World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

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Study of acute and subacute toxicity of the aqueous extract of the rhizomes of *Anchomanes difformis* Blum Engl. (Araceae) in rats

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World Journal of Biology Pharmacy and Health Sciences, 2023, 14(01), 001–008

Publication history: Received on 07 February 2023; revised on 10 March 2023; accepted on 13 March 2023

Article DOI: https://doi.org/10.30574/wjbphs.2023.14.1.0039

Abstract

The aim of the study was to evaluate the acute and subacute toxicity of *Anchomanes difformis* rhizomes extract. The qualitative and quantitative chemical analyzes of the phenolic compounds from *Anchomanes difformis* rhizomes aqueous extract. were carried out by CCM and spectrophotometry. The acute toxicity study was conducted on female swiss mice at doses of 2000, 3500 and 5000 mg / kg per os. The subacute toxicity study was carried out in Wistar rats quatidianly treated with 400 mg / kg per os of the aqueous extract for 28 days. Control lots received 10 ml / kg. Chemical analyzes revealed the presence of polyphenols and flavonoids. The acute toxicity study of the aqueous extract did not result in any deaths. The LD50 is greater than 5000 mg / kg. The subacute toxicity study showed weight gain in rats and various organs: uterus, heart, kidneys, liver, and lungs. On biochemical parameters, aqueous extract significantly increase Creat, Asat, Alat levels, but not Gly and Chol. In hematological parameters *Anchomanes difformis* aqueous extract caused increase in WBCs, IDR-CD, PCT and decrease in PLT, GMV, IDR-DS.

Keywords: Acute; Subacute; Toxicity; hematological; biochemical; *Anchomanes difformis*; Rat

1. Introduction

Anchomanes difformis is a plant used in traditional Congolese medicine and well well known by traditional therapisst (Makemba et al., 2017). Many people believe that natural and traditional products, they are safe, easier to collect or buy from traditional herbalists and healers, either in their raw forms; or in the form of traditionals preparations (Aouadhi et al., 2010). Several toxicity case are associated with the use of plants (Fu et al., 2008). Some of plant treatments cause side effects. Like all medicines, medicinal plants should be used with caution (Ybert et al., 2007). Plants are a valuable source of natural drugs, as they are less toxic, and nearly 80 % of people in developing countries use them to treat several diseases (Sangaré, 2003).The objective of this study is to evaluate the acute and subacute toxicity of *Anchomanes difformis* aqueous extract.

2. Materials and methods

Plants material: The plant material is essentially rhizomes of *Anchomanes difformis* wich was collected at Makana village (Pool Department) under the supervision of Bouesso Henriette(health tradipractitioner) who knows the village and

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the plant. The sample was dried at room temperature (25°C) and reduced to powder in the laboratory of Physiology and Experimental Physiopathology of the Faculty of Sciences and Technologies of Marien Ngouabi University (Brazzaville- Congo).

Animal material: Wistar rats of three to four months old with average weight 172 g, and female swiss mice aged 3 months with average weight 23 g raised in a well-lit room, under standard conditions (± 25.12h ligh / dark cycle) in the laboratory of Physiology and Experimental Physiopathology of the Faculty of Sciences and Technologies. They were regularly fed with standard food.

Preparation of the extract: 40 g of *Anchomanes difformis* rhizomes powder is placed in 400 mL of distilled water and heated for 30 min at 100°C in a flask heater then filtered. The obtained filtrate is concentrated in an oven at a temperature of 60 °C. The dry residues obtained are kept in labeled and hermetically sealed glass vials to avoid any possible denaturation of the extract.

2.1. Determination of polyphenols and flavonoids

2.1.1. Total Polyphenols

0.1mL of plant extract, 0.9 mL of Folin ciolalteu and 0.2 mL of Na2CO3 (20 %) are mixed. The mixture will be incubated in the dark for about 40 min and raiding is taken at 725 nm with a spectrophotometer.

2.1.2. Total Flavonoids

250 µL of extract + 1mL of distilled water is mixed at time t = 0 min, 75µL of a NaNO2 solution (5%), after 5min 75 µL of Al2Cl3 (10%), 6min 500 µL of NaOH (1N) and 2.5 mL of distilled water are added the spectrophotometer reading was taken at 510 nm.

2.2. Acute toxicity in mice

The acute toxicity of aqueous extract of the rhizomes of *Anchomanes difformis* was carried out according to the OECD 2008a method, in swiss mice for 14 days. Twenty (20) female mice were divided into four groups of 4 mice each. The mice of groups 1, 2, 3 and 4 respectively received 0.5 mL of distilled water / 100 g for the control; 2000, 3500 and 5000 mg / kg of the extract for the test groups. The general condition of the animals (mobility, somnolence, piloerection, ptosis, sensitivity and mortality) was observed every 30 minutes for 6 hours and the weight evolution was noted. The mortality rate was determined after 72h (Boumba et al., 2018; N'goka et al., 2019).

2.3. Subacute toxicity

To assess the tolerance of the rhizomes extract of *Anchomanes difformis* for long- term use, a subacute toxicity study was conducted according to OECD Test Guideline 407 (OECD, 2008b). It was conducted on wistar albinos rats divided into two (2) groups as follows: group 1, receiving 10 mL / kg body weight of distilled water (control); group 2 receiving 400 mg / kg body weight of *Anchomanes difformis* aqueous extract. The treatment was given daily for 28 days. Rats were fed and hydrated and weighed every 2 days. Prior to treatment, the rats were fasted for 24h before the experiment.

2.4. Effect of aqueous extracts on organ weight

At the end of 28 days of treatment with the aqueous extract of rhizomes of *Anchomanes difformis* and before dissection, the rats of each batch were anaesthetized with ethyl ether. The different organs: kidneys, lungs, uterus, liver and heart were carefully removed and weighted with a precision balance. Then a macroscopic observation of the color and size of these organs was made.

2.5. Effect of aqueous extracts of *Anchomanes difformis* on biochemical and hematological parameters

After 28 days of treatment, the animals were anaesthetized with ethyl ether. For each animal, about 2 mL of blood was collect from the ophthalmic vein in two types of tubes: EDTA tubes for analysis of hematological parameters and dry "Vacutest" tubes for analysis for biochemical parameters. The latter were centrifuged at 4000 rpm for thirty (30) minutes using a centrifuge. The plasma obtained was collected and stored in Eppendoff tubes before performing the biochemical analyses.

2.6. Determination of biochemical parameters

The biochemical parameters were measured at the National Public Health Laboratory in Brazzaville using a Technicont RA- 1000. The parameters assayed are: Alamine amino transfers (ALAT), Aspartate amino transfers (ASAT), Total cholesterol (Chol- total), Creatinine (CRE), Glycemia (GLY), (Nsonde Ntandou et al., 2015).

2.7. Determination of hematological parameters

Hematological parameters were measured at the Public Health Laboratory in Brazzaville. The blood count formula is composed of Red blood cells (RBC), with blood cells (WBC), Hemoglobin (HGB), Hematocrit (HCT), Mean Corpuscular Hemoglobin Concentration (MCHC) and Platelets (PLT). The determination of each hematological parameter was done according to conventional methods (Peneme, 2017; Nkoua, 2018).

2.8. Statistical analysis

The results obtained are expressed as mean ± DSM for n=4; n=5 per group, using Microsoft Excel Windows 13 software. The results obtained in the test groups were compared with the negative control group using Student's t test. The significance level being set at $p < 0.05$.

3. Results

3.1. Determination of polyphenols and flavonoids

The results of quantitative spectrophotometric analysis of hydroethanolic extract of *Anchomanes difformis* rhizomes (Figure 1) showed the extract to contain quantitatively total polyphenols and flavonoids.

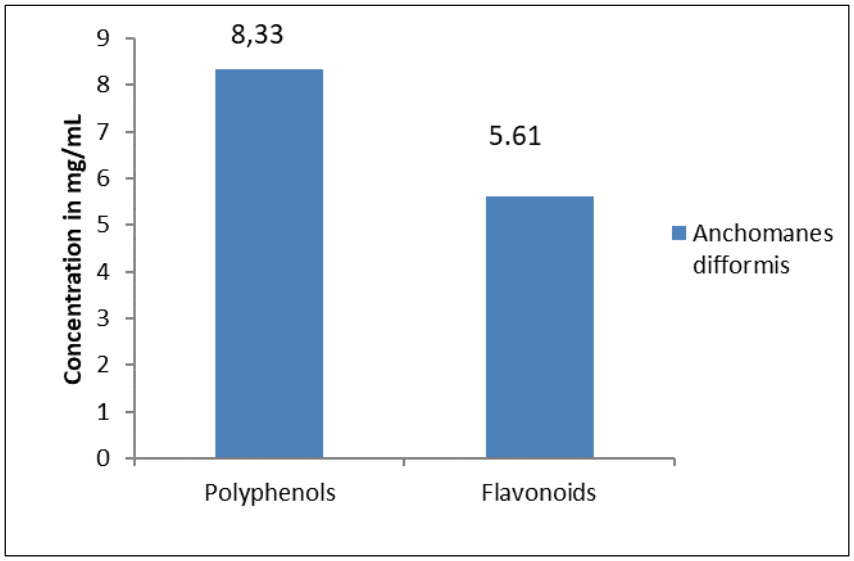

Figure 1 Total polyphenol and flavonoid content of aqueous extract of *Anchomanes difformis* rhizome

3.2. Toxicity study

3.2.1. Acute toxicity

Administration of aqueous extract of the rhizomes of *Anchomanes difformis* at dose of 2000, 3500 and 5000 mg/kg *per os* in mice did not alter the general condition and behavior of animals as control mice. (Table 1). No mortality was observed up to 72h. The LD_{50} is there for greater than 5000 mg/kg.

The aqueous extract of *Anchomanes difformis* caused an increase in the weight of the animals at these dose levels compared to the negative control (Figure 2). This extract did not cause any mortality.

Table 1 Effect of aqueous extract of rhizomes of *Anchomanes difformis* on behavior in mice

Parameters	Treatments			
	Control (0.5mL/100g)	**2000 mg / kg**	**3500 mg/ kg**	**5000 mg / kg**
Number of animals	4	4	4	4
Drowsiness	N	N	N	N
Vomiting	N	N	N	N
Ptosis	N	N	N	N
Pilo erection	N	N	N	N
Mortality	0	0	0	0

N= normal; 0= no mortality

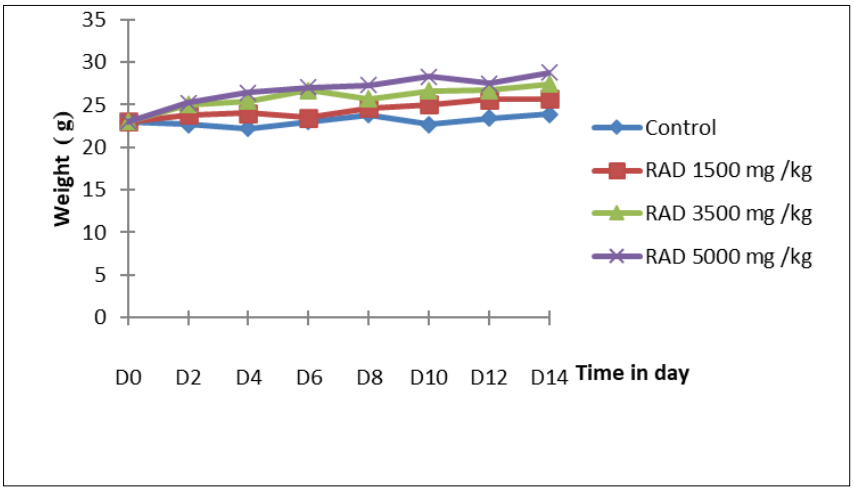

Figure 2 Effect aqueous extract of *Anchomanes difformis* on weight development in mice

Results express as mean ± DSM ; RAD: aqueous extract of *Anchomanes difformis* rhizomes.

3.2.2. Subacute toxicity

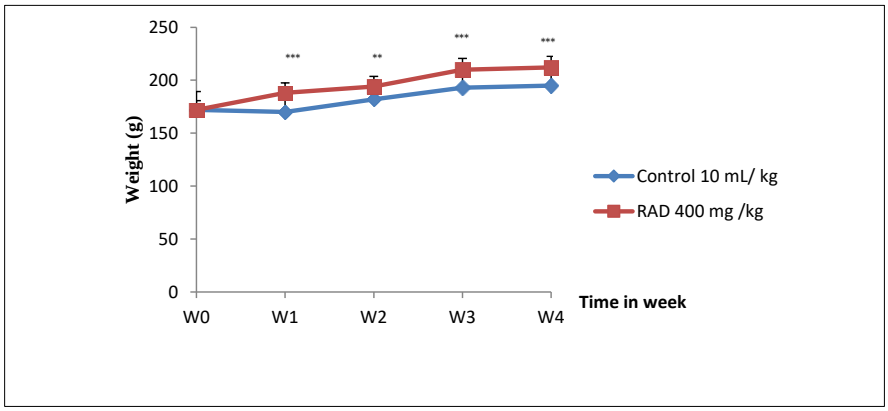

Figure 3 Effect of aqueous extract of rhizomes of *Anchomanes difformis* on weight evolution.

Results expressed as mean ± SD, n= 5; ***P< 0.001; **P< 0.01 ; RAD: aqueous extract of *Anchomanes difformis* rhizomes.

Daily administration of *Anchomanes difformis* extract at dose of 400 mg/kg for 28 days did not cause death in the rats. The general condition and behavior of animals were not altered compared to control rat.

The weight changes of rats during the 28 days of the subacute toxicity study are presented in figure 3. Rats treated with aqueous extract of *Anchomanes difformis* rhizomes showed a weight gain over the control group.

Table 2 shows the relative masses of the organs removed at the end of 28 days study. From this table, it can be seen that the extract causes significant variations in the masses of the organs. But it was found that organs such as: liver, lungs increased significantly in mass under the effect of aqueous extract of rhizomes of *Anchomanes difformis* compared to control rats. Macroscopic observation of these organs in treated rats showed variations in color compared to controls. The *Anchomanes difformis* treated rats showed light red colored organs as compared to those treated with distilled water.

Table 2 Effect of aqueous extracts of *Anchomanes difformis* on the weight evolution of organs at D_{28}

Organs(g)	**Treatments**	
	Control	**400 mg kg (RAD)**
Uterus	1.37 ± 0.12	1.62 ± 0.1*
Heart	0.57 ± 0.52	0.81 ± 0.59*
Right kidney	0.45 ± 0.44	0.64 ± 0.12*
Left kidney	0.44 ± 0.8	0.61 ± 0.72*
Liver	5.19 ± 0.41	6.12 ± 0.28*
Lung	1.06 ± 0.23	2.01 ± 0.52*

Results expressed as mean ± SD, n=5; *P<0.05.

Table 3 shows a decrease in blood glucose levels following administration of the aqueous extract of *Anchomanes diffomis* rhizomes. The aqueous extract of *Anchomanes difformis* rhizomes, showed significant decrease in decrease in creatinine (Create), Aspartame amino transfers (Asat), and Amine amino transfers (Alat). However we found a no significant difference in blood glucose (Gly) and cholesterol (Chol) compared to controls.

Table 3 Effect of aqueous extract of *Anchomanes difformis* rhizomes on biochemical parameters at D_{28}

Biochemical parameters	**Treatments**	
	Controls (10mL/ kg)	**400 mg kg (RAD)**
Creat (g/l)	11.70 ± 1.42	4.11 ± 1.60**
Asat (ui/l)	173.33 ± 1.38	120.39 ± 0.55*
Alat (ui/l)	103.48 ± 0.81	49.22 ± 0.71***
Gly (g/l)	1.08 ± 0.53	0.95 ± 0.07^{ns}
Chol (ui/l)	500.44 ± 0.06	477.72 ± 1.42^{ns}

Results expressed as mean ± SD, n=5; *P< 0.05, **P< 0.01, ***P< 0.001; ns: not significant

The aqueous extract of *Anchomanes difformis* rhizomes causes the increase in with blood cells (WBC), red blood cells (RBC), platelets (PLA), IDR-CV, PCT and a decrease in mean corpuscular volume (MCV), IDR-DS (Table 4). The changes in hemoglobilin (HB), mean corpuscular hemoglobilin concentration (MCHC), mean platelet volume (VPM), Hematocrit (HT) were no significant compared to the control lot.

Table 4 Effect of aqueous extracts of *Anchomanes difformis* rhizomes on hematological parameters at D_{28}

Hematological Parameters	**Treatments**	
	Distilled water (10mL/ kg)	**RAD (400 m g kg)**
GB (uL)	$6.05.10^3 \pm 0.55$	$11.55.103 \pm 1.62^{**}$
GR (uL)	$4.41.10^6 \pm 0.51$	$7.45.106 \pm 0.41^{*}$
PLAT (uL)	$366.10^3 \pm 1.06$	$326.51.103 \pm 1.20^{*}$
HB (g/dl)	12.6 ± 0.07	12.7 ± 0.43^{ns}
CCMH (g/dl)	33.6 ± 0.28	34.4 ± 0.56^{ns}
VGM (fL)	83.15 ± 0.35	**$49.6 \pm 0.28^{*}$**
IDR-DS (fL)	37.6 ± 1.62	**$28.8 \pm 1.67^{*}$**
VPM (fL)	8.8 ± 0.35	8.75 ± 0.35^{ns}
TCMH (pg)	28.25 ± 0.41	17 ± 0.42^{ns}
IDP	15.85 ± 0.63	14.45 ± 0.63^{ns}
HCT (%)	37.88 ± 1.40	36.9 ± 1.40^{ns}
IDR-CV (%)	12.9 ± 0.46	**$16.95 \pm 0.49^{*}$**
PCT (%)	0.156 ± 0.21	**$0.288 \pm 0.21^{*}$**

Results expressed as mean ± SD; n=5, *P< 0.05; **P< 0.01.

4. Discussion

The aqueous extract of *Anchomanes difformis* revealed the considerable presence of polyphenols and flavonoids. These results are in agreement with those of Oluwasesan et al, (2019) who found the same chemical groups with *Anchomanes difformis* oil.

In the acute toxicity study of *Anchomanes difformis* in mice, no mortality was observed. We observed a non-significant increase in weight in the animals.

These results are similar to those obtained by Oghale Ovuakporie and al, (2015) who also observed the absence of toxicity of this plant, working on the aqueous extract of the leaves of *Anchomanes difformis*.

The subacute toxicity study was carried out for 28 days, the dose of 400 mg/kg was retained because it is therapeutically effective and less than one tenth of the LD50 (Makemba –Nkounkou et al., 2017; Nsonde Ntandou et al., 2017).

Compared to control group, the significant increase in weight of rats after administration of aqueous extract of *Anchomanes difformis* was observed from de second week to the last week. The aqueous extract of *Anchomanes difformis* rhizomes are said to contain steroidal substances Djiguibet, (2009); Makemba and al, (2017); Nsonde ntandou and al, (2017), which could be responsible for the increase in weight of the rats compared to controls. The aqueous extract of *Anchomanes difformis* rhizomes at a dose of 400 mg/kg significantly increases the weight of the liver, lungs, uterus and kidney.

This increase in organ weight is an indicator of the anabolic effects of the chemicals consumed by the animal (Djiguibet, 2009). The change in organ weight is an indication of toxicity after exposure to a toxic substance (Raza and al., 2002; Otmani and al., 2016). These aforementioned organs showed a light red coloration for those treated with aqueous extract of *Anchomanes difformis* rhizomes.

The increase in liver weight may be related to congestion by reservation of blood in the liver Rasekh and al, (2008); Otmani and al, (2016). These findings could be attributed to histopathological changes in rats characterized by congestion, tubular necrosis and renal abscesses.

These abnormalities in liver and kidneys tissues could be explained by the presence of the chemicals present in aqueous extracts of *Anchomanes difformis* rhizomes administered in rats (Badianga, 2011; Seiba, 2012). These chemicals are likely to increase kidney weight and cause hyperplasia of the stomach, when administered with the extract (Eklund, 1975).

The aqueous extract of *Anchomanes difformis* rhizomes does not significantly reduce glycaemia compared with control rats.These results corroborate those of Ovuakporie et al, (2015) who also did not find an effect on glucose in the same conditions. However, they disagree with those obtained by Adeyeni et al, (2015), on the subacute toxicity of the ethanolic extract of *Anchomanes difformis* which showed a significant decrease in blood sugar. This difference could be explained by the difference in nature and in quantity of the chemical groups which constitute these two types of extracts because of the difference in solubility between water and ethanol. This difference may also be due to the influence of the environment on the nature of the samples used.

The aqueous extract of *Anchomanes difformis* also promoted significant decrease in creat, Alat. due to the presence of its secondary metabolites.

The hematological parameters of the aqueous extract of *Anchomanes difformis* rhizomes showed significant increase in white blood cells (WBC) and significant decrease in blood platelets (BP) compared to the controls. These results agree with the work of Egwurugwu and al, (2017) who worked on the hydroethalic extract of *Anchomanes difformis* rhizomes of Nigerian sample. Ho ever, they found an increase in blood platelet count (LTP). The aqueous extract of *Anchomanes difformis* promoted a significant increase in red blood cells (RBCs), IDR- CD, PCT compared to controls. The significant decrease in mean corpuscular volume (MCV), IDR-DS in animals treated with aqueous extract of *Anchomanes difformis* rhizomes as compared to the control lot. This decrease could be attributed to the hemolysin and saponoids present in this extract (Yaldasheva and al.; 2005; Makemba and al., 2017). This reduction could be attributed to the presence of tannins in the extract; the latter having inhibitory effects on blood cell synthesis or hematopoiesis (Boussahel, 2011).

5. Conclusion

Qualitative spectrophotometric analyzed of *Anchomanes difformis* a revealed the presence of total polyphenols and flavonoids.

The experimental study of acute toxicity of aqueous extract of *Anchomanes difformis* did not cause any mortality. *Anchomanes difformis* is no toxic plant and the DL_{50} is greater than 5000 mg/ kg.

Subacute toxicity of aqueous extract of *Anchomanes difformis* rhizomes in rats for 28 days revealed the toxic indices, color change and increase in organ weight. The aqueous extract of *Anchomanes difformis* rhizomes influenced the biochemical and hematological parameters.

Compliance with ethical standards

Acknowledgments

The authors are grateful to Madame Bouesso Henriette(health tradipractitioner) for plant collection and to Professor Jean-Marie MOUTSAMBOTE for botanical identification of plant. We are also grateful administrative authorities of Faculty of Sciences and Technologies and National Institute for Research in Health Sciences (IRSSA), for availing their facilities and time for some aspects of this study.

Disclosure of conflict of interest

There is no conflict of interest between the authors involved in this work.

Statement of ethical approval

The ethical rules published by International Association for the Study of Pain (Zimmermann, 1983) were respected.

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