

Toxicological Evaluation of *Aspilia Africana* Leaf Extracts in Mice

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Abstract: Problem statement: The degree of toxicity of crude extracts of *Aspilia Africana* (Bush marigold) leaf on the basis of routes and dosages of administration were investigated. 204 healthy, male Swiss albino mice (20-25g) were used in three consecutive studies. **Approach:** In the first and second study, oral and intraperitoneal doses of 100, 500, 1000, 2000 and 4000 mg kg⁻¹ body weight of aqueous, chloroform or ethanolic extract were administered to mice. 0.2 mL of distilled water was given to the control group as placebo. Mortality and behavioural changes were monitored at 1, 2, 4, 6, 24, 48 and 96 h post administrations. In the third study, higher doses of 4,000, 8,000, 12,000, 16,000 and 20,000 mg kg⁻¹ body weight aqueous or ethanolic extract were orally administered to fresh groups of mice. **Results:** Results revealed that the degree of toxicity of *Aspilia africana* leaf was extractant, dose and route of exposure responsive. Signs of behavioral toxicity; nervous and respiratory disorders and piloerections fluctuated in mice. Results indicated that the medium Lethal Dose (LD₅₀) was greatest for the aqueous extract and least for the chloroform extract. Oral exposure had significantly greater LD₅₀ (p<0.001, Av. value = 8,194.84 mg kg⁻¹) compared to intraperitoneal exposure with an average of 232.55 mg kg⁻¹. **Conclusion:** These findings support the common practice of oral administration of either aqueous or ethanolic extract of *Aspilia africana* as a medicinal decoction in herbal medicine. The study concludes that oral administration of up to 10,000 mg kg⁻¹ body weight of aqueous and ethanolic extracts of *Aspilia africana* leaf are safe for human and animal use.

Key words: *Aspilia africana*, behavioural effects, leaf extracts, route of exposure, respiratory disorders, reproductive risks to animals, focusing attention

INTRODUCTION

The identification of more than 20,000 medicinal plant species of tropical origin by the World Health Organisation (Gullece *et al.*, 2006) has contributed immensely to the advancement of Complementary and Alternative Medicine (CAM). Currently, the identification, validation and formulation of animal management and health care policies are the focus of renewed interest in most ethnoveterinary researches (Shapoval *et al.*, 1994; Izzo *et al.*, 1995; Souza *et al.*, 2002). These studies include the diversification of traditional plants to include some weed species (Njoroge *et al.*, 2004; Oluyemi *et al.*, 2007; Schultes, 1994) had earlier opined that focusing attention on the plants used by indigenous people is the most effective method of identifying plants that may contain useful bioactive substances.

In most rural communities in Africa, primary health care delivery services remains dependant on the

use of several medicinal plants since synthetic drugs have proven to be prohibitively expensive and unavailable (Ngari, 2010). Nevertheless, studies on the efficacies of these tropical plants as new sources of natural, effective, cheaper and potentially less toxic drugs has not been completely explored (Njoroge *et al.*, 2004; Barbieri *et al.*, 2002).

Aspilia africana (Asteraceae) is a perennial herbaceous plant with long history of traditional medicinal and agricultural uses (Carew *et al.*, 1980). The plant is commonly known as haemorrhage or iodine plant due to its ability to stop bleeding. Traditional communities in Africa and Asia use this herb as a bactericide, anti-inflammatory, astringent and wound healing agents (Akobundu, 1987; Gill, 1992; Adeniyi and Odufowora, 2000). Oral decoction of the leaves is reported to relief febrile headache (Gill, 1992), quicken delivery in females (Obute and Adubor, 2007) as well

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as to cure other ailments including lumbago, sciatica and stomach disorders (Biser, 1998).

Several studies revealed that various leaf extracts of *Aspilia africana* have varying biological activities. (Macfoy and Cline, 1990) reported that the ethanolic extract could exhibit in vitro gastroprotective, anti-inflammatory and antibacterial effects. (Dimo *et al.*, 2002) showed that its methanolic extract reinforced the vascular smooth muscle contraction. Aqueous extract of *Aspilia Africana* leaf have shown varying degree of biological activities against bacteria, fungi and yeast (Taziebou *et al.*, 2007). Okoli *et al.* (2007) also reported that its ethanolic extract caused extracellular Ca²⁺ dependent increase in vascular tone.

Recent studies however indicated some concern on the health risk of the extensive use of *Aspilia africana* plant. Oluyemi *et al.* (2007) showed that intraperitoneal administration of the methanolic extract of *Aspilia africana* leaf to wistar albino rats caused significant delay in their estrus cycles and severe damages to their uterine tissues. (Eweka, 2009) similarly revealed that an oral administration of the aqueous extract of *Aspilia Africana* leaf could distort the histology of the ovaries in female wistar rats, thus impairing fertilization. These studies suggest that *Aspilia africana* plant could be toxic as well as pose some health and reproductive risks to animals.

This present study was designed to evaluate the effects of route of administration and varying doses of different extracts on the degree of toxicity of *Aspilia africana* leaf and their impact on the behavioural responses in Swiss albino mice.

MATERIALS AND METHODS

Identification of plant material: Fresh leaves of *Aspilia africana* were collected in January, 2010 from local gardens at the University of Calabar and authenticated by Mr. Frank Apejaye of the Department of Botany, University of Calabar. A voucher specimen (No.252) was deposited at the herbarium section of the Department of Botany, University of Calabar for further reference.

Preparation of extract: The leaves were sorted to remove any contaminants, dead matter and sand particles. They were air-dried for 96 h and the dried leaves were ground to fine powder to form *Aspilia africana* leaf meal using a hammer mill fitted with a 1mm screen. 100 g of leaf meal was measured into three conical flasks and soaked in 600 mL of distilled water, 80% ethanol and chloroform solution,

respectively for 48 h at room temperature. Each mixture was filtered into 250 mL conical flask with Whatman filter paper no.1. The filtrates were then concentrated in vacuo (40°C) to produce gel-like aqueous (AeAaL), ethanolic (EeAaL) and chloroform (CeAaL) extract of *Aspilia africana* leaf, respectively. The percentage extract yields were also determined. Appropriate stock solutions were prepared by serial dilution (Dapper *et al.*, 2007) on the day of experiment.

Experimental protocol: Two hundred and four (204) healthy, male Swiss albino mice (20-25 g⁻¹) bred at the Experimental Laboratory of the Department of Zoology, University of Calabar, Calabar were used for the acute toxicity test Table 1. The clinically healthy mice were kept under standard environmental conditions at temperature of 27-29°C and 12 h photoperiod. For three (3) days, the animals had free access to water and were fed rat chows (Vital Feeds Nigeria Limited) ad libitum to enable them acclimatize to their metabolism cages.

Acute toxicity test was performed according to the Organization of Economic Co-operation and Development's guidelines for testing of chemicals (OECD, 2004). To investigate the effects of route of administration (intraperitoneal or oral administration) on the degree of toxicity of each *Aspilia africana* leaf extract, three studies were conducted (Lorke, 1983).

Table 1: Distribution of mice for acute toxicity tests

Route/dose of administration (mg/kgBw)	Number in aqueous experiment	Number in chloroform experiment	Number in ethanolic experiment
Intraperitoneal administration 1st study			
0	4	4	4
100	4	4	4
500	4	4	4
1000	4	4	4
2000	4	4	4
4000	4	4	4
Total number of mice used in the first experiment			72
Oral Administration (mg/kgBw) 2nd study			
0	4	4	4
100	4	4	4
500	4	4	4
1000	4	4	4
2000	4	4	4
4000	4	4	4
Total number of mice used in the second experiment			72
Oral administration (mg/kgBw) 3rd study			
4,000	4	4	4
8,000	4	4	4
12,000	4	4	4
16,000	4	4	4
20,000	4	4	4
Total number of mice used in the third experiment			60
Grand total number of mice used in the three experiments			204

In the first and second studies, 24 mice were randomly assigned to six groups of four animal each. The mice were housed individually in metabolism cages. Each group was administered one of the following doses; 100 mg kg⁻¹, 500, 1000, 2000 and 4000 mg kg⁻¹ of aqueous, chloroform or ethanolic extract of *Aspilia africana* leaf either intraperitoneally (first study) or through oral administration (second study). The mice in the control group were orally administered 0.2 mL of distilled water as placebo. Therefore, in each experiment, a total of 72 mice were studied Table 1. The maximum volume of extracts administered was 0.4 mL⁻¹ mouse.

Following administration, each mouse was returned to its cage and allowed access to feed and water.

The mice in cage were observed 1, 2, 4, 6, 24, 48, 96 h and once daily thereafter over 14 days for clinical signs of toxicity. Percentage mortalities were converted to probits (a probability unit) and plotted against the log₁₀ of dose of each extract LeOr, 1994. Regression lines were also fitted by the least square method and the confidence limits for respective medium lethal dose (LD₅₀) values were calculated.

Oral administration of between 100 and 4,000 mg kg⁻¹ of the three *Aspilia africana* leaf extracts (except chloroform at 4,000 mg kg⁻¹ did not result to no mortality in mice, thus the third study was initiated.

The third study involved the random assignment of a total of 60 mice to 5 groups of 4 animals per leaf extract. Each group was orally administered one of the higher doses (4,000, 8,000, 12,000, 16,000 and 20,000 mg kg⁻¹) of either aqueous, chloroform or ethanolic extract of *Aspilia africana* leaf. The procedures adopted in the first two studies to establish the LD₅₀ of each extract were repeated.

RESULTS

The patterns of mortality in mice administered the various *Aspilia africana* leaf extracts were dose-dependent Fig. 1 and 2. Intraperitoneal and oral exposures to *Aspilia africana* leaf extracts revealed highly significant (p<0.001) influences on percentage mortality.

From the probit analysis Table 2, medium Lethal Dose (LD₅₀ i.p) ranging from 100-398.11mg kg⁻¹ was established in mice exposed intraperitoneally to *Aspilia africana* leaf extracts. The acute oral LD₅₀ of *Aspilia africana* leaf Table 2 was indicated at 12,589.25

(AeAaL), 1,995.26 mg kg⁻¹ (CeAaL) and 10,000 mg kg⁻¹ (EeAaL).

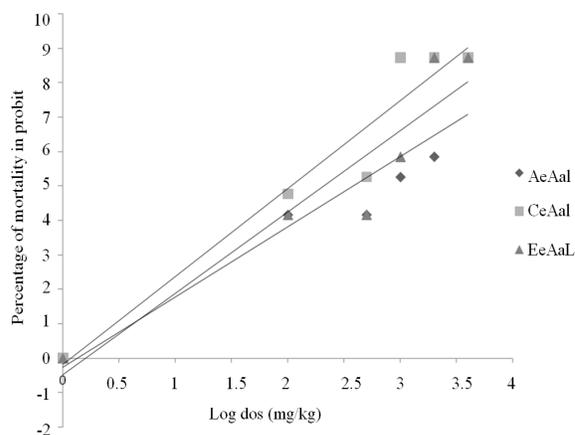


Fig. 1: Acute toxicity test following intraperitoneal (i.p) administrations of *Aspilia africana* leaf extracts AeAaL-aqueous extract of *Aspilia africana* leaf, CeAaL-chloroform extract of *Aspilia africana* leaf, EeAaL-ethanolic extract of *Aspilia africana* leaf

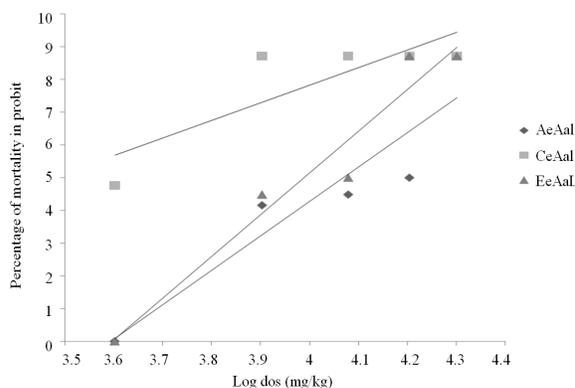


Fig. 2: Acute toxicity test following oral administrations of *Aspilia africana* leaf extracts AeAaL-aqueous extract of *Aspilia africana* leaf, CeAaL-chloroform extract of *Aspilia africana* leaf EeAaL-ethanolic extract of *Aspilia africana* leaf

Table 2: Determination of the medium lethal doses (LD₅₀) of *Aspilia africana* leaf extracts in mice

Extract	Intraperitoneal administration			Oral administration		
	Prediction equation	LD ₅₀ (i.p) mg/kg	R	Prediction equation	LD ₅₀ (oral) mg/kg	R
Aqueous	Y = -0.261+2.034X	398.11	0.939	Y = -37.741+10.511X	12,589.25	0.936
Chloroform	Y = 0.192+2.555X	100.00	0.966	Y = -13.854+5.421X	1,995.26	0.842
Ethanolic	Y = -0.482+2.362X	199.53	0.941	Y = -45.686 + 12.715X	10,000.00	0.973

Table 3: Rate of lethality in mice administered *Aspilia africana* leaf extracts

Dose (mg/kg)	Mortality (%)		
	AeAaL	CeAaL	EeAaL
Intraperitoneal administration			
0	0.0	0.0	0.0
100	20.0	40.0	20.0
500	20.0	60.0	20.0
1000	60.0	100.0	80.0
2000	80.0	100.0	100.0
4000	100.0	100.0	100.0
Oral administration			
0	0.0	0.0	0.0
100	0.0	0.0	0.0
500	0.0	0.0	0.0
1000	0.0	0.0	0.0
2000	0.0	0.0	0.0
4000	0.0	40.0	0.0
8000	20.0	100.0	33.0
12000	33.0	100.0	50.0
16000	50.0	100.0	100.0
20000	100.0	100.0	100.0

No clear sign of toxicity was observed in mice orally exposed to 100-4,000 mg kg⁻¹ of aqueous or ethanolic extracts of *Aspilia africana* leaf Table 3 whereas, a mortality of 40% was recorded in mice orally exposed to 4,000 mg kg⁻¹ of the chloroform extract. 100% mortality was reached following oral administration of 8,000, 16,000 and 20,000 mg kg⁻¹ of chloroform, ethanolic and aqueous extract, respectively of *Aspilia africana* leaf Table 3. Intense behavioural changes were also dose-dependent.

Behavioral response in Mice: No clear behavioral changes were observed in mice that received low doses of extracts (100-500 mg kg⁻¹), compared to the control group. Changes in behavior became noticeable in the groups that received 2,000 mg kg⁻¹ oral exposure of chloroform extract. 15 m after administration, some of the animals became erratic for 5-10 sec and remained immobile for 30 m. 6 h post administration, all the mice resumed feeding.

Mild signs of intoxication were observed at a dose range of 4,000-8,000 mg kg⁻¹ oral administration. Apart from behaviors exhibited at low doses of the extracts, biting and vigorous face wiping with forelimbs were noticed. These behaviors continued for 10-20 m. Thereafter, the mice became immobile, paralyzed and laid on their abdomen with limbs stretched. Piloerection occurred and the animals became cold. Mortality was recorded 8 h after administration.

At a dose of 12,000-20,000 mg kg⁻¹, marked behavioural changes occurred. Respiration became forced and irregular within 15-20 m of administration. This led to spasm and death (mortality) began 45 m

post administration. Necropsy revealed the lungs became haemorrhagic with extreme pulmonary oedema.

DISCUSSION

Intraperitoneal exposure showed that mortality could reach 100% when mice are exposed to 1,000 mg kg⁻¹, 2,000 and 4,000 mg kg⁻¹ of chloroform, ethanolic and aqueous extract of *Aspilia Africana* leaves, respectively. Hashemi *et al.* (2008) earlier reported that oral administration of 2,000 mg kg⁻¹ of aqueous extract of some selected herbs was non-toxic to birds. Mice that received 100-500 mg kg⁻¹ of aqueous or ethanolic extracts of *Aspilia africana* leaf had the lowest mortality. Mortality remained significantly ($p < 0.05$) higher in those that received equivalent doses of the chloroform extract. The reason for high mortality in mice exposed to chloroform extract could be associated with liver damage as suggested by Kent (1998) when chloroform gas was administered to rat.

The present findings indicated that oral exposure to aqueous and ethanolic extracts of *Aspilia africana* leaf below 4,000 mg kg⁻¹ appeared to be non-toxic probably because the circulatory levels of the toxic substances were metabolically lowered below the lethal threshold thus inhibiting its lethal effect (Kent, 1998). The acute toxicity test showed that the chloroform extract was of higher toxicity in mice than the ethanolic extract while the aqueous extract was the least toxic. There were indications of significant effects of the route of administration of the *Aspilia africana* leaf extracts. In all extracts, intraperitoneal administration was of higher toxicity, even at doses as low as 500 mg kg⁻¹, compared to higher oral doses of up to 12,000 mg kg⁻¹.

The higher values obtained for LD₅₀ under oral administration further indicated that oral administration of *Aspilia africana* leaf extracts could be safe for human and animal use. The aqueous extract seemed to be of relatively lower toxicity based on its higher LD₅₀ value. This further revealed that aqueous extract of *Aspilia africana* leaf was relatively of low toxicity compared to the ethanolic extract, while chloroform extract appeared to be the most toxic of the three extract forms. This observation supports the common practice of oral administration of either aqueous or ethanolic extract of *Aspilia africana* as a medicinal decoction in herbal medicine (Sofowora, 1993).

Previous studies had reported oral LD₅₀ values of 894 mg kg⁻¹ (Okoli *et al.*, 2007) and 6,600 mg kg⁻¹ (Taziebou *et al.*, 2007) for methanolic and aqueous extracts of whole plant and leaf of *Aspilia africana*. Other studies reported acute oral LD₅₀ of 810, 2,220, 4,590, 980 and 1,562 mg kg⁻¹ for cinnamaldehyde, beta-ionone, thymol (Lee *et al.*, 2004) and *Adansonia*

digitata leaves, respectively. These reports implied that the LD₅₀ of a plant material was dependent on the route of administration, the principles present as well as the types of extractant used.

The relatively high toxicity of CeAaL could suggest that metabolic detoxification was inhibited due to the rapid and extensive rate of absorption into the animals' bodies. The high cost of chloroform and concerns about its biohazard to humans and animals (Kent, 1998) justify its limited use in folk medicine.

These findings and other field studies support the insinuations that *Aspilia africana* leaf is of low to moderate toxicity (>5,000 mg kg⁻¹) based on the recommendation of Olson (2007). Recent studies have shown some toxic effects of AaL on animal physiology which have been reported to include; reductions in uterine tissue and duration of estrus cycle as well as antioviulatory effects in female rats (Oluyemi *et al.*, 2007; Taziebou *et al.*, 2007; Okwuonu *et al.*, 2007). However, no report on such effect on farm animals has been published.

Behavioural responses observed were consistent with the findings of Eno *et al.* (2000). Intraperitoneal administration was marked with intense behavioural changes at as low as 500 mg kg⁻¹ of extracts due to rapid rate of absorption into the blood stream.

CONCLUSION

The differences in the degree of toxicity exhibited in mice exposed to the aqueous, chloroform and ethanolic extracts of *Aspilia africana* leaf suggested that they could have different effects on the physiology of animals. The oral exposure is the safer route of administration of crude extracts of *Aspilia africana* leaf. Results from the LD₅₀ studies revealed that the chloroform extract of *Aspilia africana* leaf was relatively more toxic compared to the aqueous and ethanolic extract forms. These findings support the common practice of oral administration of either aqueous or ethanolic extract of *Aspilia africana* as a medicinal decoction in herbal medicine. Based on the results from this study, it is therefore recommended that oral administration of up to 10,000 mg kg⁻¹ body weight of aqueous and Ethanolic extracts of *Aspilia Africana* leaf are safe for human and animal use.

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