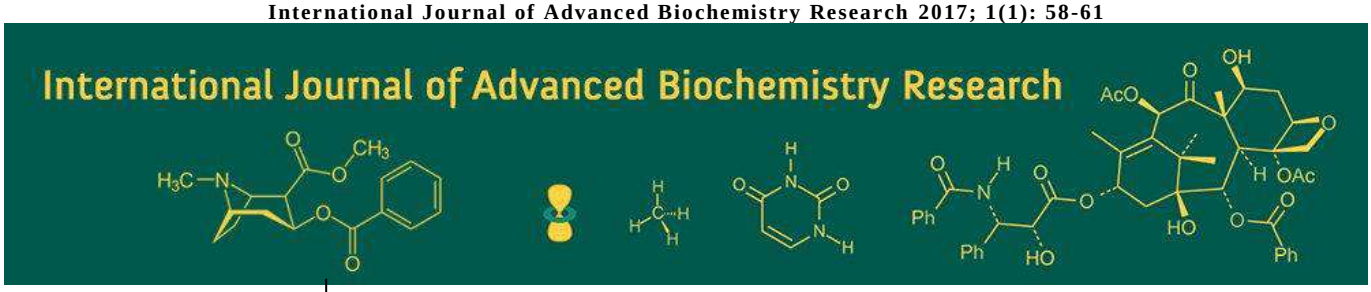

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Toxicity profile of *Asparagus africanus* root ethanolic extract in albino rats

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Abstract
In traditional medicine, *Asparagus africanus* is a plant with medicinal properties used for the treatment of numerous ailments. The toxicity profile of *Asparagus africanus* root ethanolic extract was determined in this study using animal model (Albino rats). *Asparagus africanus* extract was administered orally to Albino rats once in acute toxicity study and daily for twenty-eight days in sub-chronic toxicity study. Animals in the control group were administered distilled water (5 ml/kg) and in the treatment groups, 100, 200 and 400 mg/kg of the plant extract was administered to the animals. In the acute toxicity study, the median lethal dose of the plant extract was determined whereas in the sub-chronic toxicity study, the effect of extract on body weight, biochemical (serum alanine transaminase, aspartate transaminase, creatinine, cholesterol and triglyceride) and haematological (red blood cells, hematocrit and white blood cells) parameters were investigated. The results of these study showed that *Asparagus africanus* root ethanolic extract caused no death of animals in the acute toxicity studies and the median lethal dose of the extract was greater than or equal to 5000 mg/kg. In the twenty-eight days study (sub-chronic toxicity study), there was an increase (significant at $p< 0.05$) in the level of red blood cells and no change in the concentration of biochemical parameters in the treated groups when compared with the untreated group. In conclusion, the results of this study suggest that *Asparagus africanus* root ethanolic extract at the doses orally administered caused no adverse effects in Albino rats.

Keywords: *Asparagus africanus*, root, toxicity, serum, blood, albino rats

Introduction
Plants with medicinal properties have long been used for the treatment and management of diseases. Approximately 80% of people worldwide use medicinal plants for the needs of their health care [1]. The recent increase in use of plants with medicinal properties for the treatment of ailments has led to increase in studies on safety and possible toxicity of some plants used as herbs for therapeutic purposes [2, 3]. Intake of a plant that has a toxic substance can lead to numerous symptoms which include stomach ache, diarrhea, vomiting, kidney damage and death in some cases [4].
Asparagus africanus Lam. is a plant with medicinal properties found in tropical Africa including Nigeria, Namibia and Zimbabwe. The plant is commonly known in Nigeria as Shekan Bera (in Hausa) and Aluki in (Yoruba) [5, 6, 7]. *Asparagus africanus* belonging to the family liliaceae is used in ethno-medicine for treating malaria, gonorrhoea, syphilis [6, 8], diarrhea [9], pile and stomach ache [10]. The root of *A. africanus* is used for the treatment of epilepsy, hypertension and chronic gout [11, 12].
Due to the use of *Asparagus africanus* in traditional medicine for the treatment of numerous diseases, the toxicity profile of *Asparagus africanus* root ethanolic extract was determined by carrying out acute and sub-chronic toxicity study of the plant extract using animal model (Albino rats).

Materials and Methods
The root of *Asparagus africanus* was gotten from a farm at Bauchi road, Jos, Plateau State, Nigeria. The plant was authenticated at the Herbarium in Botany Department, Obafemi Awolowo University (O.A.U), Ile-Ife, Osun State, Nigeria.

Extraction

Asparagus africanus root was washed with a clean tap water and then oven dried at 40 ºC. The dried plant sample was ground into powder, soaked in ethanol (70%) for three days (72 hours) and filtered using Whatman no.1 filter paper. The filtrate was concentrated into a solid paste using a rotary evaporator. A freeze drier was used to dry the plant extract before it was stored in a refrigerator (4 ºC) prior to the study [13].

Animals

The experiment using the animals (Albino rats) was carried out using the approved guidelines of Pharmacology Department, Faculty of Pharmacy O.A.U, Ile-Ife, Osun State, Nigeria after obtaining the Albino rats from Pharmacology Department.

Albino rats (male and female) used for this study weigh between 150 – 180 g. They were kept in polypropylene rat cages that are ventilated. Broiler's mash was used for feeding the animals and they had free access to water. The animals were allowed to get used to the environment for fourteen days before starting the study.

Acute toxicity study

The acute toxicity of *Asparagus africanus* root ethanolic extract (carried out in two phases) was determined in Albino rats after administration of the extract via oral route [14]. Nine albino rats were divided into three groups of three rats each in phase 1. Animals in groups 1, 2 and 3 were administered 10, 100 and 1000 mg extract/kg body weight once. They were then observed at 10, 30, 60 and 120 minutes and at 4, 6 and 24 hours for signs of toxicity which include distress in respiration, convulsion, vomiting, diarrhea and mortality. The animals were further observed daily for fourteen days. In phase 2, fresh set of three animals were used and they were divided into three groups with a rat each. Animals in each group were administered the plant extract at 1600, 2900 and 5,000 mg /kg once and were observed as in phase 1. The median lethal dose (LD_{50}) was determined at the end of the acute toxicity study.

Sub-chronic toxicity study

Twenty-four Albino rats of both sexes were divided into four groups of six rats each. Group 1 was orally administered 10 ml distilled water/kg body weight once daily for twenty-eight days. Animals in group 2, 3 and 4 were orally administered 100, 200 and 400 mg extract/kg body weight respectively once daily for twenty-eight days [13]. The animals had free access to water and food although the experiment and they were observed daily for symptoms of toxicity. The animals were sacrificed under diethyl ether anaesthesia on the 29th day after taking food and water after taking food and water away from them for 24 hours. The blood was collected from the Albino rats by cardiac puncture into plain bottles (5 ml) and K+EDTA bottles.

Blood samples in plain bottles were centrifuged at 2500 rpm for 25 minutes after allowing it to stand for 5 minutes and the serum was collected for biochemical analysis which include aspartate aminotransferase, alanine aminotransferase [15], creatinine [16], total cholesterol [17, 18] and triglyceride [19] concentration determination. Haematological analysis which includes haemoglobin, hematocrit, red blood cell count and white blood cell count was carried out using blood samples in K+EDTA bottles. The percentage change in body weight was calculated for day 7, 14, 21 and 28 was calculated. The volume (ml) of water and weight (g) of food consumed by the animals in each group were taken daily and the change in food and water intake was calculated for day 7, 14, 21 and 28 [13].

Statistical analysis

One-way analysis of variance and post hoc comparison (Bonferroni t-test) Post hoc were used to determine the 95% level of significance at $p < 0.05$ (Primer version 3.01).

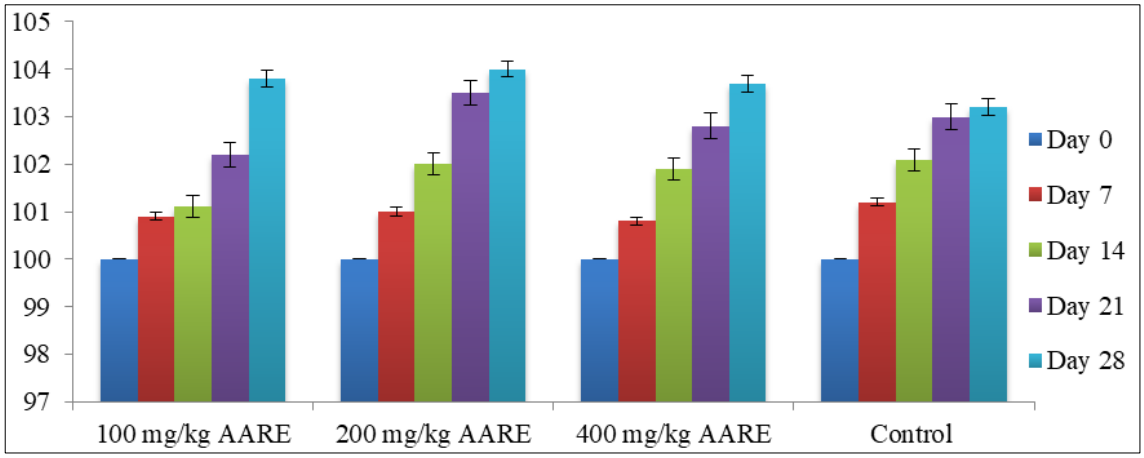
Results

Acute toxicity and median lethal dose of *Asparagus africanus* root

Asparagus africanus root ethanolic extract caused no mortality of Albino rats and no signs of toxicity such as mortality, convulsion, diarrhea and changes in fur colour were observed in the Albino rats. The median lethal dose (LD_{50}) of the plant extract was greater than or equals to 5000 mg/kg via oral rout of administration.

Effect of *Asparagus africanus* root ethanolic extract on change (%) in body weight (g)

Asparagus africanus root ethanolic extract caused no significant ($p < 0.05$) change (%) in the body weight of Albino rats when compared with the control (Fig. 1).



Values are expressed as Mean ± SEM; n = 6. AARE: *Asparagus africanus* root ethanolic extract

Fig 1: Effect of *Asparagus africanus* root ethanolic extract on body weight (g) of Albino rats

Effect of *Asparagus africanus* root ethanolic extract on food (g) intake

Asparagus africanus root caused no significant ($p< 0.05$) change in food intake by Albino rats when compared with the untreated animals (control) as shown in Table 1.

Table 1: Effect of *Asparagus africanus* root on food (g) intake by Albino rats

Asparagus africanus root	Day 7	Day 14	Day 21	Day 28
100 mg/kg	25.2 ± 1.3	27.8 ± 2.0	29.6 ± 1.9	32.1 ± 0.9
200 mg/kg	26.0 ± 1.0	28.7 ± 1,8	30.3 ± 1.8	33.0 ± 1.2
400 mg/kg	26.6 ± 1.3	28.9 ± 1.9	30.5 ± 2.0	33.9 ± 1.1
Control	25.7 ± 1.7	27.9 ± 1.8	30.9 ± 1.7	34.0 ± 1.3

Values are expressed as Mean ± SEM; n = 6

Effect of *Asparagus africanus* root ethanolic extract on water (ml) intake

The plant extract caused no significant ($p< 0.05$) change in water intake by Albino rats when compared with the untreated animals (control) as shown in Table 2.

Table 2: Effect of *Asparagus africanus* root on change in water (ml) intake by Albino rats

Asparagus africanus root	Day 7	Day 14	Day 21	Day 28
100 mg/kg	68.2 ± 2.1	69.0 ± 2.2	70.2 ± 1.5	71.9 ± 2.0
200 mg/kg	66.1 ± 2.4	67.8 ± 2.0	68.2 ± 1.8	70.0 ± 2.1
400 mg/kg	70.0 ± 1.9	71.1 ± 1.8	72.1 ± 1.7	73.0 ± 1.9
Control	66.7 ± 2.0	68.0 ± 2.0	69.3 ± 1.4	70.9 ± 2.2

Values are expressed as Mean ± SEM; n = 6

Effect of *Asparagus africanus* root ethanolic extract on haematological parameters

The *Asparagus africanus* root ethanolic extract caused an increase in the level of red blood cells (significant at $p< 0.05$) and there was no change (not significant at $p< 0.05$) in the level of packed cell volume, haemoglobin and white blood cells of Albino rats when compared with the untreated animals (control) as shown in Table 3. The increase in the level of red blood cells caused by the extract was dose dependent [the higher the dose of the extract the higher the increase in red blood cells] (Table 3).

Table 3: Effect of *Asparagus africanus* root on haematological parameters in Albino rats

Asparagus africanus root	Hematocrit (%)	Haemoglobin (g/dl)	Red blood cell (x 10^6/µl)	White blood cell (x 10^3/µl)
100 mg/kg	47.9 ± 2.0	13.2 ± 1.7	17.2 ± 0.8*	3.8 ± 1.7
200 mg/kg	49.0 ± 2.2	13.9 ± 1.9	18.9 ± 0.5*	3.9 ± 1.9
400 mg/kg	51.3 ± 1.9	14.1 ± 1.4	20.0 ± 0.7*	4.0 ± 1.5
Control	43.8 ± 1.6	13.0 ± 1.1	14.9 ± 0.8	4.1 ± 1.8

Values are expressed as Mean ± SEM; n = 6. *Significant at $p< 0.05$ when compared with the control

Effect of *Asparagus africanus* root ethanolic extract on biochemical parameters

Asparagus africanus root ethanolic extract caused no change (not significant at $p< 0.05$) in serum alanine transaminase, aspartate transaminase, creatinine, cholesterol and triglyceride concentration in Albino rats when compared with the untreated animals (control) as shown in Table 4.

Table 4: Effect of *Asparagus africanus* root ethanolic extract on biochemical parameters in Albino rats

Parameters	100 mg/kg AARE	200 mg/kg AARE	400 mg/kg AARE	Control
ALT (U/L)	35.2 ± 0.9	35.4 ± 1.0	36.0 ± 1.1	36.7 ± 1.3
AST (U/L)	81.1 ± 2.0	82.0 ± 2.7	82.8 ± 2.4	82.7 ± 2.3
CRT (µmol/l)	21.3 ± 1.1	22.7 ± 1.4	23.1 ± 1.7	22.5 ± 1.9
Cholesterol (mmol/l)	7.4 ± 1.2	7.0 ± 1.4	6.7 ± 1.3	7.1 ± 1.6
Triglyceride (mmol/l)	3.2 ± 1.9	3.0 ± 1.6	2.9 ± 1.4	3.1 ± 1.5

Data are expressed as Mean ± SEM; n = 6. AARE: *Asparagus africanus* root ethanolic extract; ALT: Alanine transaminase; AST: Aspartate transaminase; CRT: Creatinine.

Discussion

In this study, *Asparagus africanus* root ethanolic extract caused no toxic effect in the Albino rats throughout the acute toxicity studies. The plant extract caused no signs of toxicity such as tumour, diarrhea, vomiting, convulsion, mortality and changes in the skin, eyes and fur colour. The LD_{50} (median lethal dose) of *A. africanus* root extract was ≥ 5000 mg/kg which suggest that at the highest dose (5000 mg/kg) administered *Asparagus africanus* root is not toxic after single oral administration to Albino rats. Previous acute toxicity study carried out on butanol fractionated root extract of *Asparagus africanus* reported that the extract has high safety profile when orally administered to Swiss Albino mice [20].

In the sub-chronic toxicity studies, *Asparagus africanus* root ethanolic extract caused no change (not significant at $p< 0.05$) in body weight (Fig. 1), food intake (Table 1), water intake (Table 2) and biochemical parameters [alanine transaminase, aspartate transaminase, creatinine, cholesterol and triglyceride] (Table 4) when compared with the control. In haematological parameters, the plant extract caused a significant ($p< 0.05$) increase in the level of red blood cells in a dose dependent manner and no changes in the level of hematocrit, haemoglobin and white blood cells when compared with the control (Table 3). Sub-chronic toxicity study have been used for long to determine the possible damage caused by medicinal plants [21, 22, 23]. Haematological and biochemical parameters are good indicators for determining the possible toxicity and safety of medicinal plants [13, 24, 25]. The results from this study, shows that 28 days daily treatment of Albino rats with *Asparagus africanus* root ethanol extract caused no adverse effect to body weight, intake of food, intake of water, haematological and biochemical parameters of the animals.

Conclusion

In conclusion, the results from this study, suggests that *Asparagus africanus* root ethanolic extract has no toxic effect at the doses used for this study when orally

administered to Albino rats once (acute toxicity study) and daily for twenty-eight days (subchronic toxicity study).

Conflicts of interest

The authors declare that no conflicts of interest exist.

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