Science World Journal Vol 14(No 1) 2019
www.scienceworldjournal.org
ISSN 1597-6343
Published by Faculty of Science, Kaduna State University

Full Length Research Article

STUDIES ON THE ACUTE TOXICITY OF THE AQUEOUS EXTRACT OF *ALYSICARPUS OVALIFOLIUS* IN MICE

John-Africa, L. B.

Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria.
PMB 21 Garki, Idu Industrial Area, Idu, Abuja Nigeria

Author Email Address lbjafrica@yahoo.com

ABSTRACT
Alysicarpus ovalifolius (Fabaceae) is an herb used in the preparation of a tea consumed as energy booster. This study was aimed at assessing its acute toxicity profile by determining in mice the LD_{50} of an aqueous extract of *Alysicarpus ovalifolius* prepared by decoction. The oral acute toxicity of the aqueous extract of *Alysicarpus ovalifolius* (AO) was determined in mice using the Lorke's method. Animals were observed for signs of intoxication and mortality for a duration of 14 days after which organs of the animals were collected, weighed and processed for histological assessment. The extract did not produce any mortality at the doses administered. No change was recorded in the relative organ weight of isolated organs. No morphological alterations were observed on macroscopic and histologic assessment of systemic organs. The LD_{50} was estimated to be greater than 5g/kg, therefore the aqueous extract of *Alysicarpus ovalifolius* may be considered as having low potential to cause toxic effects on acute oral administration.

Keywords: *Alysicarpus ovalifolius*, LD_{50}, acute toxicity, histology.

INTRODUCTION

Acute toxicity tests evaluate the potential of a substance to cause acute toxicity in a test organism on exposure to a single dose of a substance especially at high doses and within a short period. The signs of noxious actions may manifest immediately or after a period of latency (Sass, 2000; Saganuwan, 2017). These tests provide a screening method for toxicity evaluation particularly for new unclassified substances. The LD_{50} (lethal dose 50% i.e. dose that is lethal to 50% of test subjects) is the quantifiable indicator of the capacity of an agent to cause harm and other decisive factors such as therapeutic dose and therapeutic index of test compounds can be derived from these tests (Akhila, *et al*, 2007; Barile, 2010[a]). The Information obtained from acute toxicity studies provide a basis for classification and hazard assessment of new chemical compounds thus providing the information used for cautionary statements on labels of potentially poisonous chemical substances (OECD, 2001; Damalas and Koutroubas, 2016). These data also serve as a way to compare the potencies of drugs in experimental and clinical settings (Zastrow, 2018).

Traditional medical practitioners and their herbal recipes are a prevalent source of healthcare in Sub-Saharan Africa with widespread use in adults and children for treatment and disease prevention (Agbor and Naidoo, 2016, Welz *et al*, 2018). Traditional medicines are prepared and administered in form of powders, soaps, tinctures, decoction, fresh juice, gums or resins. Herbal tea (decoction) is a common form of administration of medicinal plants. The administration of herbal preparation in the form of tea is popular probably because of the ease of self-preparation and administration. The patronage for herbal recipes is promoted by factors that include availability, low cost, belief that herbal medicines do not produce adverse effects and increased desire to enhance ability to cope with the stress of day-to-day activities. This could lead to indiscriminate consumption with the potential to cause adverse effects, as the quantity consumed is largely unrestricted. Herbal teas have been attributed with many beneficial properties ranging from sedative, anxiolytic, laxative, pain relief, weight loss, boosting energy levels but mainly for improving the general well-being of a person. Although these are generally assumed to be safe, some safety concerns have been highlighted (Ravikumar, 2014). Acute intake especially at high dose maybe characterized by debilitating side effects (Ekor, 2014).

The plant *Alysicarpus ovalifolius* (Shumach. and Thonn.) J. Leonard. Fabaceae is used in the preparation of an herbal tea commonly consumed in Northern Nigeria by persons engaged in labour intensive activities such as athletes, night watchmen, commercial vehicle drivers as well as students for its claimed ability to increase vigour, delay fatigue and increase alertness (Dukku *et al.*, 2017). However, the availability of data on the safety and toxicity profile of the products on acute or chronic consumption is limited. The tea is readily available and relatively cheap thus it is consumed indiscriminately. This study was aimed at assessing its safety on acute consumption by determining the LD_{50} of the aqueous extract of *A. ovalifolius* in mice.

MATERIALS AND METHODS

Plant Collection and Extract Preparation

The aerial parts of *A ovalifolius* were collected from Suleja, Niger State, Nigeria, in the month of August 2017. The plant was identified and authenticated by the Curator of the Herbarium in the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD) Idu, Abuja, Nigeria, where a voucher specimen (NIPRD/H/6920) was prepared and deposited for future reference.

The aerial parts of the plant were obtained by cutting with a pair of scissors about 2 cm off the ground. Sand particles and other debris were shaken off. The plant material was air- dried and pulverized to obtain a coarse powder using a pestle and mortar. A decoction of the plant material was prepared by boiling one hundred grams (100 g) of the dried powdered plant material in water for 20 min. This was allowed to cool overnight after which it was filtered. The filtrate was evaporated to dryness on a water bath to afford a dark brown solid (the extract) with a yield of 9.98 $\%^{w}/_{w}$. This was stored in a refrigerator in an air-tight container (Nafiu et al, 2017).

Science World Journal Vol 14(No 1) 2019
www.scienceworldjournal.org
ISSN 1597-6343
Published by Faculty of Science, Kaduna State University

Animals used for acute toxicity test

Swiss albino mice (25 – 29 g) obtained from the Animal Facility Centre (AFC) of the National Institute for Pharmaceutical Research and Development, Abuja, Nigeria were used for the studies. All animals were housed under ambient conditions and fed on standard rodent diet with clean drinking water ad libitum. Animals were approved for this study based on adherence to handling according to the institutional animal guidelines for care and use of animals as stipulated in the Standard Operating Procedure of the Department of Pharmacology and Toxicology, NIPRD (SOP No 05:03:02).

Acute toxicity studies

This was carried out following the method of Lorke (1983) as described by Bulus *et al* (2001). The oral route was used for extract administration. Extract was freshly prepared in distilled water and administered using an oro-gastric cannula. In the first phase, mice were randomly placed in groups of 3 mice each (n = 3). Mice from group 1 were treated with 10 mg/kg of extract, group 2 received 100 mg/kg while the animals in group 3 were administered 1000 mg/kg body weight of the extract. Another group was included which severed as control. The animals were observed for physical signs of toxicity for a period of 24 h. Doses administered in the second stage was scheduled based on outcome of stage one. Animals (n = 1) were given 1600, 2900 and 5000 mg/kg of extract. Mice were weighed on day 0, 7 and 14. All animals were monitored for a duration of 14 days after which organs that include brain, liver, kidney and heart were collected for histological examination.

Biochemical Assays

The serum was separated from non-heparinized blood and the serum biochemical parameters which include alanine amino transferase (ALT), aspartate amino transferase (AST) were analyzed using commercial Spectrum diagnostic kits (Aboulthana *et al*, 2018).

Histological Analysis

All the animals were euthanized for gross pathological examination of all major internal organs. Organs that include brain, lungs, liver, kidney, spleen, stomach, small intestine and gonads were excised, blotted of blood, weighed and observed macroscopically. The relative organ weights were calculated. The organs were then preserved in 10 % neutral buffered formalin. The tissues were prepared as described by Slaoui and Fiette (2011) and examined with an optical microscope.

Statistical Analysis of Data

Results were expressed as mean ± SEM. Significance was determined using one-way analysis of variance (ANOVA). The level of significance was set at $p < 0.05$.

RESULTS

No death was observed in all animals throughout the 14 day-duration of the study but an increase in locomotor activity was observed in treated mice (Table 1). There was no observable difference in change of body weight (Table 2) or relative organ weight (Table 3) between treated and control animals. Macroscopic observation of the animals treated with the extract showed no apparent change in appearance (colour and size) of the systemic organs when compared with the control group. The levels of hepatic enzymes analyzed were also not significantly ($P<0.05$) different from that of control (Table 4). Likewise, no morphological alterations were recorded in the cellular structures of the organs of the mice (Fig 1).

Table 1: Effect of the aqueous extract of *Alysicarpus ovalifolius* on mortality and behavioural responses observed in mice

Behavioural Response	Treatment (mg/kg)						
	Phase 1				Phase 2		
	Control	10	100	1000	1600	2900	5000
Abdominal writhes	0/3	0/3	0/3	0/3	0/1	0/1	0/1
Diarrhoea	0/3	0/3	0/3	0/3	0/1	0/1	0/1
Salivation	0/3	0/3	0/3	0/3	0/1	0/1	0/1
Hyperactivity	0/3	0/3	3/3	3/3	1/1	0/1	0/1
Lethargy	0/3	0/3	0/3	0/3	0/1	0/1	0/1
Aggression	0/3	0/3	0/3	0/3	0/1	0/1	0/1
Respiratory changes	0/3	0/3	0/3	0/3	0/1	0/1	0/1
Tremors/convulsion	0/3	0/3	0/3	0/3	0/1	0/1	0/1
Coma	0/3	0/3	0/3	0/3	0/1	0/1	0/1
Death	0/3	0/3	0/3	0/3	0/1	0/1	0/1

No. of animal with observed parameter/no. of animals used

Table 2: Effect of the aqueous extract of *Alysicarpus ovalifolius* on weight of mice

Treatment Dose (mg/kg)		Weight of animal (g)		
		Day 0	Day 7	Day 14
Control		25.10 ± 0.49	26.20 ± 0.62	27.00 ± 0.58
10	Phase 1	25.00 ± 1.00	26.00 ± 1.04	26.67 ± 1.20
100		25.33 ± 0.67	26.33 ± 0.67	27.60 ± 0.95
1000		25.77 ± 0.53	25.93 ± 0.79	27.00 ± 1.00
1600	Phase 2	25.80	27.30	28.00
2900		25.90	27.30	28.00
5000		25.60	26.30	27.00

Values are presented as mean ± SEM (n = 3; phase 1), No significant difference between control and treated groups in phase 1. Values in Phase 2 were not compared due to absence of measure of variability

Science World Journal Vol 14(No 1) 2019
www.scienceworldjournal.org
ISSN 1597-6343
Published by Faculty of Science, Kaduna State University

Table 3: Effect of the aqueous extract of *Alysicarpus ovalifolius* on relative organ weights in mice.

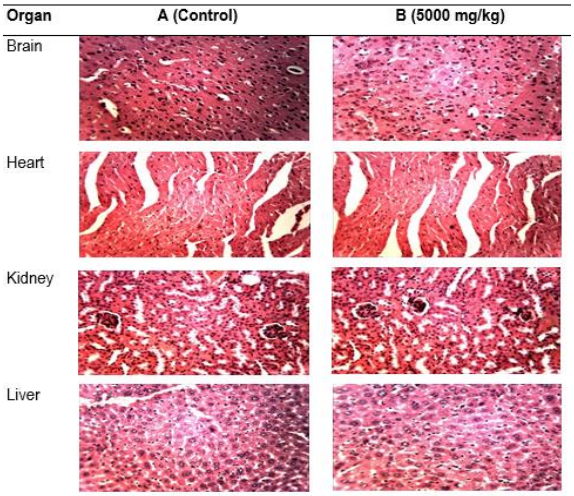
Treatment		Systemic Organ			
Dose (mg/kg)		Brain	Heart	Liver	Kidney
Control		1.52 ± 0.08	0.69 ± 0.03	4.78 ± 0.60	0.96 ± 0.06
10	Phase 1	1.51 ± 0.04	0.62 ± 0.02	4.91 ± 0.03	0.96 ± 0.06
100		1.50 ± 0.12	0.64 ± 0.06	4.88 ± 0.97	0.97 ± 0.13
1000		1.51 ± 0.02	0.65 ± 0.06	4.85 ± 0.41	0.87 ± 0.11
1600	Phase 2	1.53	0.70	4.84	0.89
2900		1.51	0.67	4.78	1.02
5000		1.52	0.66	4.95	0.97

Values are presented as mean ± SEM (n = 3; phase 1), No significant difference between control and treated groups in phase 1. Values in Phase 2 were not compared due to absence of measure of variability

Table 4: Effect of the aqueous extract of *Alysicarpus ovalifolius* on biochemical parameters of the mouse

Treatment		Serum parameter	
Dose (mg/kg)		AST (IU/L)	ALT (IU/L)
Control		68.8 ± 1.84	85.33 ± 9.33
10	Phase 1	73.52 ± 4.45	85 67 ± 5.24
100		71.19 ± 4.62	86.67 ± 8.01
1000		71.02 ±2.67	83.33 ± 9.53
1600	Phase 2	70.65	88.90
2900		67.20	97.35
5000		69.25	79.85

Values are presented as mean ± SEM (n = 3; phase 1), No significant difference between control and treated groups in phase 1. Values in Phase 2 were not compared due to absence of measure of variability. AST = Aspartate aminotransferase, ALT = Alanine amino transferase.



Pictomicrograph of the brain, heart, kidney and liver: A (Control) and B (5000 mg/kg) showing normal cellular architecture after single oral treatment with aqueous extract of *A. ovalifolius* (H&E x400)

Figure 1: Effect of the aqueous extract of *Alysicarpus ovalifolius* on histology of organs of the mouse

DISCUSSION

In this study the index of acute toxicity which is the LD_{50} was not calculated as no death was recorded, however it was estimated to be greater than 5g/kg. According to Lorke, LD_{50} values greater than 5,000 mg/kg are of no practical toxicological interest. In addition to estimation of the LD_{50}, other goals of toxicity studies include elucidation of the dose(s) that cause major adverse effects to the test subjects and identification of the possible target organs of toxicity as reported by Parasuraman (2011) and Chinedu *et al*. (2013). Other objectives involve determination of the most important clinical signs attributable to exposure to high dose of the test substance, time of onset, remission and recovery from those signs, sequence and timing of events leading to death of test subjects in instance where lethality is recorded (OECD, 2001; Akhila *et al*, 2007 Barile[b], 2010).

Toxicological manifestations of an administered substance may be detected from behavioural and/or biochemical changes elicited in animals. In this test, animal presented with hyperactivity suggesting stimulant effects by the extract, however other behavioural pattern of the treated animals was similar to control group. The use of the tea is associated with behavioural and mood modifying changes (Dukku, 2017), although administration of the extract did not cause any observable changes in the histology of the brain. Analysis of diagnostic serum parameters for liver (AST, ALT) showed that the values were within normal range. Toxicities of herbal formulations manifest as liver and kidney irregularities due to the involvement of these organs with metabolism (Knights *et al*, 2013; Famsu-Foguem, 2014; Kesavarapu, 2017). In this study, no significant ($P<0.05$) difference was observed in the level of serum biochemical parameter tested, likewise no changes were seen on histological examination. This is probably because the plant extract did not cause any deleterious effects that could provoke a change in the level of these parameters as species of *Alysicarpus* have been reported to demonstrate hepatoprotective activity (Bashir *et al*,

2016). Mortality in acute poisoning by herbal remedies has been associated with heart-related toxicities (Brown, 2018), histology revealed no change in the morphology of the cells of the organs after administration of high doses of the extract. This indicates that on acute intake the plant extract probably did not cause obvious adverse effects on the organs examined.

The results obtained in this study therefor indicates that the aqueous extract of *A. ovalifolius* may be considered as not having potential to cause harm on acute oral administration.

REFERENCES

Agbor, A. M. and Naidoo, S. (2016). A review of the role of African Traditional Medicine in the management of oral diseases. *African Journal of Traditional Complementary and Alternative Medicine*, 13(2): 133 – 142.

Barile, F. A. (2010) a. Dose Response. In *Clinical Toxicology: Principles and Mechanisms*. Second Edition. Florida CRC Press, USA.

Barile, F. A., (2010) b. Descriptive animal toxicity tests. In *Clinical Toxicology: Principles and Mechanisms*, Second Edition. Florida CRC Press USA.

Bashir, M., Uzair, M. and Ahmad, B. (2016). Ethnobotanical, Phytochemical and Pharmacological Aspects of Genus *Alysicarpus*. *International Journal of Pharmacy*,8(4): 1 – 15.

Brown, A. C. (2018). Heart toxicity related to herbs and dietary supplements: online table of case reports. Part 4 of 5. *Journal of Dietary supplements*, (15(4): 516 – 555.

Bulus, T., Atawodi. S., and Mamman, M (2011). Acute toxicity effect of the aqueous extract of *Terminalia avicennioides* on white Albino Rats. *Science World Journal*, 6(2): 1 – 4.

Chinedu, E., Arome, D., and Ameh, F. (2013). A New method for determining acute toxicity in animal models. *Toxicology International,* 20(3): 224 – 226.

Damalas, A. C. and Koutroubas, S. D. (2016). Farmas' exposure to pesticides: Toxicity types and ways of prevention. *Toxics*, 4(1): Doi: 10.3390/toxics401001.

Dukku, A. M. (2017). Emerging Perspectives on Drugs of Abuse: A Focus on Gadagi Consumption in Kano, Nigeria. *Advances in Psychology and Neuroscience*, 2(2-1):7 – 14.

Ekor, M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and chalenges in monitoring safety. *Frontiers in Pharmacology,* 4:177 Doi:10.3389/fphar.2013.00177.

Famsu-Foguem, K. and Foguem, C. (2014). Adverse drugs reactions in some Africa herbal medicine: literature review and stakeholders interview. *Integrative Medicine Research*, 3(3): 126 – 132.

Kesavarapu, K., Kang, M., Shin, J.J. and Rothstein K. (2017). Yogi detox tea: A potential cause of acute liver function. *Case Report in Gastrointestestinal Medicine*, Doi.org/10.1155/2017/ 3540756.

Knights, K. M., Rowland, A. and Miners, J. O. (2013). Renal drug metabolism in humans: the potential for drug-endobiotic interactions involving cytochrome P450 (CYP) and UDP-glucoronosyltransferase (UGT). British Journal of Clinical Pharmacology. 76(4): 587 – 602.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology,* 54: 275 – 287.

Nafiu, O. M., Hamid, A. A., Muritala, H and Adeyemi, S. B (2017). Preparation, standardization and quality control of Medicinal Plants in Africa. In *Medicinal spices and vegetables from Africa: Therapeutic potentials againstmetabolic inflammatory infections and systemic diseases*. Doi:10.1016/B978-012-809286-6.00007-8 Editor V. Kuete

OECD (2001). Organization for Economic Co-operation and Development Guidelines for the testing of chemicals/section 4: Health effects test No. 423. Acute oral toxicity - Acute toxic class method.

Parasuraman S. (2011). Toxicological screening. *Journal of Pharmacology and Pharmacotherapy,* 2(2): 74 – 79.

Ravikumar, C. (2018). Review on Herbal Teas. *Journal of Pharmaceutical Science Research*, 6(5): 236 – 238.

Saganuwan S. A. (2017). Toxicity studies of drugs and chemicals in animals: an overview. *Bulgarian Journal of Veterinary Medicine*, 20(4):291-318.

Sass N. (2000). Humane end points and Acute Toxicity testing. *ILAR Journal*, 41(2):114 – 123 Doi.org/10.1093/ilar.4.2.11.

Slaoui, M. and Fiette, L. (2011). Histopathology Procedures: from tissue sampling to histopathological evaluation. *Methods in Molecular biology* (Clifton, N. J.). 691.69 – 82. Doi:10.1007/978-1-60761-849-2_4.

Welz, A. N., Emberger-Klien, A. and Menrad, K. (2018). Why people use herbal medicine: insights from a focus-group study in Germany. *BMC Complementary and Alternative Medicine*, Doi.org/10.1186/s12906-018-2160-6.

Zastrow, M. (2018) Drug receptor and pharmacodynamics. In *Basic and Clinical Pharmacology*: Edited by B. G. Katzung Fourteenth Edition. McGrawHill Education/Medical.USA