

# Effect of Extreme Consumption of Clove on the Morphology of Mice and Liver Tissue

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## Abstract

**Introduction:** Flavors are one of the main materials used in the world kitchens. Therefore, we must research their harms in the service of society. **Objective:** This study aimed to investigate the effect of too much cloves consumption on the morphological and histological structure of the mice liver. It is important to have a clear view of the excitements that play a major role in damaging organs. **Methodology:** About 20 mice were used. I was orally (1g) to ginger for 50 days. that anesthetization, separating the liver, fixing by formalin, using the routine method to preparation block and sectioning (5um) then coloured with H&E. **Results:** symptoms was decrease in weight and signs of dehydration and hair loss,. the liver appearance of severe congestion with a dark red colour, accompanied by bleeding and the appearance of necrosis in the liver. Microscopy indicated a loss of the normal structure of the liver tissue. The sections also showed the emergence of the stages of programmed death of cells. **Conclusion:** We concluded that consuming spices in large quantities will lead to toxic liver toxicity, but it is not dangerous.

**Keywords:** Clove, Liver, Mice, Morphology, Tissue

## Introduction

The spice has been used for centuries as a food preservative and medicinal plant because it acts as both antioxidants and microbes<sup>1</sup>. Decades ago, healthy eating habits were responsible for protecting against various diseases associated with poor nutrition such as obesity, cardiovascular diseases, etc.<sup>2,3</sup>.

Given the active components of cloves such as (gallotannic acid, caryophyllin, tannin, protein, fats, carbohydrates, energy, vitamin B1, B2, iron, oils, resins, and pentosantes, mineral elements, cloves are an excellent source of manganese, which is a very good source. Dietary fiber, vitamin C, vitamin K, ω-3 fatty acids, and a good source of calcium and since previous

studies have highlighted the therapeutic importance of cloves because of the beneficial elements it contains<sup>4,5</sup>, so we will discuss in this study the effect of high doses of cloves depending on The researchers presented previous studies in which they dealt with several aspects.

So the study<sup>2</sup> on the combined effect of excessive consumption of Yaji sauce consisting of (ginger, cloves, red pepper and black pepper on rabbit liver tissue), and the use of spice extracts expanded in treating some types of bacterial infections.

Therefore, this study was designed to link the individual with the common effects of excessive consumption of cloves on the phenotypic and histological changes of rat liver resulting from excessive consumption of this spice, and contribute to setting the permissible limit for its consumption and avoiding excessive of it .

## Materials and Methods

**Animals:-** This study was carried out in the laboratories of the Diyala University and the laboratories

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of Hospital, it included 20 mice weighing (30-26g).

**Animal dosing:-** The animals were administered orally by (1g) per ginger powder mixed with (10ml) of distilled water, period lasted for 50 days. The groups were divided into two groups, which included 10 mice for each group, as follows. Separating the liver by using the Pellet Wild method, then put into formalin after washing it with saline solution, for a period ranging between (12-16h). Using routine preparation and sectioning by manual rotating microtome of the type (HM325) and that coloured with Delafield haematoxylin and Eosin<sup>6</sup>.

## Results and Discussion

### Clinical symptoms

The organ that controls the various physiological processes in the body of a living organism is called the liver. In the long term, multiple injuries can cause injuries that lose the ability to regenerate<sup>7</sup>. Man has been accustomed since ancient times to using cloves in cooking for its aromatic feature or as herbal remedies instead of Chemical and medicinal treatments, as a result of their common perception of herbs that do not cause side effects according to their beliefs at the cheapest prices<sup>8</sup>.

Therefore, the current study showed the extent of the effect of cloves on the health and efficiency of the liver if taken in large doses and for long periods as it was represented by the emergence of different clinical symptoms when mice were dosed with a spice Cloves, at a concentration of (1g), showed clear signs of loss of appetite compared to before the dose, which was accompanied by a decrease in weight and signs of dehydration and hair loss, which led to the appearance of skin in most areas of the body as shown in (Fig.1).

This is not in line with study<sup>9</sup>, which showed different results in terms of weight gain in rabbits when dosed with cloves, some of which resulted in death. But similar to the results of the study<sup>10</sup> when dosing mice with Paraxol, as the behavioral changes caused by the doses 250, 350 and 450 mg / kg of body weight reflected signs of lethargy and withdrawn, poor eating and drinking water, and then partial cessation of eating them,

as well as the occurrence of vaginal bleeding and the death of some. And, it was consistent with Study<sup>11</sup>, in that the mice that took khat extract suffered a significant decrease in their body weights, indicating the effect of anorexia and liver damage due to the effect of khat on it.

### Examination of the morphology

Clinical results showed the liver is dark red in color, and the limbs are transparent and paler. The lobes of the liver appeared, varying in color as a result of hemorrhage and also eroding the liver tissue, as shown in (Fig.1), which led to the death of some of them.

This is identical to what is found in the study<sup>10</sup>, where the livers of pregnant mice exposed to glue smoke at a concentration of 2500mg/kg of body weight were noted, their irregularity. To the extent of the researcher's knowledge, he did not find a result identical to what he had reached in terms of the negative effect of cloves, because most studies dealt with its curative effect, and he did not find a similar result for necrosis in the liver in terms of appearance.

### Microscopical apperants changes:-

In general, the histological sections showed a loss of the normal structural structure of the liver tissue represented by the radial arrangement of the hepatic cords in the central lobular region and in the surrounding areas in the dosed groups, as shown in Figure (2,3,4).

The livers of mice fed with cloves showed vasodilation, hepatic sinuses and hemorrhage, due to severe congestion of the vessels between the hepatic lobules, which led to the central vein and the portal area being filled with blood components and leaching severely in the hepatic sinusoid. Also small amounts of phagocytic cells are near the blood vessels.

Seeing the presence of enlargement and thrombotic necrosis of hepatocytes, and some histological sections indicated the presence of slightly contracted liver cells in addition to the emergence of irregularly shaped hepatic cells with an acidic cytoplasm and a small fractionated nucleus representing the early stages of apoptosis, accompanied by the presence of small spherical cells with a nucleus Mono is small and the cytoplasm of

the eosinophilic dye also represents the late stages of programmed death. In other sections, the emergence of nucleated cells and the leaching of yellow droplets and their retention inside hepatocytes, as shown in Figure (2-6), and these results were in agreement with the study <sup>12</sup>, which dealt with the effect of cadmium on the liver at different doses and for different periods, and it was found that the dose on the 30th day is similar to the results of the current study; Except for the amount of inflammatory cells around the blood vessels, which were in the current study in smaller quantities. On day 45, the nuclear degeneration and loss of the cell appeared. Likewise, with study <sup>11</sup>, which dealt with the toxic effect of dried and green khat on the livers of rats, it was found that khat leads to congestion of the hepatic vessels, while study <sup>13</sup> had different results, where the components of cloves were used to inhibit cirrhosis by inhibiting free radicals and through this study it was found that eating cloves in quantities It will generate free radicals, which in turn will damage hepatocytes and damage tissue.

The reason for the emergence of enlargement of liver cells, necrosis and lymphocyte infiltration in mice that consumed high doses of cloves, because liver cells are the sites most affected by cloves if it is taken in an excessive amount, which affects the transfer of the mitochondrial membrane in the liver and causes the mitochondria to swell in the resulting liver cells. And increased biochemical markers and liver enzymes, which quickly pass through the leaky membranes of cell organelles. Consequently, the massive accumulation of

fluid in the vacuoles may lead to cell lysis and death<sup>14</sup>.

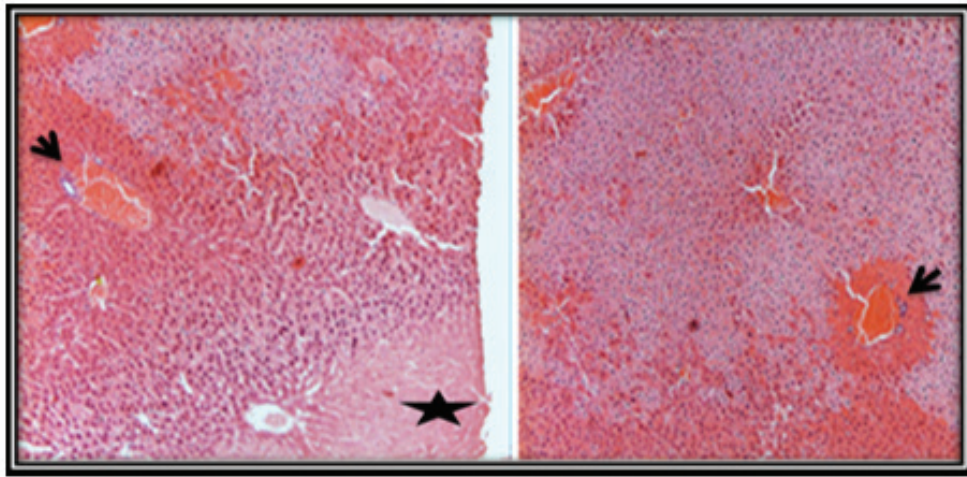
The mechanism of cell death works normally to preserve them by removing harmful or severely damaged cells. However, excessive liver apoptosis is likely to contribute to acute hepatitis by causing liver dysfunction. Natural killer cells, T cells, and macrophages are mobilized during ALI, and liver-resident macrophages act as a factor contributing to liver damage by mass production of cytokines in response to inflammatory stimuli. These inflammatory cells secrete pro-inflammatory cytokines such as tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), interleukin (IL) -1 $\beta$  and IL-6, and these pro-inflammatory cytokines play a role in promoting ALI progression <sup>15, 16</sup>.

From the researcher's point of view, it was found from this scientific evidence that the spices studied in our research have some pharmacological and chemical properties similar to those of drugs that are capable of causing liver damage and thus explain their ability to affect the observed tissue structures. Therefore, based on the results of this study, it was found that excessive consumption of cloves for long periods of time is capable of causing liver damage and therefore it should be avoided or rationalized using it.

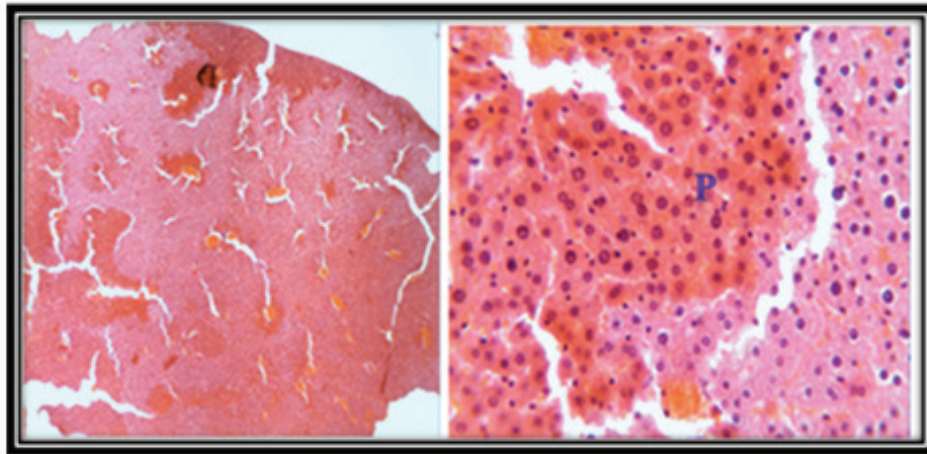
Sudden liver damage results in acute and short periods of injury, which in turn causes ill health and a higher death rate, which may be due to complex interactions of oxidative stress, apoptosis and inflammation.



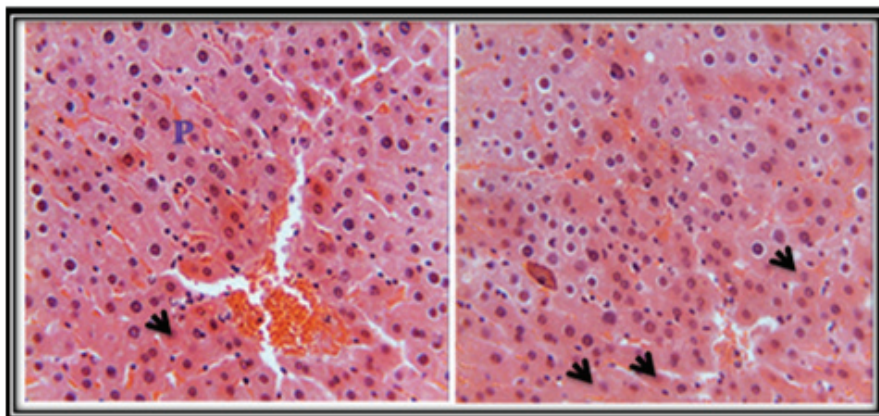
**(Figure1):macroscopically a mouse with a cloves spice showing signs hair loss, macroscopical the liver of the shielded mouse with clove spice showing erosion of the tissue and the formation of the necroses (arrow) .**



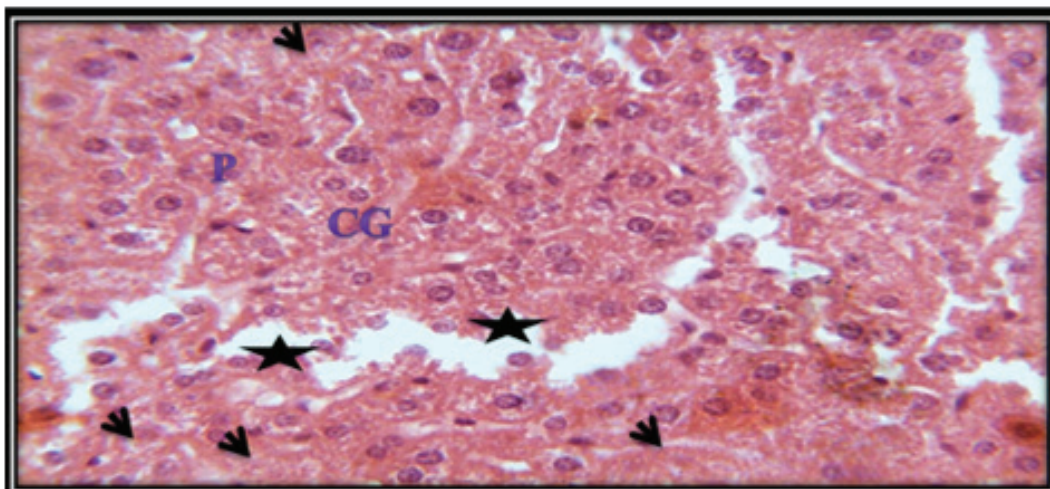
(Figure2):liver dosed with clove spice showing congested blood vessels, and infiltration little of mononuclear (kupffer cells) inflammatory cells (arrowhead), vacuole degeneration, and hepatocellular necrosis accumulated in the extremities (black star).X10



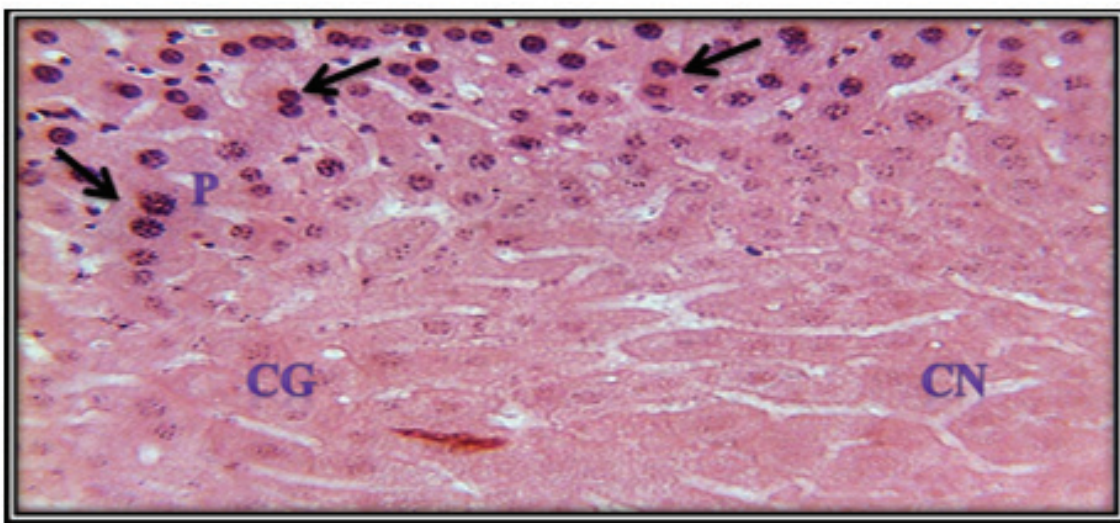
(Figure3):liver dosed with a clove spice showing the area of pyknosis and coagulative necrosis and tissue erosion and dilated blood sinusoids, Nuclear pyknosis (P) of (X10 andX40)



(Figure4):liver dosed with a spice of clove showing amyloid with infiltration, and blurred borders between the cytoplasm and the nucleus of hepatocytes (arrowhead), in addition to the appearance of bile droplets , dilated blood sinusoids and cell enlargement Hepatic, Nuclear pyknosis (P) at a power of(X40)



(Figure5):liver dosed with a spice of clove showing Blurred boundaries between the cytoplasm and the nucleus of hepatocytes(arrowhead), the star indicates areas of liver congestion and cell necrosis and dilated blood sinusoids(black star),Nuclear pyknosis (P), cytoplasmic granularity(CG) at a strength of (x40)



(Figure6):liver dosed with a clove spice showing the presence of dark areas coagulative necrosis (two phases ) and faded ones with and there is loss cellular detail (X40).Nuclear pyknosis (P), cytoplasmic granularity(CG) , cell necrosis (CN) , and many bi-nucleated cells (arrows)are also shown.

### Conclusions

To initiate, we say too much of something is dangerous, no matter how beneficial it is, cloves is well known as a food flavor worldwide with high human consumption. In conclusion, the results showed that proven that eating clove in excessive and repeated doses leads to the damage that was discovered in the livers of experimental animals. It damages the structural structure of liver tissue and the damage may reach erosion in

parallel with the experimental results and the reported cases. These results coincide with the term “Hepatotoxic “. there should be awareness

### Recommendations

1. Carry out a biochemical and physiological study to see the effect of spice
2. Conducting a study at the level of the electron

microscope to find out the effect of spice on the cellular structure

3. Additional studies are suggested using oral clove dose in food and as solution to compare toxic effects produced by the two different routes of administration.

**Ethical Clearance** - Taken from Ethics Committee of Diyala Medical College

**Source of Funding** - Self founded

**Conflict of Interest** - Nil

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