

The Effect of High Spice Consumption on the Phenotypic and Histological Structure of the Mouse Liver

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Abstract: The research aimed to study the phenotypic and histological changes of the mouse liver resulting from excessive consumption of these spices. Introduction: The current study was carried out to prove the effect of excessive consumption of nutmeg, ginger, cloves, and spices mixed on the livers of experimental mice by using a concentration of 1 g of each type on laboratory mice (Swiss albino) for 50 days, we use about 50 mice. That anesthetization, separating the liver, fixing by formalin, using the routine method then colored with H&E. Results: The results showed abnormal symptoms, the most important of which are weight loss, lethargy, hair loss, dehydration, haemorrhage in the mouth, and high temperatures with the appearance of gangrene in the ear and tail, which led to the death of some mice. With severe congestion of the liver, erosion of the hepatic tissue resulted in the loss of the normal structural composition of the liver tissue, such as dilatation of blood vessels and hepatic sinusoids, and the emergence of the initial and late stages of programmed death (apoptosis), in addition to the deposition of glycogen in the hepatocytes. Conclusion: We find that taking spices in large quantities leads to liver damage, but it is not as dangerous as the amount of spice used with the weight of the mice.

Keywords: Effect, Spice, Phenotype, Tissue, Mice, Liver.

INTRODUCTION

Since ancient times, man used plant parts in their fresh form or after drying them in some dishes of food. They were called spices, such as nutmeg, ginger, cloves, black pepper, etc...

Considering the active constituents of these spices, this study was designed to correlate the individual with the combined effects of excessive consumption of nutmeg, ginger, and cloves on the liver tissue of laboratory mice. Therefore, the research aimed to study the phenotypic and histological changes of the mouse liver resulting from excessive consumption of these spices (Fig 1), and to contribute to setting the permissible limit for the consumption of these spices and avoiding excessive consumption of them (Parthasarathy, V. A. *et al.*, 2008).

MATERIALS AND METHODS

Animals: - This study was carried out in the laboratories of Diyala University and the laboratories of the Hospital; it included 50 mice weighing (30-28g). Divided into five groups (ginger, cloves, nutmeg, mixture, and control (Fig. 2)

Animal dosing: The animals were administered orally (1g) to all the groups, mixed with (10ml) of distilled water; the period lasted for 50 days. The groups were divided into two groups, which included 10 mice for each group, as follows. The liver was removed using the Pellet Wild method,

then put into formalin after washing it with saline solution, for a period ranging between 12 and 16 h. Using routine preparation and sectioning by manual rotating microtome of the type

(HM325) and was colored with Delafield hematoxylin and Eosin (Al-Deresawi, M. S. 2018)

RESULT & DISCUSSION

The liver is considered one of the most important organs that are affected by the removal of toxic effects and various metabolites of various drugs and spices, including the used spices (Alsaman, M. 2012)

For the use of spices in many international, Asian, and Arab kitchens alike, so this study aimed to find out the extent of the damage caused by continuing to eat these products and their effects resulting from eating them on human health by conducting laboratory research on laboratory animals. Therefore, several side effects were found, and the most important what this study found is summarised as follows:

Clinical signs and Macroscopic Structure

The results of the current study showed that when mice (Swiss albino) were given nutmeg, abnormal clinical symptoms appeared; It was represented by loss of appetite and lack of nutrition compared to before the dose, which led to weight loss, and the owner of these symptoms is fatigue for short

periods, and then it regains its natural vitality in the first dose periods, and the period of lethargy increases as we progress in the dosing period, which causes the death of some of them, and this is similar to the results of the study (Al-Sadi, H. I. *et al.*, 2017) When the mice were dosed with Parkazole, the behavioral changes caused by doses of 250, 350, and 450 mg/kg of body weight showed signs of lethargy, withdrawal, poor eating and drinking water, and then partial cessation of eating them, as well as the occurrence of vaginal bleeding and death of some of them.

It was also noted a increase in temperature, especially in the head area (Dkhil, M. A., & Al-Quraishy, S. 2010; Yoshioka, M. *et al.*, 2001), and these symptoms are similar to the effect of red pepper on rabbits, according to a study (Dkhil, M. A., & Al-Quraishy, S. 2010), in addition to the beginning of the appearance of rot (Gangrene) in the ear and tail in the last stages of the dose, which caused the death of some of them As shown in (Fig. 2 A), and to the extent of the researcher's knowledge, he did not find any research matching the phenomenon of organ damage due to eating spices.

When the mice were dosed with ginger spice (Fig. 2 B), it differed from the previous one, as their ability to eat food was normal before and after the dose, and despite that, it was accompanied by a decrease in weight, and this indicates an increase in the metabolic mice in the body, in addition to an increase in activity after the dose, but during the dose, minor signs of bleeding appeared from the mouth 18 However, in a study (Nwaopara, A. O. *et al.*, 2007), different results were shown in terms of weight gain for rabbits when they were dosed with ginger.

A rise in temperatures was noted, especially in the head area, in addition to the beginning of the appearance of rot in the tips of the ear and the tail, which began in the first dose periods, and led to the fall of most of the ear as shown in (Fig. 2 B), And this was found to be identical to the study of (Kod'ousek, R. *et al.*, 2007) used carrageenan to see its effect on the heart muscle cells of the rats used, it was noted that there was necrosis in the tail of the rats due to thrombosis of blood vessels, and this is similar to the effect of ginger spice and it is believed that it is due to the occurrence of thrombosis if it is taken in high quantities. In any part of the body of animals used in previous research, one of the reasons for the occurrence of necrosis is the large production of free and

uncontrolled oxygen radicals, which leads to protein denaturation, lipid peroxidation, and the breakdown of DNA chains (Kod'ousek, R. *et al.*, 2007) When the mice were given clove seasoning, clear signs of loss of appetite appeared compared to before the dose, which was accompanied by a decrease in weight and the appearance of signs of dehydration on them. It was dosed with the spices used in the experiment, and a temperature rise was not observed. In addition, she did not show symptoms of putrefaction, but she showed symptoms of hair loss and regrowth, with a note of the appearance of the skin in most parts of the body, as shown in (Fig 2 C), which led to the death of some of them (Nwaopara, A. O. *et al.*, 2007)

Finally, the mice were dosed with a mixture of the above-mentioned spices, as the symptoms were manifested by loss of appetite and abnormal feeding compared to the period before the dose, which led to weight loss. black, and it was noticed that the color of the stool changed in the last periods of the dosage period, and it was subjected to severe dehydration, which was accompanied by bloody bleeding from the mouth during the dosage. It also had a slight temperature rise, but hair loss was observed in large quantities compared to cloves. Moreover, it was accompanied by the appearance of cracks in the neck area with bloody bleeding as shown in (Fig. 2 D), which resulted in the death of some of them.

Appearance Symptoms of the Liver

The results of the study for the liver of the samples of the experimental groups demonstrated the occurrence of macroscopic lesions compared to the control group. In the experimental group ingested with nutmeg, it was noted that severe congestion of the liver appeared in dark red and was accompanied by bloody bleeding, as shown in (Fig 3 A)

The results of the study showed that the ginger samples caused macroscopic damage to the liver of the third experimental group compared to the normal liver, as congestion was observed in some lobes of the liver and their appearance in a dark red color due to the increase in blood pooling, in addition to the pallor of their edges with the appearance of white scars on some lobes, which led to the erosion of the liver tissue. As shown in ((Fig. 3 B).

As for the clove sample, it was observed that the liver appeared in dark red with the presence of white scars, as well as the transparency and pallor

of the periphery of the liver, with a concentration of fatty matter on it compared to others. In addition, the liver lobes showed uneven areas in color as a result of bloody bleeding, and erosion of the liver tissue also appeared, as shown in (Fig. 3 C) and this is similar to the results of the study (Al-Sadi, H. I. *et al.*, 2017) when dosing rats with Barazole, as the changes brought about by doses of 250, 350 and 450 mg/kg of body weight were embodied, according to what the examinations showed. Pregnant mothers had pale liver, hemorrhaging, and the appearance of white scars on some lobes, severe congestion, and The hepatic lobes are hemorrhagic, with the accumulation of fatty substances between the lobes.

The results also showed when the experimental group was dosed with the spice mixture, the liver appeared pale, and this may be due to the occurrence of bloody bleeding. It was also observed that damage appeared on the edge of the lobes, which may be due to the effect of excessive consumption of spices combined and their absorption by the digestive system, or it may be due to the deposition of some toxic substances present in Spices if used excessively, as shown in (Fig. 3 D), as these results agree with what was stated in (Owaied, Y. H. *et al.*, 2016).

Examination of Microscopic Histopathological Changes

The histological sections showed, in general, the presence of distortion (loss of the normal structural composition of the liver tissue), represented by the radial arrangement of the hepatic cords in the central lobular region and the surrounding areas in the dosed aggregates,

The livers of mice dosed with spice (nutmeg, ginger, cloves, and the mixture) were examined histologically. It was noted that there was dilatation of the blood vessels (sinusoidal dilatation) between the hepatic lobules. This is due to the acute congestion of the veins. It was also noted that the hepatic sinusoids expanded, which led to filling the central vein and the portal area with blood components. And its infiltration into hepatic sinusoids, i.e. the occurrence of hemorrhage, which is caused by the continuous effects of hypoxia, in addition to the formation of phagocytic cells near the blood vessels as shown in (Fig. 4 A, B; Fig. 5), and this is similar to the effect of tamoxifen on liver cells. For female white mice mentioned above (Alsalman, M. 2012). These symptoms were also observed when rats were treated with uranyl nitrate (11), in addition to

that, it was consistent with (7; 10), and this is similar to the results of the study (Al-Sadi, H. I. *et al.*, 2017) when dosing rats with parasol, as changes caused by doses of 250, 350, and 450 mg/kg of body weight were recorded. Through microscopic examinations, congestion of the blood vessels between the lobules, dilatation of the sinusoids, vacuolar degeneration, coagulative necrosis of the hepatocytes, congestion of the central veins, and focal infiltration of inflammatory cells around the blood vessels.

It is also similar to a study (Luaibi, N. M., & Shafiq, A. 2016), which included dosing rats with varying concentrations (50-100-200) g / kg of the seeds of the sweet bean {Ehmar} *Foeniculum vulgare*, as it showed similar results for the dose with a weight of (100-200) g / kg in terms of expansion of hepatic sinusoids. Hepatic cells degenerate and inflammatory cells permeate with congestion of blood vessels and the appearance of necrosis in the hepatic tissue. A study (Al-Rubaye, R. H. 2017) also showed the effect of aqueous extract of coriander leaves on the histological structure of the liver of male albino mice, clear degeneration and deformation in the hepatocytes, the appearance of blood congestion, infiltration of inflammatory cells, and vacuolation in the cytoplasm of hepatocytes at a dose of 250 mg. (These results indicate that the oral dose of the extract is effective. Watery coriander leaf has hepatotoxic effects at a dose of 250 mg per kg of body weight.)

It was also noted in (ginger, clove, and mixture spices) (Fig. 6, 7,8,9), that there was an enlargement of hepatocytes (Al-Hilfy, J. *et al.*, 2010; Abbood, A. S. *et al.*, 2013) and thromboembolic necrosis of hepatocytes (Al-Sadi, H. I. *et al.*, 2017). Some histological sections showed the presence of slightly shrunken liver cells, in addition to the appearance of irregularly shaped liver cells with acidophilic cytoplasm (the intensity of the deposits of glycogen in the hepatocyte ⁽¹⁵⁾ and segmented small nuclei representing the initial stages of programmed death, apoptosis, accompanied by the presence of small spherical cells with a small single nucleus and acidophilic cytoplasm as well, and these represent the stages Delayed programmed death (Hosseini, M. *et al.*, 2011; Nwaopara, A. O. *et al.*, 2007). In other sections, bile droplet infiltration and retention inside hepatocytes appeared (Al-Hilfy, J. *et al.*, 2010; Nwaopara, A. O. *et al.*, 2007; Abbood, A. S. *et al.*, 2013; Ugwuja, E. I. *et al.*, 2016) while the current study differed with (El-

Kott, A. F. *et al.*, 2010) where it was found that the effect of aqueous ginger extract of 400 mg/kg antioxidant in the liver of diabetic rats After being injected with Alloxan, known for its ability to damage pancreatic and hepatic tissues.

From the point of view of the researcher, it was found from this scientific evidence that the spices studied in our research possess some

pharmaceutical and chemical properties similar to the classes of drugs capable of causing liver damage and thus explain their ability to influence the observed histologic structures. Therefore, based on the results of this study, it was found that excessive consumption of nutmeg, ginger, and cloves is capable of causing liver damage and should therefore be avoided.



Fig. (1): A photograph showing the types of spices used, which represent (A) nutmeg, (B) ginger, and (C) cloves.

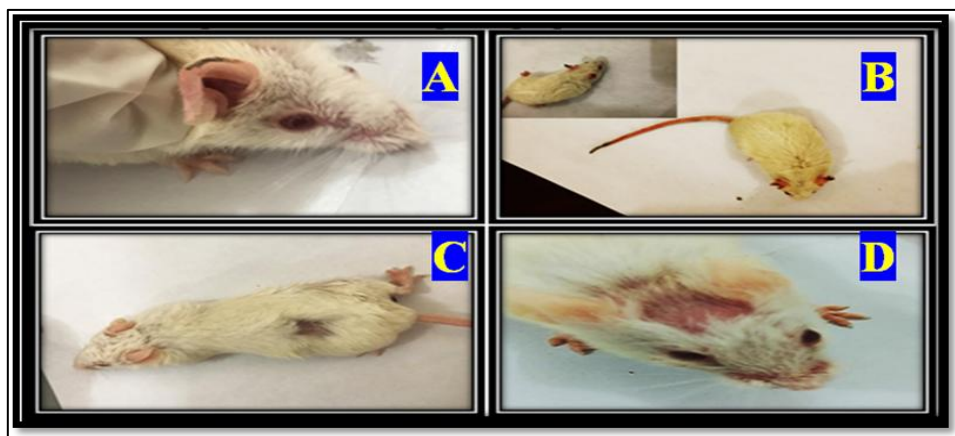


Fig. (2): Photographs of mice fed with spices (A) of a mouse fed with nutmeg (B) of a mouse fed with ginger (C) of a mouse fed with cloves (D) of a mouse fed with mixture.

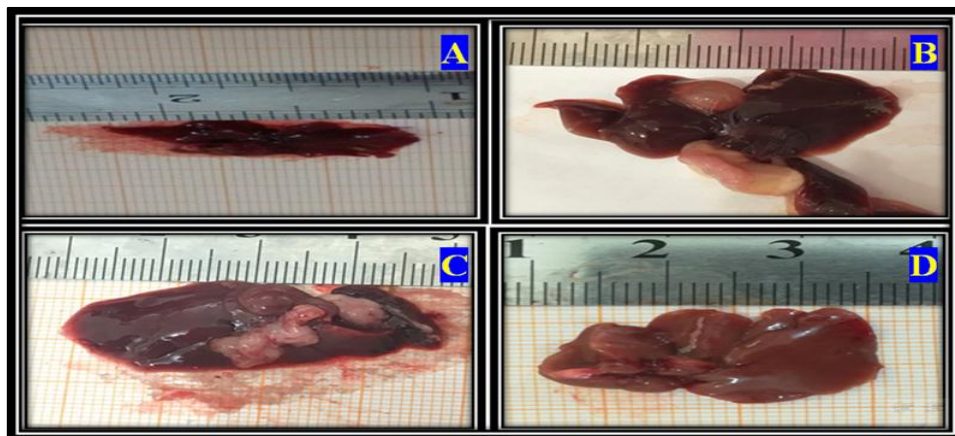


Fig. (3): Photographs showing the livers of mice dosed (A) with nutmeg (B) with ginger (C) with clove (D) with mixed.

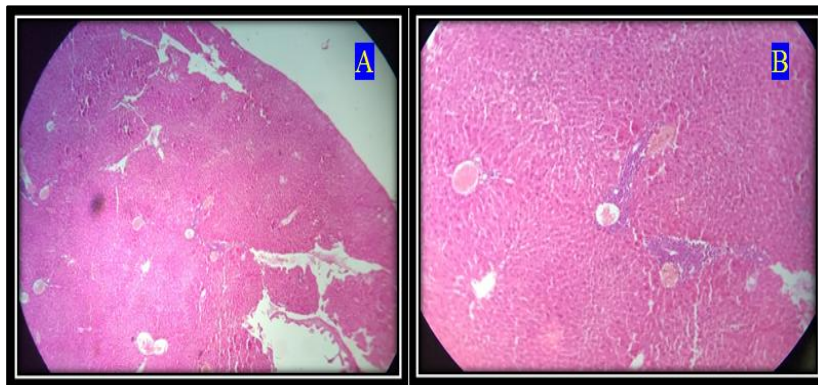


Fig. (4): shows a section of the liver tissue of a mouse dosed with nutmeg Dilated sinusoids and vascular congestion with infiltration of mononuclear cells (A) 40X magnification (B) 100X magnification.

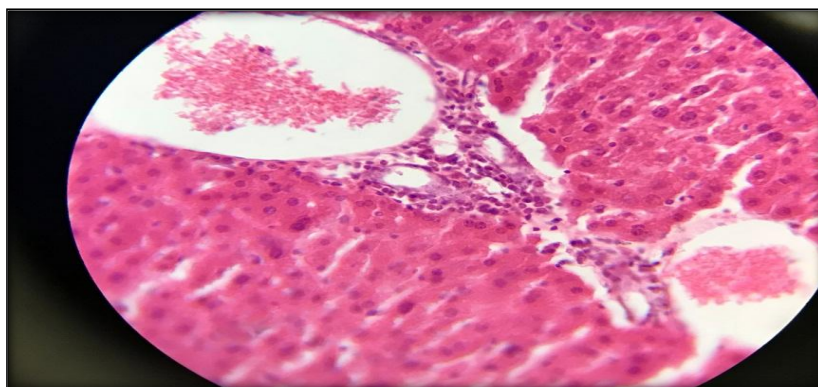


Fig. (5): shows a section of the liver tissue of a mouse dosed with nutmeg spice, in which dilatation of blood sinusoids and congestion of blood vessels with infiltration of mononuclear cells appears in addition to cellular degeneration. Magnification power 400X magnification.

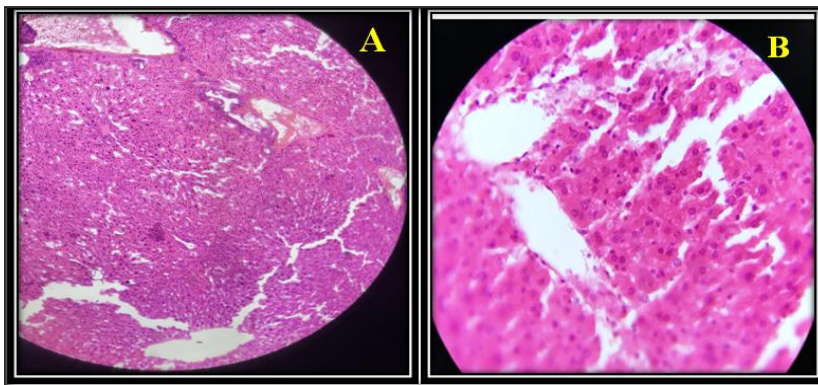


Fig. (6): A section of the liver tissue of a mouse dosed with ginger spice shows expansion of blood sinusoids and severe congestion of blood vessels with infiltration of blood components in addition to mononuclear cells (A) 100X magnification (B) 400X magnification.

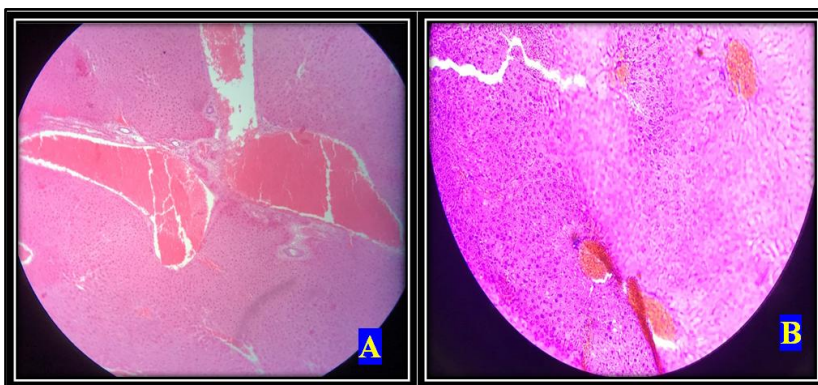


Fig. (7): shows a section of the liver tissue of a mouse dosed with cloves showing severe congestion of blood vessels with infiltration of blood components in addition to coagulative necrosis of the hepatocytes (a) 40X magnification (b) 100X magnification.

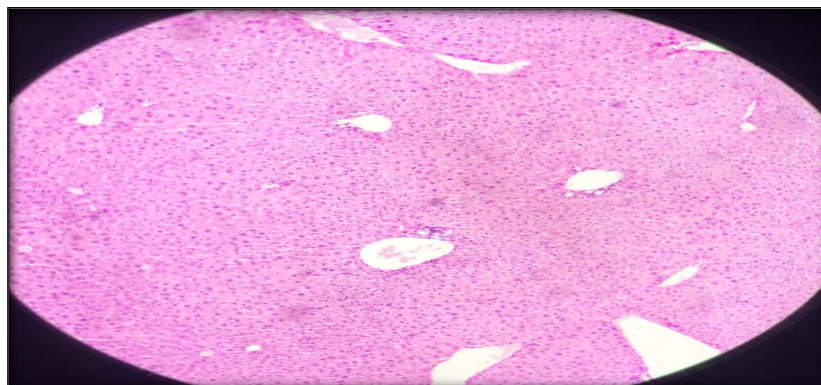


Fig. (8): shows a section of the liver tissue of a mouse dosed with the spice mixture showing congestion of blood vessels with infiltration of blood components in addition to coagulative necrosis of the hepatocytes and the appearance of areas of degenerative necrosis. Magnification power 100X.

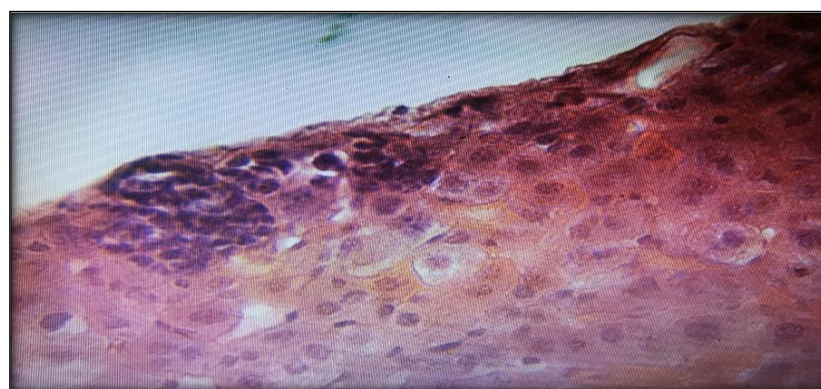


Fig. (9): shows a section of the liver tissue of a mouse dosed with the mixture showing coagulate necrosis of the hepatocytes and the emergence of areas of degenerative necrosis, as well as the intensity of the deposits of glycogen in the hepatocyte, in addition to the appearance of hepatocytes that suffer from programmed death in their initial and final stages. Magnification power 400X.

CONCLUSIONS

First of all, we say that the more something is above its natural limit, the greater its danger, regardless of its usefulness. Spices are good in terms of their components and aromatic characteristics, so human consumption of them increased, which led to this study shedding light on the extent of their danger, as the results of this study showed when eating spices. Excessively and long-term use will lead to the opposite result, which is represented in the damage of clinically dosed animals and a change in the morphological and histological structure of the liver and the damage may reach tissue erosion. Used compared to the weight of the dosed animal, so there must be awareness when using and mixing them.

REFERENCE

1. Parthasarathy, V. A., Chempakam, B. and Zechariah, J.T. "Chemistry of spices." CIBI North American office, USA.I-445 (2008) 7-78.
2. Al-Deresawi, M. S. "Methylation effect of miR-129-2 and miR-203 genes on the expression of SOX4 gene in uterine abnormalities Supervised by." *Institute of Genetic Engineering and Biotechnology for Postgraduate Studies/Iraq* (2018).
3. Alsaman, M. "EFFECT OF TAMOXIFFEN ON HEPATIC CELL OF FEMALS ALBINE MICE ON THE ELECTRON MICROSCOPICAL STUDY." *Iraqi Journal of Science* 53.1 (2012): 68-72.
4. Al-Sadi, H. I.; Al-Hmadani H. M. H.; Al-Sultan R.G. "Morphological and Histological Changes in Liver and Lung of mice and Their Embryos Administered with Parkizol Drug Uni." *Mousl; Iraq* 22.10 (2017); 1-12.
5. Dkhil, M. A., & Al-Quraishy, S. "Effects of extensive consumption of hot red pepper fruit

- on liver of rabbit." *Journal of Medicinal Plants Research* 4.23 (2010): 2533-2538.
6. Yoshioka, M., Doucet, E., Drapeau, V., Dionne, I., & Tremblay, A. "Combined effects of red pepper and caffeine consumption on 24 h energy balance in subjects given free access to foods." *British Journal of Nutrition* 85.2 (2001): 203-211.
 7. Nwaopara, A. O., Odike, M. A. C., Inegbenebor, U., & Adoye, M. I. "The combined effects of excessive consumption of ginger, clove, red pepper and black pepper on the histology of the liver." *Pakistan Journal of nutrition* 6.6 (2007): 524-527.
 8. Kod'ousek, R., Jezdinský, J., & Krajčí, D. "Histological and ultrastructural changes of cardiomyocytes in experimental rats with tail thrombosis following subplantar application of carrageenin." *Medical Principles and Practice* 16.5 (2007): 360-366.
 9. Kod'ousek, R., Jezdinský, J., & Krajčí, D. "Histological and ultrastructural changes of cardiomyocytes in experimental rats with tail thrombosis following subplantar application of carrageenin." *Medical Principles and Practice* 16.5 (2007): 360-366.
 10. Owaied, Y. H.; Ahmed, S. S.; Khamis, H. S. "Pathological study of Salmonella bongori with physical and histological change in mice." *Third scientific conference of the Faculty of Veterinary Medicine / University of Tikrit* (2016).
 11. Al-Hilfy, J., Ferial, A. M., & Al-Kaisy, K. "EFFECTS OF SOYBEAN SEEDS ON HISTOLOGICAL STRUCTURE OF LIVER OF MALE RATS *Rattus norvegicus* TREATED WITH URANYL NITRATE." *Iraqi Journal of Science* 51.4 (2010): 520-527.
 12. Luaibi, N. M., & Shafiq, A. A. "Study of Biochemical and Histological effects of fennel seeds (*Foeniculum vulgare*) on Liver in Male Rats." *Journal of the College of Basic Education* 22.96 (2016): 1-24.
 13. Al-Rubaye, R. H. "Effect of aqueous leaves extract of *Coriandrum sativum* on histological structure and liver function of male albino mice." *Ibn AL-Haitham Journal For Pure and Applied Science* 29.1 (2017).
 14. Abbood, A. S., Abdel-Hadi Gal, M., & Jassim, K. A. "The histological effect of *Aeromonashydrophila* on liver of male albino mice." *Baghdad science journal* 10.2 (2013): 24.
 15. Ulusoy, E., & Eren, B. "Histological changes of liver glycogen storage in mice (*Mus musculus*) caused by high-protein diets." *Histology and histopathology* 21.7/9 (2006): 925.
 16. Hosseini, M., KAMKAR, A. M., & Rakhshandeh, H. "Analgesic effect of clove essential oil in mice." (2011): 1-6.
 17. Ugwuja, E. I., Erejuwa, O. O., & Ugwu, N. C. "Spices mixture containing garlic, ginger and nutmeg has protective effects on the kidneys and liver of cadmium exposed rats." *Advanced Pharmaceutical Bulletin* 6.2 (2016): 271.
 18. El-Kott, A. F., El-Sayad, S. M., & Abdel-Aziz, A. M. "The effects of ginger (*Zingiber officinale*) on histology and immunohistochemistry of liver and kidney and certain haematological parameters in alloxan induced diabetic rats." *Egypt. J. Exp. Biol.(zool)* 6.1 (2010): 61-70.

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