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Acute Oral Toxicity Analysis of Methanolic Stem Bark Extract of *Warbugia ugandensis* on Atherosclerotic Lesions in Aortic Tunica Intima of New Zealand Rabbits upon Induction of Atherosclerosis

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ABSTRACT

Warbugia ugandensis is a traditional plant with multiple benefits. In Africa and Asian countries, the plant has been adopted to treat various conditions. It is commonly referred to as the green heart. On the other hand, atherosclerosis remains the leading cause of many vascular diseases. Its management is normally prolonged with patients being put on long-term care to achieve objectives of management. The aim of this study was to assess acute oral toxicity of *W. ugandensis* so as to come up with a safe dose to incorporate into the entire study. For acute oral toxicity a total of 12 rabbits were used. This study was carried out in 2 phases namely; phase I and phase II. Phase I had 9 animals that were further sub divided into 3 groups of each 3 rabbits. Phase II had 3 rabbits. The animals in all the 2 phases were observed for behavioral changes and mortality over 24 hours. The following results were obtained; in both phases, between 30 minutes to 48 hours the rabbits displayed normal activities while after 48 hours no mortality was recorded. Therefore, it was concluded that the safe dose of *W. ugandensis* for use in animal study is ≤ 5000mg/kgbwt as at this dose normal animal activity and no mortality was reported.

Keywords: Acute oral toxicity; Mortality; *Warbugia ugandensis*; Green heart; Vascular disease; Atherosclerosis; Safe dose; Piloerection.

1. Introduction

Warbugia ugandensis is a native tree of East Africa origin, commonly referred to as greenheart. It has a diversity of uses, including atherosclerosis (Orwa et al., 2009). The genus *Warbugia*, is a member of cinnamon family Canellaceae has been described as Africa's panacea. It is an evergreen tree that can grow up to thirty meters tall and seventy centimeters in diameter. It has a variant bark which is sometimes smooth or crusty, light green and a rounded crown. The leaves grow to 3-15 cm by 1.4-5 cm and appear alternately on stems and have dotted glands on their surface with no stipules (Orwa et al., 2009). The flowers are kidney-shaped and appear either lonely or in small 3-4- flowered cymes. Flowering occurring in the early part of wet season, fruiting taking place in late part of wet season and fruit may remain on the tree for quite a long time. In Kenya, *W.* ugandensis flowers in December-January with sown in May.

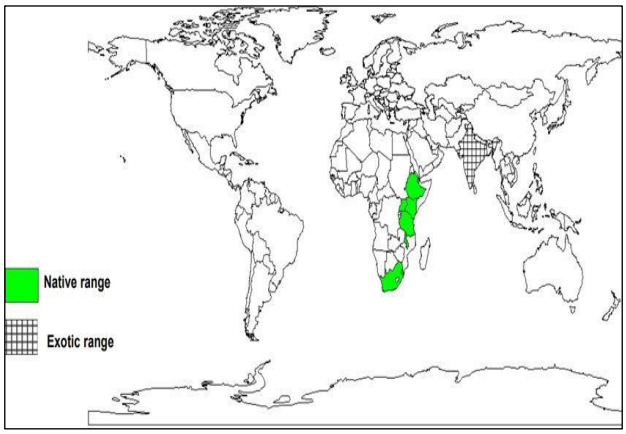

Figure 1. Species of *Warbugia ugandensis* distribution across Africa and other regions (Orwa et al., 2009)

W. ugandensis does well in coastal rainforests at altitudes of 100-2200 m with a mean annual rainfall of 1000-1500 mm (Orwa et al., 2009).

Studies done across the globe have shown that *W. ugandensis* has minimal toxicity. Though there is contradictory information about plant toxicity whereby (Mwitari et al., 2013) suggests that *W. ugandensis* has cytotoxic effects whereas (Anywar et al., 2021) indicates that its cellular assay isn't toxic. Karani et al. (2013) has reported that *W. ugandensis* at a dose of LD50 less than 5000 mg/kg showed no symptoms of toxicity or mortality and therefore it was concluded that toxicity was above 5000 mg/kg bwt. No pharmacological interactions with other drug components or plants have been documented and this calls for a general exploration of this plant in terms of toxicity in induced atherosclerosis. Due to this existing information the current study seeks to determine the safe doses of *W. ugandensis*.

1.1. Study objectives

(i) Assessment of safe dose of methanolic stem bark extract of *Warbugia ugandensis* on atherosclerotic lesions in aortic tunica intima of New Zealand rabbits upon induction of atherosclerosis.

(ii) Assessment of animal behaviour changes in Phases I and II.

2. Materials and Method

2.1. Determination of Acute Oral Toxicity of *W. ugandensis*

Acute oral toxicity was conducted so as to guide on safe doses of plant extracts as per Lorkes protocol (Chinedu et al., 2013). A total of 30 animals were used in the whole study however, 12 animals were used in assessment of acute oral toxicity while 18 rabbits were subjected to other experiments as outlined in the whole study.

Phase I: Nine animals (n=9) were utilized in this phase, in which they were assigned into 3 groups of 3 animals each. Animals in each group got plant extract as follows; Group A1, 10 mg/kg, Group B1, 100 mg/kg and finally Group C1, 1000 mg/kg. They were monitored for behavioral changes and transience for over 24 hours.

Phase II: Three animals (n=3) were used in this phase. Each group received different doses of plant extracts as follows; Group A2 1600 mg/kg, Group B2 2900 mg/kg and finally Group C2 5000 mg/kg. Animals were observed for behavioral changes and mortality for over 24 hours. Rabbits were monitored for piloerection, respiratory distress, mucosal abnormalities, somatomotor activity, salivation, diarrhea, coma, convulsions, and mortality over 48 hours. The adopted observation plan was as follows; immediately after plant extract administration, 30 minutes, 1 hour, 4 hours, 24 and 48 hours after plant extract administration. Monitoring of toxicity continued for the next 14 days, during which weighing was carried out daily. Final sacrificing of animals occurred on day 15 after an overnight fast. A necropsy was performed with help of the Vet surgeon.

2.2. Determination of LD50

The following formula was applied as per Lorke's method (Lorke, 1983) to find Lethal dose (LD50):

LD 50 = $\sqrt{D0\ x\ D100}$

where LD 50 - lethal dose at which 50% of the animals died; D0 – highest dose that didn't result in mortality; and D100 - lowest dose that didn't result in mortality.

2.3. Study design and experimental animals

This was a posttests only true experimental design in which a total of 12 pure bred New Zealand was used. Twelve male New Zealand rabbits were obtained from Nairobi university animal house department of biology. Systematic simple random sampling method was used to assign the animals in different groups. The rabbits were weighing approximately 1.8kgs. They were then allowed to acclimatize for one week with close monitoring of their health status before and during the study. They were housed in standard rat cages (one for each group) and exposed to 12-hour light/dark cycles under humid tropical conditions. Each cage was labelled with a cage card showing experiment number, date of starting the experiment, dosage level, Age, Number of animals, Species and sex. The rabbits were allowed unrestricted access to standard feed rabbits pellets obtained from UNGA Mills and water *ad libitum* throughout the experimental period. The rabbits were handled in accordance with the guidelines for the care and use of laboratory animals.

2.4. Ethical approval

Ethical approval from the Animals Ethics and Research Committee for conducting the study was obtained from Jomo Kenyatta University Agriculture Technology Institutional Scientific and Ethics Review Committee (JKU/ISERC/02316/0891). National Commission for Science, Technology and Innovation, approval number NACOSTI/P/23/28152 granted permission to conduct the research. Animals were handled in accordance with established University of Nairobi Biology Animal House handling guidelines. Ethical considerations followed included: Reducing to the fewest number of animals possible that meet my research goals and provide statistically robust data; Refinement where all animals were placed in standard polycarbonate cages measuring 30x24x18 inches to reduce stress, fighting and injury (Hungu, 2011), beddings changed daily or when soiled, 12-hour day/ light cycle, humane culling using concentrated CO_2 and sick animals cared for by an in-house veterinarian. The frail animals, were recalled from the experiments and immediately sacrificed by concentrated CO_2 euthanization. All animals used in the experiments were sacrificed using humane end points at the end of the study (Kirkwood et al., 2013). The protocol followed Guidelines for Care and Use of Laboratory rabbits in Biomedical Research, and the rabbit were only used once in the experiment (Leary et al., 2013).

3. Results

3.1. Determination of acute oral toxicity of *W. ugandensis* on white New Zealand rabbits

Acute oral toxicity of *W. ugandensis* on white New Zealand rabbits was done in two phases and in each phase different doses of *W. ugandensis* were given and its effects were monitored against time.

Phase 1

Control group: This group was subjected to 5% DMSO + distilled water for 48 hours and the effects were reported as follows;

Immediate: The rabbits displayed normal activities.

30 minutes – 48 hours: The rabbits displayed normal activities.

Mortality: After 48 hours of the experiment, no mortality was recorded therefore, mortality rate was zero.

Experimental groups

A1 group: This group had 3 rabbits which were subjected to 10 mg of *W. ugandensis* extract in 5% DMSO and effects were observed for 48 hours, during which activities and mortalities of rabbits were monitored and recorded.

Immediate: Normal activities of rabbits were recorded and no mortality was reported.

30 minutes – 48 hours: Normal activity of rabbits was noted and no mortality was reported.

B1 group: 3 rabbits were used, subjected to 100mg of *W. ugandensis* bark extract dissolved in 5% DMSO. The following was observed;

Immediate: Rabbits had normal activities and no mortality was observed.

30 minutes – 48 hours: normal activity of rabbits was displayed and no mortality was recorded.

C1 group: This group comprised of 3 rabbits that were treated with 1000mg of *W. ugandensis* bark extract dissolved in 5% DMSO for 48 hours. Rabbit activities and mortality were observed and recorded.

Immediate: Rabbits in this group displayed normal activities and no mortality was reported.

30 minutes – 48 hours: Normal rabbit activity was observed and no mortality was reported.

All the rabbits (control and experimental) in phase 1 displayed normal activities and no mortality were reported. This therefore signifies that *W. ugandensis* bark extract of 10 mg to 1000mg has no toxicity.

Phase II

A2 group: Here only one rabbit was used. It was subjected to 1600mg of *W. ugandensis* bark extract dissolved in 5% DMSO for 48 hours. The following results were obtained;

Immediate: Rabbit had normal activity and no mortality was reported.

30 minutes – 48 hours: rabbit had normal activity and no mortality was recorded.

B2 group: A single rabbit was used which was treated with 2900mg of *W. ugandensis* bark extract for 48 hours.

Immediate: Rabbit had normal activity and no mortality was reported.

30 minutes – 48 hours: normal rabbit activity and no mortality was recorded.

C2 group: One rabbit was used which was treated with 5000mg of *W. ugandensis* bark extract for 48 hours.

Immediate: Rabbit displayed normal activity with no mortality.

30 minutes – 48 hours: normal rabbit activity with no mortality.

It was observed that rabbits subjected to 1600mg up to 5000mg of *W. ugandensis* bark extract had normal activity with no mortality therefore no acute oral toxicity.

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Table 1. Comparison of dose effect of *W. ugandensis* in relation to time and mortality

		Observation							
	Dosage (Centa et al.)	**Immediate**	**30min**	**1hr**	**4hrs**	**24hrs**	**48hrs**	**Mortality (n=3)**	**Mortality Rate**
Phase I									
WUBE in 5% DMSO	A1 10 MG	NA	NA	NA	NA	NA	NA	0	0
	B1 100 MG	NA	NA	NA	NA	NA	NA	0	0
	C1 1000 MG	NA	NA	NA	NA	NA	NA	0	0
Control	5% DMSO + Distilled Water	NA	NA	NA	NA	NA	NA	0	0
Phase II									
WUBE in DMSO 5%	A2 1600	NA	NA	NA	NA	NA	NA	0/1	0
	B2 2900	NA	NA	NA	NA	NA	NA	0/1	0
	C2 5000	NA	NA	NA	NA	NA	NA	0/1	0

Key: WUBE - Warbugia Ugandensis Bark Extract, NA - Normal Activity, DMSO - Dimethyl Sulfoxide.

4. Discussion

This is a test used to obtain information on the biologic activity of a chemical and gain an insight into its mechanism of action. The information obtained is then used in hazard identification and risk management so as to produce, handle and use chemicals. Here LD_{50} value is used as a statistical derived dose (Walum, 1998). It is important to note that animals are subjected to different doses of *W. ugandensis* bark extract and their behaviors are monitored at different times and recorded.

It was observed that in phase1; both control and experimental groups, normal activities were observed at immediate and between 30 minutes to 48 hours. No mortality was observed during this period. These findings are similar to (Anywar et al., 2021; Ngugi, 2020) who established that *W. ugandensis* had no toxicity at these doses. This therefore signifies that acute oral toxicity test is prudent when evaluating safe doses of plant extract. In the present study evidence of no mortality and normal activity shown by the rabbits up to a dose of 1000mg/kgbwt of *W. ugandensis* signifies that at this dose *W. ugandensis* is safe for use as it cannot induce any effect on animals under study.

In phase II of the study, it was observed that animals subjected to 1600, 2900 and 5000mg/kg bwt of *W. ugandensis* demonstrated normal activity and no mortality was reported. These findings are in agreement with (Karani et al.,

2013) who found out that the oral acute toxicity of bark extract of *W ugandensis* at a dose of LD_{50} <5000mg/kg had no signs of toxicity or mortality. Moreover, (Ngugi, 2020) found similar results when assessing the effects of *W. ugandensis* on asthma. According to (Kathare et al., 2021) *W. ugandensis* was safe for use at certain dose conducted via acute oral toxicity. Based on the present study, it can be established that any dose between 1600 to 5000mg/kgbwt of *W. ugandensis* is safe and therefore does not pause any health effect to the rabbits under the study. The LD_{50} of *W. ugandensis* in the current study was discovered to be >5000 mg/kg body weight, making it a generally safe species according to classification by (Lorke, 1983). This concurs with a cytotoxicity test of *W. ugandensis* performed on Vero E6 by (Karani et al., 2013) MTT experiment on cells which demonstrated that it was safe for use because its CC_{50} was >250ug/ml in relation to the LD_{50} Rukunga and Simon categorization of cytotoxicity exceeded 5000mg/kg.

Based on the two phases of drug evaluation, it can be noted that *W. ugandensis* is safe for use at any dose up to 5000mg/kgbwt. These could be due to the fact that the levels of phytochemical components present at this dose or within these doses are not sufficient enough to cause both histological and physiological harm to animals. It is prudent to note that any dose that exceeds confirmed acute oral toxicity is not safe as this could potentially be harmful to important organs that are involved in drug pharmacokinetics. However, Bark extracts, leaves, and young shoots of *W. ugandensis* have been utilized for years without experiencing any negative effects (Kirkwood et al., 2013; Kokwaro, 2009).

5. Conclusion

The safe dose of *W. ugandensis* for use in animal study is ≤ 5000mg/kgbwt as at this dose normal animal activity and no mortality was reported. Therefore, further studies can be done on the Assessment of pharmacokinetics and dynamics of the safe dose of *Warbugia ugandensis*.

Declarations

Source of Funding

This study did not benefit from grants from any non-profit, public or commercial funding agency.

Competing Interests Statement

The authors have declared that no competing financial, professional or personal interests exist.

Consent for publication

The authors declare that they consented to the publication of this study.

Authors' contributions

Both the authors took part in literature review, analysis, and manuscript writing equally.

Ethical Approval

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