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Evaluation of the Acute in Vivo Toxicity of the Total Aqueous Extract of *Desmodium adscendens* Leaves with Hepatoprotective Potential

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Abstract | **Original Research Article**

This study aimed to evaluate the toxicological potential of the total aqueous extract of *Desmodium adscendens* leaves (TAEDa) in experimental rats to validate its traditional use in the treatment of liver disorders. Distilled water and TAEDa at doses of 2000, 4000, and 5000 mg/kg bw were administered to four different groups of rats, observed for 14 days to assess acute toxicity. After 14 days, no abnormal behavioral or physical signs were observed. At the end of this same period, the extract at doses of 4000 and 5000 mg/kg bw significantly increased the relative weight of the livers. Conversely, the relative weights of the kidneys, lungs, and heart decreased considerably after administration of the extract at the maximum dose of 5000 mg/kg bw. At the end of the experiment, biochemical analyses showed that transaminase and creatinine levels had increased, whil

Keywords: *Desmodium adscendens*, acute toxicity, extract, biochemical analyses.

1. INTRODUCTION

Toxicity is defined as the set of adverse effects, including morphological and functional damage in a living organism, caused by a substance introduced in a single, relatively high dose or in small, repeated doses over a long period (Nnanga *et al.,* 2020).

It remains a highly effective means of determining the harmful nature of a substance before proceeding to the regulatory stage. Toxicity is applied to several products of different kinds. Among these are medicinal plants, which possess significant therapeutic potential (Sone *et al.,* 2021). For the treatment of diseases, the World Health Organization (WHO) now recommends the use of plants whose efficacy and safety are guaranteed (WHO 2002). Plant reserves now constitute a vital basis for access to primary healthcare for populations in Africa, particularly in Côte d'Ivoire (Gurib-Fakim, 2006). However, despite the consistent use of plants in healthcare, very little information is available on their toxicological properties (Sone *et al.,* 2021). This is the case for Desmodium adscendens, a plant regularly used to treat hepatitis by populations in Côte d'Ivoire. Its anti-hepatitis effect has already been demonstrated by Dosso *et al.,* (2024). They showed remarkable results in its ability to decrease the concentrations of biochemical parameters, particularly transaminases, and to stabilize blood levels after hepatitis induction with paracetamol.

The present study aims to evaluate the acute toxicity of the total aqueous extract of *Desmodium adscendens* leaves in *Wistar* albino rats.

2. MATERIALS AND METHODS

2.1. Biological Material

2.1.1. Plant Material

The plant material consisted of *Desmodium adscendens* leaves harvested in Daloa (central-western Côte d'Ivoire) in March 2024.

2.1.2. Animal Material

The rats were obtained from the laboratory animal facility of the Animal Physiology Department at the Biosciences Training and Research Unit of the Félix Houphouët-Boigny University of Abidjan in Cocody. These animals were raised under standard conditions in the same facility.

2.2. Methods

2.2.1. Sampling, Identification, and Grinding of Plants

After harvesting, the fresh leaves were immediately transported to the laboratory where they underwent the following successive operations: sorting, washing with tap water, draining, and weighing. They were subsequently identified at the National Floristic Center in Cocody. The leaves of *Desmodium adscendens*, weighing 4.3 kg, were shade-dried for three weeks until a dry mass of 3.6 kg was obtained. They were then ground in a Moulinex grinder to obtain a fine powder. This powder was transferred to a glass container and stored away from air and moisture until the extraction stage.

2.2.2. Preparation of the aqueous extract of *Desmodium adscendens* leaves

One hundred grams (100 g) of leaf powder were decocted in 1 liter (1 L) of water. The decoction was stirred magnetically for 72 hours, then filtered, and the residue was rinsed with the same solvent (water). The filtrates were evaporated in an R-205D rotary steamer under reduced pressure. After evaporation, the extract was weighed, and the yield was calculated using the formula (Abebe *et al.,* 2014): Yield = (Mass of extract obtained / Mass of powder) x 100.

2.2.3. Animal experimentation

For this experimental study, non-pregnant female albino Wistar rats weighing an average of 155 ± 23 g were selected. These rats underwent a three (3) day acclimatization under standard animal facility conditions. Specifically, the animals were housed in cages containing wood shavings, provided with free access to water and food, and subjected to alternating 12 hours of light and 12 hours of darkness (Lien *et al.,* 2001; Sone *et al.,* 2021). The toxicity of the total aqueous extract of Desmodium adscendens leaves was assessed using a method derived from the OECD's line 425 dose adjustment method (OCDE, 2008). The animals were divided into four (4) groups of 6 animals each as follows:

Group 1, control receiving 1 ml of distilled water at a dose of 10 ml/kg body weight (bw)
Group 2, total aqueous extract of *Desmodium adscendens* (TAEDa) at a dose of 2000 mg/kg bw
Group 3, total aqueous extract of *Desmodium adscendens* (TAEDa) at a dose of 4000 mg/kg bw
Group 4, total aqueous extract of *Desmodium adscendens* (TAEDa) at a dose of 5000 mg/kg bw.

The extract was dissolved in distilled water using a homogenizer. Subsequently, after a 16-hour fast, the extract was administered by gavage in a single dose to the animals at doses of 2000, 4000, and 5000 mg/kg bw. After administration of the different doses of the extract, the animals were placed under observation to assess external signs of toxicity (diarrhea, fatigue, signs of pain, difficulty moving) as well as mortality between the first two hours and the first seven days. Body weight was recorded on the day of administration and every two days thereafter for a period of 14 days. After a 12-hour fast and on day 15, the animals were anesthetized by intraperitoneal injection of 2 ml of ether. Venous blood was collected from the retro-orbital sinus of each animal's eye using a sterile Pasteur pipette and transferred to heparin tubes. These tubes were capped and left to stand for 6 hours, then centrifuged at 3000 rpm for 15 minutes. The supernatant (serum) was collected and stored in labeled dry tubes at 4°C for biochemical analyses (transaminases, urea, creatinine) according to the method described by Meite *et al.,* (2017). The organs (kidneys, liver, and heart) were removed after dissection, cleaned in physiological saline (9% NaCl), dewatered, and weighed.

2.3. Statistical Analyses

Data were processed using Excel 2010 (USA) and GraphPad version 8 (GraphPad, San Diego, USA) for statistical analyses. Values were presented as mean ± standard error of the mean (SEM) in graphs. The Mann-Whitney U and Kruskal-Wallis nonparametric tests were used for intra- and inter-group comparisons. Ordered analysis of variance (ANOVA) and Duncan's post-hoc test were used to compare means between the different groups. The significance level was set at $p < 0.05$.

3. RESULTS

3.1. Yield

The yield obtained was 18.6% from 100 g of leaf powder.

3.2. Acute toxicity

After 14 days of observation, no deaths were observed in rats treated with the extract at different doses of 2000, 4000, and 5000 mg/kg body weight. The LD50 would therefore be greater than 5000 mg/kg.

3.2.1. Effects on Behavioral Parameters

The behavioral parameters assessed were pain and noise response, locomotion, social interaction, aggression, coat condition, and fecal status. Oral administration of single doses of 2000 mg/kg, 4000 mg/kg, and 5000 mg/kg of the total aqueous extract of *Desmodium adscendens* leaves did not show any significant changes in behavior or physical appearance. The animals appeared to be in good health. No major signs of toxicity, such as changes in pain and noise response, mortality, or fecal status, were observed (Table 1).

Table 1: Effects of the total aqueous extract of Desmodium adscendens leaves on behavioral parameters in rats over 2 hours on day 7 following administration. N: normal compared to the control; D: slightly decreased; A: none; P: not aggressive

Groups	Dose (mg / kg)	Pain response	Reaction to noise	Number of deaths	Locomotion	Social interaction	Aggressiveness	Coat condition	State of feces
Normal group	0	N	N	A	N	N	P	N	N
TAEDa	2 000	N	N	A	N	N	P	N	N
TAEDa	4 000	N	N	A	N	N	P	N	N
TAEDa	5 000	N	N	A	N	N	P	D	N

3.2.2. Effects on Relative Organ Weight

Table 2 shows the effect of the extract on the relative weight of organs. The following were observed:

- A significant increase in the relative weight of the liver in the groups of animals treated with the extract at doses of 4000 and 5000 mg/kg bw;
- A significant decrease in the relative weight of the kidneys, lungs, and heart in the animals treated with the extract at a dose of 5000 mg/kg bw.

Table 2: Effects of the total aqueous extract of *Desmodium adscendens* leaves on the relative weights of organs as a function of dose

Groups	Parameters			
	Liver	**Kidney**	**Lung**	**Heart**
Normal group	3.6 ± 0.1	0.7 ± 0.04	0.9 ± 0.11	0.4 ± 0.03
TAEDa - 2000	3.8 ± 0.3	0.7 ± 0.06	0.8 ± 0.07	0.4 ± 0.04
TAEDa - 4000	4.8 ± 0.4*	0.6 ± 0.05	0.8 ± 0.09	0.3 ± 0.02
TAEDa - 5000	5. ± 0.42*	0.4 ± 0.03*	0.6 ± 0.08*	0.2 ± 0.02*

3.2.3. Effects on Biochemical Parameters

Table 3 shows the variation in biochemical parameters according to the different groups. At doses of 2000 and 4000 mg/kg, the total aqueous extract did not cause any significant variation in these parameters. At the maximum dose of 5000 mg/kg, treatment with the extract resulted in a significant increase in transaminases (AST and ALT) and creatinine. Blood urea nitrogen (BUN) levels did not change significantly at this same dose.

Table 3: Effects of the total aqueous extract of *Desmodium adscendens* leaves on biochemical parameters according to the doses

Groups	Parameters			
	Uremia	**Creatininemia**	**ALT**	**AST**
Normal group	26.2 ± 4	3.4 ± 2	25.4 ± 4	76.5 ± 9
TAEDa - 2000	27.1 ± 5	4.3 ± 3	28.6 ± 6	80.1 ± 5
TAEDa - 4000	28.5 ± 4	5.5 ± 3	37.9 ± 4	89.4 ± 7
TAEDa - 5000	30.1 ± 6	7.1 ± 4*	58.2 ± 5*	158.6 ± 11*

4. DISCUSSION

Total aqueous extraction of Desmodium adscendens leaves (EATDa) showed a relatively high yield. This yield could be explained by the choice of distilled water as the solvent. This result contradicts the findings of Ndomou *et al.,* (2014).

Administering high doses did not visibly affect the behavioral and physical parameters of the rats. These results are consistent with those obtained by Boumba *et al.,* (2018) and Etamé *et al.,* (2017), who demonstrated similar effects of the wine extract of Carica papaya seeds at the same dose.

EATDa at doses of 4000 and 5000 mg/kg bw caused a significant increase in the relative weight of the livers compared to the livers of the control group rats. These results are consistent with those obtained in the studies by Nnanga *et al.,* (2020). This study also showed a significant decrease in the relative weights of the heart, kidneys, and lungs at a dose of 5000 mg/kg bw. These results are consistent with those of Sone *et al.,* (2021), who made the same observations in their studies.

A significant increase in transaminases (ALT and AST) and creatine was also noted at the maximum dose of 5000 mg/kg bw compared to the control group.

This increase in transaminases would indicate hepatocyte damage, according to Shittu *et al.,* (2015). According to Lawal *et al.,* (2015), this increase in serum transaminases without signs of pathology could be explained either by autoregulation of these cells or by the short observation period of 14 days.

The results obtained from the toxicological study indicate that the LD50 of the EATDa extract is greater than 5000 mg/kg body weight. According to the OECD's 2008 Globally Harmonised Classification System, the extract can be classified as category 5 and considered non-toxic by oral administration. Studies by Sérika *et al.,* (2019) showed a high presence of polyphenols, flavonoids, and reducing sugars. The effects observed with the extract are likely due to the presence of these chemical compounds.

4. CONCLUSION

This work has shown that the total aqueous extract is non-toxic by oral administration at a dose of 5000 mg/kg or less over a 14-day observation period. It would therefore be beneficial to develop an improved traditional medicine based on this extract and to consider studies on its mechanisms of action to confirm these pharmacological effects.

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Competing interests

Authors have declared that no competing interests exist.

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