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Ameliorative Effects of Methanolic Extract of The Fruit Pulp of *Azanza Garkena* on Dexamethasone - Induced Impaired Spermatogenesis in Male Wistar Rats, 1: Effects on Haematology, Serum Chemistry and Male Hormones

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Abstract- ***The aim of this research was to study the effects of the methanolic extract of Azanza garkeana fruit pulp (MEAGFP) on dexamethasone-induced impaired spermatogenesis in male Wistar rats. The animals were divided into six groups and administered varying doses of dexamethasone and MEAGFP. After sacrifice, blood and tissue samples were collected for analysis. The results showed that the MEAGFP contained 9.39% flavonoids and resulted in higher testosterone concentration compared to the control. Similarly, the drug and its co - exposure with the MEAGFP did not cause (p≥0.05) a significant difference in blood parameters. Also, the drug and its co-exposure to MEAGFP had no significant effect (p≥0.05) on ALT, AST, urea and creatinine levels in the group receiving the higher dose in combination with the drug. Testicular width was significantly higher in certain groups, and the drug increased testicular weight and sperm abnormalities at higher doses, although not significantly, compared to other groups. The study concluded that the drug induced some cellular changes in Wistar rats, while the combination of MEAGFP and the drug led to increased seminiferous luminal cellular layers and spermatozoa. The drug caused hepatic congestion and edema, while both concentrations of MEAGFP caused tubular necrosis and cellular infiltration. However, no visible cellular changes were observed in both dexamethasone and the two different concentrations of MEAGFP.***

Index Terms ***Amelioration, Azanza garkeana, Dexamethasone, Spermatogenesis***

I. INTRODUCTION

Reproductive health is state of complete physical, mental, physiological, and social well-being of the reproductive system and its associated functions and processes (WHO, 2023a). Therefore, deteriorations in the reproductive health of affected individuals in terms of infertility and sterility adversely affects their reproductive capacities and or potentials. Infertility is the reproductive disturbances-induced temporary inability to become pregnant or produce (Gokuldas *et al.,* 2021), whereas sterility is intense case of permanent infertility (FAPM). WHO (2023b) opined that the condition affects about one in six (1 in 6) people globally thereby creating the need for its mitigation. Similarly, infertility does not only directly impact the reproductive system in men and women, it predisposes the system to other diseases such as uterine fibroids, obesity, and sexually transmitted diseases (Billups, 2019).

Male infertility may be up to 50% in some parts of the world, though there may be regional variations (Borght and Christine, 2018). Testicular dysfunction and post-testicular deficiency (ejaculatory dysfunction or obstruction to sperm delivery) remain the main cause of male infertility in humans (Borght and Christine, 2018). However, the most frequent cause of impaired spermatogenesis in humans is testicular dysfunction (Jungwirth *et al.,* 2012). Infertility could also be failure to produce offspring in monotocous animals or the production of subnormal number of offspring in polytocous animals (Momont, 2022). Hormonal imbalance, oestrus cycle disorder, and inability to conceive, as well as conceptual prenatal and perinatal death in female animals or due to issues associated with sperm production, transport, and storage, including libido disorder and the animal's inability to mount and or

mate in males are some of the causes of infertility (Momont, 2022). Reproductive aging, which is a multifactorial process involving the direct damage to reproductive organs or indirectly, on germ cells quality could also results in infertility (Comizzoli and Ottinger, 2021). According to Ottinger (2010), besides the effects of natural physiological factors, endocrine disruptors, drugs, nutrition, or stress can accelerate and negatively impact the reproductive aging. This is in addition to the possible role of photoperiods, nutrition, and infection on reproduction disturbances in animals (Ali *et al.,* 2019).

Some drugs have the propensity to induce gonadal damages resulting in either infertility or sterility, depending upon the extent of such damages (Pody and Walker, 1985). Such drugs may include steroid hormones, anesthetics, cardioactive drugs, analgesics, histamine antagonists, and antimicrobials have as well as heavy metals and ethanol (Pody and Walker, 1985), which are either recreational, over-the-counter, or prescription drugs used in proper dosages and routes or misused (Rayburn *et al.,* 2018).

Therefore, concerted efforts must be made towards ameliorating the deterioration in reproductive health of affected animals, especially in males, using whatever cheaper and readily available resources such as *Azanza garkeana.* (also known as Goron Tula, African chewing gum, silky snot), (Sulieman, 2019). It is a deciduous shrub that grows to a height of 3-15 m . It grows naturally in tropical regions of Africa especially at1700 m above sea level with total annual rainfall of between 250 and 1270 mm (Orwa, 2009). In Nigeria, it grows mainly in a town called Tula in Kaltungo Local Government Area of Gombe State, North-East Nigeria (Bukar *et al*., 2021). The traditional use of *A. garckeana* for libido enhancement in both males and females exist (Maroyi, 2017), suggesting its possible aphrodisiac property. Given that male reproductive health has generally received only a little attention over the years (Hawkes and Hart, 2000; ESF, 2010), the aim of this study was to investigate the ameliorative effects of Miracle fruit, *A. garckeana* (F.hoffm.) Exell & Hillc, on steroid-induced impaired spermatogenesis in male rats. The earlier folkloric claims that the plant enhances sexual appeal and desire for sexual intercourse, which are both characteristics of its acclaimed aphrodisiac and fecundity potentials, formed the basis for the present study in male Wistar rats that underwent dexamethasone administration- induced testicular damage.

II. MATERIALS AND METHODS

Ethical Approval

Ethical approval was obtained from the University of Abuja Ethical Committee on Animal Use, with approval number: UAECAU/2024/002.

Preparation and Collection of Plant Materials

Azanza *garckeanea* fruits were purchased from Tula town, Kaltungo Local Government Area, Gombe State, Nigeria, and identified/authenticated at the Herbarium of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria, with voucher no: ABU07276. The experimental fruit was dried at room temperature at the laboratory of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, before pulverization into a fine powder for further use. Absolute methanol was used to extract the pulverized *A. garckeanea* fruit pulp via the Soxhlet extractor to obtain the filtrates, which was dried to constant weights over water baths at 50^{o}C. The extractive yield was calculated as a percentage of the total weight of the extract relative to the total weight of the used pulverized fruits, according to El Mannoubi (2023).

Experimental Design

Acute toxicity bioassay:

The acute toxicity bioassay of the MEAGFP was carried out using the Acute Oral Toxicity: Up-and-Down-Procedure (Test No.425) of OECD (2022).

Sub-chronic toxicity bioassay:

The Wistar rats were divided into six groups (Groups A - F) comprising seven rats each. The rats were administered the extract by gavage daily for 63 days and dexamethasone weekly during the same period. The various substances were given according to the experimental groups as follows:

1. Group A: Control Wistar rats were administered normal saline (1 ml/kg b.w) by gavage as described by (Bukar *et al.,* 2021).
2. Group B: Treated Wistar rats were administered dexamethasone (3.5 mg/kg b.w per day intraperitoneally) as described by (Orazizadeh *et al.,* 2010; Kumar *et al.,* 2016).
3.Group C: Treated Wistar rats were administered MEAGFP (250 mg/kg) (low dose) by gavage as described by (Bukar *et al.,* 2021).
4.Group D: Treated Wister rats were administered MEAGFP (500 mg/kg b.w.) (high dose) by gavage as described by (Bukar *et al.,* 2021).
5.Group E: Treated Wistar rats were administered dexamethasone (3.5 mg/kg bw/day intraperitoneally) and MEAGFP (250 mg/kg b.w) (low dose) by gavage
6.Group F: Treated Wistar rats were administered dexamethasone (3.5 mg/kg bw/day intraperitoneally) (high dose) and MEAGFP (500 mg/kg b.w) (high dose) by gavage.

III. HEMATOLOGY, SERUM CHEMISTRY AND REPRODUCTIVE HORMONE DETERMINATION

Hematological determination:

Blood samples were collected from the Wistar rats via the ocular median canthus as described by Parasuraman *et al.* (2010). Two millilitres of the collected blood were dispensed into EDTA-containing sample bottles for full blood count using a haematology autoanalyzer (Orphee®, 5-part Mythic 60), according to the manufacturer's instructions.

Serum blood chemistry determination:
Another portion of the collected blood were dispensed into plain sample bottles and allowed to coagulate before centrifuging at 3000 rpm for 15 minutes to obtain the serum for biochemical analysis according to (Shnewer Mahdi Al-Turfi *et al.,* 2022). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, and creatinine were analysed with commercial diagnostic kits (ELITECH Group), according to the manufacturer's instructions.

Reproductive hormones determination:
Commercial diagnostic kits (ELITECH Group) were used to ascertain serum testosterone, follicle stimulating hormone (FSH), and luteinizing hormone (LH) levels in the experimental Wistar rats, according to the manufacturer's instructions.

Data analysis
Data were expressed as mean ± SEM (standard error of mean) before subjecting them to Student's t-test and a One-way ANOVA and post hoc Dunnett's multiple comparison test for statistical significance ($p<0.05$) using the GraphPad Prism programme (GraphPad Prism version 7, www.grappad.com)

IV. RESULTS

Hematology, Serum Chemistry and Reproductive Hormone Determination

Hematological Evaluations
Although dexamethasone tended towards causing polycythemia or erythrocytosis, the changes were not significant ($p>0.05$). Similarly, although erythrocytic indices tended to increase following dexamethasone exposure, the changes were not significant ($p>0.05$). The above changes notwithstanding, MEAGFP co-exposure did not significantly ($p>0.05$) alter these changes (Table 3). Dexamethasone administration induced insignificant ($p>0.05$) leukopenia characterized by neutropenia, lymphopenia, and monocytosis. Likewise, MEAGFP co-exposure did not significantly ($p>0.05$) alter these changes.

Serum Chemistry
Dexamethasone exposure induced insignificant ($p>0.05$) reduction in serum ALT, AST, and urea but caused insignificant ($p>0.05$) increase in the serum creatinine of the exposed Wistar rats (Table 4). However, MEAGFP co-exposures caused no significant ($p>0.05$) changes in these values.

Reproductive Hormone Profiles:
Results showed that LH concentration was low in the 250mg/kg of extract compared to other groups, although the difference was not significant ($p>0.05$) (Table 5). FSH values were lower in the plant extract groups and their combinations with dexamethasone compared to control and dexamethasone alone, but the differences were not significant ($p>0.05$).

Testosterone concentration in dexamethasone alone group was lower in value compared to the control although the difference was not significant. 500mg extract group showed highest testosterone level among all the groups; similarly its combination with dexamethasone showed the next ranking level of testosterone, although these differences were not significant ($p>0.05$).

V. DISCUSSIONS AND CONCLUSION

The recorded insignificant ($p>0.05$) tendency towards polycythaemia or erythrocytosis induced by dexamethasone administration might be due to the ability of corticosteroids to increase RBC, Hb, and PCV (King *et al.,* 1988), as well as ability to stimulate erythrocytosis in normal bone marrow (King *et al.,* 1988). Haskovic *et al.* (2022) has reported a transient betamethasone-induced significant ($p<0.05$) increase in the red blood cells count, haemoglobin concentration and packed cell volume in Wistar rats. However, Stoicescu (2014) and Razzaq *et al.* (2020) reported significantly ($p<0.05$) decreased RBC, Hb, PCV values in anabolic steroids and dexamethasone administered human and Wistar rats who incriminated bone marrow suppression. These reports notwithstanding, glucocorticoids reportedly do not have direct effects on RBC and platelet counts (Alan & Alan, 2018). The variable results call for further investigations into the effects of glucocorticoids, especially dexamethasone, on the haematological profile of the exposed animals. The administration of both doses of MEAGFP caused insignificant ($p>0.5$) reduction in the dexamethasone-induced insignificant ($p>0.05$) erythrocytosis. The tendency towards erythrocytosis could have also been due to splenic contraction or dehydration, according to Harvey (2012), in the exposed Wistar rats, which the MEAGFP administration showed the propensity to mitigate.

Dexamethasone administeration also tended to induce insignificant ($p>0.05$) reduction is serum ALT, AST, and Urea with insignificantly ($p>0.05$) increased creatinine levels in the exposed fish. However, Jackson *et al.* (2008), Hasona & Morsi (2019) and Razzaq *et al.* (2020) reported markedly increased ALT and AST in dexamethasone- administered Wistar rats. Therefore, the findings of the present study need further investigations. However, MEAGFP administration produced insignificant ($p>0.05$) variable results in the exposed Wistar rats.Dexamethasone administration caused an insignificant ($p>0.05$) reduction in testosterone level compared to the control. The finding might be due its ability to alter hypothalamo-pituitary-gonadal axis functions and induce changes in concentrations of some key reproductive hormones and cause systemic paternal/maternal and foetal effects (Suter & Schwartz, 1985; Hardy *et al.,* 2005; Shannon & John, 2010). This is because dexamethasone is a potent steroid (Guerrero *et al.,* 2011). The administration of exogenous corticosteroid (like dexamethasone) suppresses ACTH production where the extent of such suppression depends upon the particularly administered drug (MacDonald, 2000).

Corticosteroids mimic sex steroids as regards their effect on the pituitary gland. Therefore, the lowered testosterone level might be due to a negative feedback effect on the pituitary gland. However, our findings also showed that the extract alone has the potential to elevate serum testosterone in males as well as ameliorate the effect of dexamethasone in serum testosterone of the exposed Wistar rats. This observation seems to be in agreement with the report that stated that due to the presence of flavonoids, *A. garckeana* seed can reduce cancer by interfering with the enzymes that produce estrogen, for example flavonoids inhibit estrogen synthetase, an enzyme that binds estrogen to receptors in several organs (Okwu, 2005; Ajayi, 2014). The summary of this report is that *A garkeana* may have anti-estrogenic properties. Anti -estrogenic drugs have been reported to improve fertility in both males and females. A typical ant- estrogenic drug used for ovulation induction is Clomiphene citrate. Clomiphene citrate (Clomid®) is a popular brand name and nickname for generic clomiphene citrate. The U.S. Food and Drug Administration (FDA) approved this oral fertility medication for use in women who are unable to become pregnant (Masanori *et al.,* 2018).

It affects the hormone balance within the body and promotes ovulation. It is approved only for use in women (Masanori *et al.,* 2018). It's sometimes prescribed off- label as an infertility treatment in men (Masanori *et al.,* 2018). Clomiphene citrate is an anti-

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estrogen that indirectly stimulates secretion of gonadotropic hormones (follicle- stimulating hormone and luteinizing hormone) from the anterior pituitary gland by blocking estrogen receptors in the hypothalamus, which increases the release of GnRH (ElSheikh *et al.,* 2015 Wiehle *et al.,* 2014_ Masanori *et al.,* 2018).). This increase in plasma gonadotropic hormone levels is thought to induce improved testosterone secretory function of Leydig cells and spermatogenic dysfunction in men with low plasma testosterone (ElSheikh *et al.,* 2015, Wiehle *et al.,* 2014).

Table 1: Effects of methanolic extract of *Azanza garckeana* fruit pulp on dexamethasone exposure on the haematological profile of adult male Wistar rats over a 56-day exposure period.

Parameters	Dexamethasone (Dexa)			Dexamethasone and methanolic extract of *Azanza garckeanea* fruit pulp (MEAGFP)				
	Control	Dexa (3.5 mg/kg)	P-value	Dexa (3.5 mg/kg)	MEAGFP (250mg/kg)	MEAGFP (500mg/kg)	Dexa (3.5mg/kg) + MEAGFP (250mg/kg)	Dexa (3.5mg/kg) + MEAGFP (500mg/kg)
RBC (x 10^{12}/L)	8.74±0.22	9.07±0.19	0.2779	9.07±0.19	6.88±1.79	8.31±1.39	7.75±1.34	6.96±1.80
	147.0±4.39	150.4±1.43	0.4716	150.4±1.43	117.7±30.53	133.3±22.29	128.7±22.28	115.1±29.81
Hb (g/I)	0.47±0.01	0.48±0.00	0.3529	0.48±0.00	0.40±0.10	0.46±0.07	0.44±0.07	0.38±0.10
PCV (I/I)	14.7±0.44	15.04±0.14	0.4716	15.04±0.14[a]	41.17±10.65[a]	13.33±2.24	12.87±2.23	11.51±2.98
MCV (ft)	47.43±1.21	48.43±0.68	0.4864	48.43±0.68[a]	12.24±3.17[a]	46.0±7.70	44.71±7.82	38.71±10.01
MCH (pg)	270.3±39.97	269.3±39.69	0.9863	269.3±39.69	212.4±54.90	247.4±41.30	246.4±41.12	212.00±54.78
MCHC (g/I)	8.96±0.76	7.77±0.99	0.3616	7.77±0.99	7.84±2.26	9.39±1.84	7.31±1.25	9.01±2.65
WBC (x 10^9/L)	0.37±0.06	0.24±0.02	0.0677	0.24±0.02	1.01±0.28	0.96±0.27	1.42±0.63	0.31±0.09
Neut. (x 10^9/L)	6.93±0.84	4.76±0.63	0.0634	4.76±0.63	6.03±1.78	7.22±1.48	4.93±0.98	7.04±2.24
Lymph (x 10^9/L)	1.59±0.33	2.76±0.56	0.0971	2.76±0.56[abcd]	0.10±0.03[a]	0.53±0.28[b]	0.64±0.26[c]	1.29±0.39[d]
	0.00±0.00	0.00±0.00	0.3370	0.00±0.00[a]	0.20±0.07	0.29±0.07[a]	0.15±0.07	0.02±0.02
Mono (x 10^9/L)	0.00±0.00	0.01±0.00	0.3370	0.01±0.00[a]	0.49±0.15[a]	0.33±0.13	0.16±0.05	0.26±0.12

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Table 2: Effects of methanolic extract of *Azanza garckeana* fruit pulp on dexamethasone exposure on the serum blood
Chemistry profile of adult male Wistar rats over a 56-day exposure period.

Parameters	Dexamethasone (Dexa)			Dexamethasone and methanolic extract of *Azanza garckeanea* fruit pulp (MEAGFP)				
	CHontrol	Dexa (3.5 mg/kg)	P-value	Dexa (3.5 mg/kg)	MEAGFP (250mg/kg)	MEAGFP (500mg/kg)	Dexa (3.5mg/kg) + MEAGFP (250mg/kg)	Dexa (3.5mg/kg) + MEAGFP (500mg/kg)
ALT (IU/L)	144.4±16.05	120.30±13.43	0.2712	120.30±13.43	84.71±33.34	121.9±27.8	65.43±11.66	75.29±23.27
AST(U/L)	160.7±38.82	125.9±36.73	0.5265	125.9±36.73	173.1±83.41	85.14±30.81	152.3±27.42	100.00±37.43
Urea (mmol/L)	6.43±0.19	5.51±0.21	0.8368	5.61±0.21	5.39±1.42	6.26±1.07	5.91±1.06	4.14±1.10
Creatinine (µmol/L)	68.57±2.30	71.57±2.53	0.3968	71.57±2.53[a]	26.0±6.91[a]	71.29±11.91	65.14±10.91	51.29±13.6

Table 3: Effects of methanolic extract of *Azanza garckeana* fruit pulp on dexamethasone exposure on the serum reproductive hormones profile of adult male Wistar rats over a 56-day exposure period.

Parameters	Dexamethasone (Dexa)			Dexamethasone and methanolic extract of *Azanza garckeanea* fruit pulp (MEAGFP)				
	Control	Dexa (3.5 mg/kg)	P-value	Dexa (3.5 mg/kg)	MEAGFP (250mg/kg)	MEAGFP (500mg/kg)	Dexa (3.5mg/kg) + MEAGFP (250mg/kg)	Dexa (3.5mg/kg) + MEAGFP (500mg/kg)
LH (miu/ml	0.23±0.01	0.24±0.02	0.9757	0.24±0.02	0.08±0.02	0.24±0.05	0.25±0.06	0.22±0.06
FSH (miu/ml)	0.10±0.00	0.10±0.00	0.4839	0.01±0.00	0.74±0.28	0.03±0.02	0.04±0.01	0.05±0.02
Test. (ng/ml)	2.75±0.56	2.04±1.34	0.6353	2.04±1.34	2.24±1.15	4.60±1.28	1.58±0.88	3.11±1.02

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