

## Effect of *Amomum granum paradisi* medium in sperm parameters activation *in Vitro* of infertile patients suffering from Asthenospermi

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### Abstract

In the present investigation, 165 mock male sperm samples were examined and revived. The study used Amomum extract with three different concentrations to increase the reproductive capacity and activate sperm parameters. After using the culture medium, the concentration in the swimming technique and a concentration of 50mlg\ l were demonstrated, with significant increases in the percentage of motile spermatozoa, the degree of sperm activity, and the sperm motility factor, and significant decreases in the spermatozoa concentration, the percentage of abnormal spermatozoa, and the concentration of white blood cells. As a result, the results showed a significant ( $p < 0.05$ ) increase in the percentage of motile sperm, the degree of sperm activity, the coefficient of sperm motility, a significant decrease in the concentration of sperm, and an abnormal percentage. Sperm, after using the culture medium, and concentrated in the swimming technique, and a concentration of 50mlg\ l We conclude from the current study that Amomum granum has a clear role in stimulating sperm transactions and thus has a role in increasing the chances of successful external fertilization operations.

**Keywords:** *Amomum granum paradisi*; Asthenospermi; Infertile Patient; Osibogun

### 1. Introduction

About 50% of infertility in humans is caused by male factors.

The cause of infertility in males is unclear in 40% to 50% of cases (1) It is possible to explain the pathophysiology of male infertility by many cellular abnormalities that appear at the reduced cellular and biochemical levels that cause sperm quality and quantity in the semen (2) as well as an hormonal imbalance related to reproduction. Furthermore, it possesses oligospermia is largely acknowledged as the single most common common reason for decreased male fertility(2, 4)

According to estimates from the World Health Organization, 80% of the world's population still uses plant bioactive components as folk medicine in conventional treatments. Dhanalakshmi,etal (5)

In traditional settings, medicines are typically made from a single plant or a group of plants, and the use of an appropriate plant part is crucial to their effectiveness in addition to their biological effectiveness, which is dependent on the quantity and type of secondary extract present in a crude drug (6)

In West Africa, it is a component of spices that are used to treat inflammation, cardiovascular illness, diabetes, diarrhea, and stomach discomfort (7).As an aphrodisiac and a remedy for scorpion stings and snake bites (8) (9).

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*Amomum granum paradisi*

Plantae

- Trechophayta
- Liliopsida
- Zingiberales
- Zingiberaceae
- Aframomum
- *Amomum granum paradisi* (L).
- *Aframomum melegueta* (L)

With orange lips and pinkish-orange top blooms that can turn into meaty, non-glowing pods, this plant can grow to a height of 1.5 meters. The edible, 5-7 cm long pods are filled with countless tiny, reddish-brown seeds, and they have a strong ginger and chili scent (Fig. 1). The stem's slender bark is covered in leaf-scarring scars. The leaves are densely nerved below and are around 30 cm long and 12 cm broad (8).



**Figure 1** The stem's slender bark is covered in leaf-scarring scars

It is a kind of herbaceous perennial plant endemic to coastal marshes along the coast of West Africa and is a member of the ginger family. It goes by a variety of names depending on the nation, such as Melegueta pepper (also known as Alligat pepper or Guinea pepper) in Nigeria (Yoruba). It has therapeutic and nutritional benefits and is widely used as a herbal treatment in Nigeria and other parts of the world to treat a variety of illnesses (9). It is a typical plant that is utilized as a spice in meals in the African rainforest, particularly in Nigeria. According to Houghton and Osibogun, it is a Zingiberaceae-family medicinal plant native to Nigeria (10).

## 2. Material and methods

### 2.1. The process of making the aqueous extract *Amomum granum paradisi*

The elephant fig plant's seeds were purchased from local markets. Three kilograms of the seeds were taken and dried in the laboratory's heat for at least a week before being ground into a fine powder using a crusher or an electric grinder. Next, 250 gm of the powdered aqueous extract of (elephant fig) seeds was added to a beaker with one liter of distilled water the extract then continues.

### 2.2. Preparing semen samples

After 3-5 days semen samples were obtained by masturbation and placed in sterile containers. of sexual abstinence and was handled as Shalaweh stated. et al., (11). samples were liquefied for 30 minutes at 37°C. Spermatozoa baseline concentration and motility wer assessed.SCA® Evolution Microptic's Sperm Class Analyzer, version 5.0 Spain's

Barcelona). Semen samples were then added to HTF-BSA following that. (1:5), centrifuged (300 g; 10 min), and re-suspended the pellets the HTF-BSA. Sperm suspensions ( $7.5 \times 10^6/\text{ml}$ ) were then used. combined with a stock solution of rooibos fermented extract, in water for HTF-BSA in order to achieve final concentrations of 0.10, 1.0, 10 and 100 g/ml for 37°C for 1 hour, while HTF-BSA acted as the control. Following then, various Parameters of the sperm were examined.

### 2.3. Microscopy of samples

**Sperm viability test:** An "Eosin-Nigrosin one-step staining procedure" was used for the sperm viability test (11). Five air-dried smears were created on glass slides using equal amounts of the sperm solution and the Eosin-Nigrosin stain.

Each specimen. The % viability of the slides was evaluated within 15 minutes. standard live sperm dead sperm cells picked up dye and looked gray, whereas living cells excluded the stain and appeared white.

Looked pinkish. Based on the quantity of viable sperm cells released, percentage viability was estimated. in relation to the overall number of sperm cells seen.

### 2.4. Total Antioxidant Capacity (TAC) and Malondialdehyde

#### 2.4.1. (MDA) concentration measurement in semen plasma

A TAC detecting kit was obtained from Nanjing Jiancheng Bioengineering Institute-China. According to this method, the antioxidant defense system, which consists of enzymatic and non-enzymatic antioxidants, is able to reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$ .

TAC was measured by the reaction of phenanthroline and  $\text{Fe}^{2+}$  using a spectrophotometer at 520 nm. At 37°C, a TAC unit is defined as the amount of antioxidants required to make absorbance increase 0.01 in 1 mL of serum. Free radical damage was determined by specifically measuring Malondialdehyde (MDA). MDA was formed as an end product of lipid peroxidation which was treated with thiobarbituric acid to generate a colored product that was measured at 532 nm (MDA detecting kit from Nanjing Jiancheng Bioengineering Institute-China (12).

### 2.5. Statistical

Comparisons were made using the ANOVA test for comparison of data in the control group and the experimental groups. The results were expressed as mean $\pm$ SEM (standard error of means). Significant difference is written in parentheses.

## 3. Results

The effect of the 15% and 30% and 50% *Amomum granum paradisi* aqueous extract on concentration of MDA and TAC of control and experimental groups in the broiler breeder male

**Table 1** The effect of the 15% and 30% and 50% *Amomum granum paradisi* aqueous extract on concentration of MDA and TAC

| Parameter's | Control          | 20 mlg\ L        | 40mlg\L           | 50mlg\L          |
|-------------|------------------|------------------|-------------------|------------------|
| MDA         | 0.43 $\pm$ 0.05A | 0.18 $\pm$ 0.01B | 0.15 $\pm$ 0.023B | 0.16 $\pm$ 0.09B |
| TAC         | 0.69 $\pm$ 0.08C | 0.91 $\pm$ 0.04B | 1.48 $\pm$ 0.07A  | 1.32 $\pm$ 0.05  |

Measurement of serum malondialdehyde (MDA) and total antioxidant capacity (TAC) The total antioxidant capacity (TAC) was considerably higher in G3 (1.48 $\pm$  0.07) and G2 (0.91 0.04) than in the control group (0.69 0.08). The mean concentration of malondialdehyde (MDA) was significantly lower in G3 (0.15 0.  $\pm$ 023) and G2 (18.0  $\pm$ 0.01) than in the control group (0.43  $\pm$ 0.05). (Table 1)

### 3.1. Sperm parameters

Table 1 also displays how several sperm parameters are affected by AET. AET had no statistically significant ( $p > 0.05$ ) impact on semen pH, despite the fact that semen pH increased in a dose-dependent way. Additionally, a dose-dependent increase in sperm motility was seen from 84.65% compared to 75.11% in the control group b.wt. However, the effect was substantial ( $p < 0.05$ ).

**Table 2** Effect of aqueous extract of *Amomum granum paradisi* on sperm parameters, by 3 concentrations

| After activation |                 |                 | Before activating |                                      |
|------------------|-----------------|-----------------|-------------------|--------------------------------------|
| 50 mg\l          | 40mg\l          | 20 mg\l         |                   |                                      |
| d<br>22.9±4.6    | c<br>16.2±4.2   | b<br>6.6±1.7    | a<br>30.1±5.1     | Sperm count (×106 mL <sup>-1</sup> ) |
| b<br>47.1±7.6    | b<br>39.1±6.8   | b<br>35.2±6.1   | a<br>30±4.5       | Sperm motility (%)                   |
| c<br>3.2±0.9     | b<br>2.5±0.4    | b<br>2±0.07     | a<br>1.8±0.3      | Sperm viability (%)                  |
| c<br>46.7±5.9    | b<br>44.1±5.3   | b<br>32.8± 4    | a<br>27.6±4.3     | Normal<br>Morphology(%)              |
| C<br>24.6± 2.5   | B<br>28.78± 2.3 | b<br>31.25± 3.2 | a<br>39.4±4.4     | Abnormal<br>Morphology(%)            |

#### 4. Discussion

The increase in sperm motility of the experimental groups in comparison with the control group could be due to the protective effect of the aqueous extract. These productive effects are reflected by the decrease in the level of malonaldehyde and the increase in the total antioxidant capacity. This is consistent with Hamza et al (13). Whereas, it has been proven that the aqueous extract of *Z. officinale* has led to an increase in testicular activities and an increase in sperm motility in treated mice (14).

AN (vitamin A, tannins, linoleic acid, oleic acid and alkaloids) that inhibit fat oxidation, improve testicular function, enhance fertility, and improve sperm count and testicular tissue. It improves semen parameters and has little or no effect on testicular tissue (Hwang et al, 2004). Oleic acid has a positive effect on the reproductive system (15).

Phytochemically, *Amomum granum paradisi* contains phenolic compounds (phenols, sterols and flavonoids) where the antioxidant effect is attributed to the presence of flavonoids and polyphenols. , non-protein amino acids, cagogenic glycosides, alkaloids and polyunsaturated fats; vitamins (B1, B2, C, E) and trace elements/minerals such as calcium, iron, magnesium, zinc, copper and phosphorous. This is consistent with (16,17,18).

The plant also contains glycosides that possess various antioxidant properties, which have the ability to improve the fertility of the male reproductive system.

The seminal quality was affected by the administration of 60 mg/L of eugenol in fresh semen and 50 or 60 mg/L in frozen semen. Therefore, anesthesia with 40 mg/L of eugenol is recommended because, despite offering the same reproductive performance to men, it resulted in Reduce animal stress, thus avoiding possible disturbances caused by the immune system of animals by stress in reproductive management Maintaining the integrity of animal health in order to preserve reproductive performance in future semen collections, in addition to this to reduce the risk of disease and mortality.

In frozen semen samples, mitochondrial function was affected by a concentration of 60 mg/L of eugenol. It is known that ATP levels in fish sperm are only sufficient to maintain motility from a few seconds to a few minutes at most (19).

Eugenol is a phenylpropane derivative with pronounced antimicrobial, antioxidant and analgesic effects. Eugenol is a common component of *Eugenia caryophyllata* L. Merr. & Perry, *Myristica fragrans* Houtt., *Laurus nobilis* L., *Cinnamomum zeylanicum* and many other plants

It is a yellowish liquid with a clove-like odor that is poorly soluble in water and highly soluble in organic solvents. Its mechanism of antimicrobial action may be explained by its effect on bacterial outer membrane function and its impairment of bacterial metabolism and protein synthesis (20,21,22,23).

It was concluded from the current study that supplementation The results of the current study showed that supplementation of eugenol and vitamin C as an antioxidant in an aqueous extract of the plant can affect the parameters of sperm motility and enhance the quality of cryopreserved sperm in the integrity of the plasma membrane, the integrity of the acrosome membrane, and potential markers of the mitochondrial membrane in comparison with the control group in particular. In the presence of eugenol, it is soluble in water (24,25,26) while vitamin E is a fat-soluble substance (27).

Eugenol is not only susceptible to absorption into sperm plasma membranes, but also dissolves in semen extenders against animal lipids, cooling and storage (28),

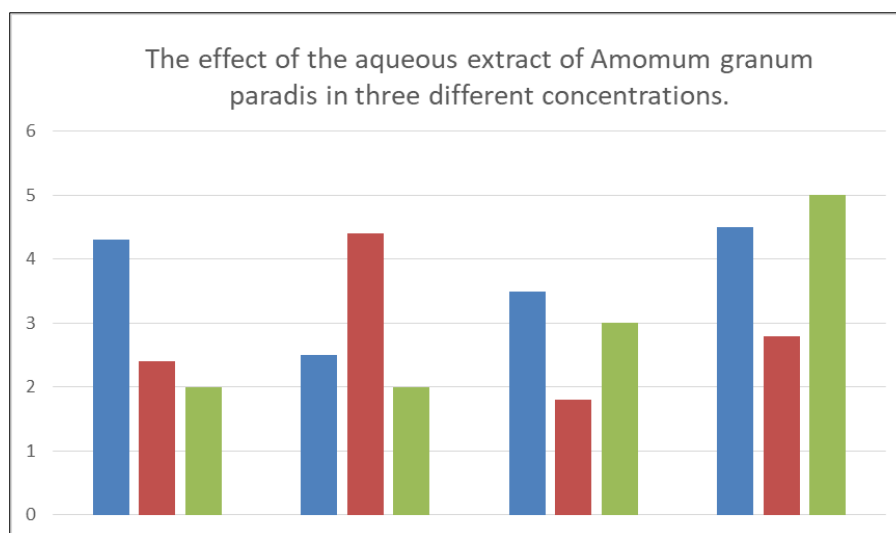
The present study indicated that the supplementation and antioxidants contained in the aqueous extract of the plant prevent lipid peroxidation in sperm by inhibiting the production of MDA. In particular, the level of MDA in the eugenol vasodilators was lower than that in the rest. Protecting sperm and maintaining its activity This means that the antioxidant activity of these antioxidants can protect sperm and improve sperm quality during preservation.

This may indicate that the evaluation was similar in both semen and spermatozoa however, while preserving both spermatogonia (29).

vitamin C against oxidative stress and morphological changes of the testicular tissues (30,31,32,33), as well as suggested by a commensurate rise in testicular and epididymal weights, as well as AET's significance in oxidative stress protection, Its propensity for reducing sperm and reproductive potential is also indicated by morphological alterations. toxicities (34, 35).

The improvement in sperm quality (semen pH, sperm motility, sperm viability, and sperm head abnormalities) and sperm count are shown to be concurrently dose-dependent in Figure 2, which may also be related to an increase in testosterone. encouraging spermatogenic cells to successfully progress through spermatogenesis and sperm maturation in the secretory activity of the accessory sex glands and the epididymis s (36, 37)

The study's findings showed that stimulating sperm for ICSI throughout a 45-minute incubation period significantly increased (P 0.05) the proportion of normal sperms. Due to their low density and lack of movement, these cells cannot reach the spermatogonia by centrifugation when the supernatant is removed, and the quantity of good sperm morphology is one of the crucial sperm criteria for ICSI fertilization to succeed (38, 39)



**Figure 2** The effect of the aqueous extract of *Amomum granum paradis*

Because the ICSI technique relies on free selection steps, Gomes and his team (2000) found that the fertilization rate decreased when sperms of abnormal shape were used compared to the control group. However, the normal shape of sperm and active motility were among the most crucial and best parameters for assessing the success of ICSI (Karpuz et al., 2007).

The 45-minute incubation period significantly improved (P 0.05) all sperm parameters, including sperm concentration, sperm motility percentage, activity level, sperm motility coefficient, percentage of normal sperm, and white blood cell concentration. The cause of this could be because, as in other cultures, the sperm adapted to the culture media during this time and benefited from its ionic salt contents, which include sodium, potassium, calcium, magnesium, phosphate, pyruvate, and lactate. These elements activate the sperm and improve the regulation of its movement and activity (40, 41, 42,43).

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## 5. Conclusion

The study used Amumum extract with three different concentrations to increase the reproductive capacity and activate sperm parameters. After using the culture medium, the concentration in the swimming technique and a concentration of 50mg\ were demonstrated, with significant increases in the percentage of motile spermatozoa, the degree of sperm activity, and the sperm motility factor. Nutritional support from a nutritionist Targeted integrative therapy. Feeding strategies it has a beneficial effect on sperm counting, movement and fertility. Modern medicine it is constantly evolving and must keep up the pioneering concept of nutritional and health benefits. In this regard, patients should be nutritionally educated. It is directed to treat male infertility from its roots problem. A healthy lifestyle should be the mainstay from management.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

No conflict of interest.

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