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Acute Toxicity of Leaf Extracts of Fermented and NonFermented*A. Annua*and *V. Amygdalina*in Rats.

Mabitine, D.M[1], Iheukwumere C.C[1], Ogbonna C.I.C[1], Gberikon,G.M[1]., Ogbonna, I.O[1]., Okoh, T[2].,Ojobo, O.A[2]., Otene, V[2].,

[1]Department of Botany, Joseph SarwuanTarka University (JOSTUM), Makurdi, Benue State.
[1]Applied Microbiology and Biotechnology Research Unit, Department of Plant Science and Technology, University of Jos, Nigeria.
[1]Department of Microbiology, J.S. Tarka University, Makurdi, Benue State.
[2]Department of Plant Science and Biotechnology, J.S. Tarka University, Makurdi, Benue State.
[2]Department of Agricultural Extension and Communication, J.S. TarkaUniversity,Makurdi, Benue State.

ABSTRACT

The safety of plant materials in the preparation of herbal drugs is vital to animal and human life. The objective of this study is to evaluate the acute toxicity of the leaf extracts of fermented and non-fermented *Artemisia annua* and *Vernonia amygdalina in* rat'smodels owing to their importance in the treatment of some diseases in Africa. Although, the non-fermented extracts of *A. annua* and *V. amygdalina* produced some deaths, the fermented ones did not show any lethal effects. LD50 of all extracts were in the range of 2264.16 to 4,113.33 indicating non-toxic status. The behavioral patterns were normal and the body and organ weights did not show significant difference (P>0.05). This infers then that the two extracts may be safe within doses which have been tested.

1.0 BACKGROUND

The safety of herbal drugs cannot be overemphasized. *A. annua* and *V. amygdalina* have been used for decades with very minimal or no lethal effects in many parts of Africa. They are commonly consumed as tea or soup in different parts of Africa as normal meal, or as preventive or curative interventions to certain diseases such as malaria, diabetes and others.

Artemisia annua: A. annua L: *A. annua*L is commonly called sweet wormwood. The plant is native to temperate Asia, but adapted globally, and belongs to the family of the Asteraceae (EI-Askary *et al.,* 2020). The plant is large, weedy, and shrubby and grows to a height above 2m, having a ribbed single-stem with alternate branches. The variety evaluated here is *A. annua* var. chiknensis with Laboratory code number (CBGE/CHINA/09/LTNGS/G) whose artemisinin content was improved upon at the center for Biotechnology and Genetic Engineering, University of Jos, Jos.

*Vernonia amygdalina:*This is a shrubby plant which grows to a height of about five (5) m high. The bitter taste on the plant has given it characteristic name as 'bitter leaf'. In different parts of the world, it is called different names however in Nigeria, it is called 'Ewuro' in Yoruba, 'Onugbu' in Igbo and 'Chuwar-doki' in Hausa.

i.Acute toxicity of *A. annua:*Absence of signs of harmfulness have been reported after consumption of 1000mg/kg bw of *A. annua* per day in rats, nor were there effects on body weight and other important parameters. Gross pathology and blood chemistry did not show any damage. Mukinda and Syce (2007) reported that the biochemical and haematological assessments of ethanolic leaf extracts (EAA) of *Artemisia annua*performed on 20 male rats. High density lipoprotein -cholesterol decreased at 100 mg/kg of EAA, low density lipoprotein -cholesterol increased, and blood sugar level also decreased meaningfully at the 100 and 200 mg/kg of EAA. Consequently, N k u i t c h o u - C h o u g o u o *et al.* (2016) reported *Artemisia annua*tea reduces alanine aminotransferase (ALAT) and possibly will be hepatoprotective unlike the artesunate toxicity which possibly results in harm on the hepatocytes and liver function.The work of Ogbonna *et al.* (2017) showed that there was no toxic effect after administration of 28 mg/ kgbw twice daily to diabetic rats.The work of Siddiqui *et al*. (2018), showed that oral administration of greater than or equals to 5000 mg/ kg bw in mice did not result in death nor post treatment behaviors depicting any sort of poisoning. *Yang et al. (2010) documented that 70 % ethanol and hot water extracts of A. annua did not produce toxic effects in mice and may validate the se of the plant in Southern Ethiopian for treatment of malaria.* In related research, On*et al*. (2023) documented that the fermented *A. annua* even at lower doses of 100 mg/ kg b.w and 300 mg/ kg bw had no significant differences on liver weight (p> 0.05) although liver weight loss was observed.

ii. Acute Toxicity of *V. amygdalina* extract: Ejiofor *et al*. (2017) reported that the extract of *V. amygdalina* did not show any form of acute toxicity nor death on experimental animals after administration of 2000 kg/ bw of the methanolic extracts of *V. amygdalina*

Acute toxicity evaluation on mice displayed absence of signs, symptoms of toxicity of death after oral administration with a dose of 2000 mg/kg body weight of the methanol extracts each of the leaf stem-bark and root of *Vernonia amygdalina* for 14 days (Ejiofor *et al.,* 2017).Akah*et al*. (2009) documented that after administration of 80, 160 and 320 mg/kg of methanol leaf extract of *V. amygdalina* to rat over 28 days, there was no sufficient toxic effect. Yeap*et al.* (2010) however reported that on administration of *V. amygdalina* leaf, in vivo and invitro toxicity was recorded.Similarly, after treatment of rats with 55 mg/ kgb.w of Chloroform extract of *V. amygdalina*7-14 days at 55 mg/ kg b.w no unpleasant consequence was reported (Asante *et al.*, 2016).This research aims at validating the safety of the extracts of the leaf of *A. annua* and *V. amygdalina*in acute toxicity test.

2.0 MATERIALS AND METHODS

2.1 Collection and identification of plant materials

The leaves of *A. annua*and *V. amygdalina*were obtained from Centre for Biotechnology and Genetic Engineering (CBGE) University of Jos, Jos Plateau State and the farm behind Modern Market Makurdi, Benue State respectively. They were rinsed with clean water, shade dried at room temperature and stored in glass bottles until needed for use.

2.2 Preparation of Extracts:

Four hundred grams (400g) of powdered (dried) leaves each plant was put in a conical flask containing 2000ml of sterile distilled water. The flask was heated with a Bunsen flame for few minutes and was allowed to cool to room temperature. It was aseptically filtered using What man filter paper (No1) to separate the residue from the filtrate.

The various extracts gotten were dehydrated hot water bath, weighed, filtered using What man No. 1 filter paper and concentrated by a rotary evaporator, and the residual extracts were finally dried. The percentage yield was obtained using dry weight, from the equation provided below. The extracts were kept and stored in refrigerator at 5 °C until use.

2.2.1 Preparation of fermented Plant samples:

Water was boiled, poured into a clean flask, left for few minutes and transferred into sterile bottles. Twenty (20g) of the leaf extract andten (10) ml of *S. cerevisiae* culture was added to the bottle, corked, sealed tight, kept in a dark environment and left undisturbed for two (2) weeks. After the 2weeks, racking was done by draining off the clear portion into another sterile container. The remaining mixture in the bottle was racked again after additional 2weeks and another 1week following similar protocol. After a total of 5 weeks, the contents in the bottle were left to age.

2.3 Evaluation of Acute Toxicity:

Acute toxicity evaluations were done using up and down method (OECD 425, 2001; Sanganuwan, 2015).

The extracts were prepared using normal saline (0.9 Sodium-chloride), dissolved in 4mls of distilled water. The various extracts used are as follows: i.Aqueous*Artemisia annua*(A. A AQ); ii. Aqueous *Vernonia amygdalina* (V. AAQ); iii. Methanol *Artemisia annua*(AAM); iv Methanol *Vernonia amygdalina* (V.AM); v.A.A AQ + V.AAQ; vi.A.AM + V.AM; vii. Fermented *Artemisia annua* (A.AF) + Fermented *Vernonia amygdalina* (V.AF).

The experimental rats were fasted overnight prior to administration (Saganuwan, 2015).

The administration was performed in batches so as to determine the dose at which the extract will be lethal to the rats (OECD 425, 2001; Saganuwan, 2015). Then the first, second and third sets of rats for aqueous extract of *A. annua* were dosed at 2000 mg/kg body weight, 3585 mg/ kgbw and 5170 mg/kg body weight respectively using a modified up and down method described by Saganuwan, (2015). After administration, the rats were closely observed to evaluate behavioral parameters for a period of 0 to 14 days and each parameter observed was recorded. Any death observed was recorded and the Lethal Dose 50 (LD_{50}) was determined by the death of the experimental animals (Apu*et al.,* 2010). However, on the 14^{th} day, the living rats were sacrificed to carry out gross pathology and histopathology on the vital organs. Consequently, the median Lethal Dose of all extracts of *Artemisia annua L*. and *V.amygdalina* were evaluated using Arithmetic-Geometric-Harmonic (AGH) method of Rough Estimation of Lethal Dose 50 (LD_{50}) (Saganuwan, 2015).

2.3.1 Determination of LD_{50}: The Lethal Dose 50 (LD_{50}) for each extract was determined accordingly (Apu*et al.,* 2010; Saganuwan, 2015). The median Lethal Dose of oral aqueous/ methanolic extracts were estimated using Arithmetic-Geometric-Harmonic (AGH) method of Rough Estimation of Lethal Dose 50 (LD_{50}) using up and down procedure of Saganuwan (2015). The organs weights, and diameter were also recorded.

2.3.2 Evaluation of Weight Gain: The rats were regularly weighed using weighing balance to determine possible weight gain or otherwise for a period of 14 days.

2.3.3 Evaluation of Behavioral Pattern: Physical parameters of general behavior such as nose-poking, skin fur, slow movement, feeding status were all assessed on daily basis.

2.3.4 Evaluation of Organ Weight: After the studies, the rats were weighed (on the morning of autopsy), euthanized by CO_2 asphyxiation, exsanguinated to reduce the inflow of blood to the organ weight (Sullivan, 2015), and subjected to complete necropsy. Following autopsy, protocol-specified organs were observed, dismembered free of fat and weighed using a calibrated balance.

2.4 Statistical Analysis of Results: Results obtained were recorded as mean ± SEM and subjected to one way analysis of variance (ANOVA) and where significant differences exist, means were separated using Fisher's LSD method. It was performed using Statistical Analysis System at 0.05 significant level ($P<0.05$).

RESULTS

The effect of the single administration of methanol and aqueous leaf extracts of *A. annua*revealed three deaths and three survivals for each extract while the LD_{50} were 4113.33 mg/ kgbw and 2792.5 mg/ kgbw respectively (Table 1).The single administration of methanol and aqueous extracts of *V. amygdalina*also revealed three deaths and three survivals for each extract at LD_{50} of 2264.16 mg/ kgb.w and 2792.5 mg/kgb.w respectively (Table 1).

Conversely, the combined aqueous extracts of *A. annua*and *V. amygdalina*recorded no deaths at LD_{50} of 2792.5 mg/ kgbw (Table 1). Also, single administration of combined non-fermented extracts of *A. annua*and *V. amygdalina*recorded no deaths at LD_{50} 2792.5 mg/kgb.w (Table 1).

At LD_{50} (2792.5 mg/kgb.w), no deaths were recorded in the single and combined fermented leaf extract of *A. annua*and *V. amygdalina*(Table 2).The results showed that except on few cases, the extracts did not show significant adverse effect on the behavioral patterns of the rats (Tables 3,4 and 5).

Effects of 2000 mg/kgbw of non-fermented extracts of *A. annua and V. amygdalina*on body weight during acute toxicity showed that although there were body weight changes in the treated groups compared to the control, there was no significant difference ($P>0.05$) (Table 6).

There were no significant differences ($P>0.05$) in body weight changes between treated groups and control with application of 2000 mg/kgbw of fermented *A. annua*and *V.amygdalina*extracts (Table 7).

Effects of fermented *A. annua*and *V. amygdalina*extracts in single and combination forms on organ weight during acute toxicity showed no significant difference in the mean values of the treatment groups ($P>0.05$) even at highest doses of 2000 mg/kg b.w and 3585 mg/ kg b.w (Table 8).

Effects of 2000 mg/kgbw and 3585 mg/kgbw of aqueous and methanol extracts (in single and combination forms) on organ weight showed that there was a significant difference along the column in the liver weight ($P<0.05$), left kidney weight ($P<0.05$, $P<0.01$). V.A M (3585 mg/kgbw), V.AAQ (2000 mg/kgbw) and V.AAQ (3585 mg/kgbw) showed that there was a significant difference($P<0.05$) compared to the control and other groups. However, there was no significant difference in the right kidney weight for all doses ($P>0.05$) (Table 9).

Table 1: Effect of Administration Methanol and Aqueous (Non- fermented) Leaf Extracts of *Artemisia annual and V. amygdalina*on rats in LD_{50} Determination

Extract	**weight before administration**	**Dose (mg/ kgbw)**	**Survival Status**
A.AM	89	2000	0
	137	3585	X
	122	2000	0
	129	3585	X
	141	2000	0
	110	3585	X
		LD_{50}=4,113.33	
A.AAQ	110	2000	0
	119	3585	0
	129	5170	X
	119	3583	0
	129	5170	X
	130	5170	X
		LD_{50}=2792.5	
V.AM	99.2	2000	0

	130	3585	X
	123	2000	0
	96	3585	X
	88.3	2000	X
	140	415	0
		LD_{50}=2264.16	
V.AAQ	125	2000	0
	111	3585	X
	110	2000	X
	88	3585	0
	100	3585	0
	118	2000	X
		LD_{50}=2676.7	
A.AAQ+V.AAQ	140	3585	0
	141	2000	0
	138	3585	0
	132	2000	0
	147	3585	0
	142	2000	0
		LD_{50}=2792.5	

Key: 0=Survival x=Death

Table 2: Effect of Administration fermented Leaf Extracts of *Artemisia annua and V. amygdalina*on rats in LD_{50} determination

Extract Status	**Weight of animal**	**Dose**	**Survival**
A.AF	140	2000	0
	138	3585	0
	173	2000	0
	154	3585	0
	147	2000	0
	148	3585	0
LD_{50} =		2792.5	
V.AF	150	2000	0
	140	3585	0
	141	2000	0
	147	3585	0
	122	2000	0
	132	3585	0
LD_{50} =		2792.5	

A.A.F+V.AF	139.28	2000	0
	154.70	3585	0
	137.57	2000	0
	146.30	3585	0
	122.92	2000	0
	138.46	3585	0

LD_{50}

=2792.5

Table 3: Effect of 2000 mg/kgbw extracts of *A. annua*and *V. amygdalina*on behavioral pattern of rats in acute toxicity behavioural pattern

Extract/Day	Skin Fur	Alertness	Touch response	Feeding	Motility	Sleep	Urination	Death
A.AM 1	N	N	N	N	N	N	N	BS
A.AAQ1	N	N	N	N	N	N	N	ABS
A.AM7	N	N	N	N	N	N	N	ABS
A.AAQ7	N	N	N	N	N	N	N	ABS
A.AM14	N	N	N	N	N	N	N	ABS
A.AAQ14	N	N	N	N	N	N	N	ABS
A.AF1	N	N	N	N	N	N	N	ABS
A.AF7	N	N	N	N	N	N	N	ABS
A.AF14	N	N	N	N	N	N	N	ABS
V.AM1	N	N	N	N	N	N	N	PRS
V.AAQ1	N	N	N	N	N	N	N	PRS
V.AM7	N	N	N	N	N	N	N	ABS
V.AAQ7	N	N	N	N	N	N	N	ABS
V.AM14	N	N	N	N	N	N	N	ABS
V.AAQ14	N	N	N	N	N	N	N	ABS
V.AF1	N	N	N	N	N	N	N	ABS
V.AF7	N	N	N	N	N	N	N	ABS
	N	N	N	N	N	N	N	ABS

Table 4: Effect of 3585 mg/kgbw extracts of *A. annua*and *V. amygdalina*on behavioural pattern of rats in acute toxicity behavioural pattern

Extract/Day	**Skin Fur**	**Alertness**	**Touch response**	**Feeding**	**Motility**	**Sleep**	**Urination**	**Death**
A.AM 1	N	N	N	N	N	N	N	PRS
A.AAQ1	N	N	N	N	N	N	N	ABS
A.AM7	N	N	N	N	N	N	N	ABS
A.AAQ7	N	N	N	N	N	N	N	ABS
A.AM14	N	N	N	N	N	N	N	ABS

A.AAQ14	N	N	N	N	N	N	N	ABS
A.AF1	N	N	N	N	N	N	N	ABS
A.AF7	N	N	N	N	N	N	N	ABS
A.AF14	N	N	N	N	N	N	N	ABS
V.AM1	N	N	N	N	N	N	N	PRS
V.AAQ1	N	N	N	N	N	N	N	PRS
V.AM7	N	N	N	N	N	N	N	ABS
V.AAQ7	N	N	N	N	N	N	N	ABS
V.AM14	N	N	N	N	N	N	N	ABS
V.AAQ14	N	N	N	N	N	N	N	ABS
V.AF1	N	N	N	N	N	N	N	ABS
V.AF7	N	N	N	N	N	N	N	ABS
V.AF14	N	N	N	N	N	N	N	ABS

Table 5: Effect of 5170 mg/kgbw extracts of*A.annua*and *V.amygdalina*on behavioural pattern of rats in acute toxicity

Extract/Day	**Skin Fur**	**Alertness**	**Touch response**	**Feeding**	**Motility**	**Sleep**	**Urination**	**Death**
A.AM 1	N	N	N	N	N	N	N	PRS
A. AAQ1	N	N	N	N	N	N	N	ABS
A.AM7	N	N	N	N	N	N	N	ABS
A. AAQ7	N	N	N	N	N	N	N	ABS
A.AM14	N	N	N	N	N	N	N	ABS
A. AAQ14	N	N	N	N	N	N	N	ABS
A.AF1	N	N	N	N	N	N	N	ABS
A.AF7	N	N	N	N	N	N	N	ABS
A.AF14	N	N	N	N	N	N	N	ABS
V.AM1	N	N	N	N	N	N	N	ABS
V.AAQ1	N	N	N	N	N	N	N	ABS
V.AM7	N	N	N	N	N	N	N	ABS
V.AAQ7	N	N	N	N	N	N	N	ABS
V.AM14	N	N	N	N	N	N	N	ABS
V.AAQ14	N	N	N	N	N	N	N	ABS
V.AF1	N	N	N	N	N	N	N	ABS
V.AF7	N	N	N	N	N	N	N	ABS
V.AF14	N	N	N	N	N	N	N	ABS

Table 6: Effect of 2000mg/kg bw Non-Fermented leaf extracts of *A.annua* and *V.amygdalina* on Body weights during acute toxicity of rats over 14 days n=3

Group	Day 1	Day4	Day8	Day12	Day14	Weight Change
Normal Ctrl	96.73±3.26^{ns}	97.33±3.35^{ns}	99.87±1.88^{ns}	98.56±3.35^{ns}	99.77±3.45^{ns}	3.04
A.AAQ	129.33±11.06^{ns}	123.97±7.48^{ns}	129.67±5.60^{ns}	130.6±6.52^{ns}	134.26±10.13^{ns}	4.93
V.AAQ	104.33±18.88^{ns}	102.43±14.47^{ns}	125.60±14.27^{ns}	128.93±9.15^{ns}	129.03±9.15^{ns}	24.7
A.AAQ+V. AAQ	115.00±5.0^{ns}	117.67±5.03^{ns}	122.67±3.51^{ns}	127.67±2.52^{ns}	131.00±1.73^{ns}	16

Values with the same superscript along the columns are not significantly different(P>0.05);

Table 7: Effect of 2000mg/kg bw of Fermented leaf extracts of *A. annua*and *V. amygdalina*on body weights during acute toxicity of rats over 14 days.

Group	Day1	Day4	Day8	Day12	Day14	Weight Change
N/ Control	160.10±2.0^{ns}	162.33±0.58^{ns}	164.00±0.00^{ns}	164.67±0.58^{ns}	166.33±0.58^{ns}	6.23
A.AF	165.00±5.0^{ns}	170.00±1.00^{ns}	169.03±0.95^{ns}	176.00±3.61^{ns}	174.07±5.18^{ns}	9.07
V.AF	156.60±2.11^{ns}	158.13±1.00^{ns}	160.13±0.23^{ns}	164.67±2.52^{ns}	168.58±4.78^{ns}	1.981
A. AF+V.AF	167.23±3.80^{ns}	160.33±0.58^{ns}	162.00±2.00 ^{ns}	166.00±2.00^{ns}	168.58±4.77^{ns}	1.58

Values with the same superscript along the columns are not significantly different(P>0.05);

Table 8: Organ weight of fermented leaf extracts of *A. annua*(A.AF) and *V. amygdalina*(V.AF)

Extract/Dose	Liver	R/Kidney	L/Kidney
Control(0mg/kg)	4.68±2.31^{ns}	0.90±0.45^{ns}	0.89±0.25^{ns}
A. A Ferm (2000 mg/kg)	4.68 ±2.31^{ns}	0.90±0.45^{ns}	0.88±0.1^{ns}
A.A Ferm (3585 mg/kg)	5.8 ± 1.9^{ns}	0.9±0.30^{ns}	0.89±2.7^{ns}
V.A Ferm (2000 mg/kg)	4.5±1.56^{ns}	0.85±0.52^{ns}	0.84±0.1^{ns}
V.A Ferm (3585 mg/kg)	5.59±1.59^{ns}	0.92±0.17^{ns}	0.91±0.11^{ns}
A. A Ferm+ V. A Ferm (20004.68±2.31^{ns} mg/kg)		0.90±0.45^{ns}	0.89.2.12^{ns}
A. A Ferm +V. A Ferm (35855.26±1.37^{ns} mg/kg)		0.13±0.31^{ns}	0.13±0.31^{ns}

Values with the same superscript along the columns are not significantly different(P>0.05)

Extract /dose	Liver	Right Kidney	Left Kidney
A.AM (2000mg/kg	4.0±0.5*	0.40±0.09^{ns}	0.59±0.17**
A.AM (3585mg/kg	3.5±0.5*	0.50±0.10^{ns}	0.33±0.15**
A.AAQ (2000mg/kg)	4.2±0.10*	0.30±0.02^{ns}	0.30±0.02^{ns}
A.AAQ (3585mg/kg)	3.99±0.19*	0.3±0.01^{ns}	0.3±0.01^{ns}
V.AM (2000mg/kg)	3.76±0.25*	0.27±0.02^{ns}	0.29±0.01**
V.AM (3585mg/kg)	3.7±0.20*	0.58±0.02*	0.47±0.15**
V.AAQ (2000mg/kg)	8.33±10.88*	0.40±0.0*	0.40±0.00**
V.AAQ (3585mg/kg)	2.09±0.01*	0.3±0.0*	0.33±0.05**
A.AM+V.AM (2000mg/kg)	3.33±1.07*	0.43±0.15^{ns}	0.60±0.20**
A.AAQ+V.AAQ (3585mg/kg)	3.83±0.29*	0.50±0.10^{ns}	0.59±0.17**

Table 9: Effects of methanol and aqueous leaf extracts of *A. annua*and *V. amygdalina*on liver and mkidney weight during acute toxicity.n=3

Mean values with ns were not significant along the columns (P>0.05).Mean value with * were significant along the column (P<0.05) ns= not significant *= significant** highly significant

DISCUSSIONS

Although outcome on LD_{50} during acute toxicity showed few deaths of rats as a result of administration of non- fermented extracts, the cause of the death may not be certain and may therefore not be directly linked to the dosage of the extracts used. To support this assertion, Siddiqui *et al.* (2018), demonstrated that oral administration of greater than or equals to 5000 mg/kg bw in mice did not result in death nor post treatment behaviors depicting any sort of poisoning. Also, Loomis and Haye (1996) classified substances with LD_{50} values between 500-5000 and between 5000- 15,000 mg/kg bw are taken as being non-toxic or slightly toxic respectively. To unveil the cause of death in acute toxicity, Synder*et al*. (2015) opined that in research studies, the cause of mortality is not frequently estimated or documented. The subject on the cause of mortality is an area of attention in toxicological pathology community. Guide lines for ascertaining the basis for death in toxicity studies have been suggested especially in carcinogenic studies. Terminal points other than death are used in rodent studies for humane, legal, experimental and practical purpose. From practical approach, tissues may be non- diagnostic within hours due to autolysis. Most often, the animals die at times when the researcher may not be available to immediately carry out diagnostic test to determine causes of death (Ray *et al.*, 2010). Autolyzed tissues are usually immediately necrotized and therefore difficult to evaluate histologically (Kane *et al.*, 2015). In addition, adequate assessment on the cause is limited because absence of standardized and consistent approach to employ (Synder*et al.*, 2015).

Since there was no death following administration of fermented extracts, this could be associated with the non-toxic nature of the different dosage of the extracts used. Ubogu et al. (2018) established that in an assessment of toxicity of 500, 1000, 2000 and 5000 mg/kgbw of fermented *Musa parasidiaca* in rats, there was no toxicity signs, or death (P>0.05).

Although Baek*et al.* (2015) reported that there was a significant reduction in body weight after treatment of non-fermented *A. annua* extract, this study has shown that the non- fermented (methanolic and aqueous) and fermented extracts of *A. annua*and *V. amygdalina* did not significantly affect the body and organ weights during acute toxicity. The reason may be associated with relative safe nature of the extracts and supported by the assertions of Carol (1995), El Hilaly et al. (2004) who reported that variations in body and organ weight are pointers of harmful effects of substances. The outcome on the single administration of fermented *A.annua* and *V.amygdalina* may be a confirmation of the absence of toxic effect of the extract as was affirmed by Ubogu et al. (2018) who established that in an assessment of toxicity of 500, 1000, 2000 and 5000 mg/kgbw of fermented *Musa parasidiaca* in rats, there was no toxicity signs, or death (P>0.05). This also compares with work of Akah and Okafor (2006), where the intra-peritoneal injection of 1122mg/ kgbw of aqueous *A. annua*in rabbit exhibited no side effects similarly, the intra-peritoneal injection of Methanol *A.annua* leaf extract at doses 80, 160 and 320 mg /kgbw after 28 days did not exhibit unusual abnormal impact on the vital organs.

The effect of various doses of non-fermented and fermented extracts of *A.annua* and *V.amygdalina* which showed that absence of any significant abnormalities in the behavioural pattern of the rats could be linked to the safety nature of the plants. This agrees with reports of Loomis and Haye on the classification substances and where substances with LD_{50} values between 500-5000 and between 5000- 15,000 mg/kg bw are taken as being nontoxic or slightly toxic respectively. Also, the safety of the extracts is supported by the work of Ubogu*et al.* (2018) and Siddiqui et al.(2018), who established that in an assessment of toxicity of 500, 1000, 2000 and 5000 mg/kgbw of fermented *Musa parasidiaca* in rats, there was no toxicity signs, questionable behavioral patterns or death.

CONCLUSION

The extracts of *A.annua* and *V.amydalina* may be considered safe for use by human beings and animals either directly or when incorporated into drugs and other useful products.

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