



## ***Nigella Sativa*-Based Protection against Uterus and Ovary-Related Histological Damages Generated by Cimetidine in Mice**

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### **Abstract**

To generate protecting effects against the histopathological changes in uteri and ovaries in mice that are caused by cimetidine, this work was initiated using the aqueous suspension of *Nigella sativa*. In this experiment, 40 female mice were recruited that were randomly divided into 4 groups. *Nigella sativa*-cimetidine group (NCG) was given orally 1000mg/kg B.W of *Nigella sativa*-based aqueous suspension (NSAS) daily that was followed by administering cimetidine at 25mg/kg B.W. *Nigella sativa* group (NG) was administered orally 1000mg/kg B.W of NSAS daily. Cimetidine group (CTG) was exposed to 25mg/kg B.W of cimetidine daily. Control group (CG) received normal saline only. The experiment was run for 76 days. After the experiment was done, animals were sacrificed to obtain uteri and ovaries from all groups. These organs were subjected to histopathological-based processing and microscopically examination. The results of applying cimetidine alone in the CTG revealed harmful changes that interfered with the normal physiological activities in these organs that were ceased after using NSAS in the NCG. The study provides valued and important information about the use of *Nigella sativa* and its extracts in generating protection against the genital-based harmful changes induced by cimetidine.

**Keywords:** *Cimetidine, Nigella sativa, protection.*

### **Introduction**

In most cases, cimetidine is used as an anti-gastric acid medicine, histamine H<sub>2</sub> receptor antagonist, to reduce the symptoms of duodenal ulcer and the Zollinger-Ellison syndrome [1,2]. Beside these uses, cimetidine also was utilized as an anti-cancer medicine that works alone or in combinations with other medicines on wide-range types of cancers [3].

The problem of over secretion of stomach acid could be reduced using cimetidine; however, because its use might be prescribed for long time, adverse effects might be generated such as gynecomastia and impotence and low numbers of sperms [4]. To reduce the impact of these adverse effects, studied have been launched globally to promote the use of cimetidine with less damages. The use of certain synthetic-based chemicals to reduce these adverse effects might generate extra side effects. Interestingly, herbal-based medications have been studied for years to remove or diminish the detrimental effects of certain medicines. *Nigella sativa* and its preparations are well-known for their special

activities in different processes such as healing, anti-microbial, anti-cancer, immune-system boosting, anti-diabetic, analgesia, and anti-inflammatory [5]. *Nigella sativa* was used to reduce the side effects of cimetidine in male mice, and it was found that *Nigella sativa* enhanced protection against histopathological changes in the tested and especially in seminiferous tubules [6]. The study, here, was designed to reveal the protecting effects against the histopathological changes in uteri and ovaries in mice that might be caused by cimetidine. These protecting processes were examined in this work using the aqueous suspension of *Nigella sativa*.

### **Materials and Methods**

#### **Experimental Design**

In this experiment, 40 female mice (6 weeks of age, 25-31gm of body weight, housed in 6×4×3 m<sup>3</sup>rooms where each 5 mice were placed in a plastic cage, and had 12:12hrs of light: dark hour ratio under 28 ±2 °C of room temperature in the College of Veterinary

Medicine, University of Al-Qadisiyah, Diwaniyah, Iraq) were recruited that were randomly divided into 4 groups. *Nigella sativa*-cimetidine group (NCG) was given orally 1000mg/kg B.W of *Nigella sativa*-based aqueous suspension (NSAS) daily that was 60 min later followed by administering cimetidine at 25mg/kg B.W.

*Nigella sativa* group (NG) was administered orally 1000mg/kg B.W of NSAS daily. Cimetidine group (CTG) was exposed to 25mg/kg B.W of cimetidine daily. Control group (CG) received normal saline only. The experiment was run for 76 days. Pure cimetidine (SDI, Iraq) at 500mg was dissolved in 200ml of distilled water (DW) that was used as 0.1ml/10gm B.Wt (not exceeded 0.3ml/mouse) to drench the mice using stomach intubation.

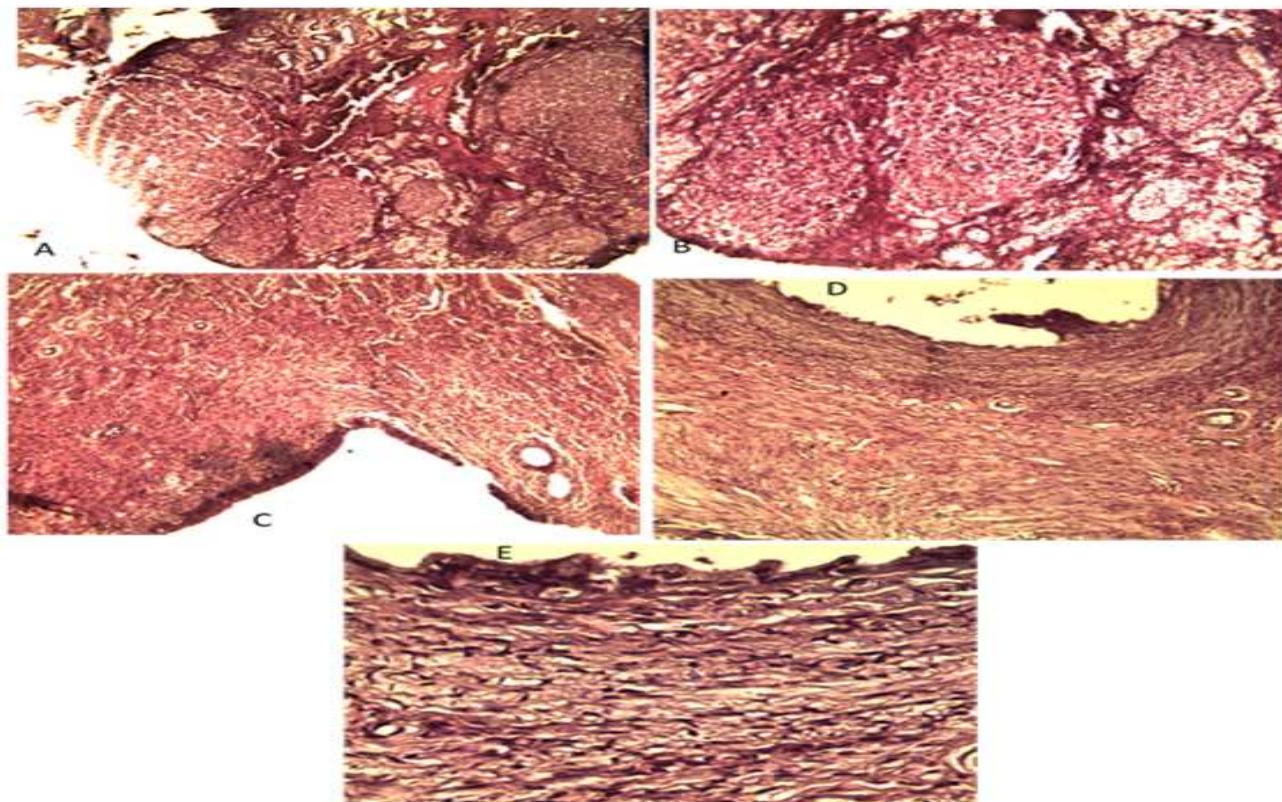
To prepare the stock solution, *Nigella sativa* at 15gm was placed in 100ml of DW that was ground in 5 to 6 intervals (60s grinding and 60s in between) under room temperature. The histopathological process was started using 1-2 cm<sup>3</sup> of uteri and ovaries to be fixed with 10% formalin solution.

The processes were performed according to [6].

## Results

The results of applying cimetidine alone in the CTG revealed harmful changes in these organs that were ceased after using NSAS in the NCG. For the CTG, the use of cimetidine alone generated detrimental effects on uteri and ovaries in the tested mice. In the ovary, it showed poor follicle development, large corpus luteum, and severe hemorrhagic process in the stroma, Figure 1A. It also revealed few primary follicles and large corpus luteum, Figure 1B.

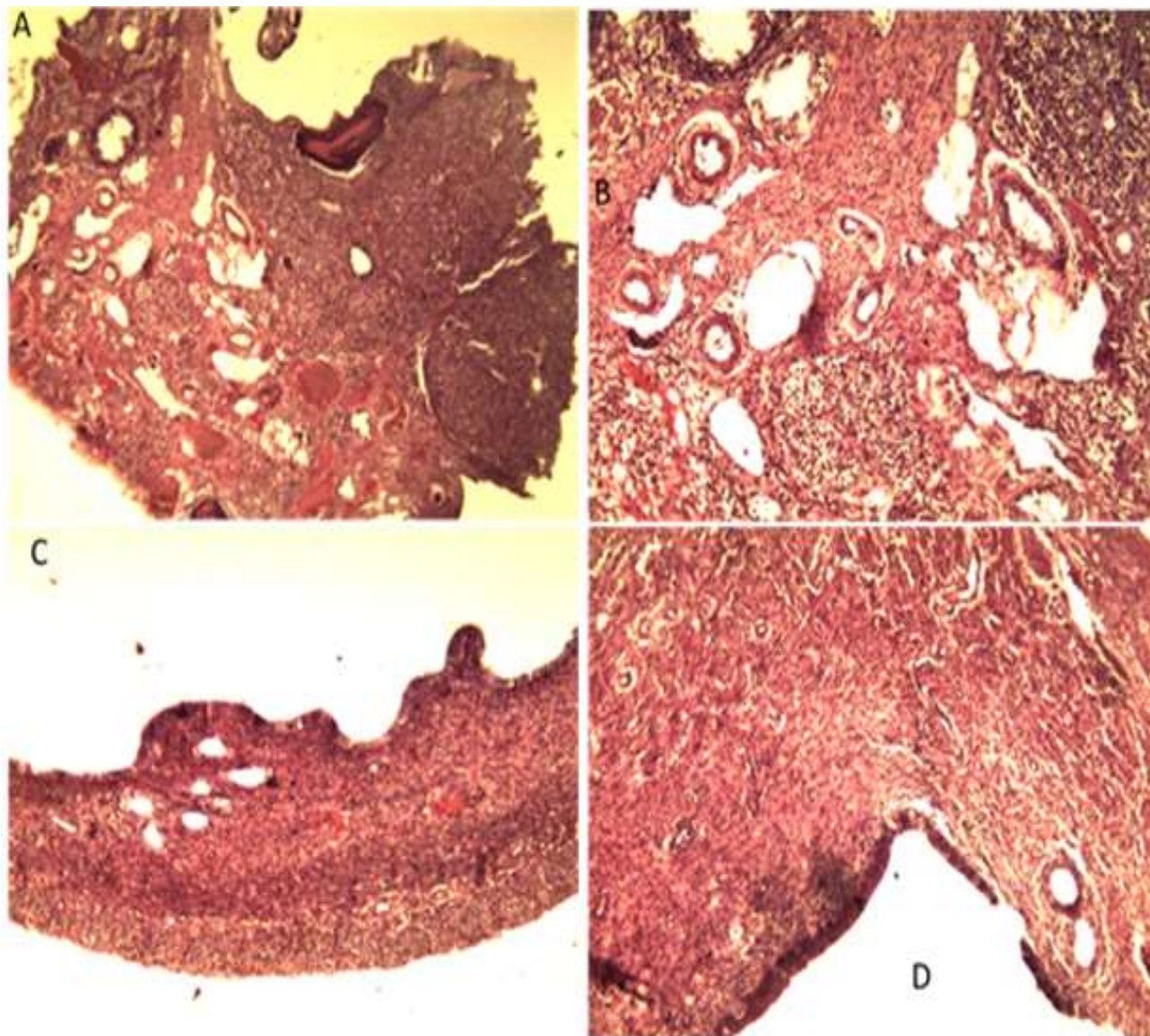
In the case of uterus, the uterus wall showed thin layer, presence of low numbers of small uterine glands, and the occurrence of severe hemorrhage, Figure 1C. In addition, the epithelial lining of uteri suffered degeneration, low numbers of small uterine glands were found in the sub-epithelial layer, and degeneration of the smooth muscle fibers, Figure 1D. Moreover, severe degeneration of the epithelial lining of uteri and low numbers of small uterine glands were revealed, Figure 1E.



**Figure 1:** Cimetidine effects only in CTG. In the ovary, it showed poor follicle development, large corpus luteum, and severe hemorrhagic process in the stroma, figure A 4X H&E. It also revealed few primary follicles and large corpus luteum, figure B 10X H&E. In the case of uterus, the uterus wall showed thin layer, presence of low numbers of small uterine glands, and the occurrence of severe hemorrhage, figure C 4X H&E. In addition, the epithelial lining of uteri suffered degeneration, low numbers of small uterine glands were found in the sub-epithelial layer, and degeneration of the smooth muscle fibers, figure D 10X H&E. Moreover, severe degeneration of the epithelial lining of uteri and low numbers of small uterine glands were revealed, figure E 40X H&E

In the case of NCG, the ovary showed moderate follicle development processes in which huge numbers of small primary and secondary follicles, large corpus luteum, and stromal hemorrhages were present, Figure 2A. In addition, presence of stromal corpus luteum and primary and secondary follicles, Figure 2B. In the uterus, the effects revealed

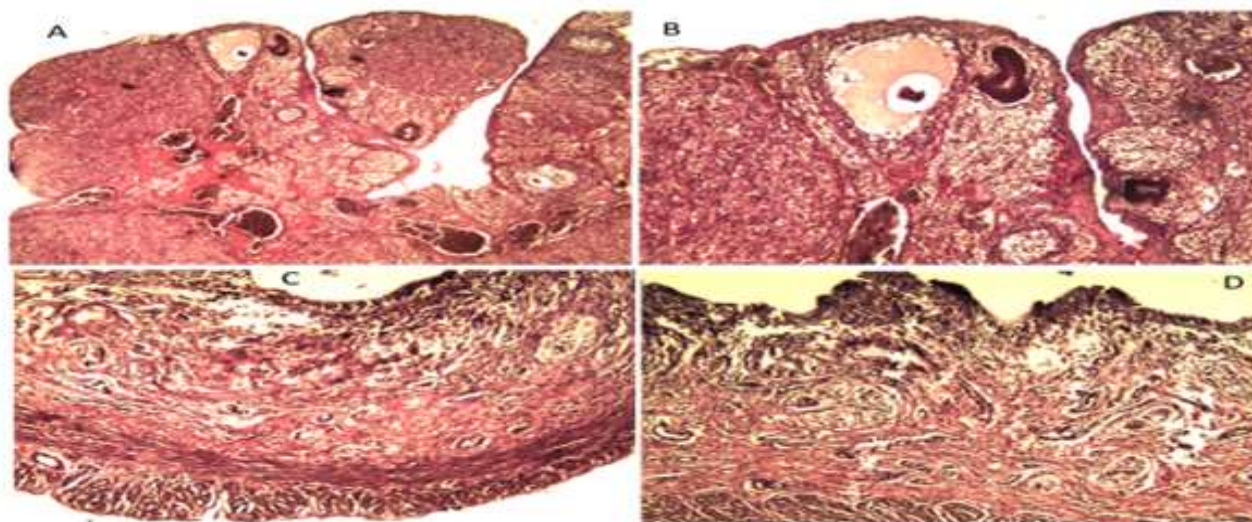
moderate numbers of sub-epithelial layer-based uterine glands, normal simple lining columnar cells, low numbers of hemorrhages, normal proliferating smooth muscle fibers, Figure 2C. Moreover, the results showed normal simple lining columnar cells and the presence of uterine glands in the sub-epithelial layers of the uteri, Figure 2D.



**Figure 2:** The effects of *Nigella sativa* plus cimetidine in the NCG. In the case of NCG, the ovary showed moderate follicle development processes in which huge numbers of small primary and secondary follicles, large corpus luteum, and stromal hemorrhages were present, figure A 4X H&E. In addition, presence of stromal corpus luteum and primary and secondary follicles, figure B 10X H&E. In the uterus, the effects revealed moderate numbers of sub-epithelial layer-based uterine glands, normal simple lining columnar cells, low numbers of hemorrhages, normal proliferating smooth muscle fibers, figure C 4X H&E. Moreover, the results showed normal simple lining columnar cells and the presence of uterine glands in the sub-epithelial layers of the uteri, figure D 10X H&E

For the use of *Nigella sativa* alone in the NG, the effects on the ovaries showed various pronounced normal signs of follicle developments of mature and secondary follicles, presence of stromal hemorrhages, and the presence of corpus luteum, figure 3A. Moreover, large mature, secondary follicles, marked corpus luteum, stromal hemorrhages were present, Figure 3B. In the case of uterus, the results demonstrated the

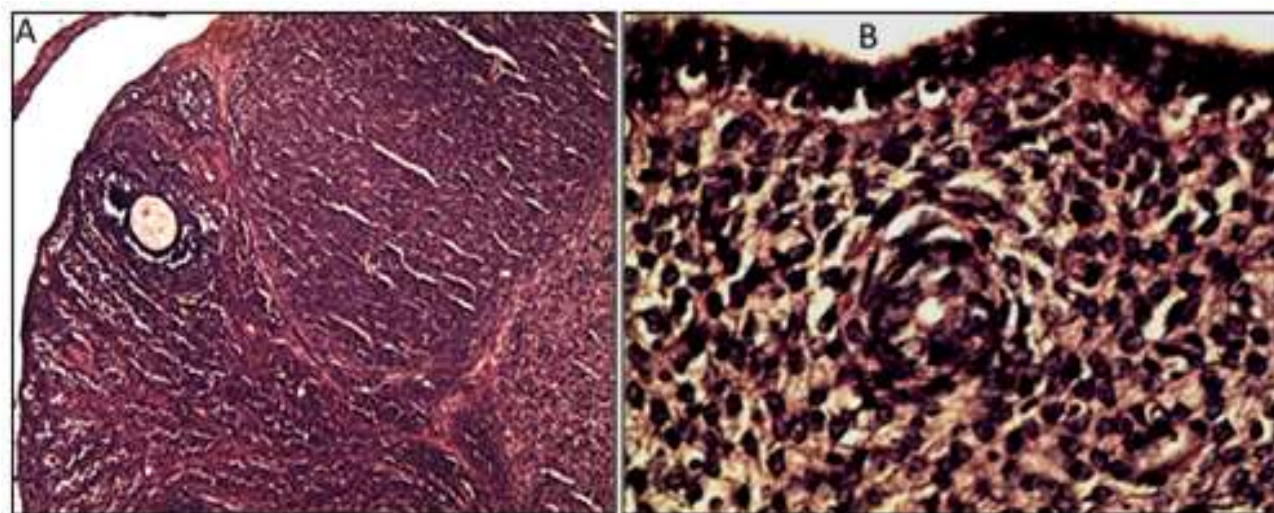
presence of normal proliferation of epithelial cells, normal thick wall, high numbers of sub-epithelial-based uterine glands, thick longitudinal and transverse smooth muscle fibers, Figure 3C. In addition, there were high proliferations in the epithelial cells, high numbers of sub-epithelial-based uterine glands, and thick smooth muscle fibers, Figure 3D.



**Figure 3:** The effects of *Nigella sativa* alone in the NG. The effects on the ovaries showed various pronounced normal signs of follicle developments of mature and secondary follicles, presence of stromal hemorrhages, and the presence of corpus luteum, figure A 4X H&E. Moreover, large mature, secondary follicles, marked corpus luteum, stromal hemorrhages were present, figure B 10X H&E. In the case of uterus, the results demonstrated the presence of normal proliferation of epithelial cells, normal thick wall, high numbers of sub-epithelial-based uterine glands, thick longitudinal and transverse smooth muscle fibers; figure C 4X H&E. In addition, there was high proliferation in the epithelial cells, high numbers of sub-epithelial-based uterine glands, and thick smooth muscle fibers, figure D 10X H&E

For the CG, there were no changes noticed in the ovary and uterus,

Figure 4A & B respectively.



**Figure 4:** The CG. A. Normal stroma is present that reveals low follicular growth with secondary follicles only and large corpus luteum 4XH&E. B. The lining of the uteri shows normal epithelial cells and profuse and mature sub-epithelial-based uterine glands 40XH&E

## Discussion

In most cases, cimetidine is used as an anti-gastric acid medicine, histamine H<sub>2</sub> receptor antagonist, to reduce the symptoms of duodenal ulcer and the Zollinger-Ellison syndrome [1,2]. Beside these uses, cimetidine also was utilized as an anti-cancer medicine that works alone or in combinations with other medicines on wide-range types of cancers [3]. The problem of over secretion of stomach acid could be reduced using cimetidine; however, because its use might be prescribed for long time, adverse effects might be generated [4]. To reduce the impact of these adverse effects, studied have been

launched globally to promote the use of cimetidine with less damages. The current study results showed detrimental effects produced by the use of cimetidine alone in the female mice. The use of this medicine was found to produce side effects that were related to nervous system in which agitation, confusion, auditory and visual hallucinations, delirium, psychosis, somnolence, and disorientation were generated [7].

The effects of cimetidine also induce changes in the permeability of membranes that were discovered by [8] who found that cimetidine affected the work of Na<sup>+</sup>/H<sup>+</sup> antiporter.

According to that, cimetidine produces side effects, and this agrees with the current study results. Here, *Nigella sativa* was successful in reducing the detrimental effects of cimetidine on female mouse ovaries and uteri. *Nigella sativa* was proved to generate beneficial and protecting effects in various researches using different toxicants[9,12].

The tartrazine, ethanol, and Aflatoxin B1 toxicities were reduced using *Nigella sativa*[13,15]. The study provides valued and important information about the use of *Nigella sativa* and its extracts in generating protection against the genital-based harmful changes induced by cimetidine.

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