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Study of the Acute Toxicity of an Aqueous Extract of the Leaves of *Senna alata* (L.) ROXB (Caesalpiniaceae) to Mammals

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Abstract | **Original Research Article**

Senna alata (L.) ROXB (Caesalpiniaceae) is a plant known and appreciated by the Ivorian population. It is commonly used to treat high blood pressure and constipation. The aim of this study was to investigate the acute toxicity of the aqueous extract of leaves of *Senna alata* leaves (EAqSa) in mammals. To do this, a phytochemical study of this extract was carried out. Acute toxicity was assessed by the intraperitoneal and oral routes. The oral toxicity tests were carried out in accordance with directive 423 of the Organisation for Economic Co-operation and Development (OECD) using three (3) lots of three (3) rats, one control lot and two (2) test lots. The control lot received 2 ml of distilled water, one test lot received 2000 mg/ kg bw of EAqSa and the second test lot received 5000 mg/ kg bw of the extract in a single dose. Toxicity tests by intraperitoneal administration are carried out using six (6) lots of six (6) mices including a control lot receiving 0.5 ml of EAqSa. The five (5) test lots received 0.5 ml of doses ranging from 750 to 2000 mg/kg bw of the extract. The animals from the different test lots were observed for twenty-four (24) hours. Qualitative phytochemical analysis revealed the presence of polyphenols, sterols and polyterpenes, flavonoids, catechic tannins, saponosides, quinonic compounds and alkaloids. Acute toxicity tests show that this extract is only slightly toxic by the intraperitoneal route and non-toxic by the oral route. The oral route of administration would be the most recommended for the pharmacological use of this extract, but should be used in moderation.

Keywords: *Senna alata*, acute toxicity, oral administration, intraperitoneal administration.

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INTRODUCTION

Senna alata (L.) ROXB (Caesalpiniaceae) is a plant found in intertropical Africa, especially in humid areas (Diallo, 2004). Several parts of the plant are reputed to be effective in treating certain diseases. The decoction of the leaves is prescribed as a drink for febrile attacks (Aké-Assi and Guinko, 1991). A bandage containing a mixture of fresh leaves and crushed root bark placed around the head treats headaches. The root decoction, mixed with salt or sugar, is used as a laxative. A light infusion or decoction of the leaves is used to treat hypertension (Adjanohoun *et al*., 2002). In Côte d'Ivoire, more than 1,421 species of medicinal plants have been identified for the treatment of various pathologies (Aké-Assi, 1991). Although Medicinal plants occupy an important place in the treatment of diseases, but they are often the cause of certain accidents due to self-medication and lack of knowledge of dosages. As the traditional use of plant extracts does not guarantee their safety (Ukwuani *et al.*, 2012), it is essential to assess their toxicity (Atsamo *et al.*, 2011). It therefore seems necessary to conduct scientific studies in order to control and understand the action of the various active compounds contained in these plants and their toxicity. The aim of this study is to enhance the value of Ivorian medicinal plants by studying the acute toxicity of the aqueous extract of the leaves of *Senna alata* while determining its main chemical compounds.

MATERIALS AND METHODS

Plant Material

The plant material consisted of leaves of *Senna alata* (L.) ROXB (Caesalpiniaceae) collected in Bonoua, south-east Côte d'Ivoire in August 2020. This plant was identified and authenticated at the National Centre of Floristics (CNF) of the Felix Houphouët-Boigny University (Abidjan, Côte d'Ivoire) under the number UCJ009126.

Animal Material

The animals used in our experiments were albino rats *Ratus norvegicus* (Muriadae) of the Wistar strain, aged eight (8) to twelve weeks, weighing between 120g and 160g and mice of the species *Mus musculus* (Muridae), of homogeneous *Swiss* parental strains, aged eight (8) weighing between 19g and 25g. The rats were used for the acute oral toxicity tests and the mice for the intraperitoneal tests. The animals were reared at room temperature. Animals were maintained on a 12:12 light/12:12 dark cycle, fed a standard chow diet, and given water ad libitum.

METHODS

Preparation of the Aqueous Extract of the Leaves of *Senna alata* (Caesalpiniaceae)

The fresh leaves were dried in the shade at room temperature. One hundred (100) g of crushed dry leaves were placed in two (2) litres of distilled water and boiled for fifteen (15) minutes. The decoctate was double filtered through cotton wool and Wattman paper (3mm). The filtrate is dried in a drying oven at 50°C for 72 hours. The aqueous extract of *Senna alata* leaves (EAqSa) is in powder form.

Qualitative Phytochemical Characterisation of the Aqueous Extract of the Leaves of *Senna alata*

This study was carried out at the Pharmacognosy Department of the Pharmaceutical and Biological Sciences Training and Research Unit of the Université Félix Houphouët Boigny in Abidjan. Qualitative phytochemical screening was carried out using a qualitative method described by Néné Bi *et al.*, (2008) and Abo (2013). It is based on specific chemical reactions that enable the different families of secondary metabolites of pharmacological and therapeutic interest to be identified.

Studies on the Acute Toxicity of the Aqueous Extract of the Leaves of *Senna alata*

Study of Acute Toxicity by Oral Administration

This study was carried out in accordance with OECD guideline 423 (OECD, 2001). This acute oral toxicity test was carried out in non-pregnant Wistar females aged between eight (8) and twelve (12) weeks and of uniform weight. Nine (9) animals were randomly divided into three (03) lots of three (03) rats each. The rats were fasted for eighteen (18) hours. The rats in lot 1 (Control) were given a distilled water solution at 10 ml/kg. Rats of lot 2 received 2000 mg/kg bw EAqSa. The extract was administered orally at a rate of two (2) mL of solution per animal. The observation time was forty-eight (48) hours. The rats were observed at thirty (30) minute intervals until the fourth (4^{th}) hour. After four (4) hours, the rats were fed water and food. Finally, the rats were observed once every twenty-four (24) hours until day fourteen (14^{th}). After 24 hours with no apparent signs of toxicity, a dose of 5000 mg/kg bw EAqSa was administered to test batch 3. The clinical signs of toxicity investigated were diarrhoea, lethargy, aggressiveness, excitability, somnolence and death.

Study of Acute Toxicity by the Intraperitoneal Route

In this study, the aqueous extract of *Senna alata* is dissolved in 9 ‰ NaCl and administered intraperitoneally to mice at single doses ranging from 750 to 2000 mg/kg bw EAqSa. The test is performed by initially injecting different doses of the test substance intraperitoneally into a number of batches of mice. Each mouse receives 0.5 ml of a single dose (evaluated in mg/kg body weight) of the substance. Mice in the control lot each also receive 0.5 ml of a 9 ‰ NaCl solution intraperitoneally. Mortality rates are determined after a 24-hour observation period. This step consists of determining two limit doses of the extract: those causing 0% and 100% mortality respectively. We will then make dilutions to determine intermediate doses.

Determination of the 50% Lethal Dose

The 50% lethal dose (LD_{50}) is the dose of substance causing the death of 50% of the mouse population studied. It is determined using a graphical method and a calculation method.

Graphical Method for Determination of the LD_{50}

The method of Miller and Tainter (1944) is the one used for the graphical determination of the LD_{50}. In this method, the percentages of dead mice are used to plot the mortality curve as a function of the logarithm of the concentrations of the product, expressed in mg/kg of body weight. The curve is obtained using the GraphPad Prism 8 programme (Microsoft, San Diego, USA).

Calculation Method for Determination of the LD_{50}

The calculation method of Dragstedt and Lang (1957) is also used to determine the $_{LD50}$. This method is based on the following assumption: Any animal that has survived a given dose of a substance administered to it would survive any lower dose of that substance. Similarly, any animal that succumbs to a given dose of a substance administered to it will also succumb to any higher dose. Thus, the percentage mortality (M%), for a given dose of the substance administered, is given by the number of dead specimens (Nm) at that dose, out of the number of dead specimens plus the number of survivors (Nv).

M % = Nm x 100 / Nm + Nv

Calculation of the LD_{50} using the Dragstedt and Lang method is based on extrapolation, i.e. finding the approximate value of the dose that corresponds to 50% mortality within an interval (X1-X2).

The formula is :

LD_{50} = [50(X2 –X1) + (X1Y2 – X2Y1)] / [Y2 –Y1]

- **X1** : lower dose for LD_{50} ;
- **X2** : upper limit of the LD_{50} ;
- **Y1** : percentage of mortality corresponding to X1 ;
- **Y2** : percentage of mortality corresponding to X2.

Treatment of Results

Graphical representations of the data were produced using Graph Pad Prism 8 software (San Diego, California, USA).

RESULTS

Qualitative Phytochemical Composition of the Aqueous Extract of the Leaves of *Senna alata*

The phytochemical screening revealed the presence of flavonoids, sterols and polyterpenes, catechic tannins, polyphenols, quinone compounds, saponosides and alkaloids in the aqueous extract of *Senna alata* leaves. Gallic tannins are absent (Table I).

Table I: Chemical composition of the aqueous extract of the leaves of *Senna alata*

Compounds researched		Tests or reagents	Results
Sterols and polyterpenes		Liebermann	+
Polyphenols		Ferric chloride	+
Flavonoids		Cyanidine	+
Saponosides		Vigorous agitation	+
Quinone compounds		Borntraegen	+
Alkaloids		Dragendorff	+
		Bouchardat	+
Tannins	catechics	Stiasny	+
	Gallic	Hydrochloric acid	-

(+): Presence of the compound **(-)**: Absence of the compound

Acute Toxicity of Aqueous Extract of the Leaves of *Senna alata* by Oral Administration in Rats

Administration of 2000 mg/kg bw EAqSa by gavage did not result in any behavioural changes in female rats. On the other hand, administration of a dose of 5000 mg/kg bw of EAqSa resulted in a decrease in motricity and grouping of the rats in one area of the cage. This phenomenon began one hour after administration of the extract to the rats and lasted approximately ten (10) minutes. No deaths were observed in any of the batches of mice after twenty-four (24) hours of observation.

Acute Toxicity of Aqueous Extract of the Leaves of *Senna alata* by Intraperitoneal Injection in Mice

Behaviour of Mice

In the first five (5) minutes, intraperitoneal injection of EAqSa at doses of between 750 and 2000 mg/kg bw caused the mice to move along the corners of the cage, stretching the trunk and rubbing the muzzle with the forepaws. The dose of 750 mg/kg bw does not influence the behaviour of the animals. At doses higher than 750 mg/kg bw, the animals moved around the cage dragging their hindquarters for 15 minutes after administration of the product. They did not eat or drink much. On the other hand, doses of 1,000 to 2,000 mg/kg bw caused jerky breathing and reduced motor skills in the mice in these batches. These animals remained huddled in a corner of the cage. The mice stopped feeding and showed signs of fatigue. Death occurred between 5 and 24 hours after intraperitoneal administration of EAqSa.

Injection of doses less than or equal to 750 mg/kg bw caused 0% mortality. However, a dose of 1000 mg/kg bw caused 33.33% mortality, 1250 mg/kg bw caused 50% mortality, 1500 mg/kg bw caused 66.66% mortality and a dose greater than or equal to 1750 mg/kg bw caused 100% mortality. The mortality rate of mice increases with the dose (Table II).

Table II: Percentage of mortality as a function of the dose of aqueous extract of the leaves of *Senna alata* administered intraperitoneally

Lots	Number of animals	Dose of EAqSa administered (mg /ml)	Number of deaths per lot	Mortality (%)	Mortality (unit probits)
1	6	750	0	0%	1.90
2	6	1000	2	33.33%	4.56
3	6	1250	3	50%	5.00
4	6	1500	4	66.66%	5.41
5	6	1800	6	100%	8.71
6	6	2000	6	100%	8.71

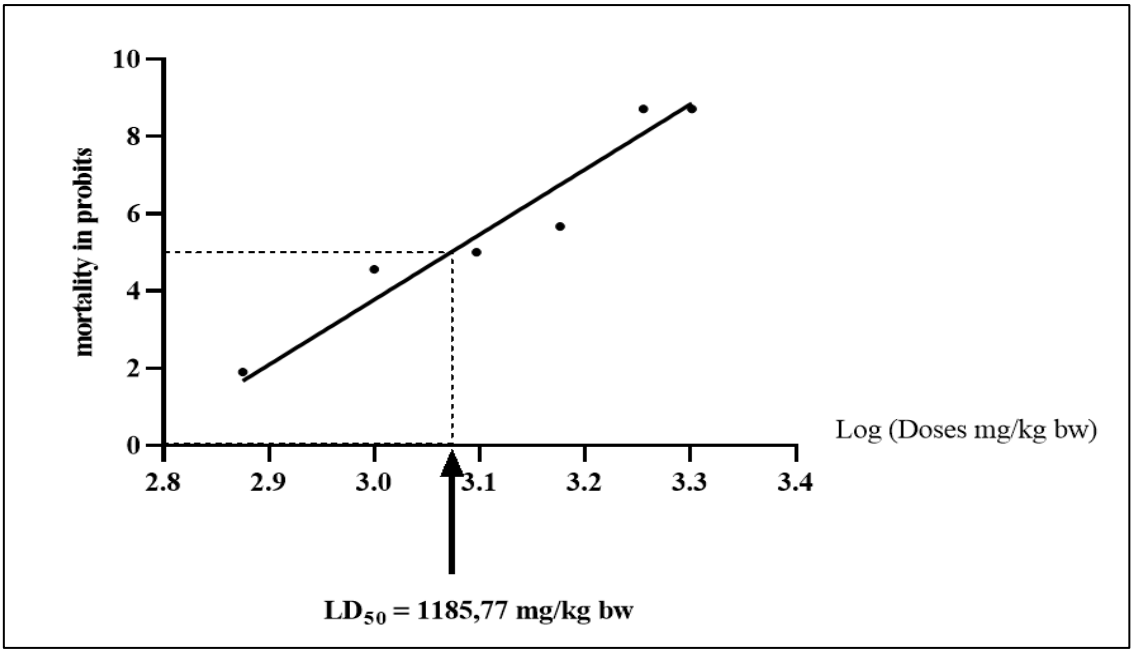

Figure: Curve of evolution of the mortality rate of mice in probits as a function of the dose of aqueous extract of the leaves of *Senna alata*

The 50% lethal dose (LD_{50}) of EAqSa determined on the linearized curve is 1185.77 mg/kg P.C

DISCUSSION

Phytochemical screening shows that the aqueous extract of the leaves of *Senna alata* contains flavonoids, sterols and polyterpenes, catechic tannins, polyphenols, quinone compounds, saponosides and alkaloids. These secondary metabolites are thought to be responsible for the potential pharmacological effects of EAqSa. Polyphenols, alkaloids, tannins and flavonoids are thought to have hypotensive properties (Prieto *et al*., 1998; Ojewole, 2005). The results of our phytochemical screening differ from those obtained by Khan *et al*., (2001), who found sterols, tannins, flavonoids, anthraquinones and saponosides in *Senna alata* (Caesalpiniaceae), but no polyphenols. This difference could be explained by a variation in the chemical composition of *Senna alata* depending on ecological factors (climatic and edaphic), i.e. the environment in which the plant was collected.

The study of the acute toxicity of EAqSa, by oral administration, carried out in accordance with OECD 423 guidelines (OECD, 2001), shows that this extract administered by gavage does not cause any death in rats for doses of up to 5000 mg/kg PC. According to the globally harmonised classification system of OECD, our extract falls into category 5 and is non-toxic by the oral route. Our results are similar to the acute and sub-acute toxicity studies carried out by Roy *et al.*, (2016), which showed that the ethanolic extract of *Senna alata* produced no toxic effects in male albino mice. An intraperitoneal toxicological study of EAqSa, using the Miller and Tainter (1944) calculation method, gave a LD_{50} of 1185.77 mg/kg body weight and a LD_{50} of 1250.075 ± 34.22 mg/kg body weight using the Dragstedt and Lang (1957) calculation method. These LD_{50} values are statistically fairly close, indicating that our results are consistent and that the methods used are credible. According to classification of Diezi (1989), a substance with a LD_{50} of between 500 mg/kg bw and 5000 mg/kg bw is classified as slightly toxic. According to this classification, EAqSa is slightly toxic by intraperitoneal injection. The acute intraperitoneal toxicity of our extract is similar to that of *Mitragyna inermis* (Rubiaceae) (Ouedraogo *et al.*, 2001) and *Justicia secunda* (Acanthaceae) (Abo, 2013), whose LD_{50} are 2250 ± 327 mg/kg bw and 810.74 mg/kg bw respectively. The aqueous extract of *Senna alata* would therefore be non-toxic by oral administration and only slightly toxic by intraperitoneal injection.

CONCLUSION

The aqueous extract of the leaves of *Senna alata* contains polyphenols, sterols and polyterpenes, flavonoids, catechic tannins, saponosides, quinonic compounds and alkaloids. Gall tannins are absent. The presence of these secondary metabolites is thought to be responsible for the pharmacological effects of EAqSa. The acute toxicity study of this aqueous extract indicates that EAqSa is slightly toxic by the intraperitoneal route and non-toxic by the oral route. Consequently, the oral route of administration would be the most suitable for its use in traditional medicine in the treatment of various pathologies, in moderation.

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