Effect of Clove Extract on the Histological Structure of the Kidneys in Swiss Mice

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Abstract: Syzygium aromaticum is a medicinal plant known for its antioxidant and anti-inflammatory properties. It is used in traditional medicine to treat a variety of conditions, but it can cause toxic effects at high doses. The study aims to effect the extract of the clove plant (Syzygium aromaticum) about the histological architecture of the kidneys in Swiss mice (Mus musculus), which took 30 days. 24 mice were used and divided into four groups according to the dose of clove extract, each group includes 6 mice. The first group (the first control experiment group), The second group received an injection of 100 mg/kg per day of the extract, the third group received 200 mg/kg per day, and the fourth group received 300 mg/kg per day of the extract. The results of the examination of the histological sections of the kidneys of animals in the injected experimental group demonstrated the presence of several abnormal histopathological alterations, including the amplation of the Bauman space, glomerular contraction, degeneration and necrosis of the cells forming the urinary tubules, degeneration of glomerular cells and cells of the parietal layer of the capsule, and the presence of congestion in the blood vessels and the formation of a blood clot with an increase in the thickness of the vascular wall, and some cellular changes such as enlargement of nuclei, cell swelling, the occurrence of vascular degeneration and cytoplasmic degeneration with infiltration of inflammatory cells, in addition to the occurrence of vascular centered fibrosis, increased thickness with density of the glomerular basement membrane.

1 INTRODUCTION

Since ages, Medicinal plants have been utilized as spices, dyes, and flavorings, in addition to their therapeutic applications by a significant portion of the global population to address various health issues, including cancers, diabetics, obesity, liver and kidney diseases, and heart diseases [1]. One of these plants is the clove plant Eugenia or LM Perry, the scientific name of which is Syzygium aromaticum (L.) is one of the types of plants that are used as a spice with common fragrances that is widely used globally In food as well as alternative medicine, as well as in traditional and folklore medicine, the oil is utilized in dentistry due to its potent analgesic and antiseptic characteristics [2]. Clove extracts and essential oil have extensive medical applications as anticancer, antimicrobial, anti-breath, antidiabetic, anti-obesity, anti-inflammation, antioxidants , antiviral and aphrodisiac, including several applications [3]. The carnation is an unopened flower bud of the clove tree, belonging to the Myrtaceae family, and is indigenous to Indonesia. It is utilized as a culinary spice worldwide and is originally from the Kings Islands (Indonesia), though it is now extensively cultivated in various tropical regions, including Zanzibar, Madagascar, Pakistan, India, and Sri Lanka for commercial purposes. Since 300 BC, Indonesia has served as a hub for Arab, Chinese, Indian, and European traders of the principal Indonesian spices, particularly the clove plant (Syzygium aromaticum L) [4]. Numerous investigations suggest that S. aromaticum L. has roughly 15-20% essential oils, while clove essential oil (CEO) is rich in phenolic compounds exhibiting different biological functions, particularly anti-fungal, anti-bacterial, insecticidal, and antioxidants capabilities. [5], [6]. The kidneys are one of the basic organs that the body needs to carry out many important functions, including maintaining balance, regulating the environment outside the cell, and emptying metabolites and toxic metabolites and drugs [7]. The kidneys are an organ that is rapidly affected by some types of drugs, i.e. the main function of the kidneys is

to maintain the state of balance and the amount of fluid in the body, and to excrete both excess water, electrolytes and waste in the blood [8]. In addition, it is a major gland that secretes some hormones, such as Erythropoietin. It facilitates the synthesis of red blood cells by activating the bone marrow, which in turn does so, and the resonance hormone Renin regulates blood pressure [9]. Due to the extreme importance of the kidneys in the sustainability and quality of life, due to their locations and functions and the lack of most previous research of the histological effects caused by extracts on them. The objective of this study was to ascertain the impact of the aqueous extract of the clove plant on the histological architecture of the kidneys in the white mouse.

2 MANUSCRIPT PREPARATION

2.1 Clove Plant Collection

The clove plant was obtained from the local markets and then cleaned by hand to get rid of impurities and then ground with a household electric grinder to obtain a fine powder and then kept in an airtight box until the powder is used.

2.2 Preparation of Aqueous Clove Extract

Prepare the extract by taking 50 grams of clove powder that has been ground and then dissolved in 500 ml of non-ionic water and then placed on a Hote plate for 72 hours to stir the mixture to dissolve the powder in water and then drain with filter paper to get rid of impurities is not solvent and then placed in the refrigerator to precipitate the precipitate and take the filtrate and put it in the center view device at a speed of 3000 cycles / minute to get rid of the remaining precipitate and then take the aqueous extract placed at a temperature chamber to evaporate the water and obtain the aqueous extract to be dissolved with distilled water and ready for injection of laboratory animals [10] .

2.3 Experimental Animals

Twenty four females were obtained from the Swiss musculus pregnant egg mice from the Institute of Embryology Research and Infertility Treatment / Al-Nahrain University, whose weight ranged from 32 to 54 g. The therapies administered were tailored to the specific requirements of the experiment:

- **Group 1:** The healthy control group.
- **Group 2:** This group received an injection of 100 mg/kg per day of the extract.
- **Group 3:** This group received an injection of 200 mg/kg per day of the extract.
- **Group 4:** This group received an injection of 300 mg/kg per day of the extract.

2.4 Clove Extract Dosage Preparation

The dose for clove extract was prepared based on the lethal half-dose (LD50) whose value in mice is 565.7 mg/kg for clove extract [11]. Where three concentrations of clove extract were selected to test the extent of its effect on mice, namely 100, 200 and 300 mg of extract / kg of the weight of the mice used in the experiment between (32-54) g. The required amount of extract was then dosed depending on the concentration (dose) by injection once a day for one month per concentration. The dosage for the specific clove extract was calculated based on the following (1) [12].

$$\frac{x}{D} = \frac{w}{1000}. \quad (1)$$

Where,

- w - the weight of the mouse used in the experiment (32–54 g);
- D - the specific dose of clove extract administered (100, 200, or 300 mg/kg of body weight);
- x - the amount of extract to be injected into each mouse, measured in milligrams.

3 RESULTS AND DISCUSSION

The kidneys work to excrete waste, acids, minerals, medicines, or metabolites of them, and because of this function may lead to histological changes in blood vessels, tubules and interstitial tissues [13], [14]. The results of the current study of the histological sections of the kidneys in the control group showed that the kidney consists of two regions, namely the cortex and the medulla. The renal cortex contains renal glomeruli with a normal shape and tufts of glomerular capillaries surrounded by the capsular space and Bowman's capsule and the proximal convoluted tubules, which have a narrow lumen and pyramidal cells with spherical nuclei and a narrow lumen as shown in Figure 1, the experimental group injected with clove plant extract at a concentration of 100 mg had histological changes in the kidneys, including

severe congestion in the renal tubules and suppuration in them, in addition to strong bleeding between the renal tubules and necrosis in some epithelial cells of the tubules and the separation of part of the cells from the basement membrane, in addition to that, it was noted in the current results that inflammatory cells combine the tubules as in Figures 2, 3 and 4.

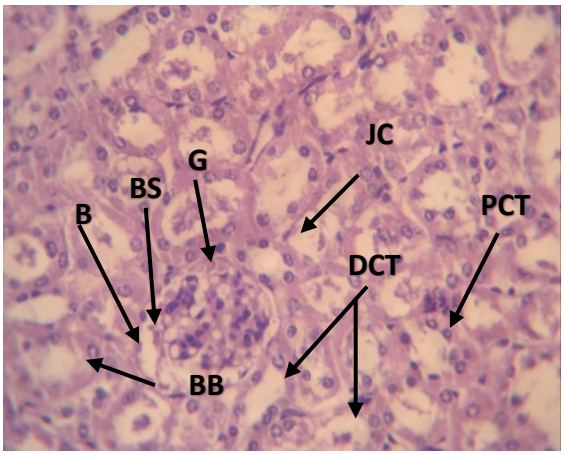

Figure 1: A transverse section of the kidney of pregnant female mice from the control group showing the cortex area. Note G glomerulus, PCT proximal convoluted tubule, JC paraglomerular cells, B Bowman's capsule, BS Bowman's space, DCT distal convoluted tubule, BB brush border stained (H&E 40X).

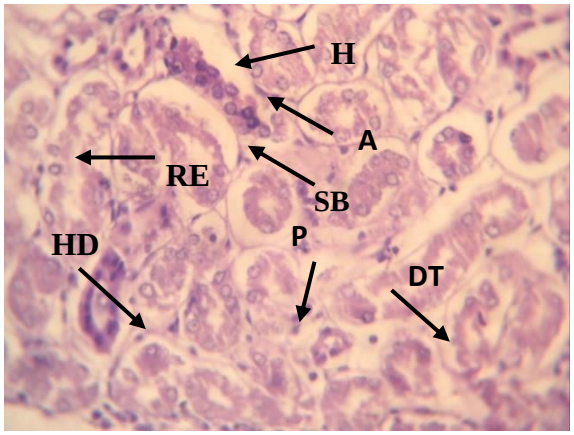

Figure 2: Section in the kidneys of pregnant female white mice experimental group injected with clove plant extract at a concentration of 100 mg / kg shows changes in the area of the cortex illustrates. H hemorrhage Re dilated tubules, SB, cell separation from basal membranes, HD alpine degeneration, P nucleolithrosis, DT destruction of some cells, inflammatory cell aggregation A, (H&E 40X).

The microscopic examination results indicated alterations characterized by the invasion of inflammatory cells. Infiltration may result from drug buildup or inflammation, as Bartoli [15] demonstrated that medications can induce systemic immune responses, resulting in inflammation and renal damage. Additionally, pre-existing renal injuries, such as fibrosis, may draw immune cells to the kidneys [16].

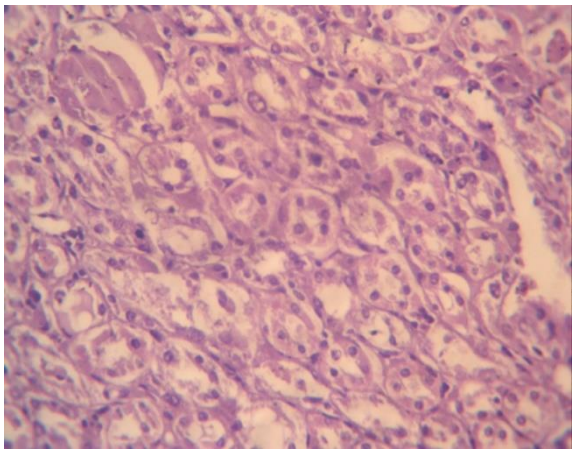

Figure 3: Section in the kidneys of pregnant female white mice experimental group injected with clove plant extract at a concentration of 100 mg/kg shows changes in the cortex area illustrates. A inflammatory cell infiltrate, V cytoplasm of the collector ducts cells, N tubule cell necrosis, P nucleololi (H&E 40X).

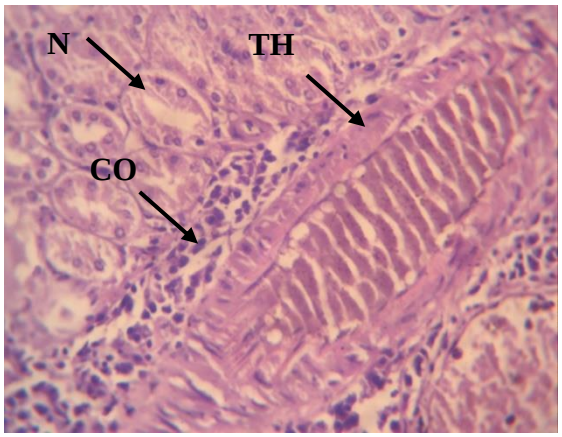

Figure 4: Section in the kidneys of pregnant female white mice experimental group injected with clove extract at a concentration of 100 mg / kg for 30 days shows vascular Vascular wall congestion and hypertrophy in the renal cortical region. CO noted acute vascular congestion, TH vascular wall hypertrophy, and N cellular necrosis (H&E 40X).

Vascular congestion in the cortical blood vessels was noted, along with hypertrophy of the vessel walls, maybe attributable to the toxic effects of clove extract or a response to inflammation resulting in augmented blood flow to the damaged region [17]-[19]. Histological sections have shown necrosis and degeneration due to the toxic effect of the extract or hypoxia, necrosis can be linked to ATP depletion, ultimately resulting in death of cells [20], [21].

Lara-Prado [22] also noted that the lack of blood flow to the kidneys may cause ischemia which increases ROS levels, modifies mitochondrial pathways, resulting in ATP depletion, cellular calcium releasing, caspase activation, and oxidative damage to lipids, DNA, and protein, eventually resulting in death of cells, as well as inhibiting antioxidant mechanisms (e.g. catalase, superoxide dismutase and glutathione peroxidase).

The examination outcomes indicated the detachment of renal tubule cells from their basal membranes, accompanied by hypertrophy and necrosis. This may stem from the influence of clove extract on the arteries, which diminishes blood flow necessary for cellular sustenance, resulting in cell death. Additionally, the extract may impact the enzymes produced by the kidney that govern arterial relaxation and contraction, such as prostaglandins [23].

The results of the current study showed that the injection of clove plant extract in pregnant female white mice at a concentration of 200 mg / kg led to pathological changes in the kidneys more severely than the previous concentration and for a period of 30 days, and these degenerative changes, which makes their cavities narrow as we did not notice the separation of some of the tubule cells from the basement membrane, and the occurrence of bloody bleeding between the renal tubules and observed enlargement and lobulation of the glomeruli so that they almost fill the capsular space, which led to the small area of the bowman space bleeding in addition to the death of some cells inside, either in some histological sections has been observed atrophy or contraction glomerulus, which led to the accumulation of cells inside it in the form of mass and the large space Bowman in it or its death and the capsule remains empty as in Figures 5, 6 and 7.

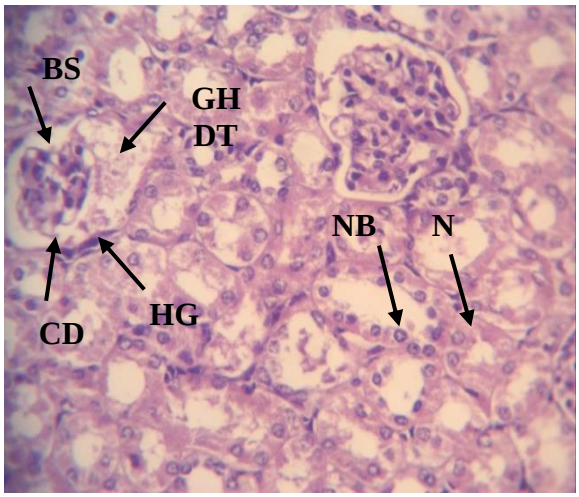

Figure 5: Section in the kidneys of pregnant female white mice Experimental group injected with clove extract at a concentration of 200 mg / kg for a period of 30 days, showing changes in the kidney cortex DT Cells are collected in the middle of the tubule N Urinary tubule cell necrosis, enlarged glomeruli HG, capsular space BS, CD cell death in the glomerulus, GH intraglomerular hemorrhage, NB nucleolary hyperplasia. Colorful (H&E 40X) .

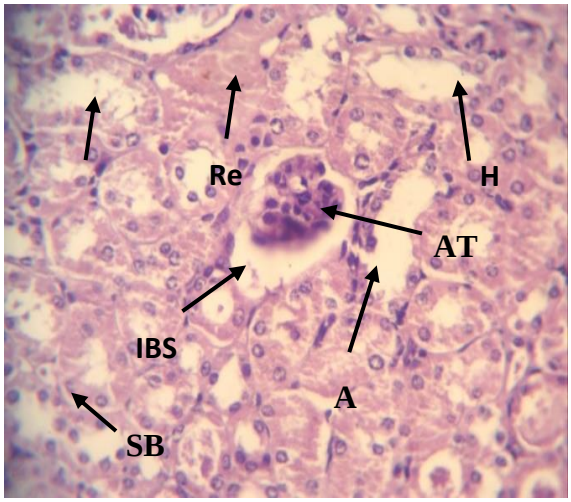

Figure 6: Section in the kidneys of pregnant female white mice experimental group injected with clove extract at a concentration of 200 mg/kg to period of one month, showing glomerular contraction (AT), inflammatory cell infiltration (A), intertubule hemorrhage (H), SB separation of cells from basal membranes, amplitude of Bowman space (IBS), dilated tubules (Re). Coloured (H&E 40X).

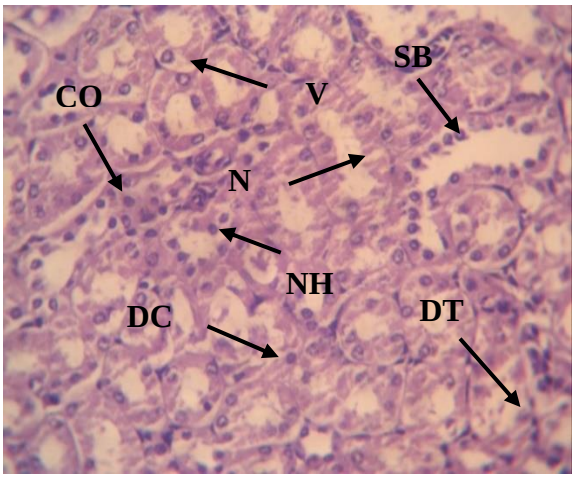

Figure 7: Cross section in the kidneys of pregnant female white mice experimental group injected with clove plant extract at a concentration of 200 mg / kg for 30 days, showing the occurrence of high degeneration of the pulposal region and renal rosids within the kidney. SB separation of cells from basal membranes, HN thickening of cell nuclei, Vtfgic cells, DT crash of some cells, N cell necrosis, CO vascular congestion, DC small size of cells, colored (H&E 40X).

The expansion of the Bowman space and the contraction of the glomeruli may result from the degeneration and demise of glomerular cells and their atrophy, or alternatively, from the influence of the extract on the hormone Angiotensin I, which stimulates mesangial cells to contract, leading to the constriction of glomerular capillary blood vessels. This cells support the capillary blood vessels, and their shrinking contributes to the narrowing of the glomerular capillary blood vessels, thereby increasing the area of the Bowman space [24]. The results of the current study showed that the injection of clove extract in pregnant female egg mice at a concentration of 300 mg / kg kidneys more severely when compared with the previous two concentrations and for a period of 30 days and these changes were kidney damage represented by severe bleeding inside the glomerulus and death of some cells in them and the occurrence of bleeding between the renal tubules and the detachment of specific cells from the basement membrane and the onset of defloration and the separation of cells and their collection inside the tubule in addition to thickening.

The death of some nuclei inside the tubules as well as the enlargement of some nuclei in the epithelial cells lining the tubules as shown in Figure 8. Uetrecht *et al*., [25] has shown that some types of drugs may affect angiotensin II, which elevates intraglomerular pressure and causes kidney damage, and that the mechanisms that contribute to injury include reduced renal blood flow, vasoconstriction of incoming glomerular arteries, or vasodilation of the outgoing glomerular arteries. The lack of blood flow works on ischemia and causes various damages such as necrosis, degeneration or cell death, and this supports what the researchers said [26], [27].

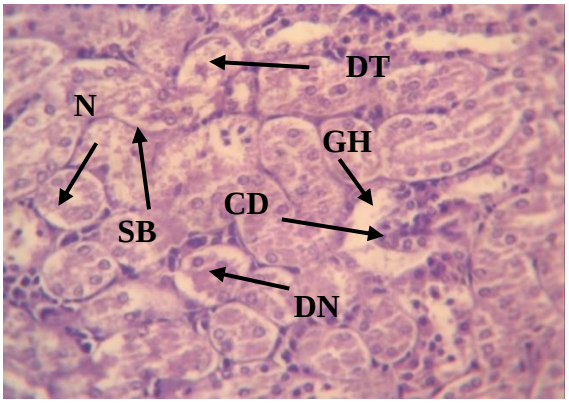

Figure 8: Cross section of kidney tissue Pregnant female white mice Experimental group injected with clove extract at a concentration of 300 mg / kg for a period of 30 days shows the occurrence of severe degenerative changes within the kidney. Intraglomerular hemorrhage GH, cell death in the glomeruli CD, SB separation of cells from basal membranes, DT Destruction of some cells, N necrosis, DN Death or decomposition of colored nuclei (H&E 40X).

The histological sections showed degenerative changes in the pulposal area in the kidney, in which there are tubules of different diameters, which include both the slim and thick Henley buttonhole, in addition to the presence of the university tubules, and these changes include bleeding between these tubules and the separation of their cells from the basement membranes and the emergence of degenerative between the cells lining the tubules and also occurs the crash of some of these cells Enlarged nuclei in most tubules and necrosis occurs between cells as we also note a small size of the tubules as shown in Figure 9.

The results from our current investigation demonstrated a rise in both the thickness and density of the glomerular basement membrane with some urine tubules, as well as an expansion for Enlargement of capsular space as a result of shrinking glomeruli and damage as glomeruli appeared accumulated cells, and atrophy glomeruli atrophy in some sections, and severe bleeding was seen inside the glomeruli and between the tubules and necrosis in the glomerulus as well as the occurrence of cell degeneration in the parietal region of the glomeruli as in the figure 10.

Acute medication-induced kidney injury is a serious problem and accounts for about 20% of cases of acute renal insufficiency among hospitalized patients [28].

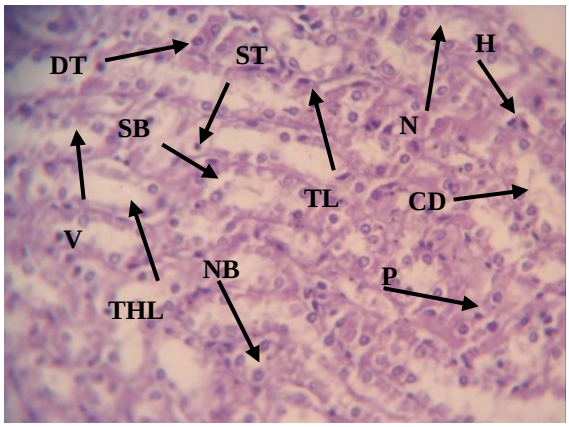

Figure 9: Clip in the kidney of pregnant white mice An experimental group injected with clove extract at a concentration of 300 mg/kg for a period of 30 days shows the pulp area of the kidney. Note THL thick Henley buttonhole, TL slim Henley buttonhole, CD combined canal, SB separation of cells from basal membranes, cell breakdown and aggregation in the center of the DT tubule, NB nucleolal hyperplasia, H intertubule hemorrhage, thickening of some P nuclei, N-cell necrosis, V-ductile tones, small size of tubules ST colored (H&E 40X).

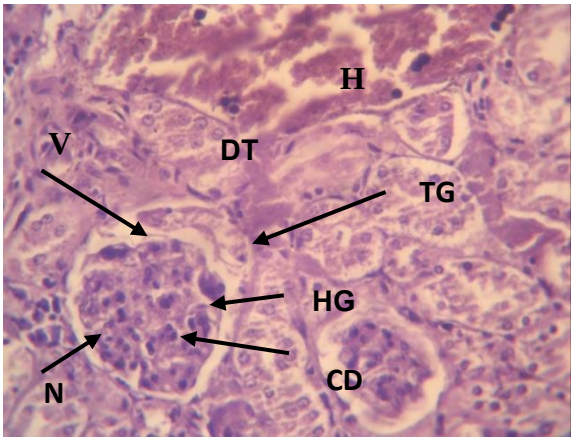

Figure 10: Section in the kidneys of pregnant female white mice experimental group injected with clove plant extract at a concentration of 300 mg / kg for a period of 30 days, shows the occurrence of TG increased thickness and density of the basement membrane of the glomeruli, H hemorrhage between tubules, DT breakdown and aggregation of cells inside the tubule, Vtfji cytoplasm cells in the glomerulus, HG intraglomerular hemorrhage, glomerular necrosis N death of some CD cells. Colored (H&E 40X).

While the results of the current study do not agree with the study Negm et al., [29], which indicated that after injection of clove extract at 300 mg/kg/day, there was a significant improvement in kidney indicators such as increased serum creatinine, blood urea nitrogen (BUN), and hyperphosphatemia resulting from adenine. Another study showed protective effects of all concentrations used, 150 mg/kg, 350 mg/kg, and 450mg/kg, of clove extract on the kidneys of white rats, due to the clove extract containing secondary metabolites in the form of saponins, tannins, flavonoids, and steroids that help repair damaged kidney cells [30].

4 CONCLUSIONS

The results of the examination of the histological sections of the kidneys of animals in the injected experimental group demonstrated the presence of several abnormal histopathological alterations, including the amplation of the Bauman space, glomerular contraction, degeneration and necrosis of the cells forming the urinary tubules, degeneration of glomerular cells and cells of the parietal layer of the capsule. The results indicate that clove (S. aromaticum) extract affects the histological structure of Swiss rats (Mus musculus), with significant dose-dependent histological changes observed. At low doses, the extract may exhibit protective effects due to its antioxidant and anti-inflammatory properties, helping to reduce oxidative damage to renal tissue. At high doses, it may lead to pathological changes such as dilatation of renal tubules, infiltration of inflammatory cells, and changes in the glomeruli, suggesting potential toxic effects at high doses. These findings underscore the importance of dose control when using clove extract to avoid negative effects on kidney health.

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