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Study of the Effect of Cardamom and Clove Aqueous Extracts on Some Sperm Parameters in Male Albino Rabbits

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Abstract

Study Background: Male infertility is an increasingly relevant health and reproductive concern, and a considerable amount of it is attributed to oxidative stress that impairs sperm during their formation and maturation. In recent years, researchers have explored naturally derived plant extracts containing antioxidant properties to improve semen quality as a relatively safe approach. Both *Cardamom* (*Elettaria Cardamom um*) and *Clove*(*Syzygium aromaticum*) are traditional spices that consist of phenols, flavonoids, and volatile oils with antioxidant and anti-inflammatory effects.

Aim: To study the effect of oral administration of aqueous extracts of *Cardamom* and *Clove*on some sperm parameters (viability, mortality and head, midpiece and tail abnormalities), as well as some biochemical and cellular blood parameters in local male white rabbit compared to control group.

Materials and Methods: Twenty-one local white male rabbits with normal clinical examinations and no evidence of any other disease process were randomly allocated to one of three equal groups (7 per group): the control group [C] received distilled water, the *Cardamom* group [H] received the aqueous extract of the seeds of *Cardamom* [HAl] and the *Clove*group [K] received the aqueous extract of the buds of *Clove*[KAl], orally for eight weeks (the complete cycle time of spermatogenesis is approximately eight weeks). To assess complete blood picture and renal functions, blood samples were taken at the end of the experiment and for semen analysis, semen was collected to evaluate motility and morphological abnormalities were determined using the eosin-nigrosin stain (200 sperm per sample). Statistical analysis was performed on the data using one-way ANOVA and Tukey's post-hoc test at $p<0.05$.

Results: The experimental treatment arms of the study demonstrated a statistically significant increase in sperm motility (2 hours after SPA: 72.5% and 75.0% versus 63.5% for control $p < 0.0001$) and a parallel statistically significant decrease in mortality rates. The white blood cell counts and urea & creatinine levels was significantly ($P<0.05$) increased, yet the counts of red blood cells, hemoglobin and packed cell volume were reduced in the *Cardamom* group. On the other hand, *Clove*group remained around control values for the same parameters with significant increase in platelet count. As for morphological deformities, the head (narrow, deformities were significantly lower in the *Clove*group, whereas, the tail (narrow and broken) deformities were microstereotactic relative increased in this group compared to the control ones, particularly for midpiece deformities.

Conclusion: Both aqueous extracts improve sperm motility, but *Clove*aqueous extract showed a safer hematological and renal profile than the *Cardamom* extract at the dose used, whereas *Cardamom* aqueous extract was associated with hematological and renal changes necessitating caution when high doses or prolonged treatments are administered. The study suggested that further multi-dose studies with hormones and histological assays as well as the active components in the extracts should be performed subsequently.

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Keywords: Cardamom; Clove; Aqueous extract; Sperm parameters; Rabbits; Oxidative stress.

1 Introduction

Infertility has become an increasingly worrying global health problem affecting 15–17.5% of couples seeking to conceive, with male factor infertility responsible for 20–30% of these cases, and contributing to an extra 50% of the total cases [1]. As per recent report by the World Health Organisation [2] , it is reported that the prevalence of male infertility varies widely across regions from 9%–15.8% in certain countries in Europe, North America and West Africa [2]. National adult age standardized annual rates exceeded 23.2% in the Western Pacific region but only reached 17.5% globally [3]. It reflects a long-running decline in sperm numbers and quality across the globe that alarmed scientists and health organizations.

This decrease is, at least in part, related to oxidative stress, defined by an imbalance between production of reactive oxygen species (ROS) and antioxidant defence capacity, which affects the male reproductive system. The most recent studies suggest that oxidative stress is found in 30% to 80% of cases of idiopathic male infertility and in 35% to 87% of cases of male fertility impairment overall [4]. The sensitivity of sperm to low-level oxidative stress arises from its particular cellular architecture; the vast majority of the cytoplasm — and thus, antioxidant defense systems — is lost during spermatogenesis leaving mature sperm with very limited capacity to repair oxidatively damaged molecules and few sources of endogenous free radicals (e.g. mitochondria and the NADPH oxidase enzyme known as NOX5). Concurrent with these metabolic changes, its plasma membrane is still composed of polyunsaturated fatty acids, rendering it highly vulnerable to lipid peroxidation [4, 5]. The imbalance is due to a series of functional and structural disorders, such as damage and loss in flexibility of the plasma membrane, fragmentation of DNA, oxidation of structural proteins essential for sperm structure which affects sperm vitality, motility and fertilisation capacity as well as contributing to an increase in the rate of morphological defects in the sperm head, midpiece and tail [6].

Antioxidant therapy, however, is not without risks as reactive oxygen species play an important role in modulating some natural physiological processes of sperm such as capacitation, acrosome reaction and hyperactivation [19,20]. This therapy has its "double-edged sword" as well; recent reviews have placed warnings on the excessive use of antioxidants that may change the redox balance and produce 'reductive stress' that interrupts physiological functions that originally require a controlled amount of free radicals. This may elucidate the inconsistencies reported with some antioxidant supplements in clinical trials [7, 8]. The other aspect indicates that the dose-response association should be studied faithfully prior to recommending any topical antioxidant plant extract.

Given the shortcomings of the conventional use of drugs in treating male infertility, their unpredictable potential adverse effects, and high cost, the scientific community shifted its interest in recent years (2024-2026) towards phytotherapy based on plant extracts characterized by high antioxidant content to be used as therapeutic and/or prophylactic complementary therapy intended to ameliorate semen quality. They contain phenols, flavonoids and volatile oils with antioxidant and anti-inflammatory efficacy, and they are easily accessible and affordable when compared to industrial pharmaceutical supplements.

For example, *Cardamom* (*Elettaria Cardamom um*), one of the most important traditional spices and the aphrodisiac in folk medicine. It contains 3–7% volatile oils, with the most active constituent being 1,8-cineole, which may comprise as much as one third of the volatile oil. Other constituents are alpha-terpineol acetate, alpha-terpineol, limonene, borneol, saponins, tannin, and flavonoids like quercetin that are reported as antioxidants, anti-inflammatories, and for enhancing local blood circulation [9,10]. *Cardamom* extracts also significantly increased the apoptotic response of germ cells in low-density lipoprotein+induced testicular injury (L) and significantly amplified the counts, motility, and morphology of sperm, as well as serum concentrations of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in diet-induced obesity [9,10]. Neal et al, report that the extract has also increased maximum testicular [11] and body weight of experimental mice [11]. In another study, it was indicated that *Cardamom* extract activates thyroid function and increases testosterone level and sperm production in hypothyroid male rats [12]. On the contrary, the action of the extracts *Cardamom* as a spermicidal activity has been observed only at high concentrations in white rats as part of an early study [13] and this may suggest the presence of a possible dose dependent biphasic effect (an intersection of this mechanism with the idea of reductive stress is quite loose, when it respects the pharmacological concept "safe dosage" for antioxidant compounds).

In the case of *Clove*(*Syzygium aromaticum*), it ranks among the richest phenol-containing plants, high in hydroxybenzoic acid, hydroxycinnamic acid, and flavonoids, and its principal phenolic component, eugenol, which can account for 70% - 90% of the oil [19]. Eugenol acetate and β-caryophyllene are the second most important. Recent DPPH and ABTS tests [14] have shown that it has a high antioxidant capacity. The protective effect of *Clove*extract on testicular tissue has

been reported in different experimental oxidative stress models. *Clove*methanol extract inhibited the expressions of vimentin and PGP9. Five of these proteins are expressed in the testicular cells of rats with experimentally induced cryptorchidism (15, 16). Further, *Clove*oil extract was reported to markedly increase sperm concentration and reduce the percentage of abnormal sperms following testicular torsion and reperfusion injury to the ipsilateral testis in rats [17]. Specifically, at the level of preserved sperm, nano-formulations of *Clove*extract significantly increased progressive motility, viability, membrane integrity, and reduced morphological abnormalities of frozen and thawed rabbit sperm [18], corroborating previous findings concerning the antioxidant effectiveness of *Clove*bud extract in preserving ram sperm [19]. Nevertheless, the contradictory results of *Clove*arise in case of its antagonistic effect at certain dosages. In male rats, *Clove*bud extract at low dose (15 mg/kg) improved testosterone levels, sperm motility and morphology, but decreasing doses showed the opposite effects on sperm parameters and induced degenerative changes in the seminiferous tubules [20,21]. The component eugenol negatively affected testicular sperm number, motility and serum testosterone concentrations in adult rats at high doses [22], while *Clove*essential oil may have protective effects on liver kidney and testis from acrylamide-induced toxicity by decreasing liver enzymes and normalizing urea and creatinine concentrations [23].

The white rabbit (Oryctolagus cuniculus) is a prevalent animal model in reproductive toxicity studies and semen quality (e.g. Hypothalamic-pituitary-gonadal (HPG) axis comparisons) as the anatomy and physiology of the reproductive system of rabbits resemble that of a large mammal as in human beings. It is also advantageous because semen can be collected with an artificial vagina and in high enough quantities to be analyzed several times without putting the animal to death, and because completeness of spermatogenesis takes less time compared to a larger animal (per unit of time spermatogenesis is slower but experiments can be completed over a reasonable experimental time frame). It is also low-cost, easy to maintain, and breed compared to other models such as primates [24,25,26]. The eosin-nigrosin stain is a classical stain used to evaluate rabbit spermatozoa for viability and morphological abnormalities from the studies of Bittie [24] to the present studies [25]. It is dependent upon the permeability of the dye to dead spermatozoa with disrupted membranes, leaving live spermatozoa without staining. To decrease inspection error, the preferred technique is to count 200 spermatozoa minimum per sample [26].

Although many studies focused on the impact of either *Cardamom* or *Clove*alone in different rodent models, there have been very few studies that compare the effects of the aqueous extracts of both plants simultaneously on sperm parameters in male white rabbits, together with simultaneous evaluation of the serum biochemical and cellular blood parameters representing the food safety for systemic use, in the available literature. This need is accentuated by the potential biphasic effect of both extracts, depending on the dose (see 4.1), and thus requires a broader assessment that moves beyond sperm parameters to blood and kidney indicators, so to test the holistic safety of the animal. Accordingly, the current investigation is designed to assess the influence of aqueous extracts of *Cardamom* and *Clove*given orally on the motility and viability rates of sperm and their morphological abnormalities (head, midpiece and tail) along with some complete blood count parameters and renal functions in local male white rabbits. This aims to develop a comparative database, which may help establish the safe and efficacious dosage of these plant extracts in future studies, and may provide a means to separate the positive effects on fertility from systemic side effects mediated by these therapeutic agents.

2 Materials and Methods

2.1 Laboratory animals and experimental design

In this study, twenty-one healthy adult male local albino rabbits were used, with similar ages and weights, after a two-week acclimatization period in standardized laboratory conditions regarding lighting, ventilation, and temperature, with free access to feed and water. The animals were randomly classified into three equal groups (7 males/group) as follows:

Code	Group	Treatment
G1 (C)	Control group	Received distilled water orally at the same volume administered to the treatment groups.
G2 (H)	*Cardamom* group	Received an aqueous extract of *Cardamom* seeds orally by gavage.
G3 (K)	*Clove*group	Received an aqueous extract of *Clove*buds orally by gavage.

The extracts were administered for 8 consecutive weeks, corresponding to an entire spermatogenesis cycle in rabbits, which allowed observation over an extended period of the actual effects of the extracts on the spermatogenic process [4,12].

2.2 Preparation of the aqueous extracts

Aqueous extracts of *Cardamom* seeds and *Clove*buds were prepared with the method of maceration (maceration: 1 g of *Cardamom* seeds or *Clove*buds in 20 ml of distilled water). The plant samples were dehydrated and finely powdered, they were then steeped in 25 ml of distilled water for 24 hours with intermittent stirring at room temperature. The extract was filtered through Whatman No.1 filter paper, and the resulting filtrate was desiccated by either using a freeze dryer or by drying overnight at a lower temperature to maintain the thermally-sensitive, active components. The lyophilized extract was refrigerated at 4 degrees C until use and the amount to be administered was dissolved daily in distilled water to give the required dose [9,14].

2.3 Collection of Blood and Semen Samples

At the end of the treatment time period, blood taken from the marginal ear vein of every animal for assessment of complete blood count (i.e. white blood cell count WBC, red blood cell count RBC, hemoglobin concentration HGB, hematocrit HCT and platelet count PLT) via an automated cell counter, blood urea B.Urea and serum creatinine S.Creatinine by spectrophotometric measurement. Semen was obtained using an artificial vagina and analyzed as soon as possible after collection.

2.4 Analysis of sperm motility and defects in morphology

Viability and mortality percentages (Life% and Died%) and the morphological defects of sperm were determined by Eosin-Nigrosin stain. A clean glass slide was used to mix a drop of semen with the stain and a thin smear was made and allowed to air dry. This was then analyzed at a magnification of 1000x using immersion oil under a light microscope. The principle of selective dye permeability states that live sperm with intact membranes are morphologically light and colorless while dead sperm with damaged or destroyed membranes absorb the dye with colors ranging from pink to dark red [24,28,29]. In each slide, at least 200 sperm were counted, and the morphological abnormalities were classified by its anatomical location in:

Category	Subtypes Examined
Head abnormalities	Compact/Coiled head and Slim/Tapered head
Midpiece abnormalities	Narrow midpiece and Cut midpiece
Tail abnormalities	Swollen tail, Double tail, and Narrow/Coiled tail

Microscopic representative samples of the studied semen smears were recorded, and the photos were taken and inserted below as Fig (1) to show the appearance of live and dead sperm after staining.

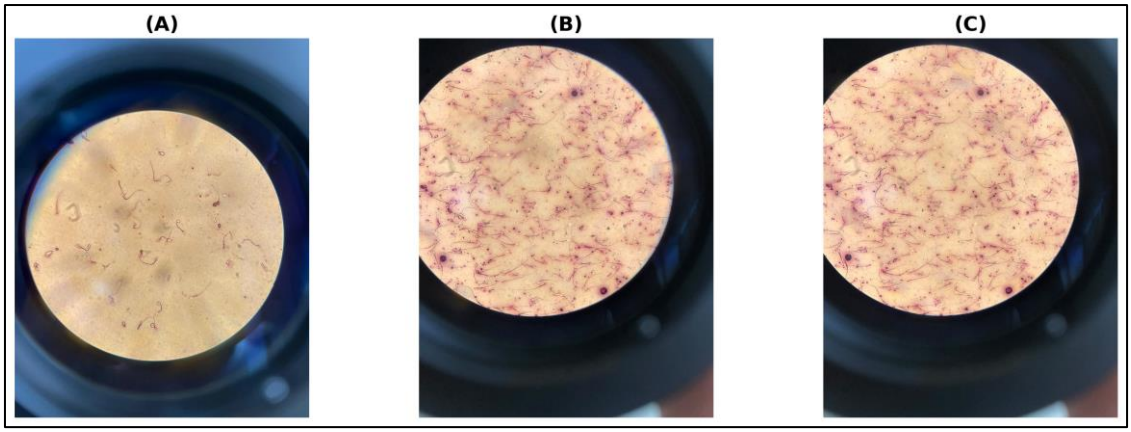


Figure 1: Eosin-nigrosin stained semen smears (X1000): Samples from three study groups showing unstained (live) and pink to red (dead) sperm, along with some sperm displaying overt morphological defects in head and tail.

2.5 Statistical analysis Statistical analysis

Data were presented as mean ± SD (standard deviation). Comparison between the three groups was performed with one-way ANOVA and where significant differences ($p<0.05$) were found, pairwise comparisons between groups were made with Tukey HSD post-hoc test. Similar groups (NS, no-significantly different) between the same row in the tables were indicated by the same Latin letters, while dissimilarities were expressed as different letters ($p<0.05$). To perform statistical analysis you can use the Python programming language (version 1.17 for SciPy package).

3 Results

3.1 Hematological parameters (Complete Blood Count)

Table(1) and Figure(2) demonstrated the differences in all complete blood count parameters which were highly significant ($p<0.0001$) among the three studied groups. Statistically, while the white blood cell count in the control and *Clove*groups were not significantly different from one another (5.60 and 5.80 × 10^3/µL respectively), the *Cardamom* group (H) showed a markedly increased, sharp rise in count (13.10 ± 1.06 × 10^3/µL), more than two-fold higher than all other remaining treatment groups. Meanwhile, the red blood cell count, hemoglobin concentration and packed cell volume decreased significantly in both H and K groups as compared to the control, but without significant differences between each other. Regarding the platelet count, it had different tendencies in both groups, as it significantly decreased following the administration of *Cardamom* compared to the control group (293 × 10^3/µL vs. 548 × 10^3/µL), and increased significantly following the administration of *Clove*compared to the control group (701 × 10^3/µL vs. 548 × 10^3/µL), with significant differences between all three groups.

Table 1: Hematological parameters of three study groups.

Parameter	Control (C)	*Cardamom* (H)	*Clove*(K)	p-value
WBC (×10^3/µL)	5.60 ± 0.79^b	13.10 ± 1.06^a	5.80 ± 0.44^b	<0.0001
RBC (×10^6/µL)	6.50 ± 0.79^a	4.84 ± 0.33^b	4.81 ± 0.33^b	<0.0001
HGB (g/dL)	14.30 ± 1.19^a	8.90 ± 0.88^b	9.40 ± 0.87^b	<0.0001
HCT (%)	45.70 ± 2.21^a	30.30 ± 1.83^b	29.40 ± 1.61^b	<0.0001
PLT (×10^3/µL)	548.00 ± 19.09^b	293.00 ± 13.18^c	701.00 ± 17.90^a	<0.0001

Values are presented as mean ± standard deviation (n=7). Columns with different letters indicate a significant difference ($p<0.05$) (Tukey's post hoc test).

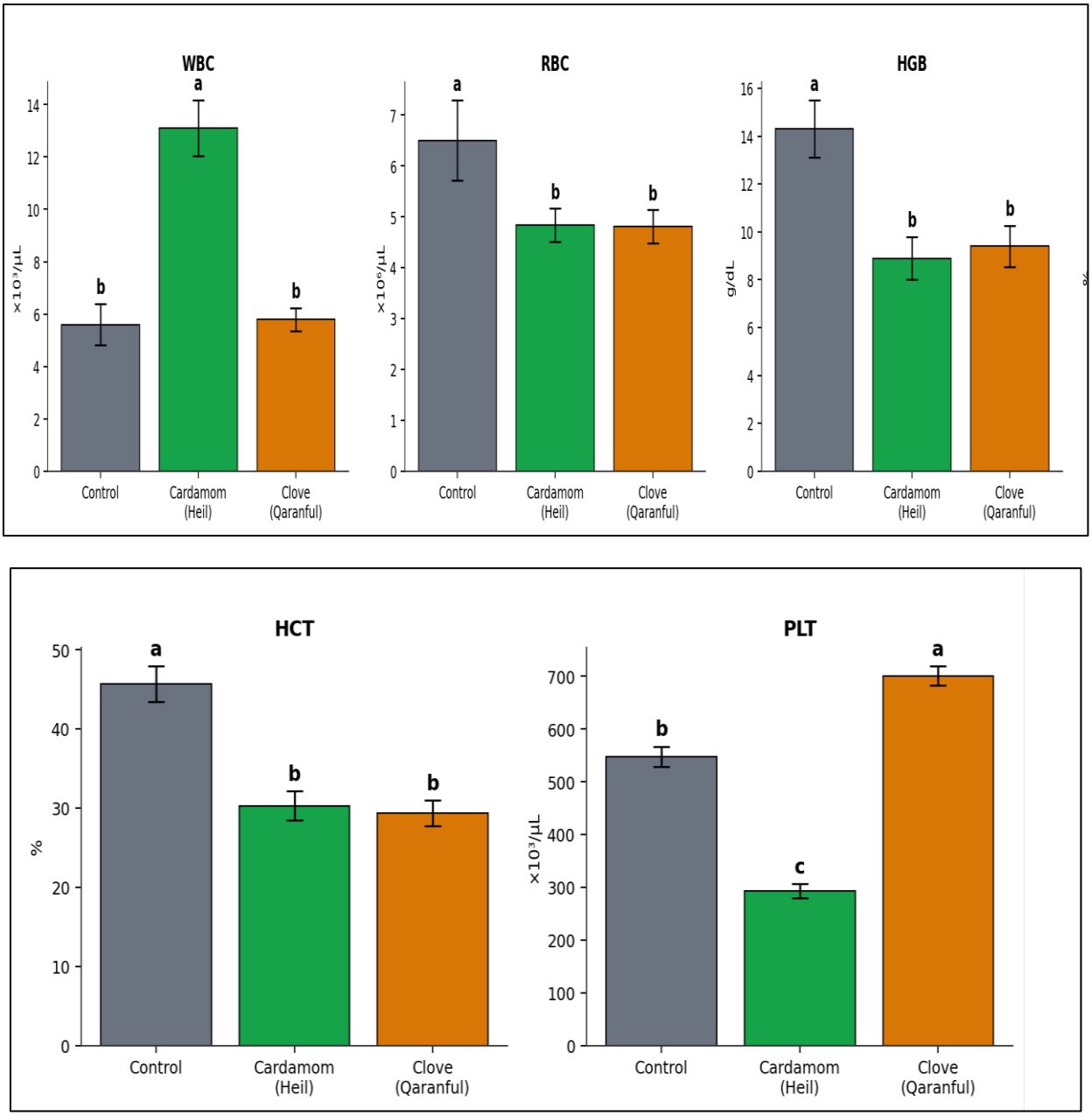


Figure 2: The hematological parameters(WBC, RBC, HGB, HCT, PLT) in the three study groups Statistical comparisons ($p<0.05$) are indicated by different letters above the columns.

3.2 Kidney functions Kidney functions

The results in Table (2) and as illustrated in Figure (3) showed a high significant increase in the mean serum levels of urea and creatinine, with a valueof ($p<0.0001$) of the day7in the *Cardamom* group as compared to the control and *Clove*groups. With it the carda group had an average of 79.00 ± 3.83 mg / dL in urea, the control group 30.00 ± 1.65 and low in *Clove*40.00 ± 3.00, with significant difference between the three groups. Compared to the control group (1.50 ± 0.22 mg/dL), creatinine increased statistically significantly more than eight times in the *Cardamom* group (13.00 ± 1.44), while in the *Clove*group, it decreased statistically significantly 5 times (0.30 ± 0.07) below control level.

Table 2: Kidney function parameters for the three study groups (Mean ± SD, n=7).

Parameter	Control (C)	*Cardamom* (H)	*Clove*(K)	p-value
Blood Urea (B. Urea) (mg/dL)	30.00 ± 1.65^{c}	79.00 ± 3.83^{a}	40.00 ± 3.00^{b}	<0.0001
Serum Creatinine (S. Creatinine) (mg/dL)	1.50 ± 0.22^{b}	13.00 ± 1.44^{a}	0.30 ± 0.07^{c}	<0.0001

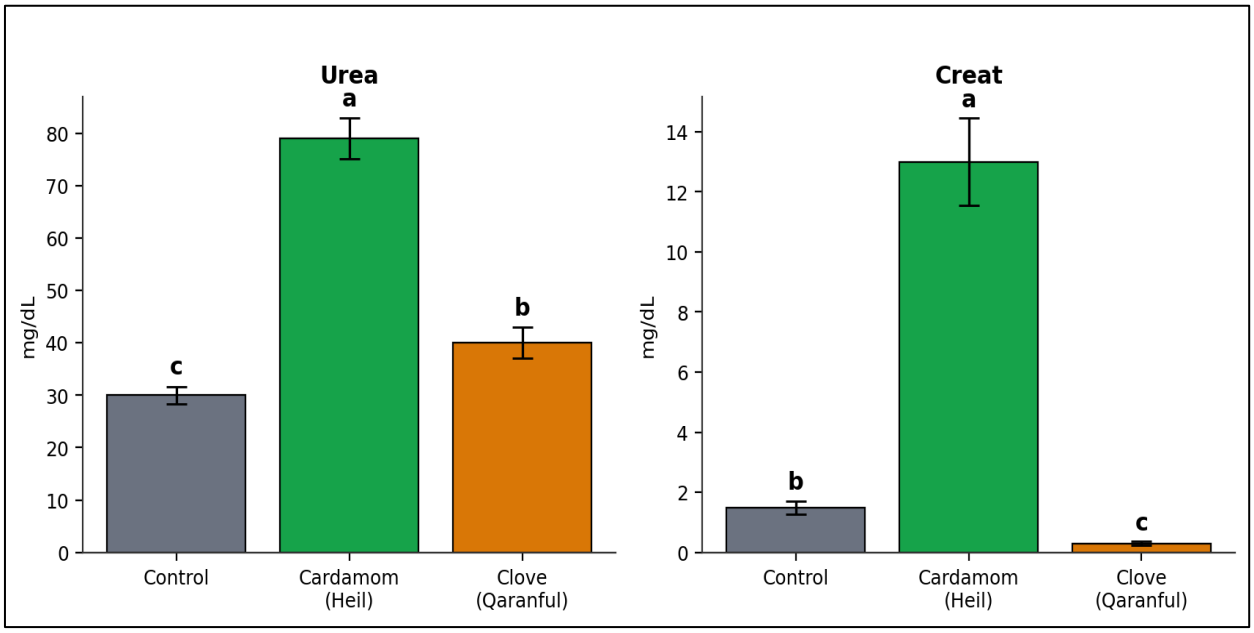


Figure 3: Serum urea and creatinine levels in the three study groups**.**

3.3 Sperm vitality and viability

Table (3) and figure (4) illustrate that there were a significant increase in sperm motility at the treatment group compared to the control (P < 0.0001), this reflected as motility rates 72.50 ± 2.74% and 75.00 ± 2.94% in the *Cardamom* and *Clove*groups respectively versus 63.50 ± 2.42% in the control groups with a non -significant difference between the two treatment groups. Accordingly, the proportion of dead spermatozoa was significantly reduced in the two treatment groups (22.50 and 25.00% in groups headed and headed K, respectively)relative to control (36.50%).

Table 3: Viability and mortality rates of sperm in the three study groups (Mean ± SD, n=7).

Parameter	Control (C)	*Cardamom* (H)	*Clove*(K)	p-value
Survival (%)	63.50 ± 2.42^{b}	72.50 ± 2.74^{a}	75.00 ± 2.94^{a}	<0.0001
Mortality (%)	36.50 ± 1.88^{a}	22.50 ± 2.02^{b}	25.00 ± 2.16^{b}	<0.0001

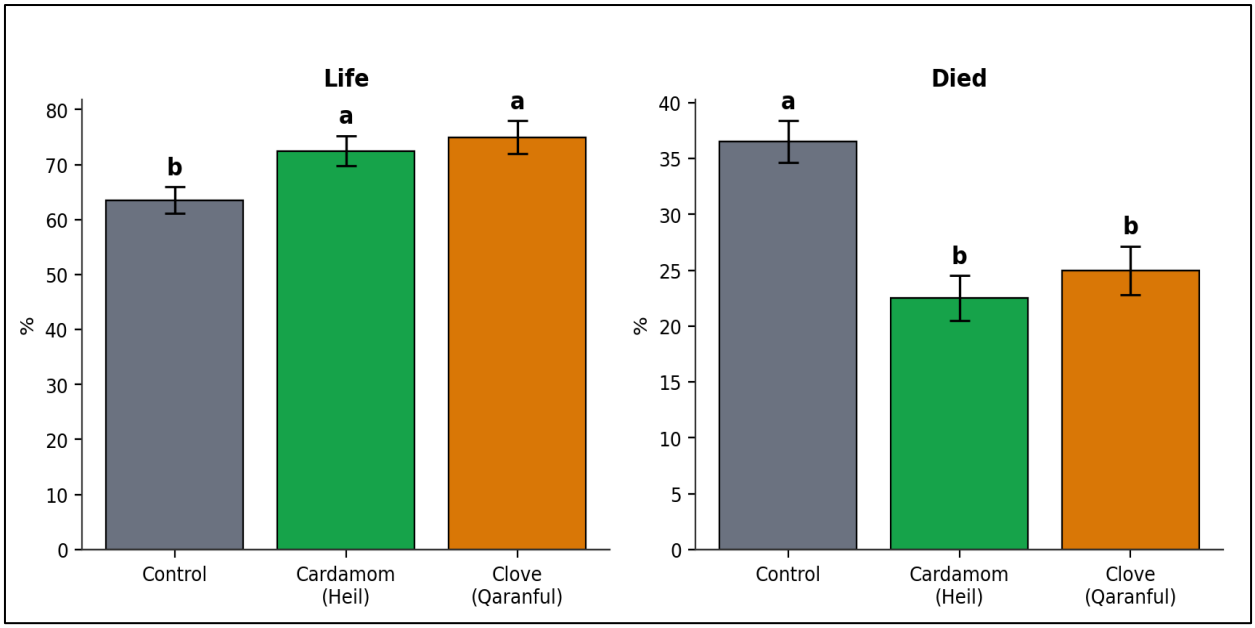


Figure 4: Percent Viability and Mortality of Sperm (%) in the three study groups**.**

3.4 Sperm morphology

Results on head abnormality (Table 4, Figure 5) showed that head detached/curled head was significantly lower in *Clove*group (2.00 ± 0.48%) when compared to control and *Cardamom* groups (3.00 ± 0.48% for each) (p=0.0011). As for the percentage of small/tapered head, there was no significant difference among groups (p=1.00).

Table 4: Sperm head abnormalities in the three study groups (Mean ± SD, n=7).

Parameter	**Control (C)**	***Cardamom* (H)**	***Clove*(K)**	**p-value**
Compact Head (%)	3.00 ± 0.48[a]	3.00 ± 0.48[a]	2.00 ± 0.48[b]	0.0011
Slim Head (%)	1.00 ± 0.22[a]	1.00 ± 0.22[a]	1.00 ± 0.22[a]	1.0000

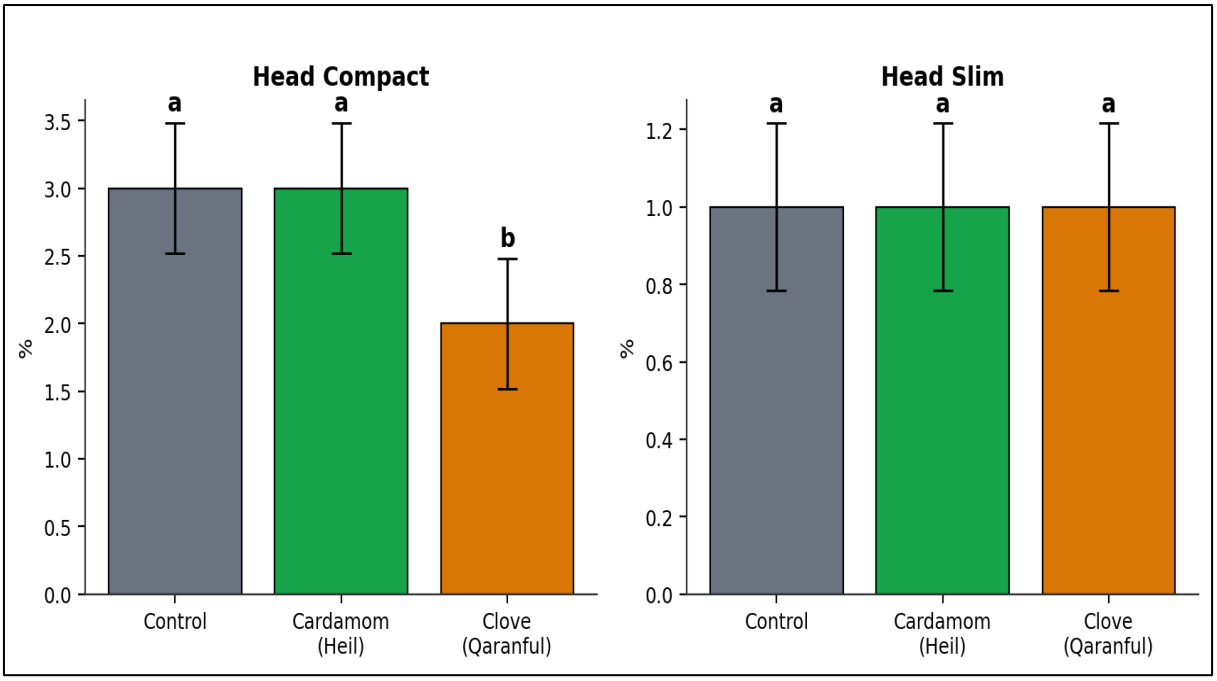


Figure 5: Head deformities (gross & fine) in the 3 study groups**.**

In terms of midpiece deformities (Table 5, Figure 6) the percentage of constriction differed highly significantly ($p<0.0001$)with the *Clove* r group (2.00 ± 0.32%) being greater than the two control and *Cardamom* groups, which were almost identical (1.00%). On the other hand, the percentage of the cut piece presents a significant ($p=0.0044$) reduce in the *Cardamom* group (5.00 ± 0.41%) compared to both groups of controls (5.50 ± 0.62%) and *Clove*(6.00 ± 0.39%), which did not differ significantly among them.

Table 5: Midpiece abnormalities of sperm in the three study groups (Mean ± SD, n=7).

Parameter	**Control (C)**	***Cardamom* (H)**	***Clove*(K)**	**p-value**
Narrow Midpiece (%)	1.00 ± 0.22^{b}	1.00 ± 0.15^{b}	2.00 ± 0.32^{a}	<0.0001
Cut Midpiece (%)	5.50 ± 0.62^{a}	5.00 ± 0.41^{b}	6.00 ± 0.39^{a}	0.0044

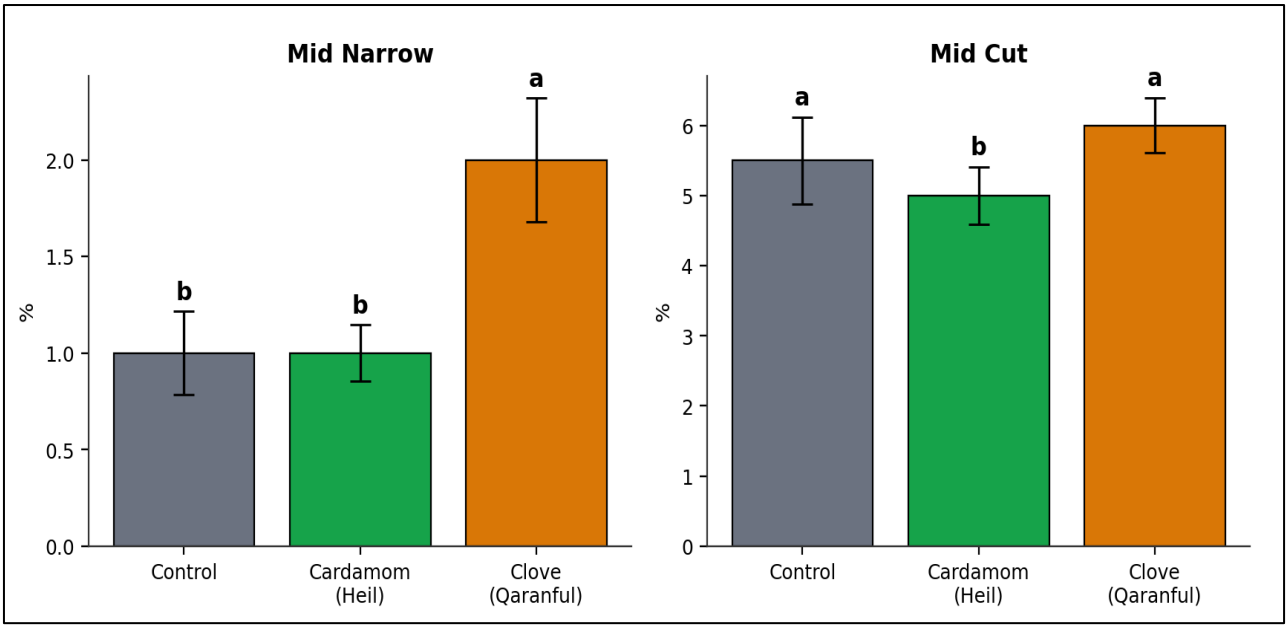


Figure 6: Median section (narrow and cut) deformations detected in the three study groups

Finally, for tail deformations (Table 6, Figure7), which showed no significant difference among the three groups in which the proportions of the enlarged (p=1.000) and double tails (p=0.0411; after Tukey correction for pairwise comparisons, none were significant for the double tail), the narrow/twisted tail proportions decreased significantly in the *Cardamom* group (2.00 ± 0.29%) compared to the two nearly equal control and *Clove*groups (3.00%) (p=0.0001).

Table 6: Sperm tail abnormalities in the three study groups (Mean ± SD, n=7).

Parameter	**Control (C)**	***Cardamom* (H)**	***Clove*(K)**	**p-value**
Swollen Tail (%)	2.00 ± 0.44^{a}	2.00 ± 0.29^{a}	2.00 ± 0.29^{a}	1.0000
Double Tail (%)	0.50 ± 0.22^{a}	0.70 ± 0.08^{a}	0.50 ± 0.14^{a}	0.0411
Narrow Tail (%)	3.00 ± 0.48^{a}	2.00 ± 0.29^{b}	3.00 ± 0.29^{a}	0.0001

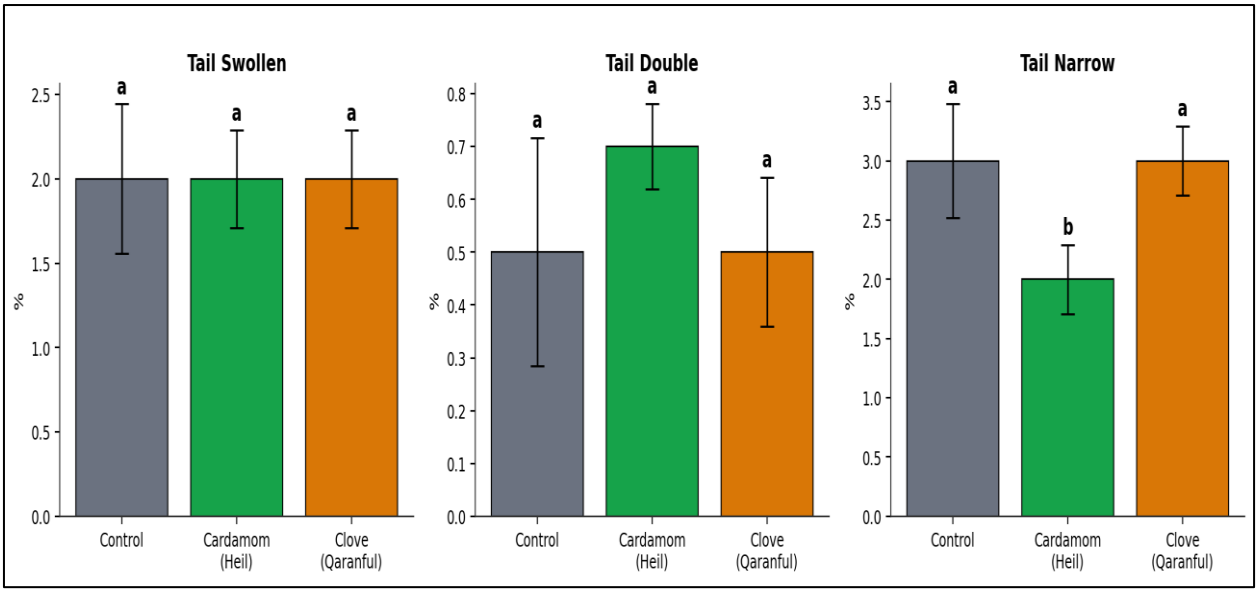


Figure 6: Tail deformities (hypertrophied, bifid, and narrow) in the three study groups.

4 Discussion

The present findings, however, showed that the *Cardamom* (AE) and *Clove*(AE) markedly enhanced sperm motility and decreased the sperm mortality rate in local white male rabbits in compare to the control group. It is aligned with previous studies on the effect of low to moderate doses of *Cardamom* and *Clove*extracts on semen parameters in rodent models [26]. The improvement is probably due to plants containing plenty of phenol, flavonoids and volatile oils with antioxidant activity [6,9,19], reducing the peroxidation of the sperm plasma membrane lipids resulting in the maintenance of membrane integrity and cell vitality. Similar to the mechanism proposed for enhancing motility and viability of frozen rabbit sperm with the addition of nano-*Clove*extract to the cryopreservation medium based on Khalil et al. [18]. This interpretation is consistent with the general physiological framework linking increased plasma membrane integrity and sperm function to reduced oxidative stress. Reactive oxygen species specifically injure these constituent polyunsaturated fatty acids in the sperm membrane and any attenuation of this oxidative injury provided either internally by endogenous antioxidant enzymes or externally by extraneous plant phytochemicals becomes evident as an increased percentage of cells with intact membranes capable of exclusion from staining by eosin-nigrosin [4,5,6].

However, the present results simultaneously showed that the two extracts differed significantly from each other regarding their hematological and renal parameters, with *Cardamom* extract producing a highly significant increase in WBC count, and urea and creatinine levels coupled with significant decrease in RBC count, hemoglobin, packed cell volume, and platelet count. This pattern implies systemic stress with possible effect on renal and bone marrow function at these doses and duration in this study. In accordance with previous studies, this conclusion hints a possible inflection point of spice extracts characterized with volatile oils. Thus, the antioxidant advantage at low doses becomes detrimental at high doses or prolonged administration periods, as exemplified by the study of Kumalasari et al. on *Clove*extract in mice [27], and by Khilari and Singh, carminative and anti-sperm activity of high concentration *Cardamom* extract [13]. This paradox: between the improvement of semen quality (sperm vitality) and the deterioration of blood and kidney profiles, in the same group of animals was elucidated on the basis of reductive stress as described in the introduction. The threshold of a safe response to bioactive components of *Cardamom* extract appears to vary between the tunica albuginea, which may derive a benefit from a modest level of antioxidant activity within its physiological tolerance level in contrast to other systemic tissues such as the kidney and bone marrow that may be particularly susceptible to elevations in the accumulation of metabolites or to the added metabolic burden from taking relatively high doses up to eight weeks without interruption. This corresponds with the general caution that excessive antioxidants may upset balances of cellular response that are skewed between tissues [28]. The significantly increased urea and creatinine (measured several times above their levels in the control group in this study) can also be partially attributed to the increased protein catabolism or direct action of some components of the extract with significant differences between samples (for example, saponins at high concentrations) on renal tubules; this needs to be confirmed histologically in the following studies.

On the other hand, the *Clove*treatment group kept the blood and kidney parameters close to the control, with a markedly increased platelet count above that of the control. This may represent a platelet supportive effect; or represent an anti-inflammatory effect that merits separate investigation. This comparatively safe profile conforms with results that showed that *Clove*extract protected against acrylamide-induced liver, kidney, and testicular toxicity in rats via recovery of elevated urea and creatinine levels [23], and with the potent antioxidant activity of *Clove*extract documented by modern GC-MS analysis [29]. Mechanistically, the difference between the two extracts is more probably due to the qualitative difference of the chemical composition of the EO; *Clove*oil dominated by a single main phenolic component (eugenol) in a relatively high and stable chemical proportion, while the bioactivity of *Cardamom* oil is dispersed in a broader mixture of a selection of terpenes and saponins among which some may show systemic efficacy and some anti-oxidant activity locally beyond the local anti-oxidant action [41]. Nonetheless, this explanation is primarily speculative, as direct quantitative chemical analysis (GC-MS/HPLC) of the extracts used in this particular study is still lacking.

For the morphological deformities, the detached head and the microcephalus head ratios were significantly lower in the *Clove*group, which corroborate the reductions of above-mentioned morphological deformities described in *Clove*extract on frozen sperm [30] and testicular sperm from torsion and reperfusion injury [31]. However, the most interesting finding was the relatively large increase in midpiece abnormalities (narrow and broken) in the same group compared to the control, which even reflects a selective effect of eugenol, the main phenolic component in *Clove*oil, on the stages of condensation of mitochondrial and formation of the mitochondrial sheath that makes up the midpiece during spermatogenesis. This is in agreement with Ribeiro et al. who found in a previous study, no significant changes in sperm morphology with sub-lethal eugenol doses but an adverse effect induced by high doses of eugenol on enzymatic and functional activity of epididymal sperm. Because the midpiece is the only portion of sperm with ATP-producing mitochondrial sheath, the selective deleterious effect on this portion (whether vital staining is otherwise improved) may not be detectable in this study of total motility and functional sperm. This emphasizes the need to validate this assessment by assessing computer-assisted sperm analysis (CASA) in further studies. In the same way, methanolic *Clove*extract on the protein expression of testicular cells in ectopic testis model also showed impartial improvement that was also associated with heterogeneity across categories of deformities as some skeletal markers improved, but the amelioration did not extend equally across all tissue components [15,16]. In contrast, the *Cardamom* group had no difference in the incidence of head malformations compared to the control group, but a substantial reduction in malformations of both the midpiece and tail in this group, partially consistent with the findings of Sirait and co-workers on improvement in sperm morphology and hormone level and apoptosis rate of spermatogenic cells by *Cardamom* seed extract in obese rats [11], and the improvement in sperm morphology and motility and sperm count [10] and in seminiferous tubule diameter in *Cardamom* seed extract-treated obese rats [18]. This partial morphological improvement was accompanied in the *Cardamom* group by the same detrimental influence on blood picture and kidney mentioned earlier reinforcing the hypothesis that the advantage of *Cardamom* extract may be limited to the testis and reproductive tissues and do not account for the overall systemic safety. This is an important aspect to keep in mind when envisaging a potential future application of this extract, as a positive effect on one parameter (e.g., sperm motility) does not justify the isolated evaluation of this parameter as adequate to assess the overall safety of the extract, nor does it replace the extensive multi-organ evaluation carried out in this study. The current study findings further relate to the emerging human clinical literature regarding male antioxidant treatment for subfertility, and the observation of a frequently nonlinear treatment response that is strongly modulated by both dose, duration of administration and patient's baseline characteristics of oxidative stress status [7,8]. Similar to those receiving higher doses of antioxidant supplements without careful medical supervision, where sometimes no effect or even deterioration of some semen parameters was noted [23, 24], the results of the *Cardamom* branch in this study suggest that the pronounced local positive effect on sperm quality cannot be generalized without further studies to the betterment of the whole organism [32]. This always entails the balancing of the desired reproductive gain versus the potential systemic harm, a hallmark of all therapies—be they industrial pharmaceutical or culinary.

5 Conclusions

Oral administration of aqueous extract of *Cardamom* and aqueous extract of *Clove*for eight weeks significantly improves the sperm motility and decreases the sperm mortality in local white male rabbits according to the present study. However, the hematological and renal profiles for the two extracts were clearly different; systemic stress at the used dose was suggested by significant hematological and renal changes (increased white blood cells, urea and creatinine and decreased red blood cells and hemoglobin) associated with the *Cardamom* extract, while without system stress the *Clove*extract maintained a profile closer to the control group (improvement in some head and tail deformities and a relative increase in the midpiece deformities). Conclusions Based on these results we indicate that further multi-dose studies with hormonal and histological evaluations, direct oxidative stress markers, and chemical identification of the

active components in both extracts should be performed before either be recommended for use to improve fertility in practice.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest regarding the publication of this manuscript.

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