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Histological and Biochemical Changes in The Uteri; *Centella Asiatica* as an Antifertility Agent

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<https://dx.doi.org/10.4314/sokjmls.v10i1.11>**Abstract**

This research investigated the adverse effect of *Centella asiatica* on the female reproductive system, particularly the uterus. The use of alternative medicine has gained more attention in recent years. Plants such as *Centella* are reported to possess several medicinal benefits, especially in nervous disorders. Predominantly known as "Gotu kola", *Centella asiatica* is found in the tropic and subtropical regions of the world. The study was experimented in forty (40) Wistar rats maintained under standard conditions. *Centella asiatica* was obtained from a local farm and extracted using ethanol maceration. Doses of the extract were scientifically calculated from the LD₅₀ and four (4) experimental groups were determined with n=10. The control was group A, while treated groups B, C, and D received 250mg, 500mg, and 1000mg/kg body weight of the extract respectively for 28 days. Half of the animals were mated to ascertain their fertility output at the end of the administration. The harvested uterus went through routine tissue processing after fixation. Some of the tissues were used for biochemical analysis of oxidative stress. Formalin-fixed tissues were stained using Haematoxylin and Eosin staining techniques. Data obtained was tested for significant differences between the control and treated groups. Results were statistically significant at $p < 0.05$. Oxidative stress parameters showed a decreased SOD and GSH with increased MDA. The pregnancy success rate of mated animals decreased in a dose-dependent manner. Histologically, there was a reduction in the number of uterine glands including desquamation of uterine epithelium in all treated groups.

Keywords: uterus, oxidative stress, reproduction**Introduction**

The global prevalence of infertility and the increasing demand for contraceptive solutions have drawn significant attention to the development and application of antifertility agents (WHO, 2021; Mascarenhas *et al.*, 2012). Traditional and modern approaches to contraception often come with side effects, ranging from hormonal imbalances to organ-specific pathologies, necessitating the exploration of alternative agents derived from natural sources. Among these, *Centella asiatica* (commonly known as Gotu Kola) has emerged as a potential antifertility agent due to its diverse pharmacological properties, including antioxidant, anti-inflammatory, and antiproliferative effects (James & Dubery, 2009; Shukla *et al.*, 2010). The pathophysiology of infertility and contraceptive interventions often involves uterine necrosis and oxidative stress. Uterine necrosis, characterized by the pathological death of uterine tissue, can result from ischaemic injury, chemical agents, or excessive inflammation (Zhao *et al.*, 2020). Oxidative stress, a condition arising from an imbalance between reactive oxygen species (ROS) production and the body's antioxidant defences is pivotal in exacerbating cellular and tissue damage in the uterus (Birben *et al.*, 2012). Together, these phenomena contribute significantly to reproductive dysfunction and necessitate targeted therapeutic strategies.

Centella asiatica has a long history of use in traditional medicine across various cultures for treating conditions such as wounds, ulcers, and memory deficits (Brinkhaus *et al.*, 2000). Recent studies have suggested that this plant exhibits bioactive compounds, such as asiaticoside, asiatic acid, and madecassoside, which may modulate reproductive functions and induce antifertility effects (Devkota *et al.*, 2010; Tiwari *et al.*, 2021). The antifertility potential of *Centella asiatica* appears to be mediated through its impact on hormonal regulation, oxidative stress mitigation, and direct effects on uterine tissue viability. Yunianto *et al.* (2017) investigated the antifertility properties of the ethanolic extract of *C. asiatica* on 32 male rats. The result showed that the treatment group had a higher percentage of infertile male (43.75%) when compared with the control (18.75%). In addition, abnormal sperm morphology and decreased sperm count was higher in the treatment group when compared with control. Furthermore, proteomic analysis was also done to determine the expression of some proteins which could play a role in male fertility. Some important proteins such as Alpha-enolase, Aldolase A, Sorbitol dehydrogenase, glutamine synthase and lipocalin were significantly down regulated or totally absent.

This study investigated the role of *Centella asiatica* as an antifertility agent, with a focus on its ability to induce uterine necrosis and modulate oxidative stress pathways. By exploring the molecular mechanisms underlying these effects, we seek to establish a scientific basis for the use of *Centella asiatica* in antifertility applications and to provide insights into its potential risks and benefits (Patil *et al.*, 2015; Chatterjee *et al.*, 2020). This understanding will contribute to the broader field of reproductive health and pharmacology, offering an alternative, natural-based approach to contraception.

In this context, the present research seeks to address the following critical questions:

1. What are the specific bioactive components of *Centella asiatica* responsible for its antifertility effects?
2. How does *Centella asiatica* influence uterine necrosis at the histopathological level?

3. What is the relationship between oxidative stress modulation and the antifertility effects of *Centella asiatica*?

Through these inquiries, we aim to bridge gaps in understanding *Centella asiatica*'s pharmacodynamics and evaluate its viability as a safe and effective antifertility agent.

Materials and Methods

Extract preparation

C. asiatica leaves were sourced from a farm in Rivers State, Nigeria. The leaves of *C. asiatica* were thoroughly cleaned, air-dried for some days to remove moisture, and ground to powder using an electric blender. The powdered leaves were thereafter macerated in 70% alcohol for 72 hours. Then, filtration was done using filter paper and the resulting filtrate was placed in a water bath for evaporation. The desired dry residue was weighed and kept in a refrigerator. Appropriate concentrations were constituted daily for administration.

Experimental Animals

A total of thirty-two adult female rats with an average weight of 142g were obtained from the Animal House of Anatomy Department, University of Benin. Cage bedding and water vessels were made available to the animals and cleaned daily. The animals acclimatized for two weeks before the commencement of administration of the extract. They were divided into four groups of eight (8) animals each. Group A served as the control while B, C, and D were the treatment groups.

Administration of Extract

The body weight of the rats was measured at the onset of this study. The values obtained were used to determine the appropriate dosage of the extract. The extract of *C. asiatica* was orally administered with the aid of an orogastric tube daily for 28 days. The four groups of animals received the following protocol:

Group A: (Control) received standard feed and water.

Group B: received 250mg/kg body weight of the extract

Group C: received 500mg/kg body weight of the extract

Group D: received 1000mg/kg body weight of the extract

Mating of Animals

A vaginal smear was collected using a 10-microlitre pipette filled with normal saline, placed on a slide, and examined under a light microscope. On the morning before mating, only animals who were in the proestrus phase of the cycle (characterized by dominant uniformly nucleated epithelial cells) were introduced to male animals. The presence of spermatozoa in the smear on the morning after mating and/or the presence of a vaginal plug was used to confirm pregnancy, and that day is taken as gestation day 0 (GD 0) (Cora *et al.*, 2015).

Histological Preparation

At the end of the experiment, the animals were sacrificed. The uterus was harvested and fixed in 10% formal saline. The tissues were processed and stained using hematoxylin/eosin dye as described by Drury and Wallington (1980). Photomicrographs of stained tissues were taken

using an Olympus microscope at magnifications of 40x and 100x.

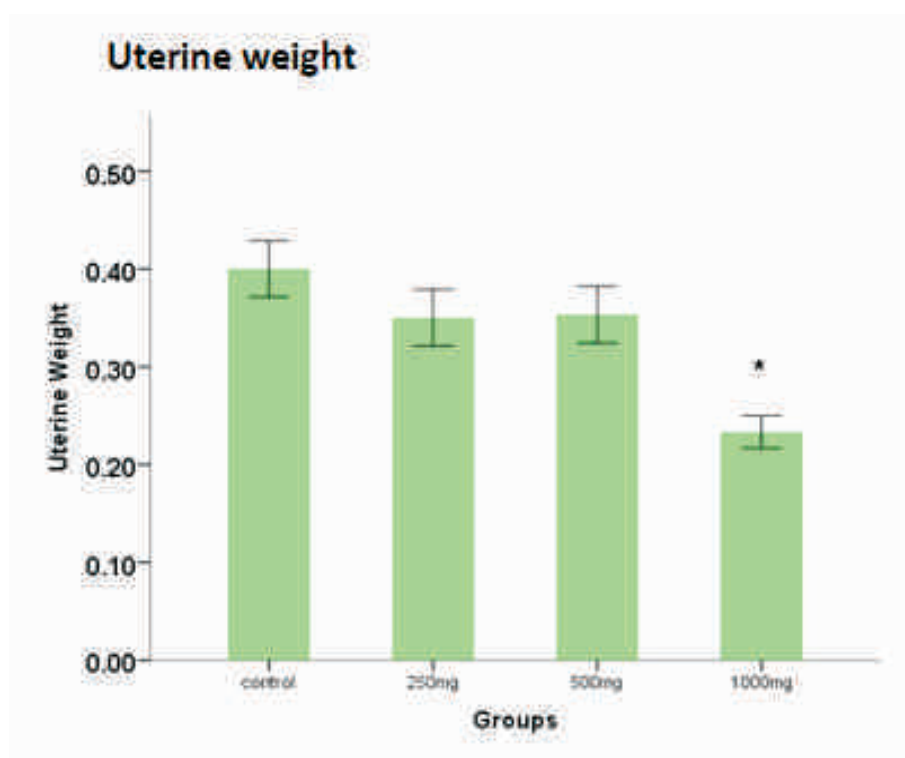
Biochemical Analysis

Uterine tissues obtained after sacrifice were placed in normal saline and processed for oxidative stress analysis. Evaluation of antioxidant enzymes in the uterus was done according to the methods previously described for malondialdehyde, superoxide dismutase (Misra and Fridorich, 1975); catalase (Cohen *et al.*, 1970), and glutathione peroxidase (Nyman, 1959).

Statistical Analysis

Data obtained was expressed as mean \pm SEM and analyzed using one-way analysis of variance (ANOVA) with the IBM statistical package for social science (SPSS) version 26 (IBM Corp., Armonk, N.Y., USA) statistical software (version 8.0.2). Graphs were used to present all results and $P < 0.05$ was considered statistically significant.

Results

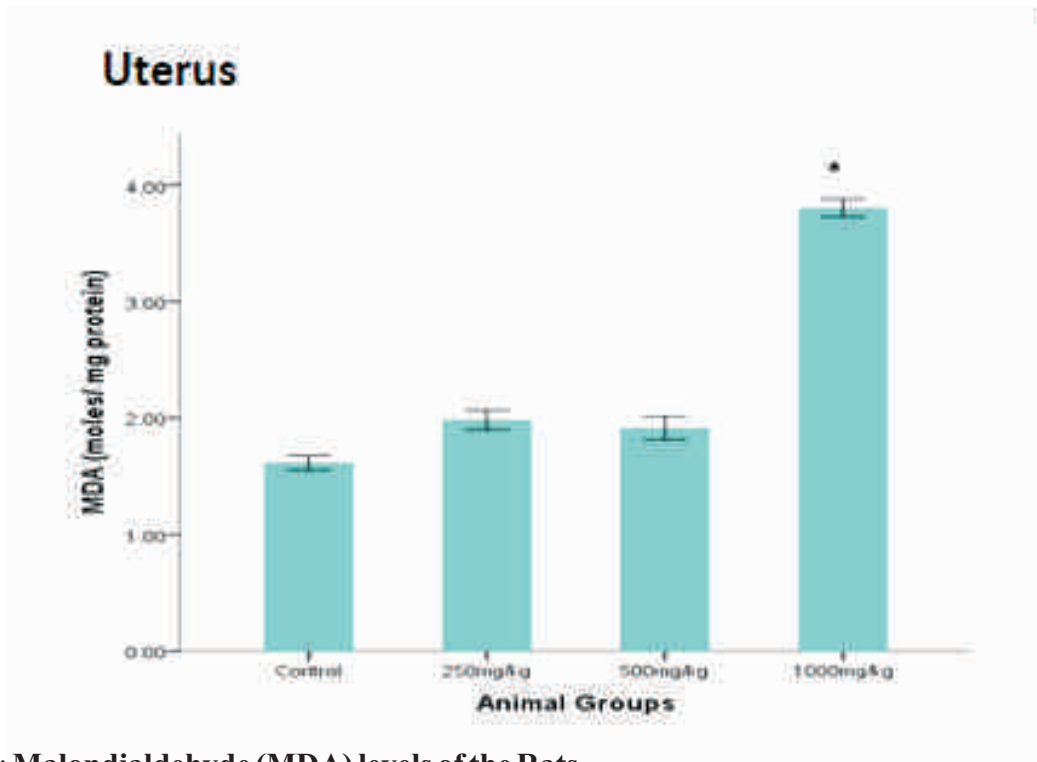


Key: *- indicates $p < 0.05$

Graph 1: Uterine weights of the Rats

The uterine weights highlighted a significant decrease in the weights of 1000mg $p = 0.002$. Groups 250mg and 500mg had no significant decrease $p = 0.217$ and $p = 0.247$ respectively.

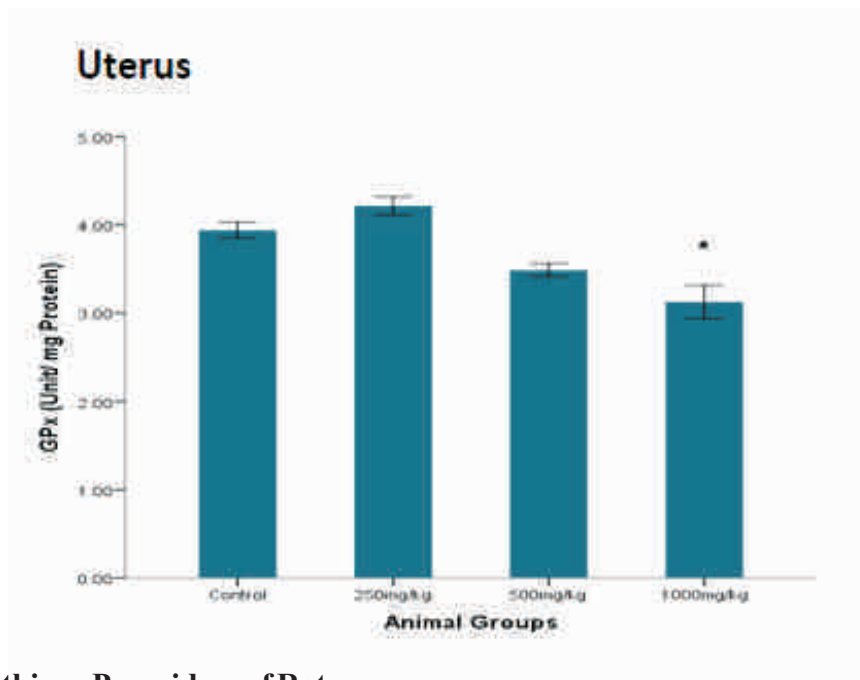
Oxidative Stress Analysis (Uterus)
Malondialdehyde



Graph 2: Malondialdehyde (MDA) levels of the Rats

A statistical increase in the group administered 1000mg p=0.039 was observed. However, the 250mg and 500mg groups had no statistical decrease p=0.758 and 0.679 respectively.

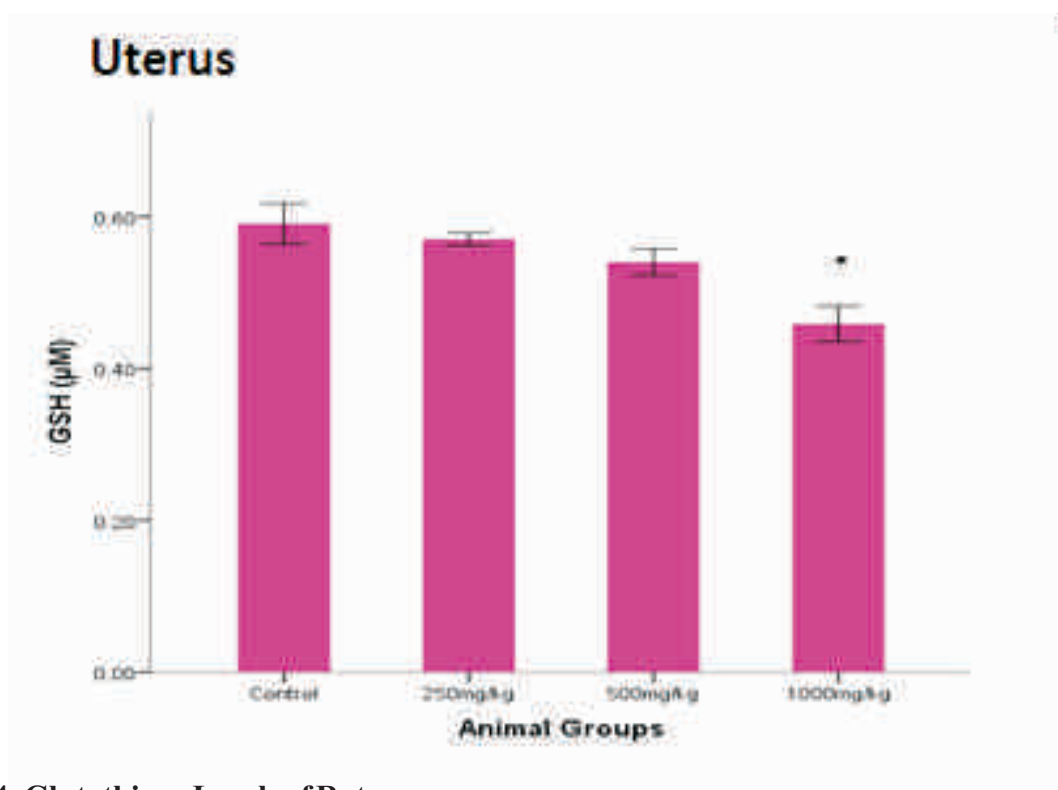
Glutathione peroxidase



Graph 3: Glutathione Peroxidase of Rats

A statistical decrease in the group administered 1000mg p=0.039 was observed. Groups 250mg, and 500mg had no statistical decrease p=0.431 and 0.56 respectively.

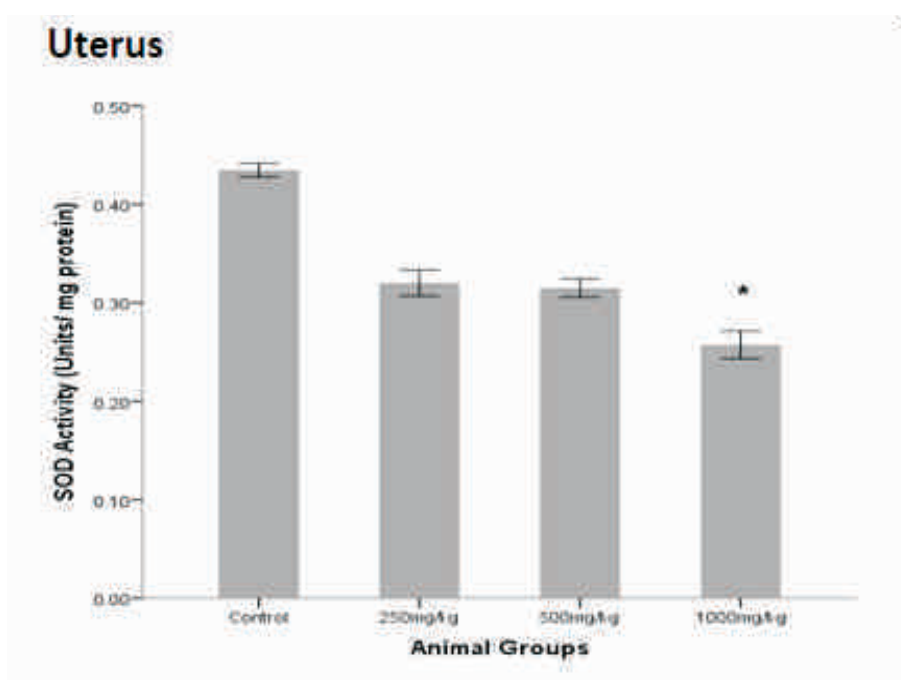
Glutathione



Graph 4: Glutathione Levels of Rats

A statistical decrease in the group administered 1000mg $p=0.040$ was observed. However, the 250mg and 500mg groups had no statistical decrease $p=0.748$ and 0.430 respectively.

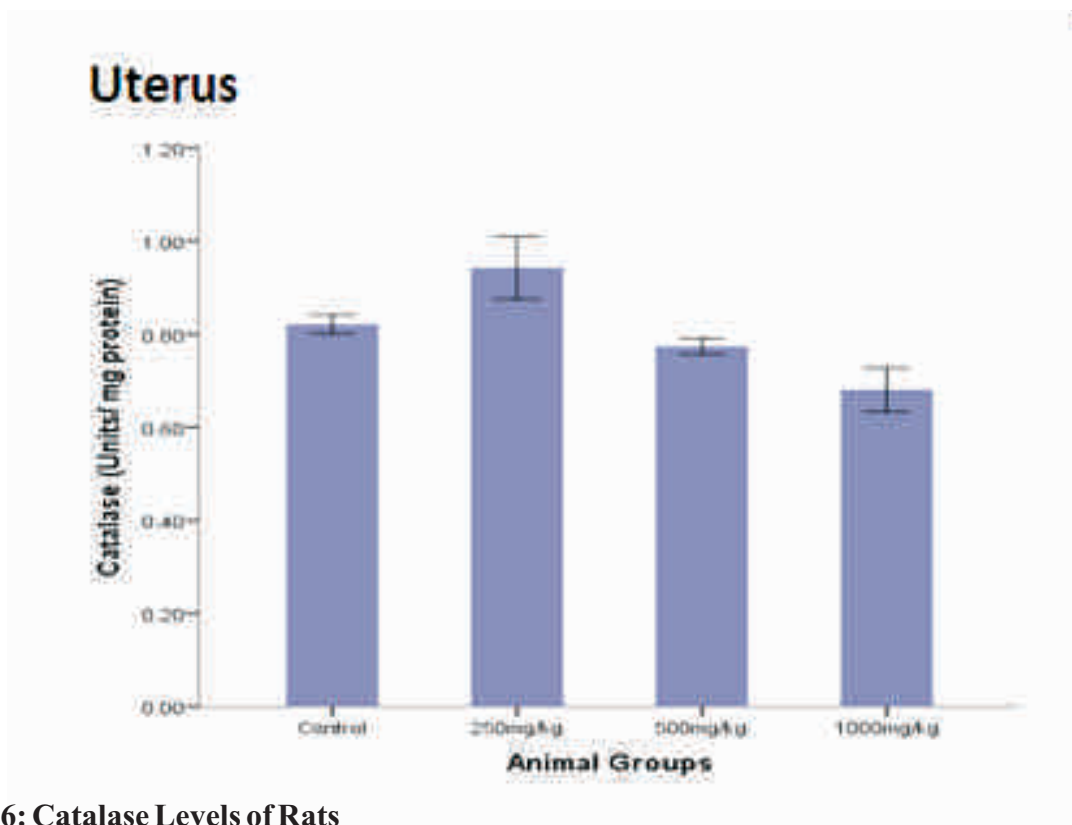
Superoxide Dismutase



Graph 5: Superoxide Dismutase Levels of Rats

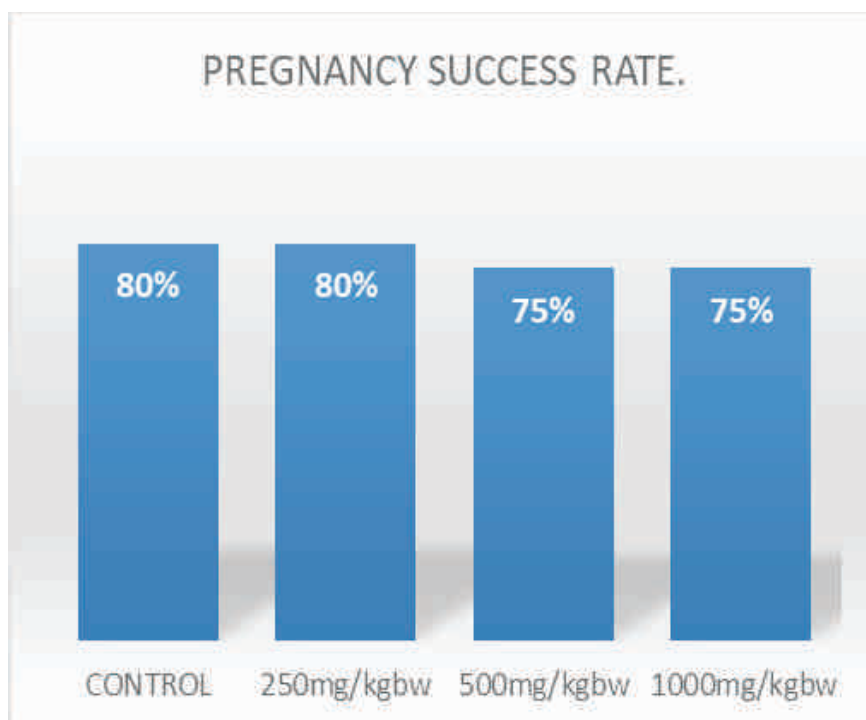
There was a statistically significant decrease in the SOD in the group administered 1000mg $p=0.004$. However, the 250mg and 500mg had no statistical decrease $p=0.058$ and 0.491 respectively.

Catalase



Graph 6: Catalase Levels of Rats

No statistically significant decrease in the level of catalase in all treated groups was observed $p=0.416$, $p=0.382$, and $p=0.065$ for 250mg, 500mg, and 1000mg respectively.



Graph 7: Pregnancy Success Rate in the Rats

Pregnancy success rate: this was highest in controls and low dose (250mg) with a value of 80%, while the 500mg and 1000mg had a value of 75% each.

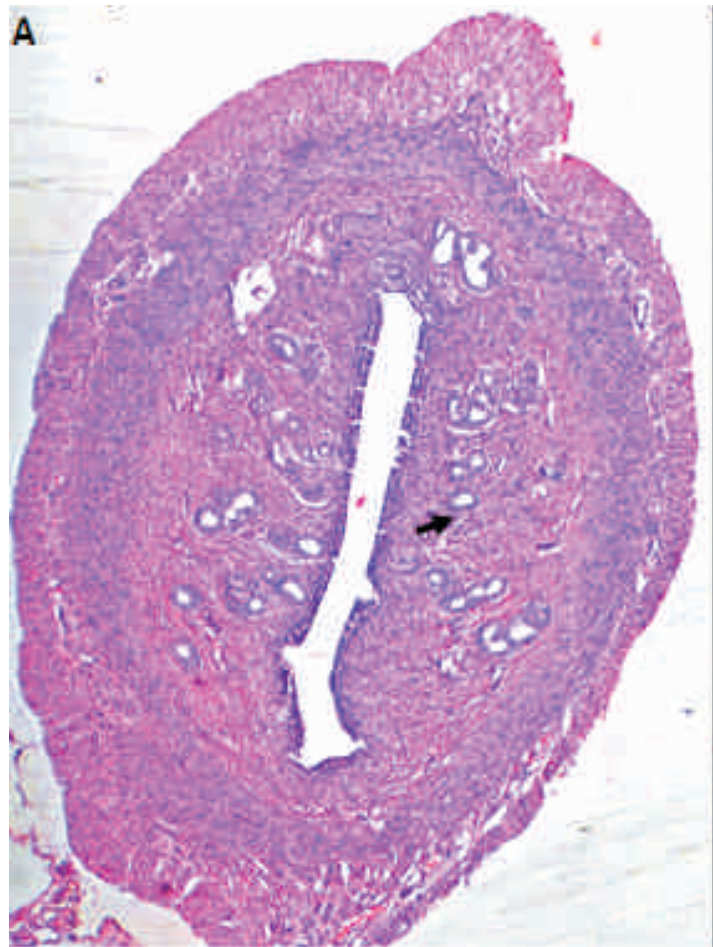


Plate 1: Photomicrograph of Uterus 40x: A-control shows normal uterine architecture with abundant uterine glands (arrow) and thickened endometrial lining

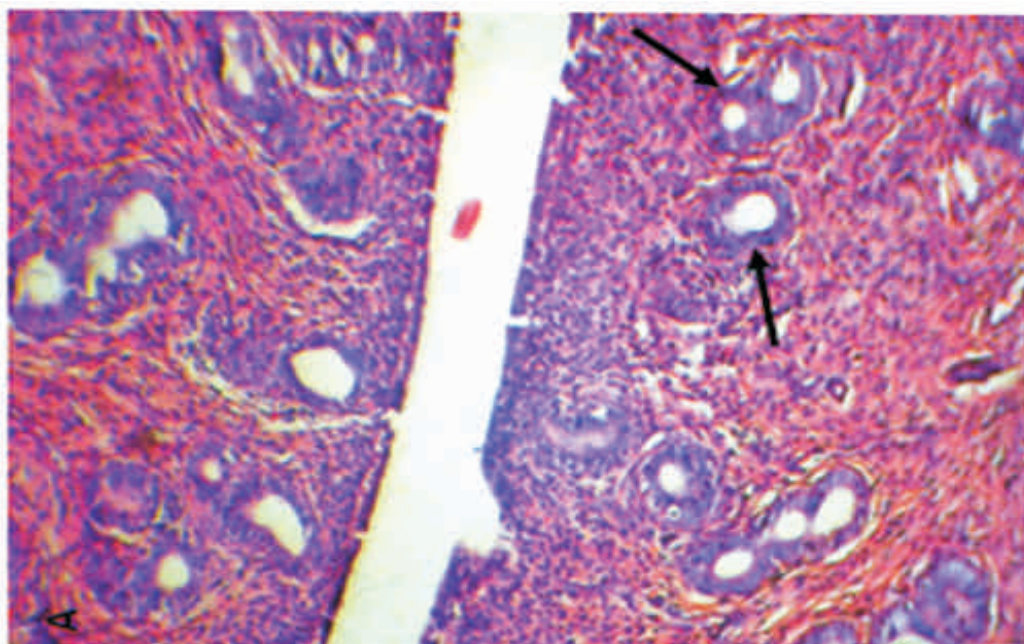


Plate 2: Photomicrograph of Uterus 100x: A-control shows normal uterine architecture with abundant uterine glands (arrow) and thickened endometrium with regularly lined epithelium.

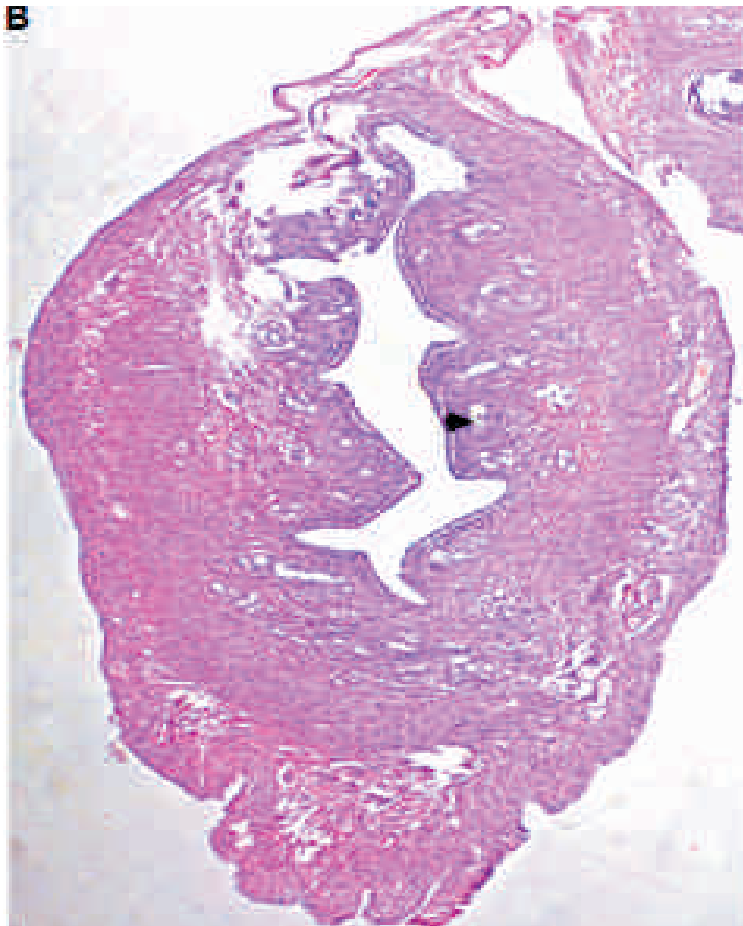


Plate 3: Photomicrograph of Uterus 40x: B- 250mg/kg dose shows reduced endometrial thickness with fewer uterine glands (arrow) as well as vacuolation in the endometrial epithelial lining.

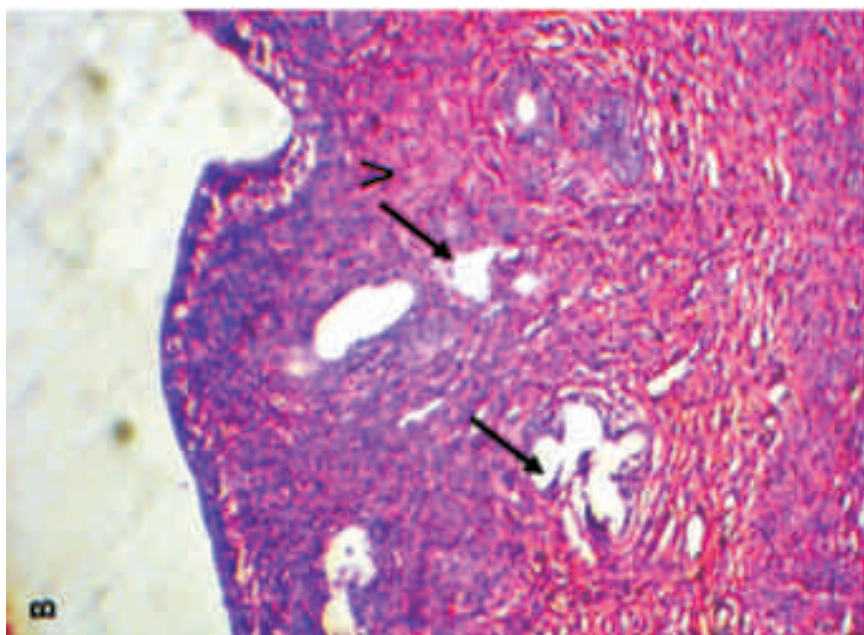


Plate 4: Photomicrograph of Uterus 100x: B- 250mg/kg dose shows reduced endometrial thickness with fewer uterine glands (arrow) as well as vacuolation (V) in the endometrial epithelial lining.

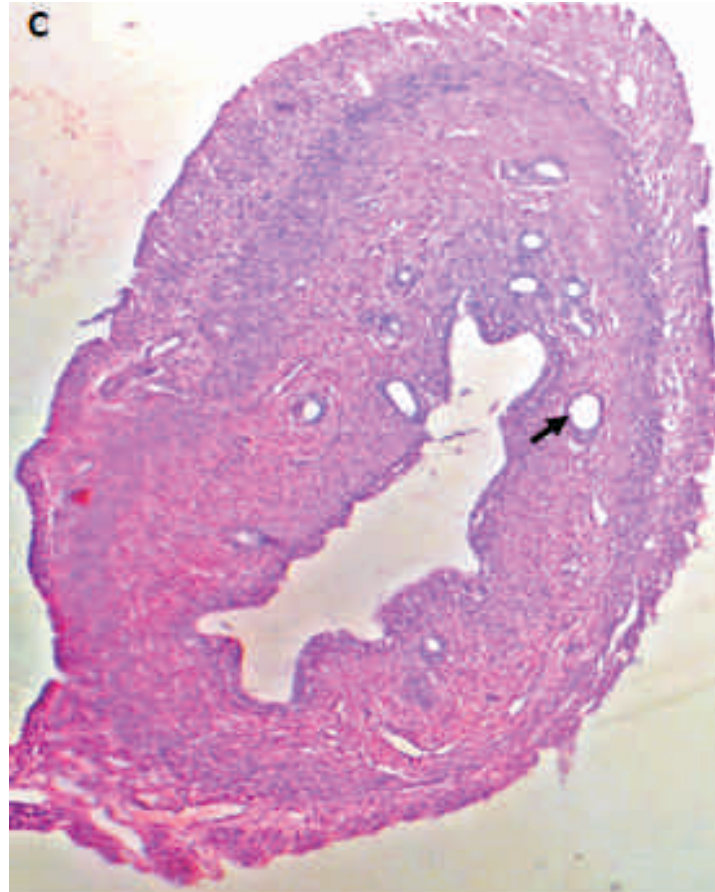


Plate 5: Photomicrograph of Uterus 40x: C-500mg/kg dose; shows reduced endometrial thickness with fewer uterine glands (arrow) and tortuous endometrial epithelium.

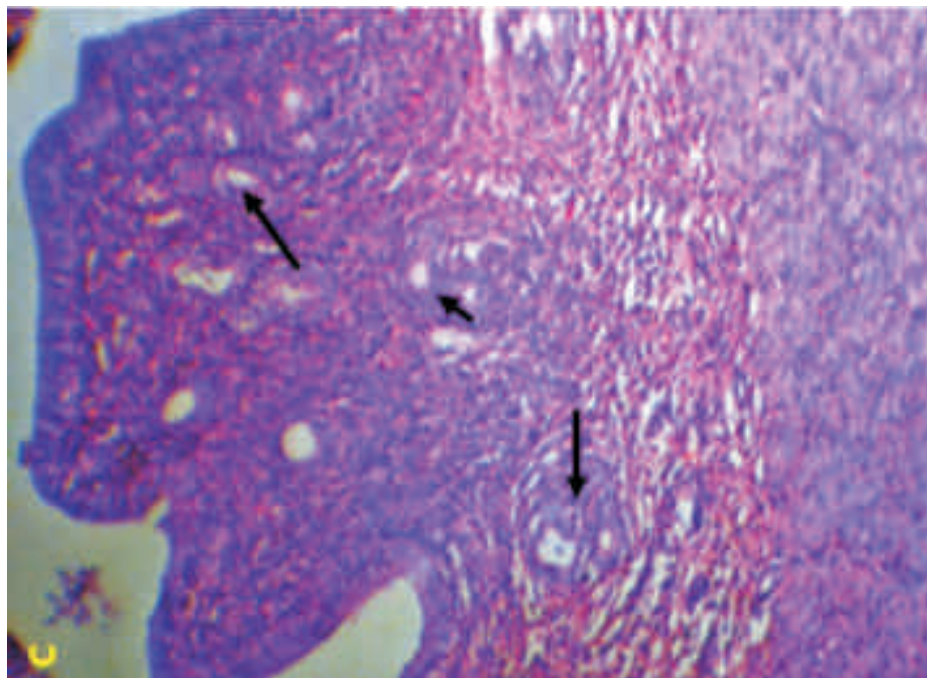


Plate 6: Photomicrograph of Uterus 100x: C-500mg/kg dose; shows reduced endometrial thickness with fewer uterine glands (arrow).

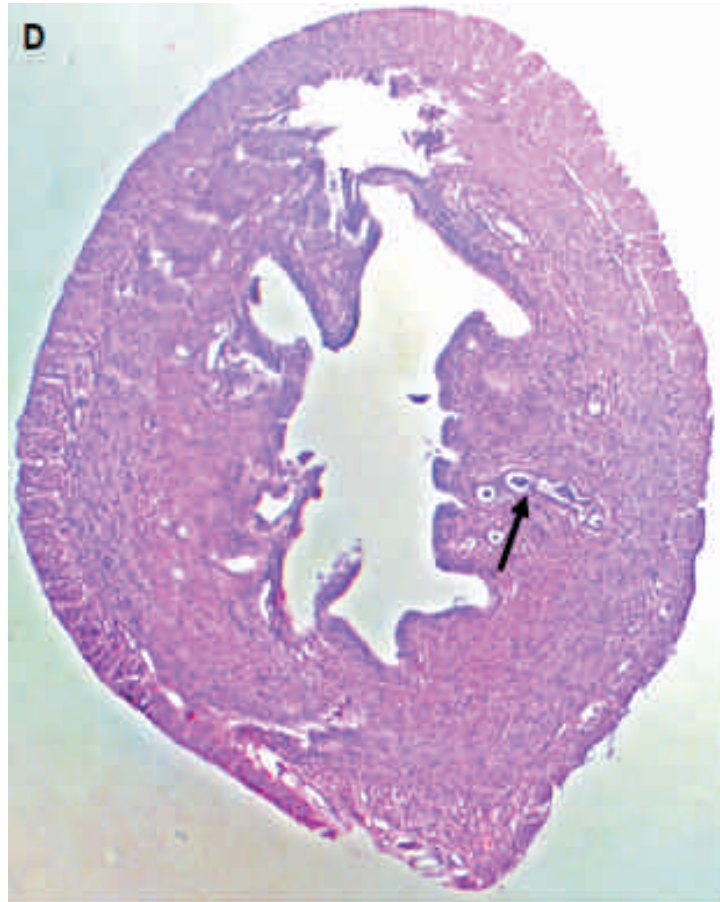


Plate 7: Photomicrograph of Uterus 40x: D-1000mg/kg dose; high dose shows reduced endometrial wall with fewer uterine glands and desquamation of the lining of the glands.

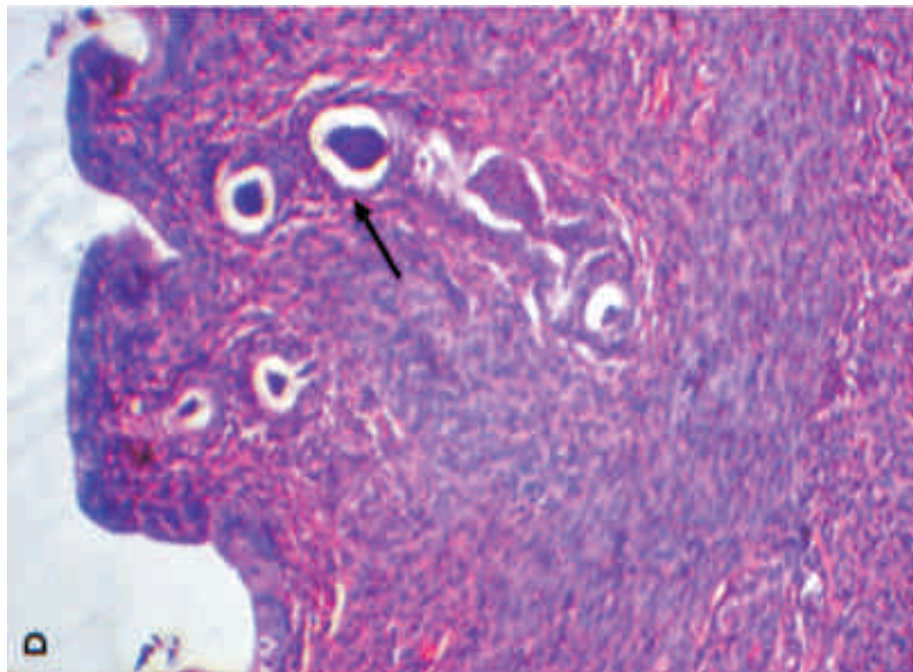


Plate 8: Photomicrograph of Uterus 100x: D-1000mg/kg dose; high dose shows reduced endometrial wall with fewer uterine glands and desquamation of the lining of the glands.

Discussion

Decreased uterine weight following administration of *C. asiatica* at a dose of 1000mg/kg bw was seen in our study. This was in contrast to Bayyinatul *et al.* (2018) who reported that a combination of leaf extracts of *C. asiatica* and *P. indica* did not cause a significant decrease in the uterine weights of treated animals at doses of 25mg-200mg/kg body weight. This contrast may be due in part to the duration of administration and the compensatory effect of *P. indica* against the cytotoxic effect of *C. asiatica* as the flavonoids in *P. indica* have been reported to increase endometrial and myometrial thickness through their antioxidant properties. The thickness of the endometrium and the mucus secreted by the uterine gland plays a significant impact on the weight of the uterus. Our study showed that both were adversely affected by the extract which is a possible reason for the decreased weight observed from this study. Additionally, weight reduction was only significant at the highest dose, emphasizing that the cytotoxic effect was due to a toxic amount of *C. asiatica*. This also agrees with Bayyinatul *et al.* (2018), who reported that a dose of *C. asiatica* at 200mg/kg bodyweight did not affect the uterine weight.

C. asiatica has been reported to be a phytoestrogen. The term "phytoestrogen" describes a class of polyphenolic compounds that are derived from dietary plants that are not endogenous. This family includes members like isoflavones and flavones (Kaempferol and quercetin). Due to structural similarities with estradiol (E2, 17 β -estradiol), the mechanism of phytoestrogen bioactivity may include interaction with estrogen receptors (ERs) (Turner *et al.*, 2007). Zhao and Mu (2011) showed that estrogenic activity affects the proliferation of stromal and epithelial cells in the female reproductive system. The triterpenoid saponins like those found in *C. asiatica* also can occupy estrogen receptors and have effects comparable to those of natural estrogen, while not having the same structure as a hormone.

The oxidative stress results showed that *C. asiatica* significantly increased the amount of malondialdehyde and reduced the level of

superoxide dismutase, catalase, and glutathione in both the uterus. This result is in contrast to Bayyinatul *et al.* (2018) who reported a significant decrease in malondialdehyde and an increase in superoxide dismutase when the combination of *C. asiatica* and *P. indica* leaf extract was administered in equal doses of 25mg-200mg/kg body weight. This difference in oxidative stress is probably due to the ameliorative effect *P. indica* against oxidative stress induced by *C. asiatica*. This may also be due to the low dose (200mg/kg bw) of the extract as that was administered compared to what was used for this current study (250 mg-1000mg/kg bw). Malondialdehyde is a highly toxic compound, a byproduct of fatty acid peroxidation. It is a reactive oxygen species and one of the biomarkers for oxidative stress. Reactive oxygen species can be both beneficial and harmful (Sies *et al.*, 2017). They are involved in most physiological activities including folliculogenesis. When oxidative damage exceeds the capacity for repair, oxidative stress occurs. Oxidative stress occurs when there is an imbalance between the antioxidants and pro-oxidants, with more of the latter. Such imbalance alters enzyme activities and molecular signaling pathways, leading to damage of the tissues (Forman and Zhang, 2021). The increased MDA and reduced SOD in this study may be an indication of increased apoptotic activity occurring in the uterus.

On the histology of the uterus, our findings revealed a reduction in the thickness of the endometrial lining as well as the number of endometrial glands in all treated groups compared to the control. The thickness of the endometrium is influenced by the action of reproductive hormones. At ovulation, an increase in progesterone and estrogen causes the endometrial lining to thicken and also increases the number of endometrial glands. The reduction in the progesterone levels seen in our study could lead to degeneration of the endometrium mimicking the menstrual phase and this could eventually result in implantation failure if fertilization occurs. This agrees with Bayyinatul *et al.* (2018) who reported decrease in endometrial thickness and glands following administration of 125mg and 200mg/kg bw of *C.*

asiatica and *P. indica*. Research on rats has demonstrated that uterine glands and their secretions are vital for the development of uterine receptivity and blastocyst implantation. They may also have an impact on the activation of blastocyst trophoblast and decidualization of stromal cells in the uterus. Likewise, in humans, uterine gland histotroph is essential for blastocyst implantation, uterine receptivity, and conceptus nourishment in the first trimester, and uterine glands are possibly associated with the decidualization of stromal cells (Filant and Spencer, 2014). The deficiency of uterine glands and the crucial secreted substances they produce, such as Lukemia Inhibitory Factor (LIF) and other factors, is thought to be the reason why blastocyst implantation failed in the uteri of Progesterone-uterine gland knockout (PUGKO) mice (Filant and Spencer, 2013). Comparably using an artificial model, mice with conditional ablation of *Foxa2* have a uterus with much fewer glands and show severe abnormalities in both blastocyst implantation and stromal cell decidualization (Jeong *et al.*, 2010).

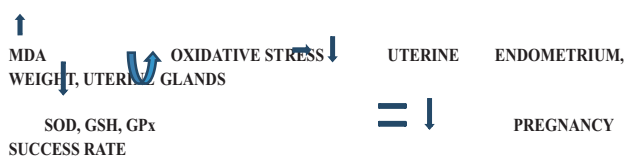
This study also assessed the pregnancy success rates of mated animals. The Pregnancy success rate is the ability to achieve conception upon mating. This parameter was evaluated, and the result showed a significant decrease in the pregnancy success rate in all treated groups, compared to the control. These findings are expected due to the aforementioned oxidative stress effects on the uterus, which could have impaired the fertility of the animals. Oxidative stress, a condition arising from an imbalance between reactive oxygen species (ROS) production and the body's antioxidant defenses is pivotal in exacerbating cellular and tissue damage in the uterus (Birben *et al.*, 2012). Together, these phenomena contribute significantly to reproductive dysfunction and necessitate targeted therapeutic strategies.

Conclusion

Our findings revealed that ethanol extract of *Centella asiatica* leaves exerts an antifertility effect on the uteri of Wistar rats through induction of oxidative stress and derailment of the histoarchitecture from its normal state, expressed as epithelial alteration and reduction in the number

of uterine glands. These effects were observed to be dose-dependent as higher doses revealed greater effects when compared to lower doses.

Mechanism of Action of Extract



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Conflict of Interest

None declared

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