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Acute and sub-acute (28-Day) oral toxicity studies of aqueous extract of *secamone afzelii* leaves in wistar rats

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Abstract
Aim of the study: The present study was carried out to evaluate the acute and subacute toxicity of the aqueous extract of *Secamone afzelii* (AESA)
Materials and Methods: Acute toxicity study was conducted in rats by using OECD 423 guidelines whereas sub-acute toxicity study was carried out in rats by using OECD 407 guidelines. In the acute toxicity study, rats were orally administered with a single dose of 2000 mg/kg and then observed individually for the first four hours, then over a period of 24 hours and at least once daily for 14 days. In the subacute toxicity studies, AESA was given orally at doses of 200 mg/kg, 400 mg/kg and 800 mg/kg body weight daily for 28 days to male and female rats respectively. General behavior, adverse effects and mortality were observed throughout the experimental period. Food intake, water intake, body weight, organ weight, hematological and biochemical parameters were evaluated.
Results: The limit dose of 2000 mg/kg did not cause any mortality or signs of acute toxicity in the rats tested during the observation period. In sub-acute toxicity tests, the results did not show any treatment related abnormalities in terms of hematological but showed biochemical parameters abnormalities. There was a significant increase ($p<0, 05$) of total protein in male rats treated with 200 mg/kg of AESA compared to control group. The male rats treated with 800 mg/kg of AESA, also indicated a significant elevation of Creatinine concentration ($p<0, 01$) compared to control group. There were no significant differences in body weight and organ weight between the control and treated groups.
Conclusion: These results concluded that the AESA did not cause any mortality and signs of toxicity in rats (acute toxicity study). The oral lethal dose of aqueous extract of *Secamone afzelii* is more than 2000 mg/kg. In the sub-acute toxicity, any mortality and signs of toxicity in female rats. But, in male rats, subacute study showed that *Secamone afzelii* at the high dose (800 mg/kg per day for 28 days) may cause renal dysfunction.

Keywords: *Secamone afzelii*, acute toxicity study, subacute toxicity study, rats, hematological parameters and biochemical parameters

1. Introduction
Medicinal plants are an effective source for both modern and traditional medicines, and about 80% of the rural populations depend on them for their primary health care's (Israël *et al*., 2010) [1]. They are used in different ways (decoction, maceration, infusion). However, the toxicity of plants from botanical data was underestimated due to the perception that herbal medicines are absolutely safe. However, severe hepatic dysfunction has been reported after ingestion of a large variety of different herbal preparations (Stickel *et al*., 2000) [2]. The growing number of herbal drug users around the globe and lack of scientific data on the safety profile of herbal products make it necessary to conduct toxicity study of herbal products (Saad *et al*., 2006) [3]. This determination is made as to highlight potential dangers for human health associated with the use of these medicines (Bamba *et al*., 2015) [4].
Secamone afzelii is used in traditional medicine for stomach problems, diabetes, colic, dysentery and also for kidney problems (Abere et Onwukaeme, 2012) [5]. This plant is also used by some traditional healers to care for pregnant women till the period of post-childbirth and to take care of the children who have some swelling (Zabri *et al*., 2008) [6]. The decoction of the entire plant is prescribed for cough, gonorrhoea, catarrh conditions and as galactogogue (Magid *et al*., 2016) [7]. It has antioxidant, anti-inflammatory and antimicrobial properties (Houghton *et al*., 2005; Mensah *et al*., 2014) [8, 9].
This study was conducted to evaluate the acute and subacute toxicity effects of the aqueous extract of *Secamone afzelii* especially on haematological and biochemical parameters in albino rats.

2. Materials and Methods

1. Materials

1.1. Collection and Identification of Plant Materials

The fresh leaves of *Secamone afzelii* were collected in Abobo (Abidjan) in 2017. The plant species was later identified and authenticated by the department of Botany, Felix Houphouet Boigny University of Abidjan. They were further dried at room temperature under the shade for two weeks and pulverized using the crushing assistance (IKAMAG RCT®). The powder of leaves obtained, constituted our sample to be analysed.

2. Methods

2.1. Preparation of the Extract

The extracts were prepared according to the method described by Yapo *et al*., 2016 [10]. Fifty grams (50 g) of plant powder were extracted in blender (the process is repeated 3 times) with one liter (1L) of distilled water. After crushing, the mixture obtained was first spun in a clean square fabric, and then filtered fifth in successively with cotton wool. The filtrate was evaporated at 55 °C for 48 hours.

2.2. Experimental animals

Wistar albino rats (70-75 g) were selected for acute toxicity studies and those weighing between 100-180 g of both sexes were also selected for sub-acute toxicity studies. They had free access to food and water and were maintained under standard laboratory conditions which included 12-hour light-dark cycle and temperature of 25-30 °C. Animals are allowed for a one week of acclimatization period prior to the study. The experimental protocol was approved by the IAEC (institutional animal ethical committee).

2.3. Acute Toxicity Studies

Acute toxicity studies of Aqueous extract of *Secamone afzelii* was carried out in female rats by using Organization for Economic Co-operation and Development (OECD) guideline 423 (OECD, 2001). Before oral administration of a single dose of the test samples, the rats were deprived of food for 3 h**.** Doses of 2000 mg/kg of the test samples were given using oral gavage to rats of Group I and Group II respectively. All the rats were observed for general behavioural changes; symptoms of toxicity and mortality after treatment for the first four (critical) hours, then over a period of 24 hours, thereafter daily for 14 days.

2.4. Su b-Acute Toxicity Studies

Sub-acute toxicity study (28-day repeated oral toxicity study) was carried out according to OECD 407 guidelines (OECD, 2008) [12]. Both sexes of rats (130-200g) were divided into four groups with 10 animals (5 males plus 5 females in each). The group I received orally 1 ml/100g body weight and served as a control group whereas group II, group III and group IV received Aqueous extract of *Secamone afzelii* at 200 mg/kg, 400 mg/kg and 800 mg/kg body weight, p.o. respectively. All the groups of rats were observed twice daily for mortality and morbidity till the completion of the experiment. All the animals were observed for clinical signs and the time of onset, duration of these symptoms, if any were recorded. Body weights of the rats in all groups were recorded once weekly during the treatment period. At the end of the experiment (on 29th day), blood samples were collected from overnight fasted rats (only water allowed) into heparinized and non-heparinized tubes for haematological analysis and biochemical analysis.

2.5. Haematological parameters

The heparinised blood was used for the analysis of haematological parameters such as haemoglobin, red blood cell count, white blood cell count, platelet count, haematocrit, were measured using fully automated hematology analyser (PE 6000).

2.6. Biochemical Parameters

The serum was separated from non-heparinized blood and the serum biochemical parameters including urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol, and total protein were analysed by Cobas Integra 400 Plus (ROCHE DIAGNOSTIC, Germany)

2.7. Statistical Analysis

Results are expressed as mean ± standard error mean (SEM). Data obtained was analysed by using one-way ANOVA followed by Dunnett test and $p<0.05$ was considered as statistically significant.

3. Results

1. Acute Toxicity Study

In the toxicity study, oral administration of the Aqueous Extract of *Secamone afzelii* (AESA) at 2000 mg/kg did not produce any deaths and clinical signs of toxicity in rats (Table 1). As there were no mortality and clinical signs of toxicity in the tested dose, LD50 value of Aqueous extract of *Secamone afzelii* was found to be greater than 2000 mg/kg.

Table 1: Effect of Aqueous extract of *Secamone afzelii* on the general appearance of the animals

Durée d'observation	½ hour	4 hours	24 hours	48 hours	1 week	2 weeks
Control	N	N	N	N	N	N
Aqueous Extract (2000 mg/kg)	N	N	N	N	N	N

N: normal

2. Sub-Acute Toxicity Study

There was no treatment related toxicity signs and mortality observed in both sexes of rats treated at 200mg/kg, 400mg/kg and 800mg/kg orally during the 4 weeks of treatment. No significant differences in body weight were observed between the initial and final body weight of the rats treated with AESA and control rats (Table 2).

There were no significant differences between control and AESA treated groups in organ weight (Table 3).

The haematological profile of treated and control group were summarized in Table 4. The results concluded that all haematological parameters such as total red blood cell count, total white blood cell count, platelet count, haemoglobin, haematocrit are normal range in both control and treated groups during the experimental period.

The data on biochemical parameters in treated and control rats were presented in Table 5. After Sub-acute administration of AESA, there was no significant difference in biochemical parameters such as AST, ALT, Urea and Total Cholesterol values between the control and AESA treated groups in both males and females ($p>0.05$, each). However, compared to the control group, only in male rats, the Total Protein significantly increased in the AESA 200 mg/kg ($p<0.05$) and the Creatinine significantly decreased in the AESA 800 mg/kg treated group ($p<0.01$).

Table 2: Effect of Aqueous Extract of *Secamone afzelii* on Body Weight Gain in Rats-Sub-Acute Toxicity Study

Treatment Group	Body weight				
	Sex	First week	Second week	Third week	Fourth week
Control	Males (n=5)	124 ± 8.5	145 ± 11	158 ± 10	187 ± 7
	Females (n=5)	133 ± 6.4	152 ± 9.1	167 ± 4.6	179 ± 3.5
200 mg/kg	Males (n=5)	140 ± 22	178 ± 25	194 ± 24	192 ± 20
	Females (n=5)	134 ± 8.4	159 ± 3.7	175 ± 5.5	172 ± 5.6
400 mg/kg	Males (n=5)	136 ± 9	180 ± 7.7	201 ± 7	204 ± 7
	Females (n=5)	132 ± 9	157 ± 10	174 ± 13	173 ± 15
800 mg/kg	Males (n=5)	130 ± 18	139 ± 22	150 ± 24	147 ± 24
	Females (n=5)	127 ± 18	136 ± 18	144 ± 23	146 ± 23

Table 3: Effect of Aqueous Extract of *Secamone afzelii* on Body Organ Weight in Rats-Sub-Acute Toxicity Study

Organs Weight (Gms)	Treatment (Dose in mg/kg)							
	Males (n=5)				Females (n=5)			
	Control	200	400	800	Control	200	400	800
Liver	3.7 ± 0.13	2.9 ± 0.45	3.5 ± 0.12	3.9 ± 0.16	2.8 ± 0.25	2.9 ± 0.13	3 ± 0.026	3.3 ± 0.18
Heart	0.27± 0.02	0.46± 0.11	0.36± 0.01	0.32± 0.02	0.34 ± 0.4	0.38± 0.02	0.37± 0.01	0.39± 0.03
Kidney	0.46± 0.03	0.59± 0.1	0.54± 0.01	0.65± 0.03	0.49± 0.02	0.53± 0.01	0.52± 0.02	0.57 ± 0.1

Table 4: Effect of Aqueous Extract of *Secamone afzelii* on Haematological Parameters in rats

Haematological Parameters	Sex	Treatment			
		Control	200 mg/kg	400 mg/kg	800 mg/kg
RBC (x106/µl)	Males (n=5)	7.5 ± 0.61	8.3 ± 0.15	7.7 ± 0.11	7.2 ± 0.31
	Females(n=5)	7.6 ± 0.69	7.9 ± 0.052	8 ± 0.18	8.5 ± 0.12
WBC (x103/µl)	Males (n=5)	15 ± 3.0	15 ± 2.6	15 ± 2.6	25 ± 2.7
	Females(n=5)	18 ± 0.90	23 ± 5.3	20 ± 2.4	22 ± 1.9
Haemoglobin(g/dl)	Males (n=5)	13 ± 0.90	14 ± 0.65	12 ± 0.46	12 ± 0.47
	Females(n=5)	13 ± 1,00	14 ± 0.12	14 ± 0.33	15 ± 0.53
Haematocrit (%)	Males (n=5)	41 ± 2.8	45 ± 2.1	39 ± 2.1	40 ± 1.4
	Females(n=5)	41 ± 3.50	43 ± 0.32	43 ± 1.2	44 ± 3.1
Platelet(x103/µl)	Males (n=5)	794 ± 12	964 ± 76	1053 ± 82	847 ± 53
	Females(n=5)	885 ± 40	862 ± 82	673 ± 164	719± 23

Table 5: Effect of Aqueous Extract of *Secamone afzelii* on Biochemical Parameters in rats

Biochemical Parameter	Sex	Treatment			
		Control	200 mg/kg	400 mg/kg	800 mg/kg
AST (U/L)	Males (n=5)	182 ± 11	206 ± 21	206 ± 27	187 ± 20
	Females (n=5)	197 ± 5.9	172 ± 4.1	179 ± 12	208 ± 24
ALT (U/L)	Males (n=5)	48 ± 3.5	52 ± 6.9	35 ± 0.88	39 ± 2.6
	Females (n=5)	38 ± 2.5	35 ± 1.3	44 ± 2.0	39 ± 5.1
Creatinine (mg/L)	Males (n=5)	1.7 ± 0.18	2.4 ± 0.41	2.4 ± 0.35	4 ± 0.58 a**
	Females (n=5)	3.2 ± 0.20	4 ± 0.27	4 ± 0.31	3.6 ± 0.41
Urea (g/L)	Males (n=5)	0.32 ± 0.03	0.31 ± 0.038	0.36 ± 0.038	0.37 ± 0.023
	Females (n=5)	0.36 ± 0.040	0.33 ± 0,03	0.4 ± 0.02	0.5 ± 0.07
Total Protein (g/L)	Males (n=5)	67 ± 1.3	74 ± 1.0 a*	65 ± 1.5	68 ± 0.88
	Females (n=5)	78 ± 2.5	79 ± 0.15	78 ± 1.6	74 ± 2.4
Total Cholesterol (g/L)	Males (n=5)	0.67 ± 0.11	0.77 ± 0.069	0.59 ± 0.055	0.75 ± 0.058
	Females (n=5)	0.64 ± 0.03	0.78 ± 0.04	0.74 ± 0.019	0.77 ± 0.08

a: compared with control; *: $p<0.05$ et **: $p<0.01$

4. Discussion

The oral route of drug administration is the most convenient and commonly used method for toxicity evaluations in pre-clinical animal models. (Pandey *et al*., 2016; Gopal *et al*., 2014) [13, 14] In the current study, in addition to acute study, subacute toxicity assay was performed to obtain data on the toxicity of the three doses of *Secamone afzelii* (200, 400 and 800 mg/kg) after 28 days repeated oral administration. The main purpose of subacute toxicity was to establish the lowest level of adverse effects and identify the specific affected organ/s by the *Secamone afzelii* after repetitive administration. The results of acute toxicity assay showed that *Secamone afzelii* at dose of 2000 mg/kg did not cause death and behavioural changes in the animals. Therefore, it can be concluded that according to OECD guidance, (OECD, 2008) [12] the aqueous extract of *Secamone afzelii* may be assigned to be the lowest toxicity class 5 (LD50> 2000 mg/kg).

Moreover, insignificant difference in vital organs' weight between the control and *Secamone afzelii* treated animals clearly demonstrates that *Secamone afzelii* at the all doses (200, 400 and 300mg/kg) did not cause any sensitivity, alteration and acute organ damage (Michael *et al*., 2007) [15].

Haematological parameters are highly sensitive markers of drug-induced toxicity (Adeneye *et al*., 2006) [16]. The results of haematological study showed no significant change in both males and females when compared with control.

The biochemical parameters evaluation regarding liver function (AST, ALT) showed no significant difference between the control and AH treated groups. Plasma levels of AST and ALT are the first and foremost indicators in

assessing liver injuries (Hassanpour-Fard *et al*., 2015) [17]. The enzymes normally present in the cytosol and are leaked out into the blood stream, when the hepatocyte plasma membrane is damaged. Monitoring plasma level of total cholesterol is important in toxicological studies due to his direct link with devastating ailments like diabetes, hypertension and cardiovascular diseases (De Lima *et al*., 2017) [19]. Compared to the control group, AESA treating did not cause any significant changes in Cholesterol level.

The low dose (200 mg/kg) of *Secamone afzelii* induced a significant increasing of total protein in plasma concentration in Males rats. This result is similar to those Musa *et al*., 2017 [20] in which we observed in female rats a significant elevation of total protein. This result is an indication that the organs were not affected however this could be done by total protein synthesis.

Kidneys play pivotal role in excretion of metabolites and drugs, and regulating blood flow and many metabolic activities, hence they are highly susceptible to damage by drugs or herbs. (Hassanzadeh-Taheri *et al*., 2016; Baudoux et Nortier, 2017) [16, 22] One of the important functions of kidneys is electrolytes balance.

However, the maximum dose (800mg/kg) of *Secamone afzelii* caused significant elevation in plasma concentration of Creatinine only in males. Numerous conditions cause elevation of Cr and uric acid in the body. The main causes for elevated plasma Cr are higher synthesis and lower excretion due to renal dysfunction or both. (De Oliveira et Burini, 2012) [23]

The results of this investigation explain absence of acute toxicity of the aqueous extract of *Secamone afzelii*; however, subacute study showed that *Secamone afzelii* at the high dose (800 mg/kg) may cause renal dysfunction.

5. Conclusion

Acute and subacute assays suggested that *Secamone afzelii* has lower toxicity. However, in subacute study and highest dose, *Secamone afzelii* revealed some signs regarding renal dysfunction as well as Creatinine increasing effects. It could be concluded that despite the many beneficial effects of *Secamone afzelii*, we should not be unaware of its unwanted effects particularly in renal function.

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