ISSN 2349-7823

International Journal of Recent Research in Life Sciences (IJRRLS)
Vol. 12, Issue 1, pp: (1-4), Month: January - March 2025, Available at: www.paperpublications.org

IMPACT OF ETHANOLIC EXTRACT OF AZANZA GARCKEANA (GORONTULA FRUIT) ON SEMEN QUALITY AND HORMONAL PROFILE OF ADULT WISTAR RATS

Ezeokafor Emmanuel Nonso[1], Udeh Ekenechukwu, Bethel[2], Dike Charles Chijioke[3], Okwuonu Ifeoma Frances[4], Nnaemeka Wuraola Serah[5], Afuberoh Francis Chukwudi[6], Nwanaga Clinton Uche[7], Anyiam Kennedy Ekenedirichukwu[8]

Dept. of Human Physiology, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi campus

Dept. of Human Biochemistry, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi campus

Dept. of Human Physiology, University of Port Harcourt

Dept. of Human Anatomy, Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli Campus

DOI: https://doi.org/10.5281/zenodo.14591704

Published Date: 03-January-2025

Abstract: **Infertility is a common problem which poses a threat and challenge in our society, which are caused by unknown origin or factors such as hormonal imbalances which reduces sperm production and functioning. The present study is aimed to investigate the impact of ethanolic extract of Azanza garckeana (Gorontula) on semen quality and testosterone level on adult male Wistar rats. A total of twenty rats were used, The rats were divided into four groups of five rats each: Group A ;served as "CONTROL" , receiving water and feed, Group B; 100mg/kg of A. garckeana extract, Group C; administered 500mg/kg of A. garckeana extract, Group D; administered 1000mg/kg of A. garckeana extract. The rats were acclimatized for two weeks before the commencement of the experiment. The ethanolic extract of A. garckeana was administered orally to Group B, C and D rats for four weeks. Semen Analysis and Testosterone level were analyzed using standard methods. One way ANOVA followed by Fisher's LSD multiple comparison was used for statistical analysis. The results revealed a significant increase in the mean Testosterone level of group B, C and D when compared to the CONTROL group, there is also a significant increase in the normal sperm cells in groups B, C, and D (p=0.014, p=0.028, p=0.004) when compared to group A. Therefore, the study showed that ethanolic fruit extract of Azanza garckeana could be used in the management of male sexual act and improved male fecundity, and should be incorporated in treatment of male infertility of different aetiologies owing to their high amount of flavonoids that could help improve infertility.**

Keywords: **Azanza garckeana, fecundity, flavonoids, hormonal imbalance, infertility, semen.**

1. INTRODUCTION

Male infertility is a significant reproductive health concern affecting couples worldwide, with approximately 15% of couples experiencing difficulty conceiving, with male factor infertility contributing to about 50% of cases [2]. Semen quality, characterized by parameters such as sperm count, motility, and morphology, plays a crucial role in male fertility [5]. Various factors such as environmental toxins, oxidative stress, and hormonal imbalances can adversely impact semen

quality [3]. Consequently, there is a growing interest in exploring natural compounds with potential beneficial effects on male reproductive health.

Azanza garckeana, commonly known as Gorontula Fruit, is a plant widely distributed in West Africa and has been traditionally used for various medicinal purposes [6]. Previous studies have reported on the phytochemical composition of Azanza garckeana, revealing the presence of bioactive compounds such as flavonoids, alkaloids, and tannins [1]. These compounds possess antioxidant and anti-inflammatory properties, suggesting potential benefits for male reproductive health.

The study aimed to determine the impact of ethanolic extract of Azanza garckeana (Gorontula Fruit) on semen quality and testosterone level in Adult Wistar rats, as the findings will provide an insight on the benefit of Azanza garckeana on semen quality and testosterone levels of the testes, which will bring an improvement to male fertility.

2. MATERIALS AND METHOD

Location

This study was carried out in the animal house, department of human physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Nnewi campus, Nnamdi Azikiwe University, Anambra State, Nigeria.

Materials

- Male wistar mice
- Saw dusts
- Laboratory coat and gloves
- Ethanol
- Beakers
- Syringes
- Measuring cylinders
- Refrigerator (Nexus)
- Azanza garckeana (Gorontula fruit)
- Normal saline
- Cages
- Canula
- Centrifuge (search tech instruments, British standard) model 80-2
- Thermostat oven DHG-90 23A, PEC medical, USA
- Neubauer Counting Chamber (England)
- Electronic weighing balance, M-methlar model M311l, China
- Mice feeds (non pelletized grower) and water

Extract Procedure

Fruits of Azanza garckeana (Gorontula kola) was purchased from Onitsha main market, Anambra State, and was washed in running tap water to remove dirt and air-dried under ambient temperature. The dried Fruits of Azanza garckeana (Gorontula kola) was milled into a coarsely powdered form using a local grinder. 250g of the dried leaf was macerated in 1000mls of 95% absolute ethanol for 48hours. It was filtered using a clean white cloth and further filtration using Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator and dried further using a laboratory oven at 45°C into a gel-like form. The extract was preserved in airtight container and kept in a refrigerator for further usage.

Experimental Design

Total of 20 Male Wistar rats were used, experimental animals were divided into four groups of five animals each as follows:

Group A received feed and distilled water ad libitum only

Group B received 250 mg/kg of EFAG

Group C received 500 mg/kg of EFAG

Group D received 1000 mg/kg of EFAG

All experimental protocol following the administration of the ethanolic fruit extract of Azanza garckeana (EFAG) was duly observed and done through oral gavage for 30 days

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Statistical Analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS version 25). An inferential statistics (ANOVA) were used, and values were presented in Mean and Standard error in mean (Mean and SEM). Data where analyzed using one-way ANOVA, followed by Post hoc LSD multiple comparison.

3. RESULT

TABLE I: Effect of ethanolic extract of *Azanza garckeana* on sperm motility and total sperm count

	Active motilesperm(%)	Non-motilesperm(%)	Totalspermcount(x10^6/L)
	MEAN±SEM	**MEAN±SEM**	**MEAN±SEM**
GroupA(control)	76.67±3.33	23.33±3.33	687.33±118.0
GroupB(100mg/kgofEAG)	90.33±2.67*	9.67±2.67*	1044.0±33.6*
GroupC(500mg/kgofEAG)	89.00±2.08*	11.00±2.08*	1013.3±55.5*
GroupD(1000mg/kgofEAG)	95.00±0.00*	5.00±0.00*	1026.3±4.33*
F-ratio	**10.856**	**10.856**	**6.420**

Data was analyzed using ANOVA followed by post Hoc LSD comparison and values were considered significant at p<0.05, *: significant, #: not significant compared to group A. EAG: EAG: ethanolic extract of Azanza garckeana

Table I result showed a significant increase in the active sperm cells in groups B, C, and D (p=0.004, p=0.006, p=0.001) when compared to group A. The non-motile sperm cells result showed a significant decrease in groups B, C, and D (p=0.004, p=0.006, p=0.001) when compared to group A. The total sperm count result revealed a significant increase in groups B, C, and D (p=0.006, p=0.009, p=0.007) when compared to group A.

TABLE II: Effect of ethanolic extract of *Azanza garckeana* on sperm morphology and testosterone level

	Normal spermcells(%)	Abnormal cells (%)	Testosterone level(ng/mg)
	MEAN±SEM	**MEAN±SEM**	**MEAN±SEM**
GroupA(control)	75.00±2.88	25.0±2.88	1.15±0.02
GroupB(100mg/kgofEAG)	86.67±1.67	13.33±1.67	1.24±0.01
GroupC(500mg/kgofEAG)	85.00±2.88	15.00±2.88	1.56±0.02
GroupD(1000mg/kg ofEAG)	90.00±2.88	10.00±2.88	1.45±0.06
F-ratio	**6.000**	**6.000**	**29.123**

Data was analysed using ANOVA followed by post Hoc LSD comparison and values were considered significant at *p*<0.05,*: significant, #: not significant compared to group A. EAG: EAG: ethanolic extract of *Azanza garckeana*

Table II result showed a significant increase in the normal sperm cells in groups B,C, and D(*p=0.014,p=0.028,p=0.004*) when compared to group A. The abnormal sperm cell result showed a significant decrease in the abnormal sperm cells in groups B,C, and D(*p=0.014,p=0.028,p=0.004*) when compared to group A. The testosterone level revealed an increase in groups B,C, and D(*p=0.110,p=0.000,p=0.000*) when compared to group A, but indicate significance in groups C and D.

4. CONCLUSION

Findings from the study revealed that Testosterone indicated a high statistically significant difference in mean in groups B, C and D when compared to group A. The physiology linked to the high testosterone level in groups administered with 100mg/kg, 500mg/kg and 1000 mg/kg of ethanolic fruit extract of A. garckeana, is the flavonoids and phenols, which has the potency of regulating FSH and LH hormones through a complex pathway not fully understood. However, the report of [4] demonstrated a significantly higher testosterone level following ingestion of Azanza garckeana against formalin-induced testicular dysfunction. [7] reported a significantly higher testosterone levels following administration of Azanza garckeana against Bisphenol-A testicular toxicity, which agrees to the study findings.

The study findings showed that administration of the ethanolic fruit of A. garckeana revealed a significantly higher mean of active sperm motility in groups B, C and D while decrease levels of non-motile sperm cells in groups B, C and D when compared to control group. The physiology linked to the improved active sperm motility and lowered none-motile sperm cells could be presence of flavonoids. However, spermatogenesis increases as indicated in the active motile sperm could result from increase testosterone level as shown above following up-regulation of testosterone production following FSH and LH regulation that could improve the active sperm motility. [4] Showed an increase in sperm motility following administration of Azanza garckeana against formalin induced testicular dysfunction, which aligns to the study findings. [7] Showed similarity to the study findings revealing a significantly higher active sperm cells following different extraction of A. garckeana. The study revealed that administration of ethanolic extract fruit extract of Azanza garckeana causes an improved level of semen quality as indicated in sperm motility, morphology, and total sperm count as well as hormonal level (testosterone).

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