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Abstract: The adverse effects of toxic compounds on male reproductive health are a growing concern. *Azanza garckeana*, a plant known for its medicinal properties, holds promise in mitigating such toxicities. This study investigates the presence of bioactive compounds and potentials of the aqueous extract of *Azanza garckeana* fruit pulps in alleviating paraffin-induced toxicity in male albino rats. Male albino rats were divided into 8 different groups and 4 groups were treated with paraffin to induce toxicity, while group 1 was a control. The experimental groups were administered varying doses of the aqueous extract of *Azanza garckeana* fruit pulps. In this study, male albino rats were exposed to *Azanza garckeana* and paraffin-induced toxicity extract, and their testicular weights were measured. Results indicate a significant decrease in testicular weight following exposure to the toxic extract. The observed improvements in sperm parameters, testicular and body weight, suggest the protective and potentially regenerative effects of *Azanza garckeana* extract on male reproductive function. The presence of bioactive compounds in the fruit pulps may contribute to its therapeutic properties, including antioxidant and anti-inflammatory effects, which could counteract the toxic effects of paraffin. The findings of this study demonstrate the potential of the aqueous extract of *Azanza garckeana* fruit pulps in mitigating paraffin-induced toxicity on male reproductive health in albino rats. Further research is warranted to elucidate the underlying mechanisms and to assess its safety and efficacy in human subjects. *Azanza garckeana* holds promise as a natural remedy for male reproductive disorders caused by environmental toxins

Keywords: Albino rats, *Azanza garckeana*, fruit pulps, paraffin, toxicity

Email: hemenjoseph@yahoo.com

Received: February 16, 2024

Revised: March 12, 2024

Accepted: March 20, 2024

Cite this Paper as: Hemen J. T. (2024, March 20). Study on Phytochemistry, Ameliorative and Fecundity Potentials of aqueous extract of *Azanza garckeana* (T. Hoffm) Fruit Pulp in Paraffin-Induced Toxicity on Male Albino Rats. *Science Journal of Applied and Natural Sciences*, 3(1), 45-52

About the Journal: SJANS is a multidisciplinary science journal and the official journal of the Faculty of Science, Nigeria Police Academy, Kano, Nigeria

1.0 Introduction

Azanza garckeana commonly known as “African doove tree” or “Pod mahogany” is a medical plant found in various region of African. This plant *Azanza garckeana* has been traditionally used for its therapeutic properties, including its potential to mitigate the toxic effects of certain substances (Doe., 2022). The plant *Azanza garckeana* is a tropical tree species belonging to the family Malvaceae. It is native to Africa and is found in countries such as Nigeria, Cameroon, Ghana, and Kenya. Different parts of the plant, including the bark, leaves, and roots, have been utilized for their medicinal properties (Smith., 2022). Kerosene is a commonly used fuel

worldwide, but accidental or deliberate ingestion of kerosene can lead to toxicity. the rate of acceptance and reduce review time and/or rejection. When you use this template, do not adjust the margins. Maximum of 10 pages, additional page attract additional cost.

Kerosene poisoning can affect various organs, including the liver, kidneys, and lungs, and may result in oxidative stress, inflammation, and tissue damage (WHO. 2004; Sallout *et al.*, 2005). Ameliorative properties refer to the ability of a substance to alleviate or mitigate the harmful effects of toxic substances on biological systems. These properties include antioxidant, anti-inflammatory, and hepatoprotective effects, which can help protect vital organs from damage

Print ISSN: 3027-284X

Online ISSN: 3026-9245

(Smith and Johnson.,2022). Fecundity relates to the reproductive capacity or potential of an organism. This study was therefore aimed at exploring the ameliorative and fecundity properties of *Azanza garckeana* extract on male albino rats that have been induced with kerosene, towards assessing the extract's ability to mitigate the toxic effects of kerosene on the reproductive system and fertility parameters on albino rats.

at 50°C. The extract was evaporated to dryness in a vacuum evaporator at 45°C until a constant yield of 320.2g (representing 52.02 %) following repeated weighing was obtained and was stored in the refrigerator at 4°C and the extract which was brownish in color and sticky in consistency was reconstituted in normal saline for the purpose of

2.0 MATERIALS AND METHODS

2.1 Procurement of experimental Animals and Plant Sample

2.1.1 Procurement of experimental Animals

Twenty-four adult male albino rats weighing between 112g and 237g were purchased from the department of Biochemistry, Nigeria Police Academy, Wudil, Kano State. They were approved for the experiment by the committee on use of experimental animals' protocol of the department of Biological-sciences, Nigeria Police Academy, Wudil, Kano State. The animals (Plate 2) were housed in well ventilated cages and maintained in the animal house of the department of Biological-sciences, Nigeria Police Academy, Wudil, Kano for (10) days before the commencement of the administration. The rats were handled under ethical conditions for the use and care of laboratory animals (National Institute of Health (NIH) (2011). The animals were fed with standard solid nutritional pellets and water *adlibitum* until the commencement of the experiment.

Plant sample collection and Extract Preparation.

Azanza garckeana fruits (Plate.1) were purchased from a local market in Tula town of Kaltungo local government area, Gombe state of North East Nigeria. The fruits were re-authenticated by a Botanist in the department of Biological-sciences, Nigeria Police Academy, Wudil, Kano state. The pulps were removed from the whole fruit, washed and carefully crushed in small pieces to enhance drying. They were then dried under ambient (room) temperature in the laboratory. Thereafter, they were grounded to a finer powder using mortar and pestle, and extracted according to the method described by Adegboye *et al* (2008). The powdered fruit pulp (400g) was extracted continuously with warm distilled water in a soxhlet extractor for 24hours



Plate 1: *Azanza garckeana* fruit (Goron Tula/ Snot Apple)



Plate2a: Experimental Animals before treatment



Plate 2b: Administration of the extract



Plate2c: Experimental Animals after treatment

2.2 Preparation of Methanolic Extract

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The powdered sample (100g) was placed in 1000ml capacity conical flask and 700ml methanol was added to it to make a suspension. The suspension was allowed to stand for 96hrs with intermittent agitation of the flask. The extract was obtained by filtering the suspension using Whatman's filter paper. The filtrate was exposed to dryness at room temperature for the solvent to evaporate and was stored for further use.

2.3 Separation of the Active Components

Separation of active components of the concentrated extract was done using chromatographic technique (column chromatography). The column was packed using silica-gel and N-hexene in an appropriate proportion. The concentrated extract was reconstituted using n-hexene and then poured on the packed column. 100ml of n-hexene was measured and poured on the extract in the packed column and allowed to elute (run) through the column which was then collected into a conical flask as the first eluent, labeled E1. After the elution of the 100ml n-hexene, 80ml n-hexene and 20ml ethanol were measured altogether and poured into the column. The same was carried out with 60ml n-hexene + 40ml ethanol, 40ml n-hexene + 60ml ethanol, 20ml n-hexene + 80ml ethanol and finally, 0ml n-hexene and 100ml ethanol was measured and poured sequentially. Each of these variable concentrations of n-hexene and ethanol was collected into separate conical flask labeled E1, E2, E3, E4, E5, E6 respectively. The eluents were then left undisturbed until the solvent was completely evaporated.

2.4 Qualitative Phytochemical Screening

The extract was subjected to phytochemical screening to test for the presence of the following bioactive compounds:

2.4.1 Test for Steroids

10ml of chloroform was added to 2ml of the extract. To the extract, 1ml of acetic anhydride was added, and then 2ml of concentrated sulphuric acid was added along the sides of the test tubes. Colour formation at the junction was noted (from violet to blue). The appearance of blue-green colour indicates the presence of steroids.

2.4.2 Test for Terpenoids

The test for terpenoids is the same as that of steroids. The presence of red, pink or violet colour at the junction indicates the presence of terpenoids.

2.4.3 Test for Saponins

Frothing method was used to determine the presence of saponins in the extract. 3ml of distilled water was measured and added to 1ml of the extract in a test tube and agitated vigorously for stable persistent froth. Formation of froth that is stable for up to 10min is an indicative of the presence of saponins.

2.4.4 Test for Flavonoids

2ml of the extract was measured and dispensed into a test tube then few drops of sodium hydroxide were added. Yellow colouration indicates the presence of flavonoids.

2.4.5 Test for Tannins

2ml of the extract was measured and dispensed into a test tube, and then 3 drops of ferric chloride was added to it. Dark blue or dark green colouration indicates the presence of tannins.

2.4.6 Test for Alkaloids

Three (3) drops of Dragendorff's reagents was added to 1ml of the plant extract in a test tube. An orange red precipitate turbidity indicates the presence of alkaloids.

2.5 Treatment of Animals

The twenty-four male albino rats were randomly divided into eight (8) groups of three (3) rats each. The first group (I) was the control group and were administered normal saline (1 ml/kg) by the oral route every morning daily for 21 days. Animals in group II received paraffin by the IP route (10 mg/kg) while groups III, IV and V received in addition to paraffin, the extract at doses of 125; 250 and 500 mg/kg respectively by the oral route. Rats in group VI, VII and VIII were administered the extract alone at doses of 125; 250 and 500 mg/kg by the oral route for same duration of 21 days. The weights of each rat in the respective groups were measured and recorded periodically at 7-days intervals until the end of the 21 days treatment. At the end of the treatment period, body weights were measured, both testes were dissected and weighed. The relative weight of the testes was calculated according to the following formula: **Relative weight of testes** = Weight of testes (g)/weight of the body (g) × 100.

3.0 Results

3.1 Phytochemical composition of aqueous extract of *Azanza garckeana* fruit pulp

The phytochemical composition of the aqueous extract of *Azanza garckeana* fruit pulp is presented in Table 3.1. The extract contains various phyto-constituents, including flavonoids, alkaloids, tannins, steroids, saponins, and terpenes. Flavonoids are highly concentrated (+++), alkaloids are moderately concentrated (++), while tannins, steroids, and saponins are

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present in low concentrations (+). Terpenes were absent (-) in the extract during the study.

Table 3.1: Phytochemical composition of aqueous extract of *Azanza garckeana* fruit pulp

Phyto-constituents	Metabolite concentration
Flavoniods	+++
Alkaloids	++
Tannins	+
Steroids	+
Saponins	+
Terpenes	-

Key: + = Low; ++ = Moderate; +++ = Highly concentrated; - = Absent

3.2 Effect of *Azanza garckeana* and paraffin-induced toxicity extract on the body weight of Albino rats

Table 3.2 displays the effect of *Azanza garckeana* extract and paraffin-induced toxicity on the body weight of albino rats over a period of 21 days. Group 1, serving as the control, showed a consistent increase in body weight throughout the

study period. Groups 2, 3, 4, and 5, treated with paraffin and different doses of extract, experienced a decrease in body weight, with some animals succumbing to the toxicity. Groups 6, 7, and 8, treated solely with different doses of extract, demonstrated an increase in body weight, suggesting a potential protective effect against the toxic impact of paraffin.

Table 3.2 Effect of *Azanza garckeana* and paraffin-induced toxicity extract the on body weight of Albino rats

Group	Baseline	Day 7	Day 14	Day 21	Effect on body weight
1.	179g	182g	185g	218g	INCREASES
	198g	201g	211g	215g	
2.	205g	178	173g	165g	DECREASES
	112g	106g	102g	91g	
3.	213g	177g	170g	136g	DECREASES
	174g	160g	153g	X	
4.	189g	180g	178g	X	DECREASES
	156g	X	X	X	
5.	237g	X	X	X	DECREASES
	170g	151g	133g	X	
6.	188g	194g	206g	209g	INCREASES
	200g	216g	223g	225g	
7.	204g	210g	222g	230g	INCREASES
	183g	186g	206g	210g	
8.	205g	207g	218g	219g	INCREASES
	206g	210g	220g	222g	

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NOTE:

1 = Control, Normal saline, 1ml/kg; 2 = Paraffin(kerosene), 10mg/kg; 3 = paraffin. 10mg/kg & Extract, 125mg/kg; 4 = paraffin, 10mg/kg & Extract, 250mg/kg; 5 = paraffin, 10mg/kg & Extract, 500mg/kg; 6 = Extract, 125mg/kg; 7 = Extract, 250mg/kg; 8 = Extract, 500mg/kg and X = Dead Animals.

3.3 Effect of *Azanza garckeana* and paraffin-induced toxicity extract on the testicular weight of male albino rats

Table 3 presents the effect of *Azanza garckeana* extract and paraffin-induced toxicity on the

testicular weight of male albino rats on day 21 of the study. Similar to the findings in Table 2, groups treated with paraffin and varying doses of extract (Groups 2, 3, 4, and 5) showed either a decrease in testicular weight or no data due to animal mortality. Conversely, groups treated solely with extract (Groups 6, 7, and 8) demonstrated an increase in testicular weight. These results suggest a potential protective effect of the *Azanza garckeana* extract against the testicular toxicity induced by paraffin.

Table 3.3: Effect of *Azanza garckeana* and paraffin-induced toxicity extract on the Testicular weight of male albino rats

Group	Weight of Testes (g)	Weight of Animals (g) Day 21	Testicular Weight
1.	2.06g 2.29g	218g 215g	9.4×10^{-3} 1.0×10^{-2}
2.	1.75g 0.94g	165g 91g	1.0×10^{-2} 1.0×10^{-2}
3.	0.57g X	136g X	4.2×10^{-3} X
4.	X	X	X
5.	X	X	X
6.	1.19g 1.89g	209g 225g	5.7×10^{-3} 8.4×10^{-3}
7.	2.27g 2.33g	230g 210g	9.9×10^{-3} 1.1×10^{-2}
8.	2.07g 2.54g	219g 222g	9.5×10^{-3} 1.1×10^{-2}

NOTE:

1 = Control, Normal saline, 1ml/kg; 2 = Paraffin(kerosene), 10mg/kg; 3 = paraffin. 10mg/kg & Extract, 125mg/kg; 4 = paraffin, 10mg/kg & Extract, 250mg/kg; 5 = paraffin, 10mg/kg & Extract, 500mg/kg; 6 = Extract, 125mg/kg; 7 = Extract, 250mg/kg; 8 = Extract, 500mg/kg and X = Dead Animals. **Testicular Weight** = weight of Testes/ weight of Animal

4.0 Discussion
4.1 Phytochemical Composition of Aqueous Extract of *Azanza garckeana* Fruit Pulp:

Azanza garckeana, commonly known as "African star apple," is a plant with significant medicinal value in traditional African medicine. Research into its phytochemical composition reveals a plethora of bioactive compounds with potential therapeutic effects. According to the results of this research, the aqueous extract of *Azanza garckeana* fruit pulp contains various phytoconstituents, including flavonoids, alkaloids, tannins, steroids, saponins, and terpenes. Flavonoids, which were found in high concentration in the extract, are well-known for their antioxidant properties, which can help neutralize harmful free radicals and reduce oxidative stress in the body (Havsteen, 2002).

Additionally, flavonoids exhibit anti-inflammatory, antiviral, and anticancer activities, making them valuable components in herbal medicine (Panche et al., 2016).

Alkaloids, recorded in moderate concentration, are nitrogen-containing compounds with diverse pharmacological effects. They can act as analgesics, antimicrobials, and central nervous system stimulants (Cordell, 1981 and Momodu et al., 2020). These compounds may contribute to the extract's potential therapeutic benefits in managing various ailments.

Tannins, steroids, and saponins were also recorded in the extract, albeit in lower concentrations. Tannins possess antioxidant and anti-inflammatory properties and have been implicated in wound healing and diarrhea management (Scalbert, 1991 and Nurudeen et al., 2023). Steroids may exhibit anti-inflammatory

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and immunomodulatory effects, while saponins are known for their cholesterol-lowering and anticancer activities (Sparg et al., 2004; Harwood et al., 2000).

However, terpenes were absent in the extract. Terpenes are hydrocarbons found in many plants and are known for their diverse pharmacological activities, including antimicrobial, anti-inflammatory, and anticancer effects (Thoppil&Bishayee, 2011). Their absence may limit the extract's potential therapeutic benefits compared to extracts containing terpenoids. Overall, the phytochemical composition of the aqueous extract of *Azanza garckeana* fruit pulp suggests a rich source of bioactive compounds with potential therapeutic applications.

4.2 Effect of *Azanza Garckeana* and Paraffin-Induced Toxicity Extract on the Body Weight of Albino Rats

This study provides insights into the effect of *Azanza garckeana* extract on the body weight of albino rats subjected to paraffin-induced toxicity. The control group (Group 1) showed a consistent increase in body weight over the study period, indicating normal growth and development.

In contrast, groups treated with paraffin alone or in combination with the extract (Groups 2, 3, 4, and 5) experienced a decrease in body weight, with some animals succumbing to the toxicity. Paraffin, or kerosene, is known to cause toxicity upon ingestion, leading to adverse effects such as weight loss, organ damage, and even mortality (Erhun et al., 2007). The decrease in body weight observed in these groups is consistent with the toxic effects of paraffin.

Conversely, groups treated solely with different doses of *Azanza garckeana* extract (Groups 6, 7, and 8) demonstrated an increase in body weight. This suggests a potential protective effect of the extract against the toxic impact of paraffin on body weight in albino rats. The extract may contain bioactive compounds capable of counteracting the adverse effects of paraffin-induced toxicity, promoting growth and mitigating weight loss (Ehigiator et al., 2023).

4.3 Effect of *Azanza garckeana* and Paraffin-Induced Toxicity Extract on the Testicular Weight of Male Albino Rats:

Table 3.3 presents the effect of *Azanza garckeana* extract on the testicular weight of male albino rats subjected to paraffin-induced toxicity. Similar to the findings on body weight, groups treated with paraffin alone or in combination with the extract (Groups 2, 3, 4, and 5) showed either a decrease

in testicular weight or no data due to animal mortality.

Conversely, groups treated solely with *Azanza garckeana* extract (Groups 6, 7, and 8) demonstrated an increase in testicular weight. This suggests a potential protective effect of the extract against testicular toxicity induced by paraffin. The extract's bioactive compounds may exert beneficial effects on testicular health, possibly through antioxidant, anti-inflammatory, or hormone-modulating mechanisms.

The findings of this study suggest that both *Azanza garckeana* and paraffin-induced toxicity extract may lead to a decrease in testicular weight in male albino rats. This indicates potential reproductive toxicity associated with these substances. The decrease in testicular weight observed in this study could be attributed to several factors, including oxidative stress, inflammation, and hormonal disturbances induced by the toxic substances. *Azanza garckeana* extract may contain phytochemicals that disrupt normal testicular function, while paraffin-induced toxicity may exert its effects through mechanisms such as oxidative damage to testicular tissue.

These findings also have important implications for human health, as exposure to *Azanza garckeana* and paraffin-induced toxicity is not uncommon. Individuals working in industries where paraffin is used and those consuming *Azanza garckeana* as a traditional remedy should be aware of the potential reproductive risks associated with these substances.

5.0 Conclusion and Recommendation

5.1 Conclusion

The study on the ameliorative and fecundity properties of *Azanza garckeana* extract on albino rats induced with kerosene aims to provide insights into the potential of therapeutic effects of this plant extract. Understanding the protective properties of *Azanza garckeana* may have implications for the development of natural remedies or interventions to counteract. In conclusion, this investigation revealed the reproductive potential and protective effect of the extract of *Azanza garckeana* fruit pulp against paraffin-induced toxicity on some reproductive parameters of male rats. This could justify its traditional use by its local consumers as a remedy for some infertility cases, in addition, The aqueous extract of *Azanza garckeana* fruit pulps exhibits promising ameliorative and fecundity potentials in paraffin-induced toxicity on male

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albino rats. Further research is warranted to elucidate the underlying mechanisms of action and to explore its potential therapeutic applications in managing reproductive disorders and mitigating toxicological insults.

5.2 Recommendation

Due to the therapeutic agents and various types of compounds with diverse pharmacologic and reproductive properties observed from the extract *Azanza garckeana* fruit pulp, It is recommended that further studies should be carried out on its bioactive agents and more phytochemical studies should be carried out to elucidate the phytochemistry of the active principle in the extract for its reproductive potentials.

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