Greener Journal of Biomedical and Health Sciences

Vol. 7(1), pp. 73-79, 2024

ISSN: 2672-4529

https://gjournals.org/GJBHS

DOI: https://doi.org/10.15580/gjbhs.2024.1.111924170

Effects of *Syzygium aromaticum* (Cloves) extract on some reproductive hormones and oxidative stress markers in female wistar rats

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ARTICLE INFO

Article No.: *111924170*
Type: *Research*
Full Text: *PDF, PHP, HTML, EPUB, MP3*
DOI: *10.15580/gjbhs.2024.1.111924170*

Accepted: *21/11/2024*
Published: *30/11/2024*

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Keywords: *Cloves, estrogen, progesterone, supraoxide, Malondiadehyde, wistar rats*

ABSTRACT

Syzygium aromaticum **which is commonly called cloves is one of the most commonly used spices around the world including Nigeria as preservative, medicine as well as in the preparation of various spicy rich dishes. Therefore, the present study was conducted to find out the effects of Cloves extract on some reproductive hormones and oxidative stress levels in female wistar rats. Twenty (20) female Wistar after acclimatization for two weeks were randomly divided into four groups with five rats in each group respectively. Group 1 was the control group and received distilled water daily, Group 2 were the low dose group which received clove extracts 1ml/rat/day, Group 3 were moderate dose which received clove extract of 1.5ml/rat/day and Group 4 were high dose clove extract group which received 2ml/rats/day. This administration was done once daily by oral gavage for a period of 28 days. The animals were weighed twice a week and their weights were recorded to determine change in body weight of the rats. At the end of the experiment blood samples were collected for analysis. The results obtained showed increase in estrogen and progesterone levels and also there were a change in superoxide dismutase (SOD) and malondialdehyde (MAD) levels across the treated groups but showed no statistically significant elevations compared to their respective control groups. Thus, our findings indicate that high doses of cloves may have an impact on reproductive hormone levels in female Wister rats.**

INTRODUCTION

Clove (*Syzygium aromaticum*) is one of the most commonly used spices around the world including Nigeria in Africa as preservative, medicine as well as in the preparation of various spicy rich dishes. The clove tree is a perennial tropical plant which grows to a height of about 10- 20meters, having large oval leaves and crimson flowers in numerous groups of terminal clusters. Clove bud is the dried, unopened, nail-shaped, flower bud of the evergreen *Syzygium aromaticum* tree. Clove bud is aromatic, a stimulant and carminative and is used for dyspepsia and gastric irritation (Ahmed et al, 1986). In addition to its flavoring, pungent and fragrant properties as well as culinary uses, the clove bud and its oil have an abundance of medicinal and recreational uses. The use of clove bud in folk medicine as diuretic, odontalgic, stomachic, tonicardiac, aromatic condiment and condiment with carminative and stimulant activity has been reported (Boulos 1983). It is also used in dentistry as a natural analgaesic and antiseptic due to its possession of eugenol; its major component (Rathi et al., 2012). Clove oil; an essential oil derived from the dried flower buds, leaves and stems of *Syzygium aromaticum* tree has a spicy aroma which makes it a sought after ingredient for food flavoring, fragrance formulations as well as in the production of soaps and detergents. The insecticidal properties of clove oil have been studied against lice, weevils, moth caterpillars, beetles and cockroaches (El-Raouf et al., 2019). It has also been found to possess mosquito-repelling properties (Saurabh et al., 2024) and (Bahmanpour et al., 2015) Clove oil contains several compounds with eugenol been the major component. Other compounds that have been determined to be present in clove oil include β-caryophyllene, eugenyl acetate, α-humulene and others. The oil of clove has also been reported to be a good remedy for bronchitis, the common 2 cold cough, fever and sore throat. Clove buds extracts and especially its oil have been reported to possess anti-oxidant, anti-fungi, antiviral, anti-microbial, anti-diabetic, anti-inflammatory, anti-thrombotic, anesthetic, pain-relieving and insect repellent properties (Pramod et al., 2010) and (Vaney et al., 2024) Some Polyphenols such as Gallic acid, Ellagic acid, Quercetin glycoside, Ellagic acid derivative and some unidentified phenolic compounds have been characterized and reported to be present in clove buds(Ahmed et al., 2016) The reproductive health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity, all matters relating to the reproductive system and its function and processes. Reproductive health implies that people are able to have a satisfying and safe sex and they have the capability to reproduce and the freedom to decide if, and how often to do so. (According to WHO). In women the reproductive health depends on maintaining coordinated responses of network of endocrine signals that functions primarily to ensure successful procreation but also have other wide- ranging influences on the female body, including not only secondary reproductive tissues such as the breast, but also nonreproductive tissues such as the bone, brain and cardiovascular system. Reproductive health is a crucial aspect of overall wellbeing, and any disruption or imbalance in the reproductive system can have significant implications for fertility and overall health in females. Understanding the effects of natural substances, such as cloves, on the reproductive parameters is therefore important for both clinical and research purposes. Previous studies have demonstrated the estrogenic properties of certain phytochemicals found in cloves, such as eugenol and flavonoids. Estrogen is a key hormone in the regulation of female reproductive functions and is involved in a wide range of processes, including the growth and development or reproductive organs, follliculogenesis, ovulation, and maintenance of pregnancy. The intricate relationship between natural substance and reproductive health has 3 been a subject of growing interest in scientific inquiry. Understanding the effect of graded doses of cloves on the uterus and fallopian tubes is of particular importance in elucidating potential implications for reproductive health. The uterus, a central organ in female reproductive system plays a pivotal role in fertility and gestation, while the fallopian tube facilitates the crucial early stage of embryonic development investigating the impact of cloves on these vital components offers valuable insight into then influence on female reproductive physiology.

MATERIALS AND METHODS

Animal care

A total of twenty healthy wistar rats weighing 150-200grams were used in the study and they were acquired from laboratory animal house of University of Port Harcourt. The animals were housed in a hygienic and well ventilated compartment maintained under standard environmental conditions and fed with normal rodent chew and allowed access to drinking water for fourteen days to allow acclimatization before the commencement of the experiment.

Preparation of extract

A total five grams (5gm) of the powdered herbs and 20mL of distilled water were added into conical flasks. The flasks were covered with a wooden cork and contents of the flasks were mixed thoroughly. The flasks were placed at shaker adjusted at 100 rpm overnight. The mixtures were then filtered through a muslin cloth and centrifuged at 2000rpm for 5 min and supernatant was transferred into sterile falcon tube after filtration and stored at 4°C in refrigerator.

Ethical Approval

Ethical consideration was gotten from the Board of The Department of Human Physiology Bayelsa Medical University, Yenagoa, Bayelsa State.

Experimental procedure:

After two weeks of acclimatization, the rats were weighed and assigned to their respective groups. The twenty-adult female wistar rats were allocated into four groups (1, 2, 3 and 4) of five animals each. During the experiment, Group 1 served as the control group receiving no extract while Groups 2, 3 and 4 each received 1ml, 1.5ml and 2ml each day respectively for a period of 28 days by oral administration and the weight of the animals were recorded twice a week. During the treatment period, the rats were closely monitored for any signs of distress or adverse effects. Group 1 (Control group) receiving no cloves just distilled water. Group 2, received low dose of cloves extract. Group 3, received moderate dose of clove extract while Group 4, received high dose of clove extract.

Collection of samples

At the end of the experimental period, the animals were allowed to fast for 12 hours and thereafter they were sacrificed. The organs from the rats were harvested and blood samples were collected by cardiac puncture using sterile needles and syringes. Portion of blood were transferred in a sterile plain sample bottles and used for the determination of oxidative parameters and hormonal analysis. Blood samples will be analyzed for levels of estrogen, progesterone using ELISA or similar techniques. And for the oxidative stress, tissue samples will be used to measure oxidative stress markers such as superoxide dismutase, malondialdehyde levels as a lipid peroxidation marker.

Statistical Analyses

Version 21.0 of the IBM Statistical Product and Service Solutions (SPSS) software was used to analysis the quantitative data obtained from the present study. One-way analysis of variance (ANOVA) followed by LSD Post Hoc tool were used to establish statistical significance, and ($P<0.05$) indicated the threshold for statistical significance. Mean and standard error of the mean (SEM) were used to represent the values.

RESULTS

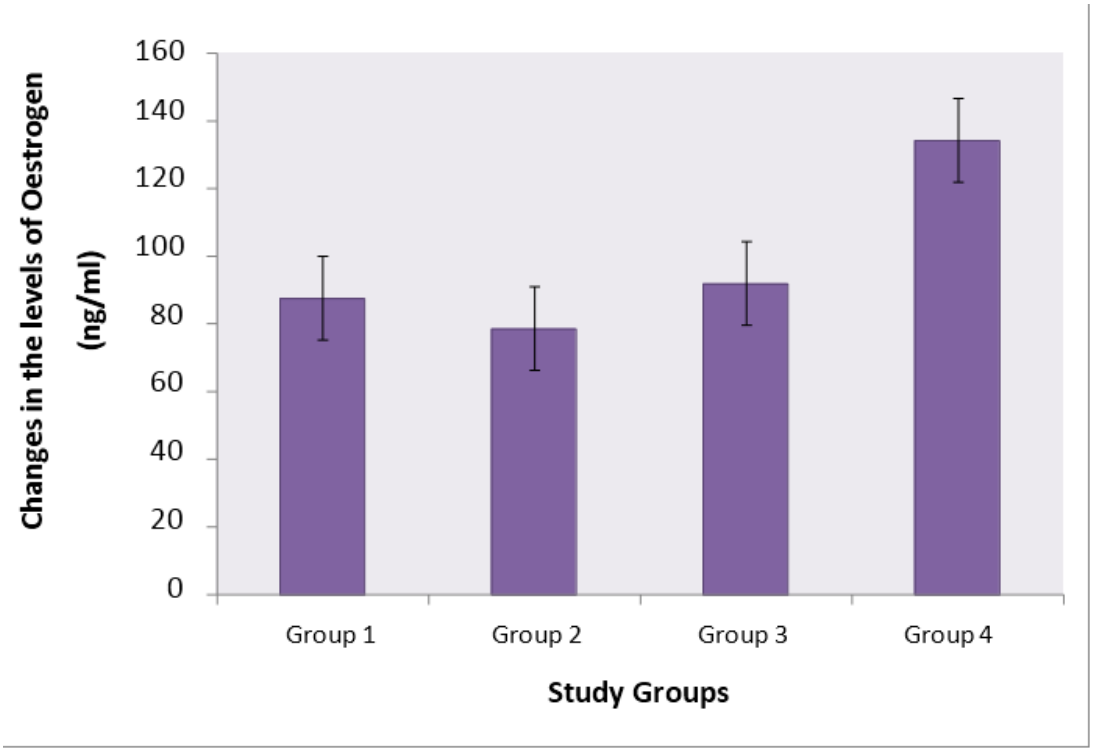

Figure 4.1: Effects of *Syzygium aromaticum* Extract on oestrogen level in female Wistarrats

Values represent mean ± SEM, n=3; [a] Significant at $p<0.05$ compared to Group 1; [b] Significant at$p<0.05$ when compared to group 2; [c] Significant at $p<0.05$ when compared to group 3.
Key: Group 1: Control
Group 2: Low Dose
Group 3: Medium Dose
Group 4: High Dose

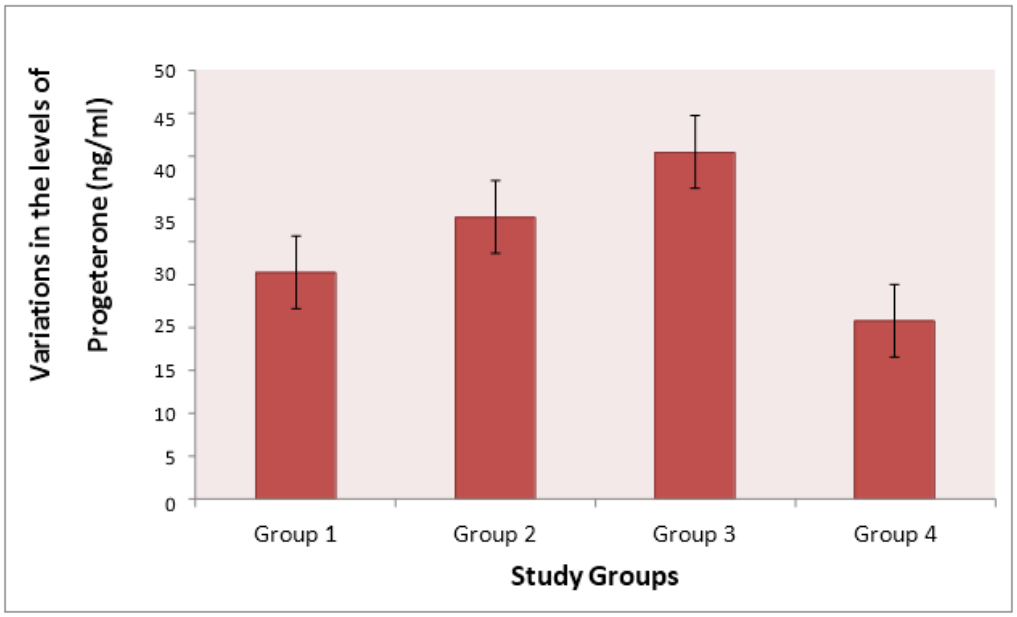

Figure 4.2: Effects *Syzygium aromaticum* Extract on progesterone level in female Wistar rats

Values represent mean ± SEM, n=3; **a** Significant at p<0.05 compared to Group 1; **b** Significant atp<0.05 when compared to group 2; **c** Significant at p<0.05 when compared to group 3.

Key: Group 1: Control
Group 2: Low Dose
Group 3: Medium Dose
Group 4: High Dose

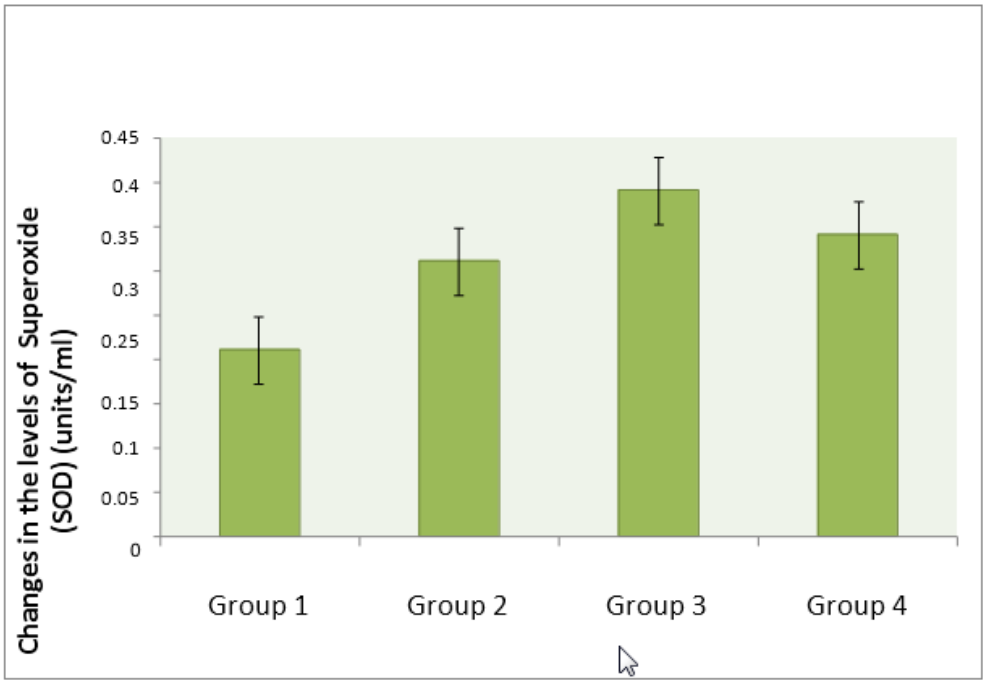

Figure 4.3: Effects of *Syzygium aromaticum* extract on superoxide dismutase (SOD) level infemale Wistar rats

Values represent mean ± SEM, n=3; **a** Significant at p<0.05 compared to Group 1; **b** Significant atp<0.05 when compared to group 2; **c** Significant at p<0.05 when compared to group 3.

Key: Group 1: Control
Group 2: Low Dose
Group 3: Medium Dose
Group 4: High Dose

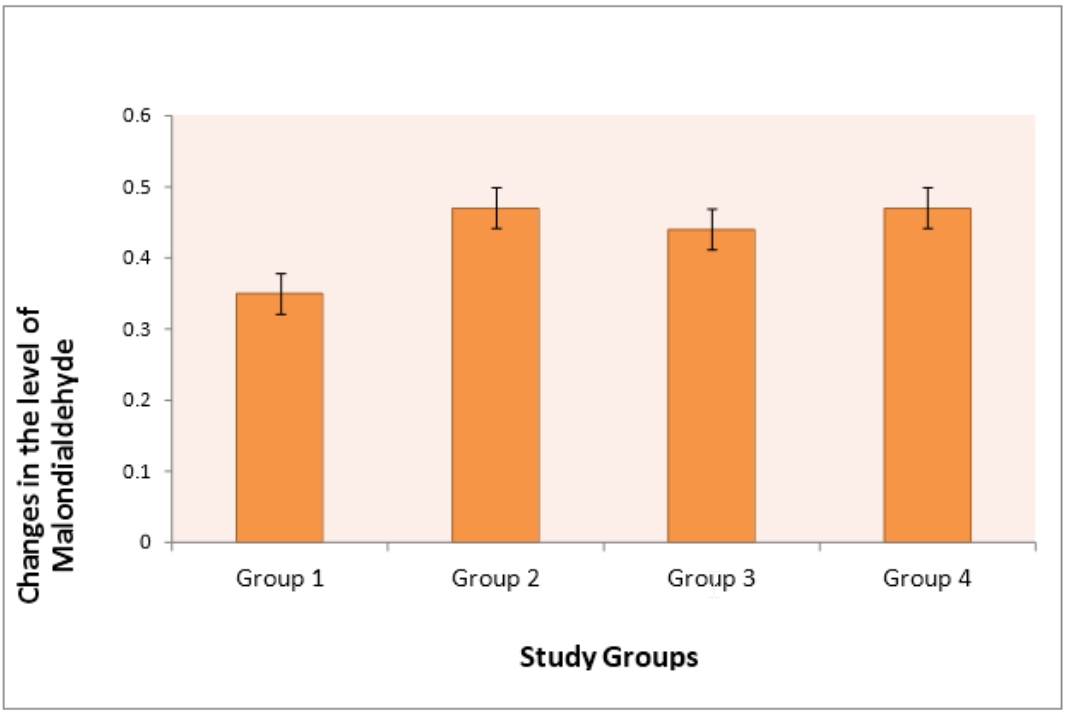

Figure 4.4: Effects of *Syzygium aromaticum* extract on malondialdehyde (MAD) level infemale Wistar rats

Values represent mean ± SEM, n=3; **a** Significant at p<0.05 compared to Group 1; **b** Significant atp<0.05 when compared to group 2; **c** Significant at p<0.05 when compared to group 3.

Key:
Group 1: Control
Group 2: Low Dose
Group 3: Medium Dose
Group 4: High Dose

Table 4.1: Effects of *Syzygium aromaticum* Extract on reproductive hormone and oxidativeStress Markers in female Wistar rats

Groups and Treatments of Study Animals	Oestrogen (ng/ml)	Progesterone (ng/ml)	Superoxide(SOD) (units/ml)	Malondialdehyde (MAD) (nm/ml)
Group 1: Control	87.66 ± 11.84	26.50 ± 2.65	0.21 ± 0.02	0.35 ± 0.04
Group 2: Low Dose	78.67 ± 10.10	32.96 ± 1.85	0.31 ± 0.03	0.47 ± 0.02
Group 3: Medium Dose	92.00 ± 10.39	40.56 ± 10.42	0.39 ± 0.05	0.44 ± 0.04
Group 4: High Dose	134.33 ± 8.25 a, b, c	20.83 ± 4.07 c	0.34 ± 0.07	0.47 ± 0.07

Values represent mean ± SEM, n=3; **a** Significant at p<0.05 compared to Group 1; **b** Significant atp<0.05 when compared to group 2; **c** Significant at p<0.05 when compared to group 3.

DISCUSSION

The results showed that the progesterone levels increased significantly and when it increases it encourages reproduction because it helps in the constriction of the uterus as well as the movement of sperm through the fallopian tube and it plays a role during the normal menstruation cycle. According to (Jessie and Micheal, 2023) progesterone plays a vital role in the maintenance of the uterus during pregnancy. As such, an important issue of concern regarding the loss of this steroid hormone correlates with miscarriage and pre-term labor. Progesterone throughout pregnancy is to maintain and decrease levels of vascular tone in the myometrium; it also influences the production of inflammatory makers (Khalid *et al*., 2023). Also, the estrogen levels increased significant. When estrogen levels increase in the female reproductive system, several physiological changes occur. Estrogen is a hormone that is primarily responsible for the development and regulation of the female reproductive system. One of the main effects of increased estrogen levels is the thickening of the uterine lining, known as the endometrium. This prepares the uterus for possible implantation of a fertilized egg. Additionally, estrogen also stimulates the growth and development of the ovarian follicles, which contain the eggs, and triggers the release of luteinizing hormone (LH), which is necessary for ovulation to occur (Mahmood, 2015). In the menstrual cycle, estrogen levels rise during the first half, known as the follicular phase, and peak just before ovulation. This increase in estrogen levels leads to the maturation and release of an egg from the ovary (Kumar *etal*., 2007). Furthermore, estrogen also plays a role in the regulation of secondary sexual characteristics such as breast development, body hair distribution, and regulation of the menstrual cycle (Kumar *et al*., 2007). Another study on female rats reported that treatment with clove extract increased the weight of the ovary and uterus indicating estrogenic effect (Suleyman *et al*., 2015; Siddiqui et al, 2011).

For the oxidative stress markers according to the results shown above they were significantly low,so this simply means that cloves actually serve as a good antioxidants and it also helps to cause stability and maintain the pregnancy or fertility. According to (Janka Vašková *et al*., 2023) oxidative stress (OS) has an important role in female reproduction, whether it is ovulation, endometrium decasualization, menstruation, oocyte fertilization, or development and implantation of an embryoin the uterus. Antioxidants are very crucial for proper female reproductive functions. They play apart in the metabolism of Oocytes, in endometrium maturation via Nrf2 and NF - kB, and in the hormonal regulation of vascular actions. Antioxidants can directly scavenge radicals and act as acofactor of highly valuable enzymes of cell differentiation and development, or enhance the activity of antioxidants enzymes (Janka Vaskova *et al*., 2023).

The data's on Figure 4.1, 4.2, 4.3 and 4.4 shows that the oestrogen level of the treated groups were seen to be significantly ($P<0.05$) elevated in Group4 (treated with high dose) when compared to groups 1, 2 and 3 respectively. Amongst the treatedgroups, the pattern of variation was observed to be dose-dependent.

The variation in progesterone levels in the treated rats were marginally ($P>0.05$) different when compared to that of Group 1 (untreated/control group). However, group 4 (treated with high dose)was found to have progesterone level that was significantly ($P<0.05$) lower when compared to thatof group 3 (treated with medium dose).

Considering the changes in super oxide dismutase (SOD) and malondialdehyde (MAD) levels across the treated rats, non-statistically significant ($P>0.05$) elevations were observed when compared to those of their respective control groups, but these elevations did follow any uniform pattern.

CONCLUSION

The present study revealed that the effects of the treatment on reproductive hormone and oxidativestress markers in female Wistar rats were dose-dependent. The high dose treatment resulted in significant elevations in oestrogen levels and a significant decrease in progesterone levels when compared to the other treatment groups. However, there were no statistically significant changes in superoxide dismutase (SOD) and malondialdehyde (MAD) levels across the treated rats when compared to their respective control groups.

ACKNOWLEDGEMENT

We acknowledge Dr.T.M .Bekinbo and Mrs. Precious Wadioni for their priceless support.

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Cite this Article: Aduema, W; Fente, AE (2024). Effects of *Syzygium aromaticum* (Cloves) extract on some reproductive hormones and oxidative stress markers in female wistar rats. *Greener Journal of Biomedical and Health Sciences*, 7(1), 73-79, https://doi.org/10.15580/gjbhs.2024.1.111924170.