



## HISTOLOGICAL ASSESSMENT FOR THE EFFECT OF BACON USE ON RAT TESTES

Quassy M. Younis

Department of Food Sciences College of Agriculture  
University of Tikrit - Tikrit -Iraq  
Quassymonther@gmail.com<sup>1</sup>

Farooq M. Kamel

Department of Food Sciences College of Agriculture  
University of Tikrit - Tikrit -Iraq

Mahfoodh Kh. Abdullah

<sup>3</sup>Department of Animal production - College of Agriculture  
University of Tikrit Tikrit Iraq

### ABSTRACT

Male white (laboratory) rats were used at a rate of (24) rats, ages ranged between (1.5-2) months, and average weights ranged between (150-200) grams. The animals were obtained from the animal house / College of Veterinary Medicine - Tikrit University, The carcinogen dissolved in olive oil was dosed to rats by oral administration at a rate of 1 ml and in two doses, each dose 0.5 ml of the carcinogen. The second dose is given three days after giving the first dose.

Another group of male rats was injected with 1 ml in the scrotum and in two doses. In each dose 0.5 ml of the carcinogen dissolved in acetone is injected according to the method mentioned, and the second dose is injected three days after the first dose.

Animals were fed laboratory-made sauces from beef containing 156 mg/kg of sodium nitrate to kg of minced meat for forty-five days with daily follow-up of the animals' behavior The results showed the appearance of external tissue changes after seven days of dosing with the carcinogenic substance, while tumors appeared after 72 hours of injecting the carcinogenic substance.

Leydig, with the increase of the bundles forming the sperm accompanied by an increase in the blood vessels in comparison with the rats that did not eat the bacon, The stages of the sperm development appeared in the seminiferous tubule and they are smooth in shape and the basement membrane thickens with the spread of the sperm bundles in a radial shape resembling a flame, While the results of the tissue sections of the rats that ate bacon showed a hyperplasia of the spermatogenetic cells with the destruction of all the spermatogenetic cells and the appearance of inflammatory fibrinous edema and hyperplasia of Lydic cells. Accompanied by the atrophy of the cells of your entire hand.

The results also showed that the interaction between the consumption of bacon and alcohol led to the beginning of atrophy in the seminiferous tubules in the stages of spermatogenesis of the cells and the enlargement and degeneration of primary and secondary spermatocytes with an increase in the decomposition of sperm and the accumulation of decomposing blood in the blood vessels accompanied by atrophy of Lydeik cells, as shown by histological sections. Another is that the seminal tube contains



a limited number of cells that form sperm, with some of them degeneration, atrophy and contraction of Lydic cells, and a thickening of the basement membrane, which indicates that there is an effect of bacon containing sodium nitrate, which affects the myoglobin pigments in meat and turns it into methemoglobin pigments that encourage oxidative processes and the formation of carcinogenic free radicals, The results also showed the clear effect of the interaction between eating bacon and alcohol, where alcohol acts as a catalyst for activating the formation of free radicals and a decrease in immune factors, especially white blood cells.

**Keywords:** Carcinogenic substance, 127 Dimethylbenzene anthracene, sodium nitrate, bacon, alcohol, male rats, testicular cancer.

## Introduction

The global meat industry has experienced many critical events related to food safety, disease outbreaks and environmental concerns such as global warming. Meat and meat products are becoming an increasingly important and integrated part of global diets among all consumer groups (Halweil & Nierenberg, 2008).

These structural changes are clearly reflected in the production, consumption and sale of meat products, as in the past decades, Europe was the main producer of meat (39%), followed by America (31%), Asia (21%), and Africa (5%), but after 2011 Asia became A major meat producer with 41% of world production, India ranks first in the production of buffalo meat, the United States of America leads the way in beef and poultry, and China produces mutton and goats (FAO Report, 2014).

It has been observed in various studies that individuals who consume large amounts of red or processed meat commonly combined with more energy-rich food products including sugar and alcoholic beverages, and reduce intake of vegetables and vitamins are at higher risk of developing cancer (Oostindjer et al., 2014).

Boskovic et al., (2015) have stated that red meat is not carcinogenic in itself, but cancer is affected by cooking practices, diet and patterns in which meat is consumed as well as avoiding browning on the outside of the meat or charring of the meat during preparation, or during baking and boiling. and cooking. Nitrates when added to meat products to extend their shelf life have negative effects on human health and there is a need to use more natural additives, such as plant extracts that are used as antimicrobial and antioxidant, have positive effects on the safety and quality of meat as well as extending its shelf life. The addition of garlic or curcumin has protective effects against cancer.

Eating red meat at a rate of more than 100 g / day leads to an increase in the risk of cancer by 12%, while for people who eat meat in their daily meals at a rate of 90 g / day with eating grains and vegetables, the increase in the risk of infection is 7% (Vieira et al., 2017).

Domingo (2017) indicated about the possibility of red and processed meat being considered carcinogenic, as the International Institute for Research on Cancer (IARC) classified the possibility of red meat being registered as carcinogenic, while the institute confirmed that processed meat is one of



the truly carcinogenic foods. The center has urged the completion of studies on the effect of red and white meat on the development of cancerous cells.

Kopp et al., (2018) reported that eating red meat can reduce bone marrow cells and cells that help increase the body's immunity, which helps the development and growth of cancer cells in the body.

## Materials and Methods

### Raw meat samples

Beef samples were obtained from the local Qasaba market stores in the city of Tikrit and were minced three times for the purpose of preparing them for the manufacturing process, then adding the ingredients to them and giving them to the rats.

### Manufacture of bacon and packed in membranes

0.5% salt with 156 mg/kg of sodium nitrate was added to a kg of minced meat at a rate of three times in the mincing machine and mixed well and then packed in the trays (intestines) and the sandwiches were dipped in boiling water for 3-5 minutes and then the sandwiches were cooled directly for the purpose of reducing heat.

### Storage in the freezer for the purpose of use

The Sun stored the miniatures filled with bacon in the freezer at a temperature of -18 ° C for 45 days from 11-1-2020 until 15-12-2021 for the purpose of preservation and given to rats at different intervals.

### Reparing the experimental animals

Adaptation was made to the rats on the new conditions in which they live for five days before the beginning of the experiment for acclimatization to the new conditions. The rats were fed a prepared standard meal, which is an integrated mixture as shown in the preparation of the standard diet, and then the other groups were fed on the experimental ration containing on cured meats.

### Preparing experimental animals

Male white (laboratory) rats were used at an average of (24) rats, ages ranged between 2-1.5) months, and average weights ranged between (200-150) grams. The animals were obtained from the animal house / College of Veterinary Medicine - Tikrit University. The laboratory animals were placed in cages. Plastic, taking into account that the space is to the extent that guarantees freedom of movement, and sawdust was used to brush the floor of the cage, in order to secure the greatest amount of moisture absorption, as the sawdust is changed periodically to maintain the cleanliness of the cage, as it was emphasized to provide drinking water stored inside sterilizable plastic bottles. , while providing food by the open method. And setting the temperature of the rats' room at 22° C, humidity rates 50%, and regulating the lighting cycle so that it is 12 hours of light and 12 hours of darkness (Bashandi, 2003).

### Preparation of 5% ethanol alcohol solution, which is offered for drinking, for the purpose of knowing the interaction between bacon and alcohol



A 5% ethanol alcohol solution was prepared by combining 5 ml of ethanol with 95 ml of distilled water and then fed to female rats.

## Preparation of the carcinogen

100 ml of the carcinogenic substance, <sup>127</sup> Dimethylbenzene anthracene, was dissolved in 20 ml of olive oil

## Dosing rats with the carcinogen

The carcinogen dissolved in olive oil was dosed according to the method indicated to the rats via oral administration at the rate of 1 ml and in two doses, each dose 0.5 ml of the carcinogen. The second dose was given three days after giving the first dose (Saravanan et al., 2016).

## Injection of the carcinogen

Male rats were injected with 1 ml into the scrotum and in two doses. In each dose, 0.5 ml of the carcinogen dissolved in acetone is injected according to the method mentioned, and the second dose is injected three days after the first dose (Takeshi et al., 2012).

## Histological examinations

Histological studies of the testicles were carried out immediately after slaughtering the animals. The samples were washed with diluted saline solution to remove blood, fixed with 10% formalin solution, then washed with running water and transferred into containers containing 70% alcohol for the purpose of preparing wax samples for them.

## Tissue diagnosis

Tissue diagnosis was carried out using an optical microscope (Olympus Japan) and the changes in the studied tissues were identified in the tissue laboratory of the College of Veterinary Medicine / Tikrit University.

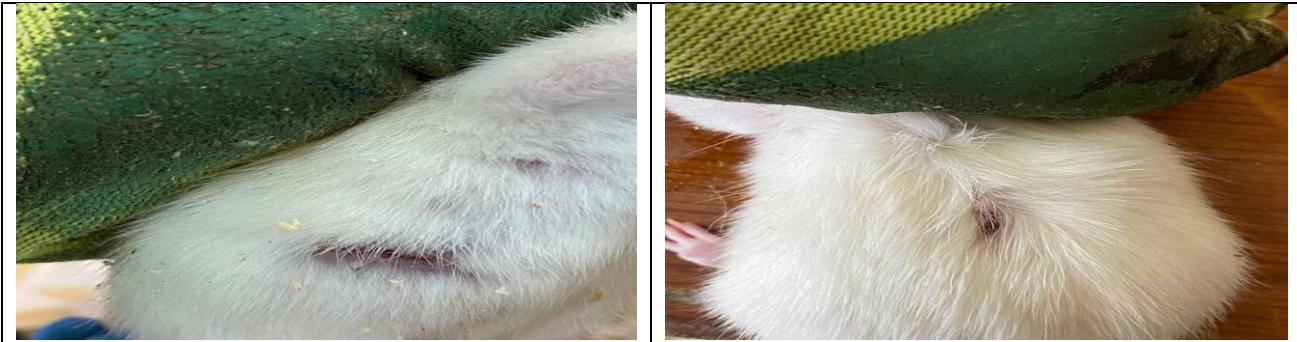
## Visual and behavioral monitoring

The rats were monitored during the 45-day trial period to follow up the external macroscopic changes of the rats, general shape, weight - ears - tail and skin - legs - hair - eyes - activity and movement.

## Results and Discussion

First: - Follow up on the appearance of skin cancer

Through daily monitoring of the experimental animals, signs of skin necrosis began to appear in the animals that were fed and dosed with the carcinogenic period a week after the feeding and dosing process, while the animals that were injected with the carcinogen showed signs of infection and necrosis in the injection areas after seventy-two hours and how much is shown in the pictures (1) and image (2) respectively

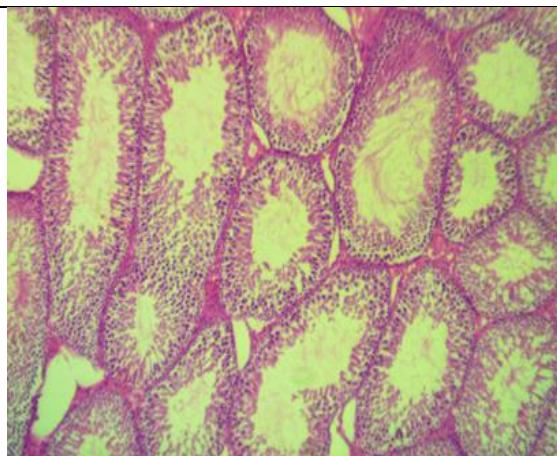


**Picture (1)** The appearance of infection in the skin of male rats that were injected with the carcinogen seven days after the start of feeding



**Picture (2)** The appearance of the infection in the skin of male rats that were injected with the carcinogen after seventy-two hours.

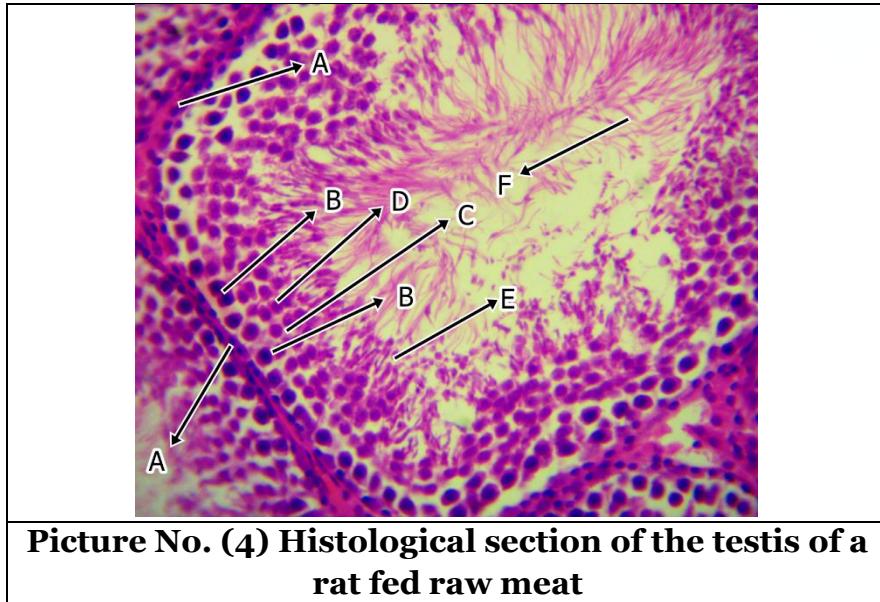
## Second: Histological examinations:



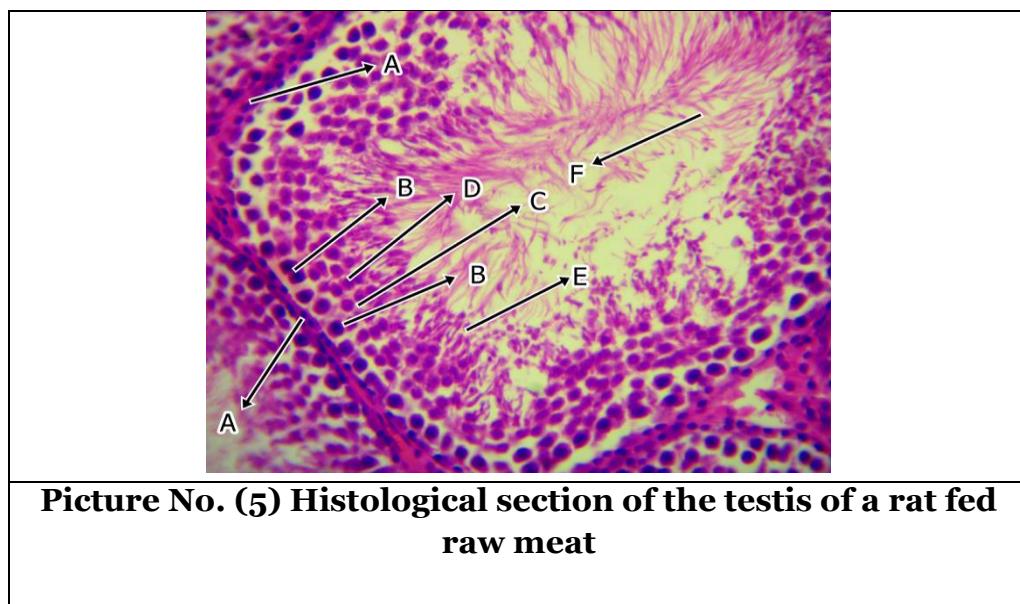
**Picture No. (3)** Histological section of the testis of a rat fed with bacon



Picture No. (3) shows the histological section in rats fed bacon, in which the testis parenchyma is noted with many coiled seminiferous tubules (A) in the stages of sperm development while they are developing normally (H&E, 40X).

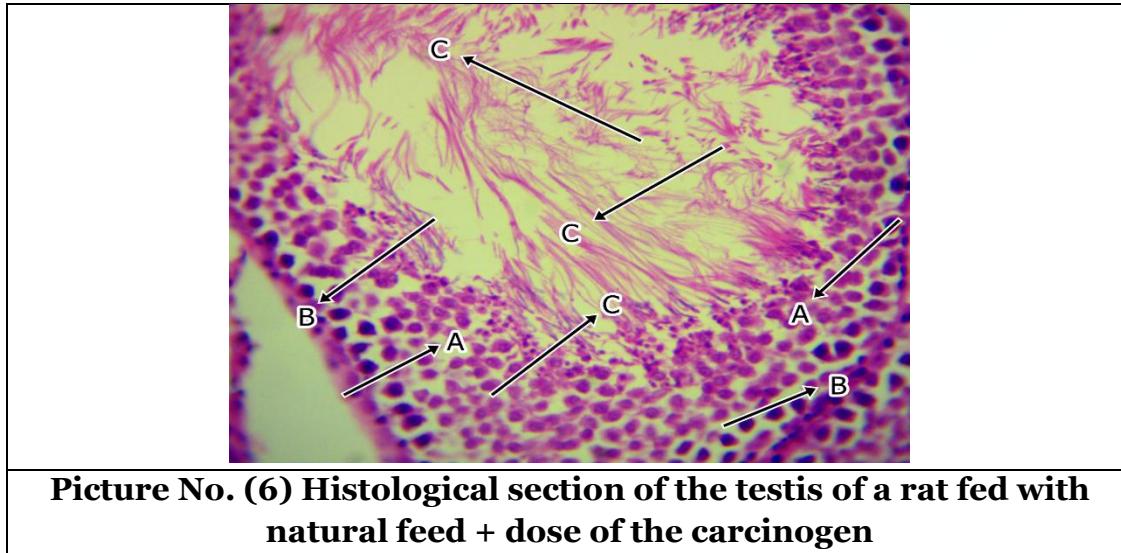


Picture No. (4) shows the histological section in rats fed uncured meat, in which the seminiferous tubule is observed with thickening in the basement membrane (A), spermatogonia (B), primary spermatocytes (C), secondary spermatocytes (D), The spermatids (E), the sperm (F), and they are in good shape and there are no abnormalities in them (H&E, 40X).



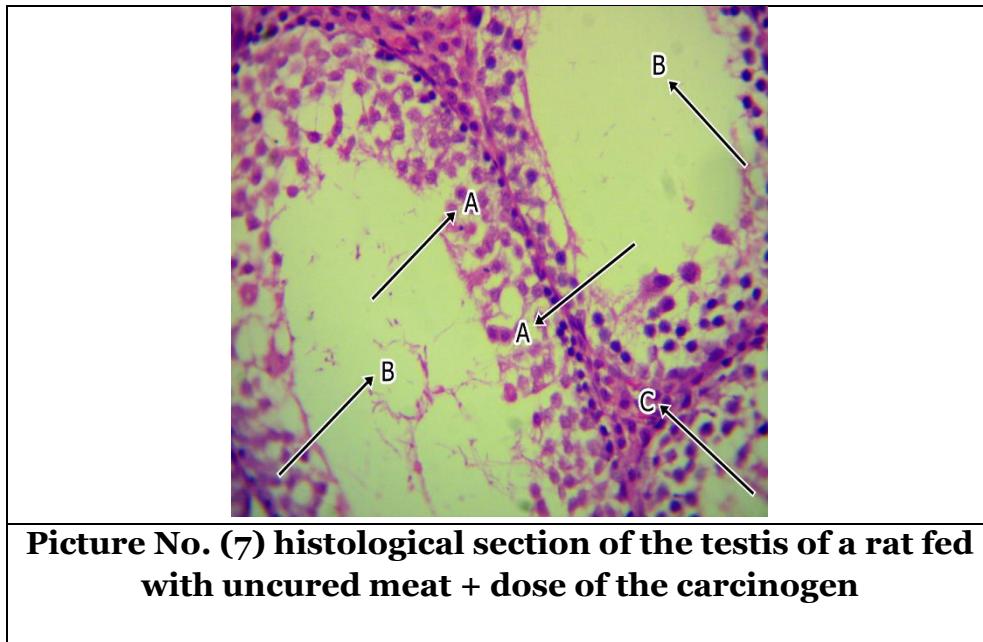


As picture No. (5) shows the histological section in rats fed raw meat, in which the coiled seminiferous tubules are observed in the different stages of sperm development and a congested blood vessel (A), the basement membrane thickens (B) (H&E,40X).



**Picture No. (6) Histological section of the testis of a rat fed with natural feed + dose of the carcinogen**

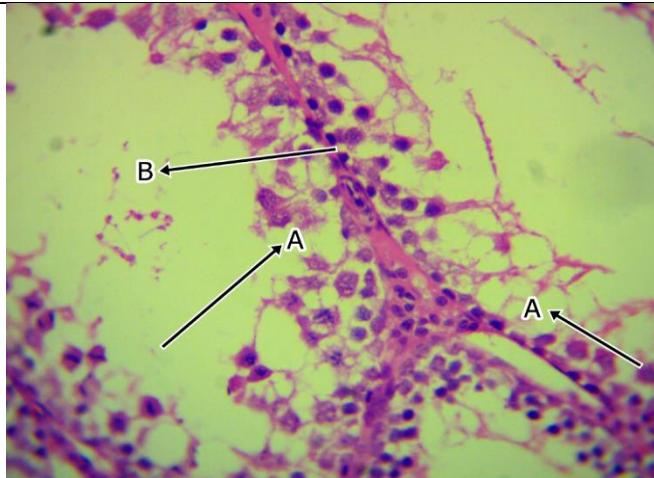
Picture No. (6) shows the histological section in rats that were fed natural forage with the carcinogen ingestion, in which the stages of sperm development in the seminal tubule are noted and they are smooth in shape (A), the basement membrane thickens (B), the sperm bundles in the form of flame (C) H&E, 40X).



**Picture No. (7) histological section of the testis of a rat fed with uncured meat + dose of the carcinogen**

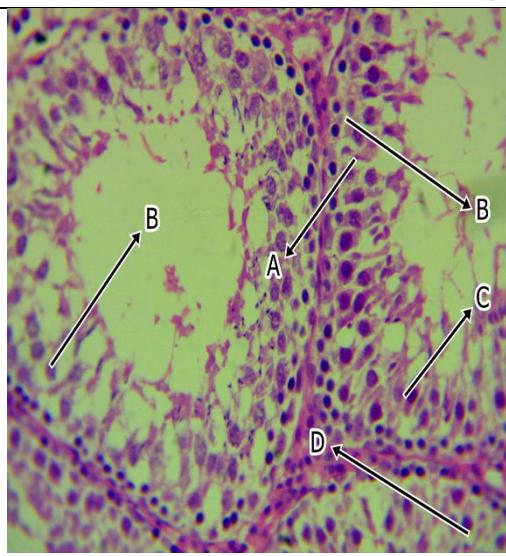


Picture No. (7) shows the histological section in rats that were fed uncured meat with the carcinogen ingestion, in which the coiled seminiferous tub was noted, and the degeneration of many stages of sperm development (A), azoospermia (B), atrophy of Lydic cells (C) (H&E,40X).



**Picture No. (8) Histological section of the testis of a rat fed with uncured meat + dose of the carcinogen)**

Picture No. (8) shows the histological section in rats fed uncured meat with the carcinogen ingestion, in which the degeneration of most of the sperm-forming cells was observed (A), the basement membrane thickened (B), the interstitial tissue thickened (C) (H&E,40X).

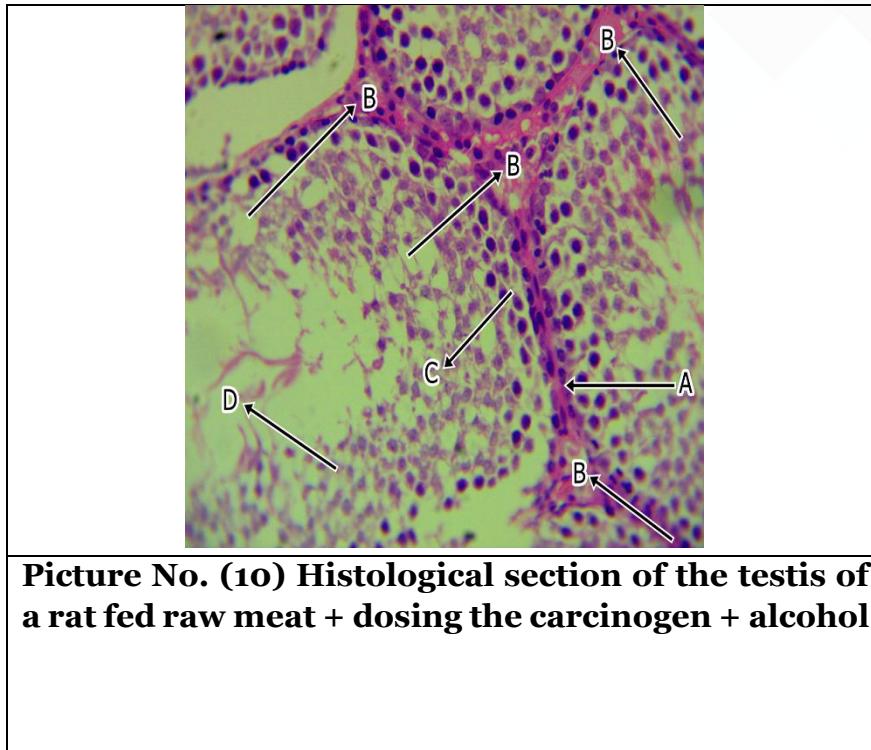


**Picture No. (9) Histological section of the testis of a rat fed raw meat + dosing the carcinogen + alcohol**

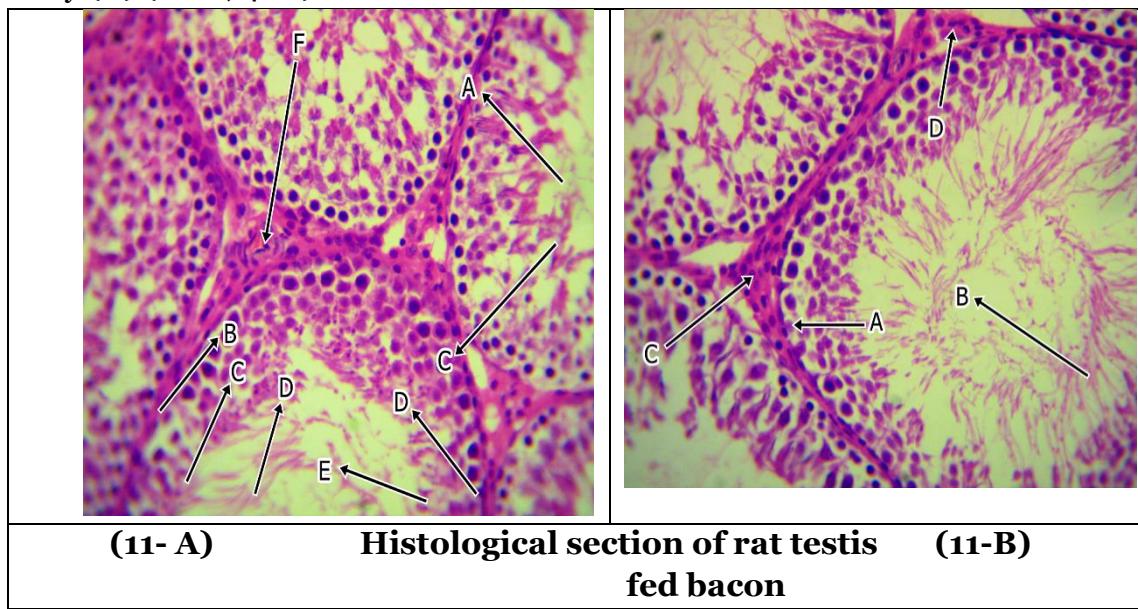
Picture No. (9) shows the histological section in rats that were fed uncured meat with the carcinogen dosed and given alcohol. It was noted that the necrosis and degeneration of sperm cells in the various



stages of their development (A), disappearance of sperm (B), hyperplasia of primary sperm cells (C), atrophy Leydig cells and their nuclei agglutination (D) (H&E,40X).



Picture No. (10) shows the histological section in rats fed raw meat with the carcinogen and given alcohol. It was noted that the basement membrane lining the seminiferous tubules thickened (A), hemolysis in the interstitial blood vessels (B), degeneration of most of the sperm-forming cells (C), Spermogenicity (D) (H&E, 40X).



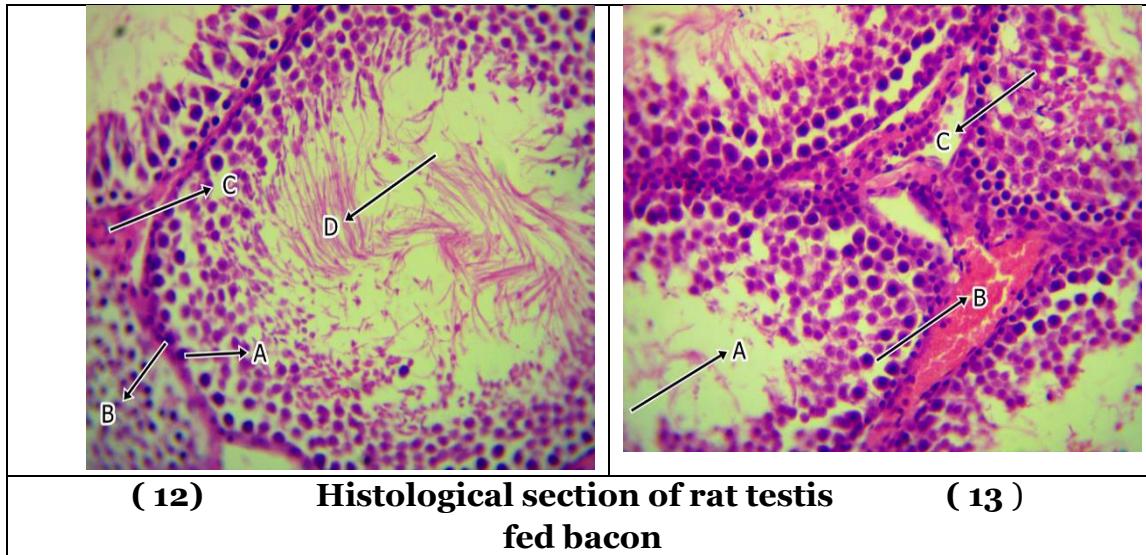


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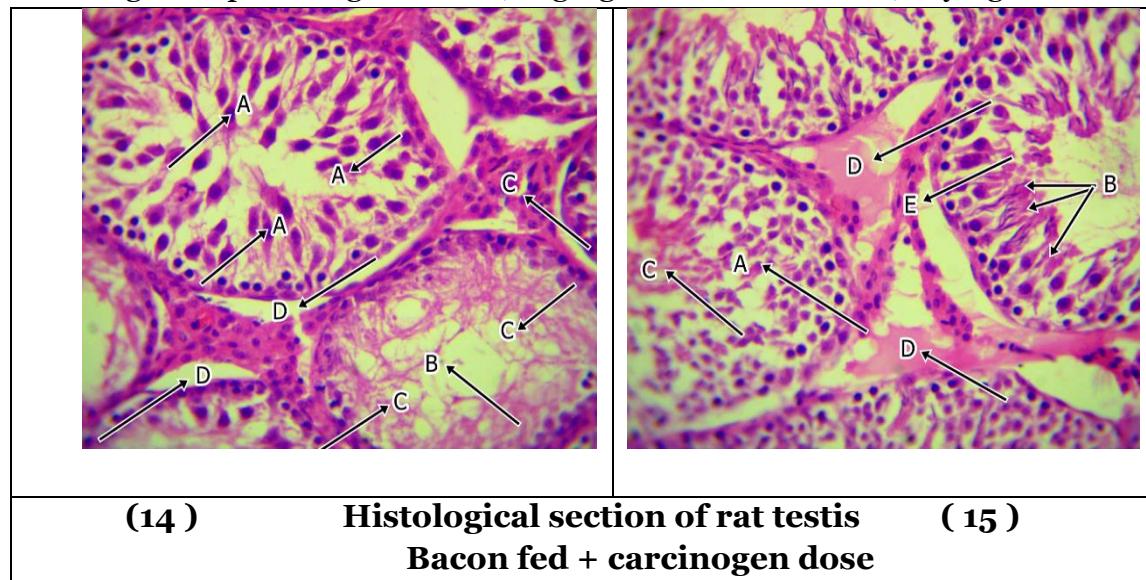
Picture No. (11A) shows the histological section in rats fed bacon, in which the seminiferous tubules are observed, in which the spermatids (A) based on the basement membrane, primary spermatocytes (B), secondary spermatocytes (C), spermatids (D) Sperm (E), Leydig cells (F) (H&E, 40X).

Picture No. (11b) shows the histological section in rats fed bacon, in which the seminiferous tubules of the testicles are observed in the stages of sperm development (A), sperm (B), Leydig cells (C), blood vessels (D) (H&E,40X).



Picture No. (12) shows the histological section in rats fed bacon. The stages of sperm development are noted in the seminiferous tubule (A), the basement membrane thickens (B), Lydic cells (C), sperm bundles (D) H&E,40X. ).

The picture (13) shows the histological section in rats fed bacon, in which the seminiferous tubules are observed in the stages of spermatogenesis (A), engorged blood vessels (B), Leydig cells (C) (H&E,40X).





Picture No. (14) shows a histological section in rats fed bacon with the carcinogen ingestion, in which spermatogenesis (A) hyperplasia is observed, all spermogenic cells are destroyed (B), inflammatory edema of fibrinous (C), hyperspasm of cells Your hand (D) H&E, 40X .

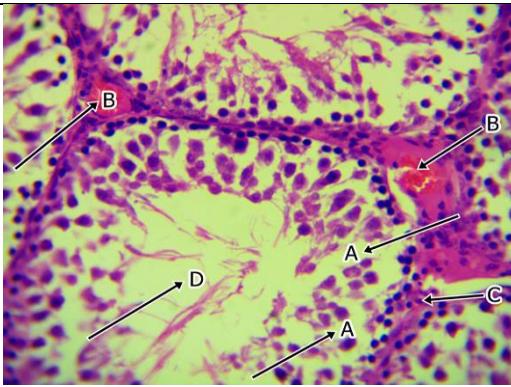
Picture No. (15) shows the histological section in rats fed bacon with the carcinogenic substance ingestion, in which the seminiferous tubules are noted in the stages of sperm development of cells (A), hyperplasia and degeneration of primary and secondary spermatocytes (B), sperm lysis (C), blood Vascular degeneration (D), Lydic cell atrophy (E) H&E, 40X).



**Histological section No. (16) in the testis of a rat fed bacon + carcinogen dose + alcohol**

Picture No. (16) shows a histological section in rats fed bacon with the carcinogen and given alcohol. It is noted that the spermatic tube contains a limited number of sperm-forming cells with some degeneration (A), atrophy and shrinkage of Leydig cells (B), thickening of the basement membrane (C), Degeneration of sperm (D) (H&E, 40X).

## Histological sections of the testicle when injected with the carcinogen



**Histological section No. (17) in the testis of a rat fed on natural feed + injection of the carcinogen**

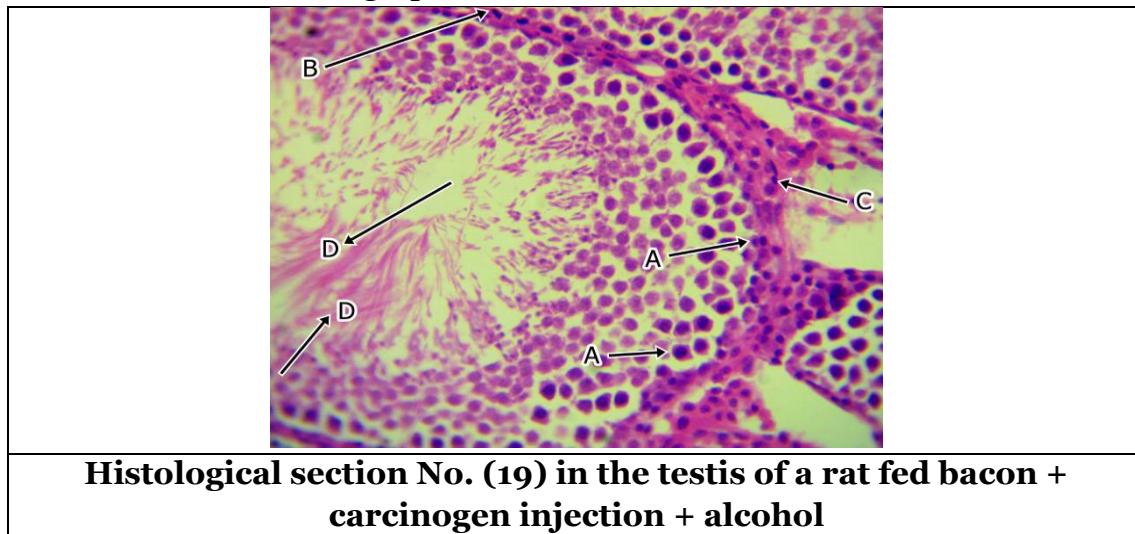


**Histological section No. (18) in the testis of a rat fed with natural feed + injection of the carcinogen**



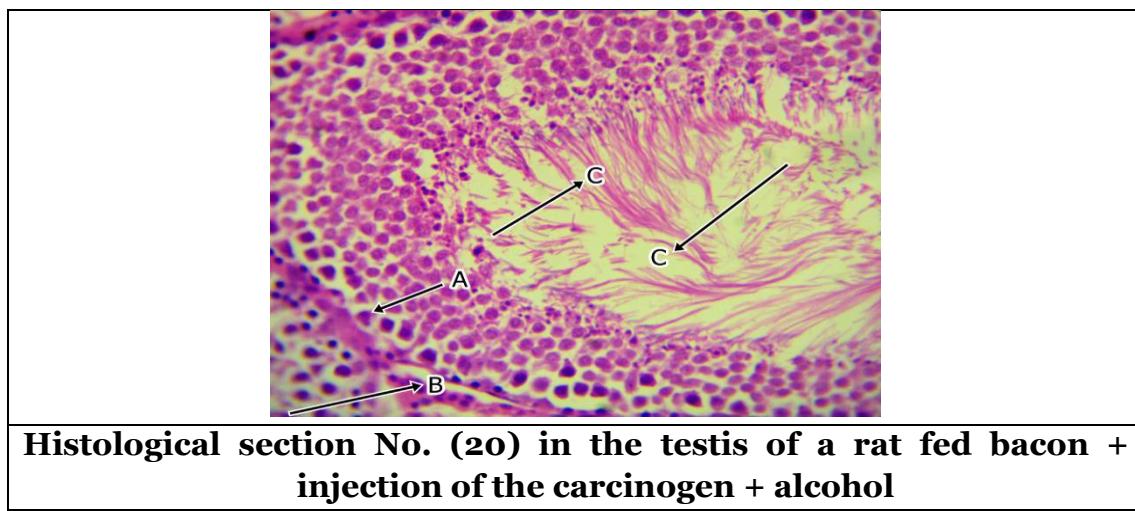
Picture No. (17) shows a histological section in rats fed a normal forage with the injection of the carcinogen, in which hyperplasia of spermogenic cells (A) is observed in the lumen of the seminiferous tubules, hyperemia in blood vessels (B), atrophy of Leydig cells (C), Azoospermia (D) (H&E,40X).

Picture No. (18) shows the histological section of the rats fed the natural feed with the injection of the carcinogen, in which hypertrophy of the stages of sperm development is observed (A), hyperemia in the interstitial blood vessels (B), and oligospermia (C) (H&E,40X ).



**Histological section No. (19) in the testis of a rat fed bacon + carcinogen injection + alcohol**

Picture No. (19) shows the histological section in rats that were fed bacon with the carcinogen injected and given alcohol. The seminal tubules are observed in the stages of sperm development and hyperplasia (A), basement membrane thickening (B), Leydig cell hyperplasia. (C), Flame-shaped Sperm Packs (D) H&E, 40X).



**Histological section No. (20) in the testis of a rat fed bacon + injection of the carcinogen + alcohol**

Picture No. (20) shows the histological section of rats fed bacon with the carcinogen injected and given alcohol. In it, the spermatic tubule and hyperplasia of all sperm-producing cells (A), Leydig cells (B), and flame-shaped sperm bundles (C) H&E are noted, 40X).



These results are consistent with the findings of Pavlova et al., (2013). He indicated that the interaction of nitrite with hemoglobin and the production of metamyoglobin reduces oxygen transport, which leads to damage and a decrease in the number of sperms in samples that received doses of nitrite oxide NaNO<sub>2</sub>.

As mentioned by Alalwani, (2014). Giving male rats monosodium glutamate leads to damage to the seminiferous tubules.

As pointed out by Ismaeil et al. (2017). When they studied the effect of injecting sodium nitrite into the testes of rats on their physiological activity, it was also observed that there was a degeneration of sperm-producing cells with congenital malformations of the testes of rats in the seminal ducts and tubes.

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