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Research paper

Preliminary Phytochemical Screening and Acute Oral Toxicity Evaluation of Aqueous, Ethanolic, and Methanolic Extracts Of Thespesia Garckeana Fruit Pericarp in Wistar Rats

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Abstract

The increasing reliance on medicinal plants for primary healthcare necessitates scientific validation of their safety and bioactive potential. This study evaluated the preliminary phytochemical composition and acute oral toxicity profile of aqueous, ethanolic, and methanolic extracts of Thespesia garckeana fruit pericarp in Wistar rats. Standard phytochemical screening methods were employed to detect major secondary metabolites, while acute toxicity was assessed using Lorke's method across two phases, considering dose variation, gender, behavioural responses, and mortality. Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, steroids, carbohydrates, and trace cardiac glycosides, with methanolic extract exhibiting the richest phytochemical profile. Extraction yield was highest in methanol (44.02%), followed by ethanol (39.09%) and aqueous extract (36.95%). Acute toxicity evaluation showed no mortality at doses up to 1000 mg/kg across all extracts in phase I. In phase II, no mortality was observed even at the highest dose of 5000 mg/kg, indicating an LD_{50} greater than 5000 mg/kg for all extracts. Behavioural observations showed mild transient effects such as reduced activity and sniffing, with no significant gender-related differences. The findings suggest that T. garckeana fruit pericarp extracts are relatively safe and rich in bioactive compounds, supporting their ethnomedicinal use and potential for further pharmacological investigations.

Keywords: *Thespesia Garckeana; Phytochemical Screening; Acute Toxicity; LD_{50}; Medicinal Plants; Wistar Rats; Solvent Extraction; Body Weight.*

1. Introduction

Plants are the renowned cradle of the traditional medicine system that assuages human diseases and promotes health for thousands of years [1], [2]. Medicinal plants remain a cornerstone of healthcare systems in developing countries, with over 70% of the global population relying on herbal remedies [3]. In Nigeria, economic constraints and limited healthcare access have further increased dependence on traditional medicine. Thespesia garckeana (commonly known as "Goron Tula") is widely used in African ethnomedicine for treating ulcers, wounds, and inflammatory conditions [4]. T. garckeana is variously known as tree hibiscus in English, azanza, snot apple, "goron tula" (Hausa-Nigeria), "morojwa" (Bostwana), "Thespesia garckeana" (South Africa) [5,6]. Despite its widespread application, scientific validation of its phytochemical constituents and safety profile remains limited. Natural products are important sources for drug development. The amounts of bioactive natural products in natural medicines are always fairly low. Today, it is very crucial to develop effective and selective methods for the extraction and isolation of those bioactive natural products [7]. Phytochemicals such as flavonoids, tannins, and alkaloids are known for their antioxidant, anti-inflammatory, and tissue-repair properties [8]. However, the safety of herbal extracts must be established before pharmacological application. Acute toxicity testing provides essential data on safe dosage, hazard classification, and potential adverse effects of medicinal products before further pharmacological development [9], [10]. The conventional extraction methods, including maceration, percolation, and reflux extraction, usually use organic solvents and require a large volume of solvents and a long extraction time [7]. Toxicity could be described as the relative ability of a substance or a chemical to cause adverse effects on living organisms; it also explains the extent to which biologically active substances elicit harm or cause mild to severe damage to an organism [11]. Although medicinal plants may have relatively fewer side effects when compared to synthetic drugs, studies have shown that they may cause acute toxicity in children, even with fatal outcomes [12]. Toxicity testing has evolved considerably over the past decades with

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increasing emphasis on scientifically robust and ethically responsible approaches that incorporate the principles of reduction, refinement, and replacement (3Rs). Acute oral toxicity assessment remains an essential component of preclinical safety evaluation for pharmaceuticals, chemicals, and herbal products. Contemporary international guidelines, including the Organization for Economic Co-operation and Development (OECD) Test Guideline 425 (Up-and-Down Procedure), promote the use of sequential dosing strategies that minimize animal use while providing reliable hazard identification and toxicity estimates [1]. Although alternative protocols are now widely adopted, classical methods such as the Lorke procedure continue to be employed for preliminary toxicological screening because of their simplicity, practicality, and suitability for estimating acute toxicity and median lethal dose (LD_{50}) values in experimental studies. Different methods of toxicity testing have been used over the years, which have also received regulatory approval by the regulatory body [13], with some having slight modifications. Despite the authenticity regulations, they also have their merits and demerits. The 3Rs alternatives depicts the reduction approach implies that the number of animals employed in a given test should be minimized while still maintaining consistency and accuracy with scientific practices that would yield convincing and valid results [14], refinement approach which is geared towards providing better welfare to animals by minimizing pain (by using appropriate anesthetic and analgesics), distress and provision of a suitable environment for animals [15] and the replacement approach that involves methods other than the use of animals, including, in vitro and in silico approaches [16]. Implementation of the 3Rs principles has significantly reduced the number of animals as well as the reduction in drug failure rate in the discovery and development pipeline [17]. In recent times, toxicity has been reported in experiments involving laboratory animals, including mice, rats, rabbits, and guinea pigs, manifesting various behavioral symptoms as parameters for their toxicological symptoms [18], [19]. The year 1992 – 2002 was characterized by the fixed dose procedure (FDP) in 1992 [20], 1996: Acute toxic class (ATC) method, i.e., OECD 423 in 1996 [20, 21]. Up and down procedure (UDP) in 1998 [22], incorporation of the LD_{50} tests into the Organization for Economic Cooperation and Development (OECD) guideline in 1981 [23], deletion of the conventional LD_{50} test and introduction of the OECD TGs around December 2002 [21]. The Lorke's method, introduced in 1983, involves the use of thirteen animals in 2 phases. In the first phase, nine animals are divided into three groups of three animals each and are administered 10, 100, and 1,000 mg/kg body weight of the test substance in order to establish the dose range producing any toxic effect. The number of deaths in each group is recorded after 24-hours. In the second phase, four doses of the test substance are selected based on the result of phase 1 and are administered to four (4) groups of one animal each. After 24-hours, the number of deaths is recorded, and the LD50 is calculated as the geometric mean of the highest non-lethal dose (a) and the least toxic dose (b). $LD50 = \sqrt{a \times b}$ [24], [22], [25]. This study, therefore, aimed to evaluate the phytochemical composition of T. garckeana fruit pericarp extracts, the acute oral toxicity profile across different solvents, and behavioural and gender-related responses in Wistar rats.

2. Materials and Methods

2.1. Plant collection and preparation

Fresh plant with fruit of T. garckeana was collected from Tula Baule (Coordinates 9.8709^0 N, 11.5134^0 E), Gombe State, Nigeria, authenticated, and assigned voucher number FHJ 36623 at the Federal College of Forestry, Jos, Nigeria. Matured fruits of the same plant were obtained, and the pericarps were dried, pulverized, and stored under sterile conditions in the post-graduate Laboratory, Department of Biological Science, ATBU, Bauchi, Nigeria.

2.2. Extraction procedure

Portions of 150 g of powdered sample were extracted separately in Distilled water (300ml), 80% ethanol (300ml), and 80% methanol (300ml), respectively. Extraction (Maceration method) was performed for 72 hours, followed by filtration and concentration using a rotary evaporator at 50°C according to the method of Idyu et al., with slight modification. The percentage yield for the Aqueous Fruit Pericarp Extract of T. garckeana (AETG), Ethanol Fruit Pericarp Extract of T. garckeana (EETG), and Methanol Fruit Pericarp Extract of T. garckeana (METG) were determined, after which the crude extracts were stored in separate well-labelled sterile bottles at -40 °C [26 - 28]. The percentage yield for each extract was calculated using:
Percentage yield (%) = Weight of Pure (Extract) /Impure (Powder) x 100/1

2.3. Phytochemical screening

Standard qualitative methods [29], [30] were used to detect Alkaloids, Flavonoids, Tannins, Saponins, Steroids, Glycosides, and Carbohydrates.

2.4. Experimental animal procurement and protocols

A total of 72 healthy albino rats weighing 149-200g), made up of 36 Males and 36 females, were obtained from the National Veterinary Research Institute (NVRI), Vom, Nigeria. The animals were acclimatized for 7 days, maintained with pelleted feed, clean water, and a conducive environment according to the required best practices as recommended by Tuhin [31] in the Postgraduate Laboratory of the Department of Biological Sciences, Abubakar Tafawa Balewa University (ATBU), Bauchi, Nigeria.

2.5. Acute oral toxicity test

The Lethal Dose (LD_{50}) of the aqueous (AETG), ethanolic (EETG), and methanolic (METG) fruit pericarp extracts of Thespesia garckeana was determined to ascertain the safe doses (therapeutic indices), using Lorke's model [32,33] with slight modification.
Although the present study employed the Lorke method because of its simplicity and suitability for preliminary screening, the findings are consistent with internationally accepted principles for acute oral toxicity evaluation outlined in OECD Test Guideline 425 [1]. Acute oral toxicity was evaluated using a modified Lorke method involving two sequential phases of dose administration. While the Organization for Economic Co-operation and Development currently recommends Test Guideline 425 (Up-and-Down Procedure) for acute oral toxicity testing to reduce animal usage and improve ethical compliance, the Lorke model remains an accepted approach for preliminary toxicity assessment and estimation of safety margins in phytopharmacological investigations. The present protocol was therefore selected to provide

an initial evaluation of the acute toxicological profile of Thespesia garckeana fruit pericarp extracts while maintaining consistency with comparable studies in the literature. A total of 72 albino rats (36 Males and 36 Females) were grouped and treated as follows:
Phase 1
The extracts were administered via the oral route (P.o).

- Group I: Administered with AETG (10mg/kg P.o.) - 3 male rats in cage Ia and 3 female rats in cage Ib.
- Group II: Administered with AETG (100mg/kg P.o.) - 3 male rats in cage IIa and 3 female rats in cage IIb.
- Group III: Administered with AETG (1000mg/kg P.o.) - 3 male rats in cage IIIa and 3 female rats in cage IIIb.

The rats in all the groups were denied access to food and water for 30 minutes after drug administration and subsequently monitored for mortality and behavioral (calmness, reduced appetite, sniffing, and nose poking) changes every 6 hours for 24 hours. The same protocol was followed for the EETG and METG groups.
Phase 2

- Group IV: Administered with AETG (1600mg/kg P.o.) - 1 male rat in cage IVa and 1 female rat in cage IV b
- Group V: Administered with AETG (2900mg/kg P.o.) - 1 male rat in cage V a and female rat in cage V b.
- Group VI: Administered with AETG (5000mg/kg P.o.) - 1 male rat in cage VIa and 1 female rat in cage VIb.

The rats in all the groups were also denied access to food and water for 30 minutes after drug administration and subsequently monitored for mortality and behavioral (calmness, reduced appetite, sniffing, and nose poking) changes every 6 hours for 24 hours. The same protocol was followed for the EETG and METG groups.
Lethal Dose (LD_{50}) was calculated, following Lorke's method according to Riazul et al [31].

$$LD_{50} = \sqrt{D_0 x D_{100}}$$

Where:
D_0 = Highest dose that gave no mortality
D_{100} = Lowest dose that produced mortality

2.6. Determination of the effect of intraperitoneal administration of t. Garckeana extracts on the body weight of male rats during an acute toxicity experiment

The body weight of the rats following oral administration of various crude extracts (AETG, EETG, and METG) as well as that of a control group administered with distilled water was measured using an animal weighing balance, the record taken and compared to check if there is any effect of the animal's body weight on the therapeutic index of the extracts.

2.7. Statistical analysis

Data were expressed as mean ± SEM. Analysis was performed using ANOVA with significance at $p < 0.05$.

2.8. Ethical approval

Ethical clearance with reference NHREC/21/05/2005/00718 was obtained from the Health Research Ethics Committee of the Bingham University Teaching Hospital, Jos, Nigeria.

3. Results and Discussion

3.1. Extraction yield

Methanol extract produced the highest extraction yield (44.02%), followed by ethanol (39.09%) and aqueous extract (36.95%). The higher yield obtained with methanol may be attributed to its polarity and superior ability to dissolve a broad spectrum of phytoconstituents, including flavonoids, phenolics, alkaloids, and glycosides. Previous studies have demonstrated that methanol efficiently extracts both polar and moderately polar compounds, thereby improving phytochemical recovery compared to aqueous extraction [11]. The relatively lower yield observed in aqueous extract may be due to the inability of water alone to extract certain semi-polar constituents effectively. Ethanol, which possesses intermediate polarity and lower toxicity compared to methanol, also demonstrated considerable extraction efficiency, supporting its suitability for phytopharmaceutical extraction processes [27].

Table 1: Phytoconstituents of Fruit Pericarp Extracts Sample of T. Garckeana

Constituents	AETG	EETG	METG
Alkaloids	++	+	++
Saponins	-	-	-
Tannins	+	+	-
Flavonoids	+++	+++	++
Carbohydrates	++	++	+
Steroids	+	+	+
Terpenes	-	-	-
Anthraquinones	-	-	-
Cardiac glycosides	-	+ (traces)	+ (traces)

Key: (-) = Not present, (+) = Present, (++) = Moderately present and (+++) = Highly present.

3.2. Phytochemical composition

Methanolic extract demonstrated the richest phytochemical profile among the tested extracts. The presence of flavonoids, alkaloids, tannins, steroids, and carbohydrates suggests that T. garckeana fruit pericarp contains biologically active compounds capable of exerting pharmacological effects. Flavonoids and tannins are known for their antioxidant, anti-inflammatory, gastroprotective, and wound healing activities.

Their antioxidant properties may stabilize cellular membranes and reduce oxidative stress, thereby contributing to tissue protection and low toxicity manifestations during acute exposure [8]. Alkaloids possess diverse pharmacological activities but may also induce transient behavioral changes at high concentrations due to their effects on the central nervous system. The absence of saponins and anthraquinones in the extracts may partly explain the lack of severe gastrointestinal irritation or diarrheal symptoms during toxicity assessment, since some saponins and anthraquinones are associated with gastrointestinal disturbances. The abundance of antioxidant phytochemicals, particularly flavonoids and related phenolic constituents, may also contribute to attenuation of oxidative injury induced by xenobiotics, thereby reducing toxic manifestations observed during acute exposure [34].

3.3. Acute toxicity findings

No mortality was observed in either male or female rats administered aqueous, ethanolic, or methanolic extracts at doses ranging from 10 to 5000 mg/kg body weight. Consequently, the oral LD_{50} for all extracts was estimated to exceed 5000 mg/kg under the experimental conditions. According to internationally recognized toxicological principles reflected in OECD Test Guideline 425, substances that do not produce mortality at limit doses demonstrate relatively low acute oral toxicity and are generally considered to possess a favorable acute safety profile [1]. Nevertheless, the absence of mortality following single-dose exposure should not be interpreted as evidence of complete safety, since repeated-dose toxicity, target-organ toxicity, reproductive toxicity, and genotoxicity require separate investigation. According to OECD toxicity classification criteria, substances with oral LD_{50} values greater than 5000 mg/kg are generally regarded as having low acute toxicity potential. This observation agrees with the principles of OECD Test Guideline 425, which recognizes that substances exhibiting no mortality at limit doses possess relatively low acute oral toxicity under the test conditions [1]. Comparable findings have been reported for medicinal plant extracts evaluated in traditional medicine, where high LD_{50} values were accompanied by minimal behavioral abnormalities and absence of severe toxic manifestations [35]. However, the absence of mortality in acute studies should not be interpreted as complete safety because acute toxicity testing alone cannot establish long-term toxicological effects, organ toxicity, reproductive toxicity, or carcinogenic potential. The findings suggest that the extracts possess a relatively wide safety margin during short-term oral exposure. Similar reports of low acute toxicity have been documented for several medicinal plant extracts rich in flavonoids and phenolic compounds [12], [28]. Mild transient behavioral responses such as calmness, sniffing, nose poking, and reduced activity were observed, particularly at higher doses. The present findings are consistent with published studies demonstrating that numerous medicinal plant extracts exhibit high acute safety margins despite containing diverse bioactive phytochemicals, supporting their continued investigation as potential therapeutic agents when subjected to comprehensive toxicological evaluation. These responses may reflect temporary physiological or neuro-behavioral adaptation to concentrated phytochemicals rather than overt toxic effects. Alkaloids and flavonoids have been reported to influence neurotransmitter activity and behavioral responses in experimental animals at high doses.

3.4. Body weight changes and solvent-related toxicological implications

Across all experimental groups, only slight fluctuations in body weight were observed, with no statistically significant differences ($P \geq 0.05$) between treated and control animals during phase I of the experiment. Body weight is an important toxicological parameter because toxic substances frequently cause weight reduction through appetite suppression, altered metabolism, gastrointestinal dysfunction, or organ impairment. Therefore, the absence of significant body weight loss in the present study suggests that the extracts did not produce severe metabolic or systemic toxicity during acute exposure. Maintenance of normal body weight following administration of herbal preparations is generally considered an indicator of preserved physiological function and absence of overt systemic toxicity. Similar observations have been reported for medicinal plant extracts in experimental animals, where stable body weight correlated with favorable toxicological profiles [36]. The relatively stable body weight profile observed across aqueous, ethanolic, and methanolic extracts may indicate that the phytochemical constituents extracted by these solvents did not significantly interfere with feeding behavior, nutrient absorption, or metabolic homeostasis. Methanol extract, despite possessing the richest phytochemical composition and highest extraction yield, did not produce mortality or marked body weight reduction. This suggests that the extracted phytoconstituents may possess relatively low acute toxic potential at the tested doses. Ethanol extract similarly demonstrated low toxicity, which is consistent with the widespread pharmaceutical use of ethanol as a relatively safe extraction solvent. Aqueous extracts are traditionally regarded as safer because water primarily extracts highly polar compounds and excludes several potentially toxic non-polar constituents. The lack of significant weight changes in animals treated with aqueous extract further supports the relative tolerability of traditional aqueous herbal preparations. Although no severe toxic manifestations were observed, the present findings remain limited to acute exposure. Additional investigations involving subacute and chronic toxicity studies, serum biochemical analysis, organ weight assessment, and histopathological evaluation are required to comprehensively establish long-term safety.

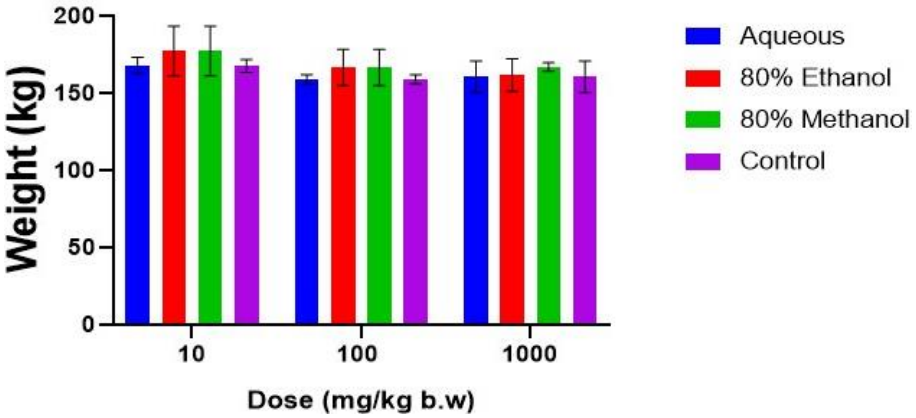


Fig. 1: Effect of Oral Administration of T. Garckeana Extracts on the Body Weight of Male Rats During Acute Toxicity Experiment.

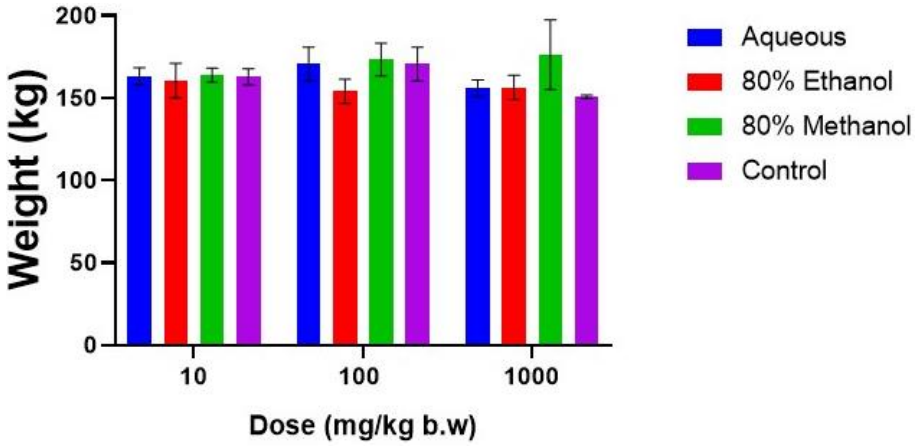


Fig. 2: Effect of Oral Administration of T. Garckeana Extracts on the Body Weight of Female Rats During Acute Toxicity Experiment

Across all groups, there were minor variations (slight fluctuations) in body weight with increasing doses, but no clear trend indicating toxicity-related weight loss or gain for the animal groups in Phase I (Figures 1 & 2). No significant change in body weight of all the rats compared with the control group (0 mg/kg). Statistical analysis showed no significant difference in body weight between treated and control groups ($P \geq 0.05$). This suggests that acute exposure to T. garckeana extracts did not significantly impact body weight for the male and female animal groups. However, Higher doses (Phase 2) were not statistically analyzed (not statistically compared), as doses ≥1600 mg/kg, variability measures were not provided ($n < 3$).

3.5. Gender-based observations

No statistically significant gender-related differences were observed in mortality, behavioral responses, or body weight changes between male and female rats administered the extracts.
The similarity in toxicological responses between both sexes may suggest comparable physiological tolerance to the extracts during acute exposure. This finding supports the possibility of broad biological applicability of the extracts across sexes. However, hormonal and metabolic differences may become more evident during prolonged exposure studies; therefore, chronic toxicity studies remain necessary.

4. Conclusion

The present study demonstrated that aqueous, ethanolic, and methanolic fruit pericarp extracts of Thespesia garckeana contain important phytochemicals, including flavonoids, alkaloids, tannins, steroids, and carbohydrates. Methanol extract produced the highest extraction yield and exhibited the richest phytochemical composition, likely due to its superior ability to extract polar and semi-polar bioactive compounds. Acute oral toxicity evaluation revealed no mortality at doses up to 5000 mg/kg body weight in Wistar rats, indicating low acute toxicity potential and suggesting an oral LD_{50} greater than 5000 mg/kg under the experimental conditions. Mild transient behavioral changes observed at higher doses were not associated with severe toxic manifestations. Furthermore, no statistically significant changes in body weight were observed between treated and control animals, indicating that the extracts did not significantly impair feeding behavior, metabolism, or physiological function during acute exposure. Since body weight reduction is commonly associated with systemic toxicity, the stability of body weight further supports the relatively low acute toxicological profile of the extracts. Nevertheless, comprehensive toxicological evaluation involving repeated-dose studies, organ histopathology, biochemical analyses, and genotoxicity assessment remains necessary before clinical application, in line with contemporary recommendations for safety evaluation of herbal medicines [10]. Although the extracts demonstrated a favorable acute safety profile with an estimated oral LD_{50} exceeding 5000 mg/kg, these findings should be interpreted within the scope of an acute toxicity study. In accordance with current international recommendations for safety assessment of herbal products, additional investigations involving repeated-dose toxicity studies, biochemical and hematological analyses, organ weight determination, histopathology, reproductive toxicity, and genotoxicity are required before definitive conclusions regarding long-term safety can be established.

Conflict of Interest

The authors hereby declare no conflict of interest.

Author Contributions

Authors OTO, RMSC, ONP, and OAT contributed to the processing, data analysis, and writing of the article. AJO and YAO did the reading. BAP did the proofreading, while SD supervised all aspects of the work.

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