSH) These results agreed with a study conducted (Elekima, & Ollor,2016) To know the effect of caromyosin dye And another study (Abdel-Aziz et al,2019) To know the effect of curcumine dye On thyroid hormones and hormone (TSH) Where a decrease in the level of hormone concentration (TSH) They attributed the reason for this decrease to oxidative stress resulting from the formation of free radicals generated by azo dyes. On the other hand, the results of the current study showed no significant change for the third group (T3) Treatment with sodium benzoate on the level of hormone concentration (TSH) The results of this study were similar to another study, which showed that when sodium benzoate was given to white male rats for a period of two weeks, there was no significant change in the level of the hormone concentration (TSH) . (Sabr& ebrahim,2015).

in addition to The results of the current study are shown in Table No.(1) Which was conducted on male rats young, there is a significant increase and at the level ($P<0.05$) In the concentration of MDA compound in the first treatment group (T1) ,the group for the second treatment (T2) And the third treatment group (T3) Treatment with the preservative sodium benzoate compared to the control group (C) significant decrease level ($P<0.05$) in compound concentration(SOD) Because of the increase in the concentration of tetrazine dye when feeding egg rats with this dye, which leads to a change in the composition of the cells and ultimately negatively affects the performance of their vital activities. As the current study showed, there was an increase in the concentration level (MDA) a drop in the level (SOD) compared to the control group in the group second (T2). And the dosed dye brown chocolate, and in the same context, it was clarified A study he conducted (Hassan& Salman,2017; Khatun et al,2021) On white rats, where a high concentration of (MDA) decrease in the level (SOD) When it treatment the chocolate brown dye, he suggested that the reason for the increase in (MDA) It is the damage to body tissues and cellular membranes that are constantly exposed to the influence of free radicals generated by the dye, which leads to the oxidation of unsaturated fats in the cell membranes, which causes consumption (SOD) and increase (MDA) The current study also showed an increase in the concentration level (MDA) and decrease in the level (SOD) Compared with the control group in the third group (T3) The dose of the preservative was sodium benzoate, and the results of this study agreed with each of

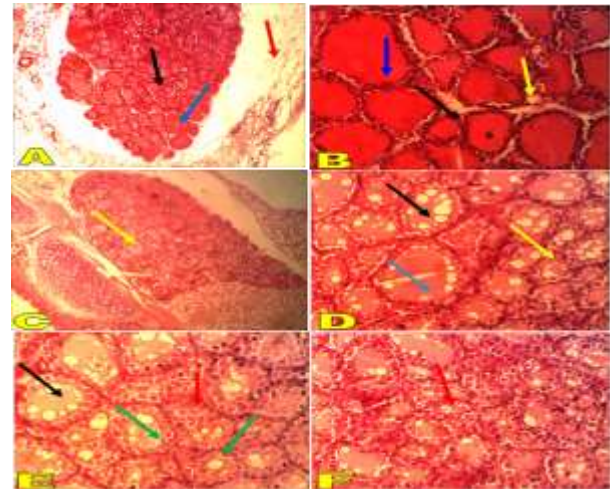
(Khan et al,2022; Radwan et al 2020; Kehinde et al,2018) Where they return the increase by level (MDA) And decrease in the level (SOD) In mice dosed with sodium benzoate, the presence of free radicals causes an increase in the level of (MDA) and consumption (SOD) And thus damage to the cells and tissues of the body and the failure of the body to perform its necessary functions and may cause defects in some parts of the body, leading to the development of dysfunction in the tissues and organs of the body.

Table 1: shows the effect of Tartrazine dye , chocolate brown dye and sodium benzoate on some biochemical parameters in young male rats.

Standards groups	T4	TSH	MDA	SOD
C	36.3267±1.44819 B	1.4633±0.11761 B	1.9540±0.32669 C	11.6640±0.38967 A
T1	20.4625±2.60623 A	0.9885±0.06991 A	6.3457±0.51932 A	7.3186±0.49954 B
T2	24.1188±4.37625 A	0.8027±0.15101 A	5.6967±0.32779 B	7.6933 ± 0.59121 B

T3	32.7115±1.84241 B	1.4894±0.10464 B	4.8157±0.50730 AB	8.9657±0.59621 B
LSD	8.880356	0.338829	1.323851	1.513556

The numbers represent averages ± standard errors



(Figure 1) Cross-section of thyroid tissue in male albino rats Picture (A & B) shows the control group, and we notice that it is surrounded by a capsule of connective tissue (red arrow) with medium sizes of thyroid alveoli. (black arrow) and it is full of colloid and is lined with a layer of cubic epithelial cells (blue arrow) and we notice a blood vessel in the alveoli (yellow arrow). As for the picture (C, D, E & F) showing the group treated with tetrazine food dye We note in it the presence of a large number of small thyroid vesicles (yellow) and bubbles in the colloidal substance (black arrow), as well as hyperplasia (red arrow) and an increase in the thickness of the layer lining the vesicles, and some vesicles are lined with more than one layer of epithelial cells (green arrow) (H& E, 10x and 40x).

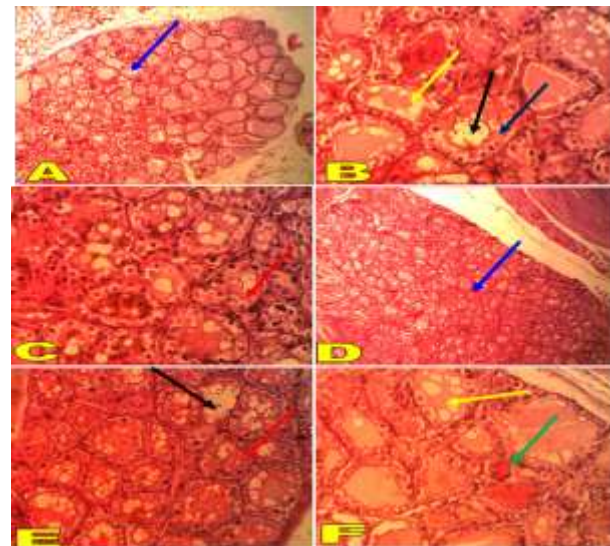


Figure (2) a cross-section of the thyroid gland, picture (A, B & C) the second treatment group, which dosed chocolate brown dye, where we notice the presence of many small vesicles (blue arrow) and some empty vesicles (black arrow) We also note the increase in the thickness of the vesicles (violet arrow) and the presence of bubbles in the colloidal substance (yellow arrow), as well as the presence of necrosis in the cells lining the vesicles (red arrow) As for the picture (D, E & F) representing the third treatment group, which was dosed with sodium benzoate, and we note the presence of many small vesicles (blue arrow) and some empty vesicles (black arrow) and the presence of bubbles in the colloidal substance (yellow arrow), as well as the presence of necrosis in the cells The lining of the vesicles (red arrow) and hyperemia (green arrow) (H& E, 10x and 40x).

Histological Study: Examination of histological slides of the thyroid gland revealed pathological changes in the first group (T1), the second group (T2) and the third group (T3). Where it was observed the presence of many small irregular thyroid follicles, as well as the presence of bubbles in the colloidal substance, some empty vesicles, hyperplasia and an increase in the thickness of the lining layer of the vesicles, and some vesicles were lined with more than one layer of epithelial cells compared to the control group. The current study agreed with the first group (T1) and the second group (T2) with (Rus et al,2010; Aboul-Soud et al,2011; Abdel-Aziz et al,2019) They attributed the reason for these changes to the ability of these pigments to bind directly in DNA and the generation of free radicals and thus oxidative stress, which causes damage and abnormalities in cells and leads to a disturbance in the metabolism process. As this study agreed with) Rani et al, 2013; Khalaf & Arafat,2015; Abd Allah, 2021)

They attributed the reasons for this change in the composition of thyroid tissue to the accumulation of preservatives as a result of excessive consumption, which contribute to the formation of free radicals, which increase oxidative stress and later turn into a toxic substance that affects the nerves.

REFERENCES

- AL-Shinnawy, M. S. (2009). Physiological Effect Of A Food Additive On Some Haematological And Biochemical Parameters Of Male Albino Rats. *Egyptian Academic Journal Of Biological Sciences. A, Entomology*, 2(1), 143-151.
- Martins, F. C., Sentanin, M. A., & De Souza, D. (2019). Analytical Methods In Food Additives Determination: Compounds With Functional Applications. *Food Chemistry*, 272, 732-750.
- Mister, S., & Hathcock, J. (2012). Under The Law, FDA Must Grant Different Standards For New Dietary Ingredients And Food Additives. *Regulatory Toxicology And Pharmacology*, 62(3), 456-458.
- Al-Harthy, A., Harib, A., Al-Shaibani, A. J., Al-Toubi, S. S., & Abukhader, M. M. (2016). Food Additives Content In Selected Snack Foods And Beverages And Public Perception Of E-Numbers In Muscat, Oman. *Athens Journal Of Health*, 4(1), 83-96.
- Bawazir, A. E. (2016). Effects Of Food Colour Allura Red (No. 129) On Some Neurotransmitter, Antioxidant Functions And Bioelement Contents Of Kidney And Brain Tissues In Male Albino Rats. *Life Science Journal*, 13(12).
- Silva, M. M., Reboredo, F. H., & Lidon, F. C. (2022). Food Colour Additives: A Synoptical Overview On Their Chemical Properties, Applications In Food Products, And Health Side Effects. *Foods*, 11(3), 379.
- Palmieri, G., Cennamo, G., & Sannia, G. (2005). Remazol Brilliant Blue R Decolorisation By The Fungus *Pleurotus Ostreatus* And Its Oxidative Enzymatic System. *Enzyme And Microbial Technology*, 36(1), 17-24.
- Sarvestani, M. R. J., & Doroudi, Z. (2022). A Comprehensive Review On Electroanalytical Methodologies For The Determination Of Carmoisine (E122). *Food Analytical Methods*, 1-10.
- Flóriano, J. M., Rosa, E. D., Do Amaral, Q. D. F., Zuravski, L., Chaves, P. E. E., Machado, M. M., & De Oliveira, L. F. S. (2018). Is Tartrazine Really Safe? In Silico And Ex Vivo Toxicological Studies In Human Leukocytes: A Question Of Dose. *Toxicology Research*, 7(6), 1128-1134.
- Masone, D., & Chanforan, C. (2015). Study On The Interaction Of Artificial And Natural Food Colorants With Human Serum Albumin: A Computational Point Of View. *Computational Biology And Chemistry*, 56, 152-158.
- Neshe, S. A., Arefin, S., Hussain, M. S., Das, A., Karmakar, P., & Hossain, M. S. (2016). Safety Evaluation Of Chocolate Brown Dye In Swiss Albino Mice. *J. Nutr. Disord. Ther.*, 6, 195.
- Kamel, M. M., & El-Lethey, H. S. (2011). The Potential Health Hazard Of Tartrazine And Levels Of Hyperactivity, Anxiety-Like Symptoms, Depression And Anti-Social Behaviour In Rats. *Journal Of American Science*, 7(6), 1211-1218.
- Elbanna, K., Sarhan, O. M., Khider, M., Elmogy, M., Abulreesh, H. H., & Shaaban, M. R. (2017). Microbiological, Histological, And Biochemical Evidence For The Adverse Effects Of Food Azo Dyes On Rats. *Journal Of Food And Drug Analysis*, 25(3), 667-680.
- Amin, K. A., & Al-Shehri, F. S. (2018). Toxicological And Safety Assessment Of Tartrazine As A Synthetic Food Additive On Health Biomarkers: A Review. *African Journal Of Biotechnology*, 17(6), 139-149.
- Al-Shinnawy, M. S., & Elkattan, N. A. (2013). Assessment Of The Changes In Some Diagnostic Parameters In Male Albino Rats Fed On An Azo Dye. *Int. J. Environ. Sci. Eng.*, 4, 85-92.
- Franco, R., Navarro, G., & Martínez-Pinilla, E. (2019). Antioxidants versus food antioxidant additives and food preservatives. *Antioxidants*, 8(11), 542.
- Gupta, R., & Yadav, R. K. (2021). Impact Of Chemical Food Preservatives On Human Health. *Palarch's Journal Of Archaeology Of Egypt/Egyptology*, 18(15), 811-818.
- Abdulmumeen, H. A., Risikat, A. N., & Sururah, A. R. (2012). Food: Its Preservatives, Additives And Applications. *International Journal Of Chemical And Biochemical Sciences*, 1(2012), 36-47.
- Nilsson M And Fagman H (2017), *Development Of The Thyroid Gland. Development* 144: 2123–2140.
- Ravichand, D. M., Sheshayamma, V., Lakshmi, K. V., & Chakradhar, T. (2005). Thyroid Hormones And Antithyroid Drugs. *Calicut Med J*, 3(4), E3.
- Spitz, D. R., & Oberley, L. W. (1989). An assay for superoxide dismutase activity in mammalian tissue homogenates. *Analytical biochemistry*, 179(1), 8-18.
- Bancroft, J. D., & Gamble, M. (Eds.). (2008). *Theory and practice of histological techniques*. Elsevier health sciences.
- Al-Rawi, K.M. and KhalafAllah, A.M. (2000). *Design and Analysis of Experiments*. National Library for Publication, University of Mosul, Ministry of Higher Education and Scientific Research, Iraq.
- Shakoor, S., Ismail, A., Sabran, M. R., Bekhit, A. E. D. A., & Roohinejad, S. (2022). Impact of tartrazine and curcumin on mineral status, and thyroid and reproductive hormones disruption in vivo. *International Food Research Journal*, 29(1).
- Elekima, I., Nwachuku, E. O., & Ben-Chioma, A. E. (2017). Effect of tartrazine orally administered on thyroid hormones and thyroid stimulating hormone of albino rats. *Ejpmr*, 4(7), 168-171.
- Abdel-Aziz, H. M., Alazouy, Z. M., Abdelfadee, K. F., & Abohashem, A. A. (2019). Effect of tartrazine on thyroid gland of male rat and ameliorating role of curcumin (histological and immunohistochemical study). *J Biochem Cell Biol*, 2(11), 2-8.
- Kurebayashi, h., fukuoka, m., nishimaki-mogami, t., minegishi, k. i., & tanaka, a. (1988). Effects of rose bengal on serum levels of thyroid hormones and thyroid peroxidase activity in male mice. *The Journal of Toxicological Sciences*, 13(2), 61-70.
- Helal, E. G., Barayan, A. W., Abdelaziz, M. A., & El-Shenawe, N. S. (2019). Adverse effects of mono sodium glutamate, sodium benzoate and chlorophyllins on some physiological parameters in male albino rats. *The Egyptian Journal of Hospital Medicine*, 74(8), 1857-1864.
- Sabr, A.N. & ebrahim,A.R.(2015). Effect of sodium benzoate in the level of thyroid stimulating hormone and the level of thyroxin hormone in mature albino male rats. *Journal of kerbala university*, 13(1) 295-299.
- Elekima, I., & Ollor, A. O. (2016). Effect of Carmoisine Orally Administered to Thyroid Hormones and Thyroid Stimulating Hormone in Albino Rats. *Int. J. Sci. Res.*, 5(10), 29-32.
- Hassan, A. J., & Salman, H. A. (2017). The Effect Study of Using Different Concentrations of Chocolate Brown Dye (Chocolate Brown HT E155) on Some Physiological Parameters and Histological Structure of Stomach and Intestine on Albino Rats. *Al-Qadisiyah Journal Of Pure Science*, 21(2).
- Khatun, A., Mondal, M., Pal, S., Parvin, S., & Paul, G.(2021) Impairment of uterine wall structure by Chocolate Brown HT in rats.
- Khan, I. S., Dar, K. B., Ganie, S. A., & Ali, M. N. (2022). Toxicological impact of sodium benzoate on inflammatory cytokines, oxidative stress and biochemical markers in male Wistar rats. *Drug and Chemical Toxicology*, 45(3), 1345-1354.
- Radwan, E. H., Elghazaly, M. M., Hussein, H. K., Aziz, K., & Barakat, A. I. (2020). The possible effects of sodium nitrite and sodium benzoate as food additives on the liver in male rats. *Journal of advances in biology*,(13), 14-30.
- Kehinde, O. S., Christianah, O. I., & Oyetunji, O. A. (2018). Ascorbic acid and sodium benzoate synergistically aggravates testicular dysfunction in adult Wistar rats. *International journal of physiology, pathophysiology and pharmacology*, 10(1), 39.
- Rus, V., Gherman, C., Miclăuș, V., Mihalca, A., & Nadăș, G. C. (2010). Comparative toxicity of food dyes on liver and kidney in guinea pigs: A histopathological study. *Annals of the Romanian Society for Cell Biology*, 15(1), 161-165.
- Aboul-Soud, M. A., Al-Othman, A. M., El-Desoky, G. E., Al-Othman, Z. A., Yusuf, K., Ahmad, J., & Al-Khedhairi, A. A. (2011). Hepatoprotective effects of vitamin E/selenium against malathion-induced injuries on the antioxidant status and apoptosis-related gene expression in rats. *The Journal of toxicological sciences*, 36(3), 285-296.
- Rani, P., Khatri, K., & Chauhan, R. (2013). Monosodium glutamate induced histomorphometric changes in thyroid gland of adult wistar rat. *Journal Of Medical & Allied Sciences*, 3(2), 67.
- Khalaf, H. A., & Arafat, E. A. (2015). Effect of different doses of monosodium glutamate on the thyroid follicular cells of adult male albino rats: a histological study. *International journal of clinical and experimental pathology*, 8(12), 15498.
- Abd Allah, A. L. (2021). Prophylactic Effect of Spirulina Versus Monosodium Glutamate Induced Thyroid Disorders in Experimental Rats. *Egyptian Journal of Nutrition and Health*, 16(1), 45-59.