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Acute and sub-chronic toxicity study of *Artabotrys aurantiacus* Engl (Annonaceae) leaves aqueous extract in rat

Patience Emambo[1], Marie Claire Tchamadeu[1*], Phillipe Belle Ebanda[1], Calvin Bogning Zangeu[1], Christian Tenezogang Takoukam[1], Modeste Nya Wankeu[1], Alain Bertrand Dongmo[1], Siméon Pierre Choukem[2]

[1]Laboratory of Biology and Physiology of Animal Organisms, Faculty of Sciences of Douala University, Po Box: 24157, Douala, Cameroon

[2]Department of Internal Medicine and Pediatrics, Faculty of Health Sciences, University of Buea, Cameroon

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Abstract

The widespread use of *Artabotrys aurantiacus* in traditional medicine for the quest for well-being aroused attention for its potential risk to human and animal health. This study points out the acute and sub-chronic toxicological profile of *Artabotrys aurantiacus* leaves aqueous extract. The extract was prepared following the traditional healer's method and orally administered to Wistar rats at a single dose of 5000 mg/kg or 50, 100, 200 and 400 mg/kg/day for 45 days for acute and sub-chronic tests, respectively. In acute toxicity, no behavioral disorder was observed in treated animals as compared to the control. The lethal dose of 50 was estimated to be higher than 5000 mg/kg and the extract was noted as slightly toxic. Similarly, no significant change in body weight nor biochemical parameters were observed after 45 days of repeated dosing of the extract. Histological study revealed that all tested doses induced no damage to the liver and kidney as compared to control. These findings indicate that the use of *Artabotrys aurantiacus* leaves aqueous extract at tested doses is not associated with any toxic effect.

* **Corresponding Author:** Marie Claire Tcthamadeu Tchamadeu ✉ marieclaire_tchamadeu@yahoo.fr

Introduction

Plants-based medicines have been the primary health care resource of indigenous people for centuries, many being documented and scientifically validated (Li *et al*., 2010). Several reports have shown that more than 50% of current orthodox drugs are derived from plant resources (Ashafa *et al.,* 2012; Madingou *et al.,* 2015). Medicinal plants were continuous draw increasing interest as they are believed to be natural, beneficial, cost-effective, worldwide available and accessible (Ojewumi & Kadiri, 2013; Neeta *et al.,* 2015). However, the folkloric usage and concerns about the potential risk for humans and animals' health indicate the need to establish their safety profile. *Artabotrys aurantiacus* Engl. Diels is a medicinal plant belonging to the Annonaceae family. It is an evergreen shrub from the genus *Artabotrys* consisting of 2,200 species. Members of this family are geographically distributed in tropical and subtropical regions in East Asia and Africa (Grover *et al*., 2002; Hashin *et al*., 2014). *Artabotrys aurantiacus* has been widely used in traditional medicine for several decades, particularly in Africa.

Various parts of the plant, including stem bark, leaves and roots, are still locally used for the management of various pathological conditions such as diabetes (Bruno, 2013). *Artabotrys* species are also employed in the manufacture of perfume due to the fragrance of the flowers, which aroma is used as flavor agents for making tea-like beverages (Seidemann, 2005; Georges *et al.,* 2011; Mishra *et al.,* 2008). Furthermore, both leaves and fruits are used as animal feed, predominantly for cattle, chimpanzees and goats (Moore, 1994; Aguilar, 2001; Marble, 2012). Pharmacological studies highlighted their cardiovascular, system-depressant, anti-fertility, anti-cancer, anti-parasitic and anti-diabetic activities (Trivedi *et al*., 1971; Cortes *et al*., 1990; Garg and Siddiqui, 1998; Murphy *et al*., 2008; Pilay *et al*., 2008; Johri *et al*., 2009; Mokoka *et al*., 2011). Knowing that medicinal plants extracts might also cause harmful effects on the body (Chanda *et al.,* 2015; Poualeu *et al.,* 2016), the present study was undertaken to evaluate the acute and sub-chronic toxicity profile of *Artabotrys aurantiacus* leaves aqueous extract in rats thereby, establishing safety limit of its usage.

Material and methods

Plant material

The fresh leaves of *Artabotrys aurantiacus* (Cameroon National Herbarium voucher specimen N°62379HNC) were collected at Obak, Center region Cameroon, in March 2020 and dried in a shadow room at ambient temperature for four weeks.

Preparation of the aqueous extract

The dried leaves were reduced into powder using the crushing machine. Four hundred grams (400g) of powdered leaves were introduced into the pot containing 5 L distilled water and heated until boiling for 30 min. After cooling at room temperature (24°C±2°C), the mixture was filtrated using Whatman filter papers n°3. The resulting solution was introduced into Petri dishes and left in an oven for 72h at 45 °C until complete evaporation of the solvent. The obtained crude extract (36.7 g) was used as *Artabotrys aurantiacus* leaves aqueous extract (AAAE) throughout the study, being stored at 4°C.

Chemicals and reagents

All chemicals used in this study were of analytical grade. Ketamine and diazepam were procured from Sigma Aldrich Co Ltd (Darmstadt Germany). Biochemical kits were purchased from SGM Labo (Roma, Italia) and used without any further purification.

Experimental animals and ethical considerations

Adult male and non-pregnant female Wistar rats of 56-70 days-old, weighing 150-170g, were procured from the animal breeding facility of the Faculty of Science, University of Douala. They were housed in a standard polypropylene cage at room temperature (24°C±2°C) and relative humidity under light and dark cycle (from 6 am to 6 pm). They had free access to a standard diet and tap water *ad libitum* throughout the study. The experimental procedure was in strict compliance with the approved protocol

by the Institutional Ethical Committee of the University of Douala (protocol approval number 3021 CEI-UDo/04/2022/T).

Assessment of acute oral toxicity

This study was carried out following the Organization for Economic Cooperation and Development guideline for testing of chemical N°423 (OECD, 2001). Briefly, six overnight fasted female rats were randomly divided into 2 groups of 3 animals each. One group, named the control group, received distilled water 10 mL/kg (*p.o*) and the other group, named the treated group, received AAAE at a single dose of 5000 mg/kg (*p.o*). After dosing, animals were closely observed for the first 30 minutes and then intermittently 4 hours to detect any change in the behavioral, neurological or autonomic profile that included tremor, twitches, respiration, sleeps, mobility and mortality. Further, animals were observed once every 12 hours for the next 14 days, being weighed on day 1, 7 and 14. At the end of the study, rats were sacrificed by cervical dislocation and vital organs (liver, lung, spleen, heart and kidney) were excised, weighed and macroscopically analyzed.

Sub-chronic toxicity evaluation

The experiment was performed following the modified OECD guideline for the testing of chemicals, line N°407 (OECD, 2008). Briefly, sixty rats of both sexes were randomly divided into 6 groups of 10 animals each (5 males and 5 females) as follows:

Group I: control group, receiving distilled water 10 mL/kg (*p.o*);
Group II, III, IV and V: treated groups, receiving AAAE at the doses (50,100,200 and 400 mg/kg/day), respectively (*p.o*);
Group VI: satellite group, receiving AAAE at the dose of 400 mg/kg/day (*p.o*);

Mortality, behavior signs (including change in the skin, fur, eyes, mucus membranes, the occurrence of secretions and excretions, autonomic activity), body weight, and food and water intake were recorded weekly throughout the experimental period. On the 46th day, animals were anesthetized using ketamine (50 mg/kg) and diazepam (10 mg/kg) and then sacrificed. Part of the blood was collected into an EDTA-containing tube for hematological analysis. Another part collected into a plain tube was centrifuged at 1500 rpm for 10 minutes and the supernatant was stored at -20°C for further biochemical parameters determination. Some organs including liver, lung, spleen, heart, kidney, aorta, thymus, ovary, epididymis and testis, were rapidly identified, dissected out and weighted. The liver and kidney were fixed in a freshly prepared 10 % formalin buffer for histological analysis. The satellite group was observed for an extra period of 14 days without receiving any extract, then sacrificed and treated as described for other groups.

Hematological analysis

The freshly collected blood sample into EDTA tubes was subjected to hematological analysis using an automated hematology analyzer (Nihon Kohden, MEK6411K). The counting principle is based on impedance variation and flow cytometry to determine the size, type and quantity of blood cells.

The cells naturally emit signals which are analyzed by the computer linked to the cytometer, making it possible to establish the leukocyte formula by giving the percentages of the different types of leukocytes, monocytes, granulocytes, lymphocytes, the number of red blood cells, hemoglobin amount, hematocrit, blood platelet count, mean corpuscular volume, mean corpuscular concentration and mean corpuscular hemoglobin level. For each sample, the results were printed and interpreted.

Biochemical analysis

All biochemical parameters including total protein, creatinine, alanine amino-transferase (ALAT), aspartate amino-transferase (ASAT), total bilirubin, alkaline phosphatase, triglyceride, total-cholesterol HDL-cholesterol and LDL-cholesterol were determined by using a spectrophotometer (GENESYS 10S UV-Vis, Madison WI 53711, USA). Serum samples were bleeding with a specific biochemical kit and proceeded as indicated by the manufacturer.

Histological analysis

The procedure described by Tarabishy *et al.* (2008), was followed for histological analysis. Briefly, sections of the liver and kidney previously fixed in formalin buffer were embedded in paraffin. 5µm of each organ was cut (Microtome, Reichert-Jung 2030) and stained with hematoxylin and eosin (H-E).

The preparation was examined under a light microscope (Olympus brand light microscope, Leitz wetzlar Germany 513) for possible alteration as compared to normal structures.

Statistical analysis

Results are expressed as mean ± SEM. The difference between the treated groups and the control group was compared using a one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. The analysis was performed using PRISMA Software (Graph Pad Software, Inc., San Diego, CA, version 5.03). P values less than 0.05 were considered significant.

Results

Effect of acute administration of Artabotrys aurantiacus aqueous extract in rats

Effect of a single dose of *Artabotrys aurantiacus* aqueous extract on animal behavior and mortality: As shown in Table 1, oral administration of AAAE at a single dose of 5000 mg/kg induced no significant change in rat behavior as compared to the control group. Also, no death was recorded and the Lethal Dose 50 (LD_{50}) was estimated to be greater than 5000 mg/kg body weight.

Table 1. Animal's behavioral change after AAAE single administration.

Behavioral parameters	Treatments	
	DW	AAAE
Tremor	N	N
Twitches	N	N
Respiration	N	N
Sleep	A	A
Mobility	N	N
Mortality	A	A

DW = Distilled water (10 mL/kg); AAAE = *Artabotrys aurantiacus* aqueous extract (5000 mg/kg); A= absent; N = normal.

Effect of a single dose of *Artabotrys aurantiacus* aqueous extract on body weight: Fig.1 shows the effect of a single dose of AAAE on rat body weight following fourteen days of observation. No significant change was recorded in treated animals as compared to the control group. Effect of a single dose of *Artabotrys aurantiacus* aqueous extract on some absolute organs weight: Visual observation of liver, lung, spleen, heart and kidneys from AAAE treated rats showed no difference as compared to control.

Further, as shown in Table 2, no significant change in organ weight was noted while comparing treated rats and the control group after 14 days.

Table 2. Organ relative weight of *Artabotrys aurantiacus* aqueous extract treated rats.

	Relative organ weight				
Treatment	Liver	Lung	Spleen	Heart	Kidneys
DW	3.10 ± 0.07	0.65 ± 0.06	0.38 ± 0.05	0.28 ± 0	0.28 ± 0.01
AAAE	3.35 ±0.05	0.59 ± 0.03	0.44 ± 0.06	0.28 ± 0	0.29 ± 0.02

DW = Distilled water (10mL/kg); AAAE = *Artabotrys aurantiacus* aqueous extract (5000 mg/kg).

Effect of sub-chronic administration of Artabotrys aurantiacus aqueous extract

Effect of repeated administration of *Artabotrys aurantiacus* aqueous extract on rats' behavior and mortality: Table 3 shows the effect of AAAE on animals' behavior and mortality after 45 days. Prolonged administration of AAAE did not induce any behavioral change in treated rats as compared to the control group. Furthermore, no death was recorded throughout the experimental period.

Table 3. Behavioral pattern of animal following 45 days repeated administration of *Artabotrys aurantiacus.*

Behavioral parameters	Treatment					
	DW	AAAE				
		50	100	200	400	400 sat
Change in skin	N	N	N	N	N	N
Fur	N	N	N	N	N	N
Eyes	N	N	N	N	N	N
Mucus membrane	A	A	A	A	A	A
Occurrence of secretion and excretion	A	A	A	A	A	A
Autonomic activity	A	A	A	A	A	A
Mortality	A	A	A	A	A	A

DW = Distilled water (10 mL/kg); AAAE = *Artabotrys aurantiacus* aqueous extract ((50, 100, 200, 400 mg/kg).

Effect of repeated administration of *Artabotrys aurantiacus* aqueous extract on body weight: As depicted in Fig. 2A and 2B, repeated oral dosing of AAAE did not induce any significant change in animals' body weight throughout the experimental period as compared to control. The body weight gain was found to be 29% and 25 % for male and female satellite rats, respectively treated with AAAE 400 mg/kg. Effect of chronic administration of aqueous extract of *Artabotrys aurantiacus* aqueous extract on food and water intake: In sub-chronic treatment, no significant difference was observed in food and water intake in female rats, while in male rats, a significant difference ($p<0.05$, $p<0.001$) was observed during the first and fourth weeks of treatment (Table 4).

Table 4. Effect of prolonged administration of *Artabotrys aurantiacus* aqueous extract on food and water intake.

	DW	AAAE50	AAAE 100	AAAE 200	AAAE 400	AAAE 400 Sat
Weeks					Food intake (g/100g of body weight)	
Female						
1	15.16 ± 0.49	15.83 ± 1.55	16.39 ± 0.86	14.45 ± 0.67	15.53 ± 0.81	16.78 ± 1.10
4	09.96 ± 0.49	10.69 ± 0.29	11.73 ± 0.28	11.94 ± 0.33	10.14 ± 0.11	11.54 ± 0.33
7	10.04 ± 0.51	10.08 ± 0.32	10.85 ± 0.61	10.36 ± 0.33	10.36 ± 0.47	10.44 ± 0.25
Male						
1	14.18 ± 1.32	14.13 ± 0.58	14.13 ± 0.61	13.51 ± 0.10	13.55 ± 1.48	13.38 ± 0.93
4	08.50 ± 0.24	11.18 ± 0.36	10.50 ± 0.39	09.74 ± 0.44	09.47 ± 0.39	09.50 ± 0.28
7	08.09 ± 0.52	09.42 ± 0.47	09.72 ± 0.34	09.44 ± 0.63	08.89 ± 0.49	09.31 ± 0.20
			Water intake (mL/100g of body weight)			
Female						
1	16.43 ± 2.00	19.68 ± 1.44	19.30 ± 2.28	23.32 ± 4.10*	18.04 ± 1.78	19.44 ± 2.79
4	16.26 ± 0.59	19.75 ± 0.81	21.32 ± 0.25	21.03 ± 0.69	16.51 ± 0.62	18.03 ± 1.08
7	15.18 ± 0.33	17.93 ± 0.95	20.21 ± 0.35	20.46 ± 0.51	17.54 ± 0.81	19.36 ± 108
Male						
1	13.97 ± 0.44	21.66 ± 2.79***	15.19 ± 1.71	15.74 ± 0.79	18.02 ± 1.37**	18.19 ± 1.71**
4	14.66 ± 1.22	16.63 ± 0.57**	16.20 ± 0.73**	15.46 ± 0.70	14.58 ± 0.58	15.61 ± 0.35
7	13.47 ± 0.47	17.66 ± 0.18	18.47 ± 0.54	14.67 ± 0.29	15.69 ± 0.96	15.69 ± 0.27

N=5; * $p<0.05$, ** $p<0.01$, *** $p<0.001$ significant difference compared to control (distilled water); DW = Distilled water (10 mL/kg); AAAE = *Artabotrys aurantiacus* aqueous extract (50, 100, 200, 400 mg/kg).

Effect of chronic administration of *Artabotrys aurantiacus* aqueous extract on absolute organ weight: Table 5 shows the variation of absolute organ weight in both female and male rats treated at all the doses of AAAE for 45 days. This result has revealed that no change in the relative organ mass weight was observed in both sexes compared to control. Effect of chronic administration of *Artabotrys aurantiacus* aqueous extract on hematological parameters. The hematological pattern of both male and female rats is presented in Table 6. At the end of the experiment, all tested doses exhibited no significant change in either parameter as compared to the control.

Table 5. Sub-chronic administration of *Artabotrys aurantiacus*.

Groups	DW	AAAE 50	AAAE100	AAAE 200	AAAE 400	AAAE 400 Sat
Organs (g)						Female
Liver	3.11 ± 0.13	2.92 ± 0.17	2.59 ± 0.14	3.01 ± 0.14	2.66 ± 012	3,43 ± 0,13
Kidney	0.23 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.28 ± 0.01	0.24 ± 0,01	0.23 ± 0.03
Heart	0.27 ± 0.01	0.26 ± 0.01	0.25 ± 0.03	0.29 ± 0.01	0.27 ±0.01	0.29 ± 0.01
Aorta	0.03 ± 0.003	0.01 ± 0.002	0.02± 0.006	0.02 ± 0.001	0.02 ± 0.002	0.03 ± 0.001
Lungs	0.56 ± 0.03	0.64 ± 0.06	0.69 ± 0.06	0.67 ± 0.03	0.68 ± 0.08	0.63 ± 0.04
Pancreas	0.32 ± 0.04	0.26 ± 0.02	0.28 ±0.05	0.35 ± 0.03	0.24 ± 0.04	0.21 ± 0.03
Spleen	0.34 ± 0.04	0.29 ± 0.02	0.30 ± 0.01	0.42 ± 0.03	0.35 ± 0.03	0.56 ± 0.09
Thymus	0.10 ±0.01	0.10 ± 0.02	0.12 ±0.01	0.14 ± 0.01	0.11 ± 0.01	0.10 ± 0.01
Ovary	0.04 ±0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ±0.03	0.04 ± 0.01	0.03 ± 0.002
			Male			
Liver	2.79 ±0.16	3.15 ± 0.22	2.56 ± 0.17	2.63 ± 0.19	2.59 ± 0.10	3.00 ± 0.27
Kidney	0.25 ±0.01	0.25 ± 0.02	0.24 ± 0.02	0.24 ± 0.01	0.25 ± 0.01	0.27 ± 0.03
Heart	0.24 ± 0.02	0.28 ± 0.01	0.28 ± 0.02	0.26 ±0.01	0.29 ±0.01	0.28 ±0.01
Aorta	0.02 ± 0.001	0.01 ± 0.002	0.02± 0.01	0.03 ±0.01	0.03 ± 0.003	0.03 ±0.003
Lungs	0.74 ± 0.04	0.63 ± 0.04	0.54 ± 0.04	0.69 ± 0.05	0.58 ± 0.04	0.63 ± 0.05
Pancreas	0.22 ± 0.02	0.22 ± 0.02	0.22 ± 0.01	0.019 ± 0.03	0.22 ± 0.03	0.22 ±0.03
Spleen	0.35 ± 0.05	0.32 ± 0.03	0.33 ± 0.04	0.31 ± 0.06	0.34 ± 0.03	0.33 ± 0.04
Thymus	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.07 ± 0.01	0.06 ± 0.01
Testis	0.47 ± 0.03	0.45 ± 0.03	0.44 ± 0.03	0.47 ± 0.03	0.50 ± 0.02	0.51 ± 0.03
Epididymis	0.18 ± 0.01	0.18 ± 0.02	0.15 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.20 ± 0.01

on absolute organ weight.

N=5; no significant difference compared to control (distilled water); DW = Distilled water (10 mL/kg); AAAE = *Artabotrys aurantiacus* aqueous extract (50, 100, 200, 400 mg/kg).

Effect of chronic administration of *Artabotrys aurantiacus* aqueous extract on biochemical parameters: The effect of AAAE on biochemical parameters is presented in Table 7. These results have not revealed any significant difference in various parameters in both sexes treated rats compared to the control group. Effect of chronic administration of aqueous extract of *Artabotrys aurantiacus* aqueous extract on the histology of liver and kidney: The microphotography analysis of liver tissue revealed no damage related to AAAE administration after 45 days of repeating dosing (Fig. 3).

Table 6. Hematological of *Artabotrys aurantiacus* treated rats after 45 days repeated dosing.

	DW	AAAE 50	AAAE 100	AAAE 200	AAAE 400	AAAE 400 Sat
Parameters						Female
White blood cells (x10³/µL)	2 ± 0.45	0.26 ± 0.01	0.25 ± 0.03	0.29 ± 0.01	0.27 ±0.01	0.29 ± 0.01
Red blood cells (x10⁶/µL)	2 ± 0.45	2.33 ± 0.26	2 ± 0.32	2.2 ± 0.37	2 ± 0.45	1.8 ± 0.89
Hemoglobin (g/dL)	14.60 ± 0.51	14.40 ± 1.21	14.46 ± 0.76	15.60 ± 0.51	14.20 ±0.3	15.80 ± 0.37
Hematocrit (%)	43.36 ± 3.50	52.36 ± 0.55	46.56± 2.59	47.56 ± 2.35	48.76 ± 1.96	45.36 ± 2.55
Platelet count (x10³/µL)	248 ± 48.75	316.4 ± 62.59	335.2 ± 61.07	269.2 ± 41.93	264 ± 60.82	341,2 ± 41.67
VGM (µm)	90.8 ± 1.11	72.6 ± 2.91	88.8 ±1.68	87.4 ± 1.82	88.6 ± 2.94	88,6 ± 1.84
TCMH (pg)	31.2 ± 0.77	26.8 ± 1.68	30.8 ± 0.66	30 ± 0.75	30.4 ± 0.54	32 ± 0.28
CCMH (g/dL)	32.4 ±0.46	32.4 ± 0.61	33±0.49	31.6 ± 0.61	33 ± 0.57	32.6 ± 0.92
Lymphocytes (µL)	0.4 ±0.17	0.5 ± 0.1	0.23 ± 0.02	0.34 ±0.1	0.26 ± 0.02	0.33 ± 0.1
Mid (µL)	0.52 ± 0.14	0.33 ± 0.02	0.48 ± 0.1	0.52 ± 0.1	0.46 ± 0.1	0.45 ± 01
Granulocytes (µL)	5.39 ± 0.18	5.04 ± 0.18	5.02 ± 0.1	3.22 ± 0.32	4.62 ± 0.72	3.72 ± 0.5
			Male			
White blood cells(x 10³/µL)	2.79 ±0.16	3.15 ± 0.22	2.56 ± 0.17	2.63 ± 0.19	2.59 ± 0.10	3.00 ± 0.27
Red blood cells (x10⁶/µL)	2.33 ±0.26	2.25 ± 0.67	2.2 ± 0.37	2.0 ± 0.32	2.0 ± 0.32	1.8 ± 0.38
Hemoglobin (g/dL)	12.33 ± 0.68	15.00 ± 1.52	14.60 ± 0.87	14.75 ± 0.49	15 .2 ±0.73	15.6 ±0.51
Hematocrit (%)	51,96 ± 0.51	45.76 ± 2.73	46.36± 2.68	45.11 ±1.93	49.36 ± 2.1	49.16 ±2.24
Platelet count (x10³/µL)	405.4 ± 18.2	159.6 ± 3.79	294 ± 54	330 ± 45	381,6 ± 33.6	303.6 ± 60
MCV (µm)	86.8 ± 3.51	89.2 ± 1.99	89.8 ± 2.69	89.25 ± 0.52	89.2± 2.79	86.4± 1.93
MCH (pg)	26 ± 0.97	31 ± 1.78	29.2 ± 0.52	29.5 ± 0.6	30.4 ± 0.61	30.2 ± 0.77
MCHC (g/dL)	32.2 ± 0.72	30.8 ± 0.33	32.4 ± 0.22	32.25 ± 0.44	32 ± 0.78	32.6 ± 0.61
Lymphocytes (µL)	0.27 ± 0.11	0.3 ± 0.04	0.24 ± 0.1	0.23 ± 0. 04	0.20 ± 0.02	0.33 ± 0.08
Mid (µL)	0.47 ± 0.08	0.62 ± 0.05	0.52 ± 0.03	0.43 ± 0.05	0.35 ± 0.06	0.45 ± 0.1
Granulocytes (µL)	4.23 ± 0.4	4.44 ± 0.44	3.97 ± 0.49	4.22 ± 0.28	4.42 ± 0.33	3.82 ± 0.61

N=5; no significant difference compared to control (distilled water); DW = Distilled water (10 mL/kg); AAAE = *Artabotrys aurantiacus* aqueous extract (50, 100, 200, 400 mg/kg), MCV=Mean corpuscular volume, MCHC=Mean corpuscular hemoglobin concentration, MCH=Mean corpuscular hemoglobin.

On the other hand, kidney structure presented a tubular degenerescency at the doses of 200 and 400 mg/kg in both sexes, which was corrected in the satellite group 14 days after the treatment was stopped (Fig. 4). No damage was observed in the other groups (50 and 100 mg/kg) compared to the control group.

Discussion

The need for the determination of the toxicological profile of *Artaboyrys aurantiacus* aqueous extract (AAAE) has been prompted by its rampant use by the population to cure various ailments. (Chanda *et al.*, 2015; Neeta *et al.*, 2015).

In this study, single oral administration of AAAE exhibited no toxicity sign (change of behavior, internal organs damages) or death in both male and female animals during the experiment. The lethal dose 50 (LD_{50}) of the tested extract was estimated to be greater than 5000 mg/kg body weight assuming that the substance is practically non-toxic (Locke, 1983; Loomis, 1996). In general, plant extract from the Annonaceae family is reported to be non-toxic such as has been observed with *Annona muricata* aqueous extract (Arthur *et al.*, 2011).

Table 7. Biochemical parameters values of treated rats in *Artabotrys aurantiacus* administration.

	DW	AAAE 50	AAAE 100	AAAE 200	AAAE 400	AAAE 400 Sat
			Female			
Total protein(g/dl)	12.18 ± 1.04	12.43 ± 2.52	11.92 ± 3.14	1172± 3.53	14.48 ± 1.31	13.31 ± 0.92
Creatinine (mg/dl)	4.4 ± 0.51	4.09 ± 0.44	3.87 ± 0.24	4.11 ± 0.51	3.89 ± 0.22	4.09 ± 0.75
Urea (UI)	0.55 ± 0.1	0.49 ± 0.19	0.48 ± 0.22	0.38 ± 0.16	0.31 ±01	0.37 ± 0.12
ALAT(UI)	59.36 ± 3.93	59.07 ± 2.04	55.52± 4.1	57.12 ± 2.29	54.42 ± 10.39	58.93 ± 8.89
ASAT(UI)	18.39 ± 4.25	18.33 ± 0.95	16.59 ± 2.66	18.16 ± 3.24	16.99 ± 3.03	17.85 ± 0.46
Total bilirubin(UI)	5.86 ± 1.65	5.58 ± 1.99	4.67 ± 1.54	5.26 ± 0.33	5.22 ± 0.82	4.55 ± 1.41
Alkaline Phos (UI)	520.02 ±36.32	463.70 ± 37.20	430.09 ± 69.07	525.58 ± 66.63	524.22 ± 41.54	494.32 ± 90.27
Triglyceride (mg/dl)	43.04 ± 3.67	46.15 ± 6.41	40.10 ± 12.08	45.61 ± 7.73	49.48 ± 9.62	44.25 ± 4.44
Total Chol (mg/dl)	44.82 ± 6.67	45.59 ± 10.66	47.67 ± 6.28	56.75 ± 6.97	50.52 ± 4.74	64.59 ± 5.95
HDL Chol (mg/dl)	20.26 ± 0.4	20.97 ± 1.94	20.55 ± 0.52	22.23 ± 1.38	21.24 ± 2.44	20.59 ± 1.28
LDL Chol (mg/dl)	24.91 ± 5.83	25.04 ± 4.86	23.31 ± 6.39	25.41 ± 5.16	24.25 ± 6.75	26.35 ± 6.09
			Male			
Total protein (g/dl)	13.44 ±1.42	13.98 ± 1.98	14.51 ± 2.19	11.04 ± 1.82	11.52 ± 1.94	12.99 ±2.46
Creatinine (mg/dl)	4.62 ±0.54	4.07 ± 0.41	4.00 ± 0.36	4.05 ± 0.39	3.82 ± 0.48	4.00 ± 0.31
Urea (UI)	0.32 ± 0.1	0.31± 0.1	0.29 ± 0.03	0.31 ±0.11	0.33 ± 0.02	0.32 ±0.02
ALAT(UI)	63.44 ± 4.33	61.98 ± 6.49	52.38 ± 4.98	57.58 ±4.99	51.59 ± 5.55	55.64 ±3.65
ASAT(UI)	21.88 ± 2.81	26.05 ± 2.82	23.85 ± 3.32	22.72 ± 1.35	24.91 ± 3.56	21.53 ± 3.12
Total bilirubin	7.41 ± 0.97	7.24 ± 1.95	6.73 ± 0.97	7.29 ±1.53	7.25 ± 2.71	6.84 ± 1.76
Alkaline phos (UI)	543.64 ± 23	501.6 ± 85	486,41 ± 44	431.83 ± 58	406.02 ± 60	459.62 ± 20
Triglyceride (mg/dl)	69.27 ± 2.34	62.08 ± 7.01	67.01 ± 9.98	64.71 ± 9,33	62.56 ± 12.65	65.89 ± 13.17
Total Chol (mg/dl)	52.85 ± 10.04	46.85 ± 3.39	55.10 ± 2.72	51.76 ± 3.56	57.03 ± 2.69	49.51 ± 2.86
HDL Chol (mg/dl)	22.45 ± 0.65	25.52 ± 1.77	20.82 ± 0.77	21.54 ± 1.65	21.28 ± 1.25	20.59 ± 1.28
LDL Chol (mg/dl)	24.92 ± 7.29	22.88 ± 3.05	23.14 ± 2.59	22.70 ± 5.40	23.24 ± 4.29	20.82 ± 3.06

N=5; no significant difference compared to control (distilled water); DW = Distilled water (10 mL/kg); AAAE = *Artabotrys aurantiacus* aqueous extract (50, 100, 200, 400 mg/kg); Alkaline phos= alkaline phosphatase; Total Chol= Total Cholesterol; HDL Chol= HDL Cholesterol; LDL Chol= LDL Cholesterol.

The high safety margin through oral route application can justify its therapeutic use in traditional medicine. Furthermore, no change in body weight, organs weight, food intake, or water consumption was observed with AAAE.

No toxicity sign (such as body weight, food and water consumption) or death was recorded during 45 consecutive days of treatment by oral administration with AAAE in the dose of 50, 100, 200 and 400 mg/kg.

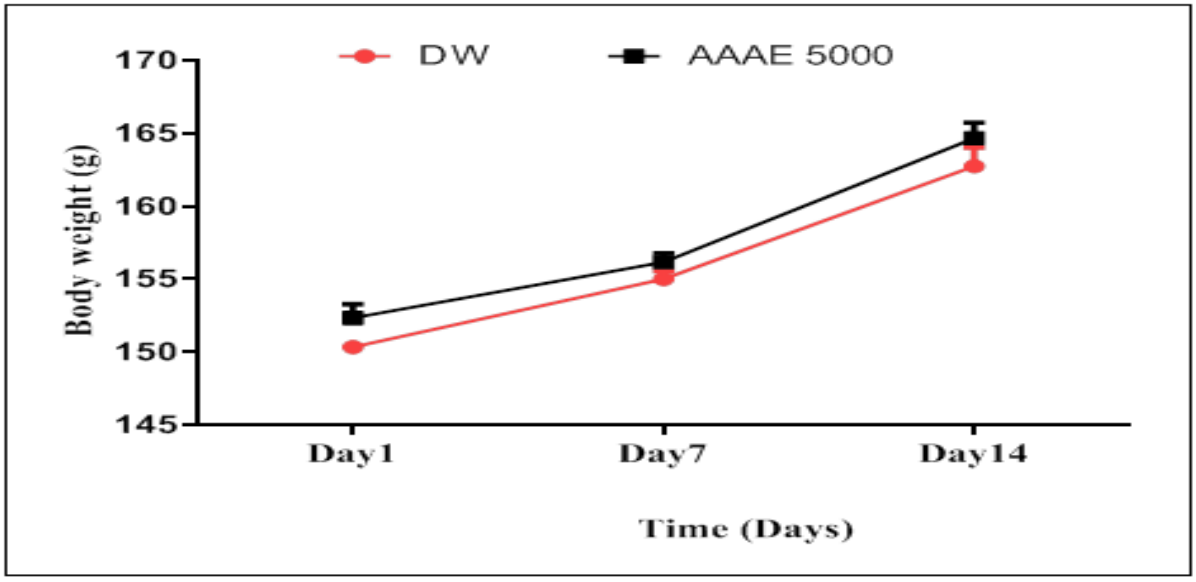

Fig. 1. Rats body weight evolution in acute toxicity test.

N=3; no significant difference compare to control (distilled water); DW = Distilled water (10 mL/kg); AAAE = *Artabotrys aurantiacus* aqueous extract (5000 mg/kg).

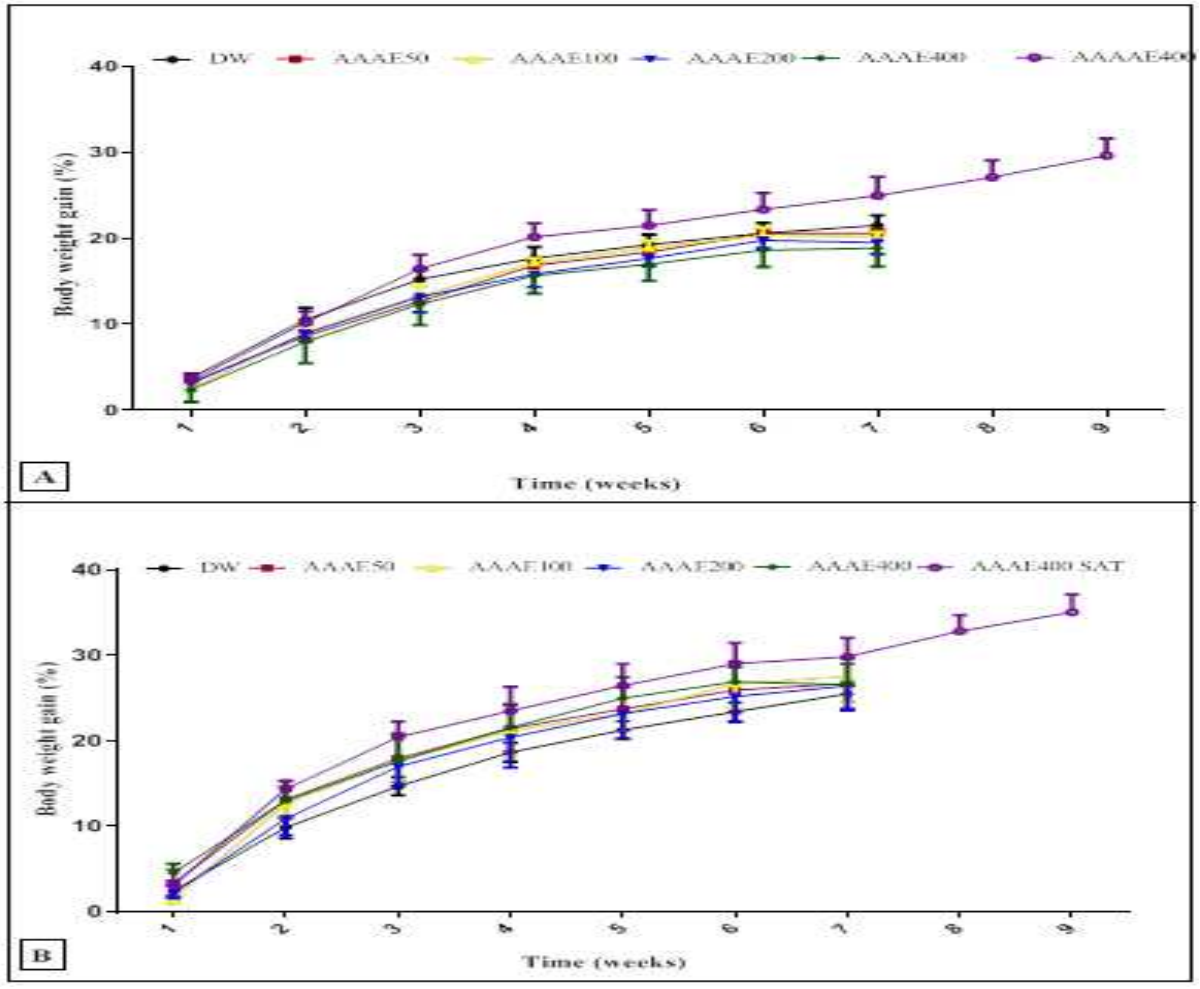

Fig. 2. Body weight spectral evolution of female (A) and male (B) rats during sub-chronic toxicity test.

N=5; no significant difference compare to control (distilled water); DW = Distilled water (10 mL/kg); AAAE = *Artabotrys aurantiacus* aqueous extract (50, 100, 200, 400 mg/kg).

In the present study, no change in relative organs weight was observed, suggesting that the aqueous extract of the plant is less toxic or non-toxic to these organs. The analysis of hematological parameters did not show any significant difference between the treated groups and the control group. This analysis is more important to assess the toxic effect of a substance because it has a higher predictive value of toxicity in humans when tests involve rodents (Olson *et al.,* 2000).

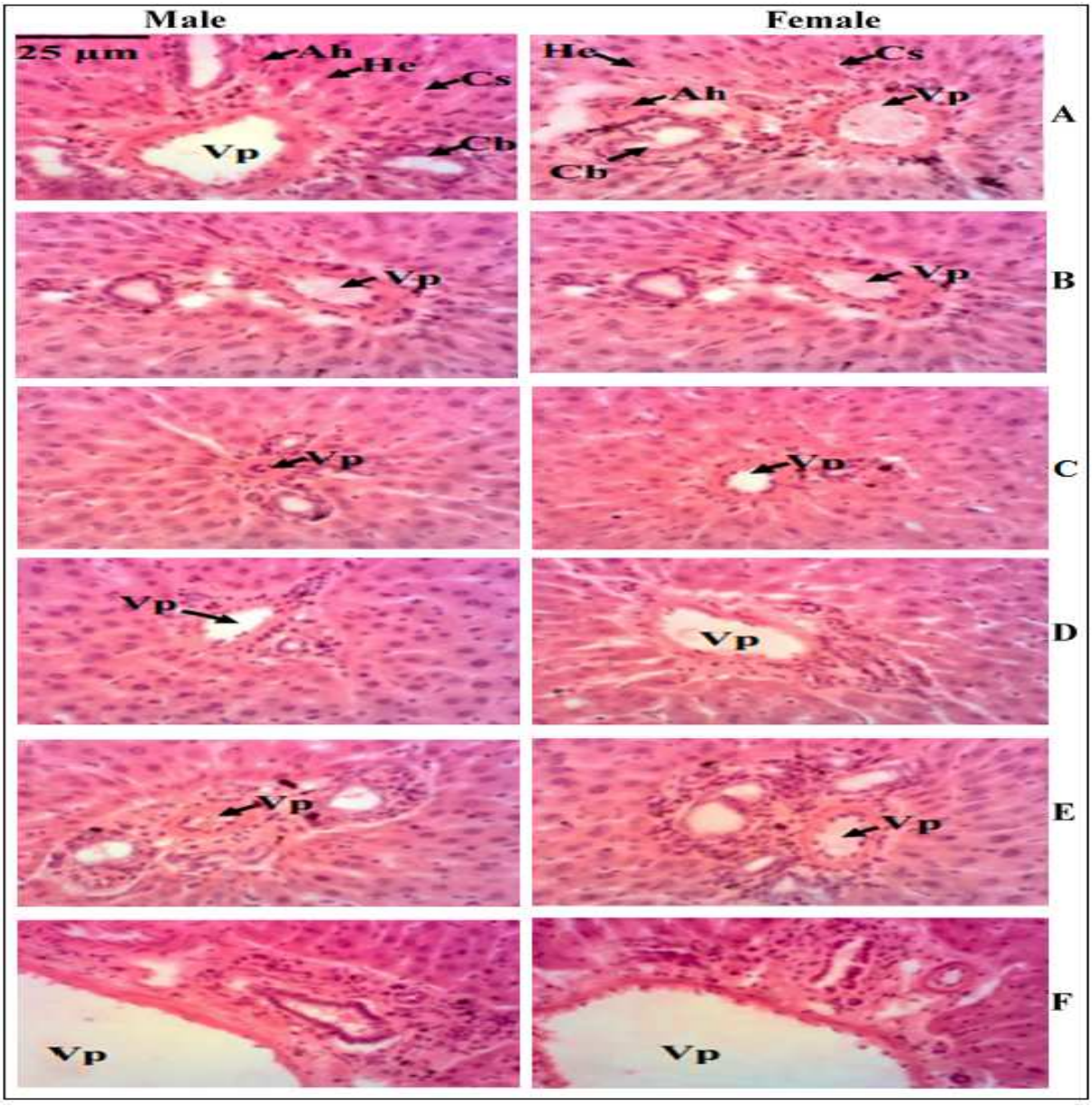

Fig. 3. Microphotography liver (HE x 100).

Vp: portal vein; He: hepatocyte; Cs: sinusoids capillary; Ah: hepatic artery; Cb: billary canicular; A= Distilled water, B= Satellite group, C,D,E,F = treated group respectively 50, 100, 200,400 mg/kg).

The biochemical indices monitored in the serum, such as the electrolytes and other substances secreted by the liver and kidney, can be used as markers for assessing the functional capacities of these organs (Tortora & Derrickson, 2006; Stephens *et al.,* 2007; Bariweni *et al.,* 2018).

The results of creatinine and urea rate which are the renal function indices, and the transaminases rate and alkaline phosphatase which are liver markers functions, have not shown any significant difference between the treated and control group. This indicated that the repeated administration of *Artaboyrys*

aurantiacus aqueous extract could not alter the normal function of these organs. Since serum proteins and lipids are also synthesized by the liver, their reduction would be the consequence of hepatic metabolism insufficiency (Kamo *et al.,* 2015). The serum protein rate, total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides have not significantly changed during the treatment indicating the normal protein hepatic metabolism and lipid metabolism (Ahmed *et al.,* 2010).

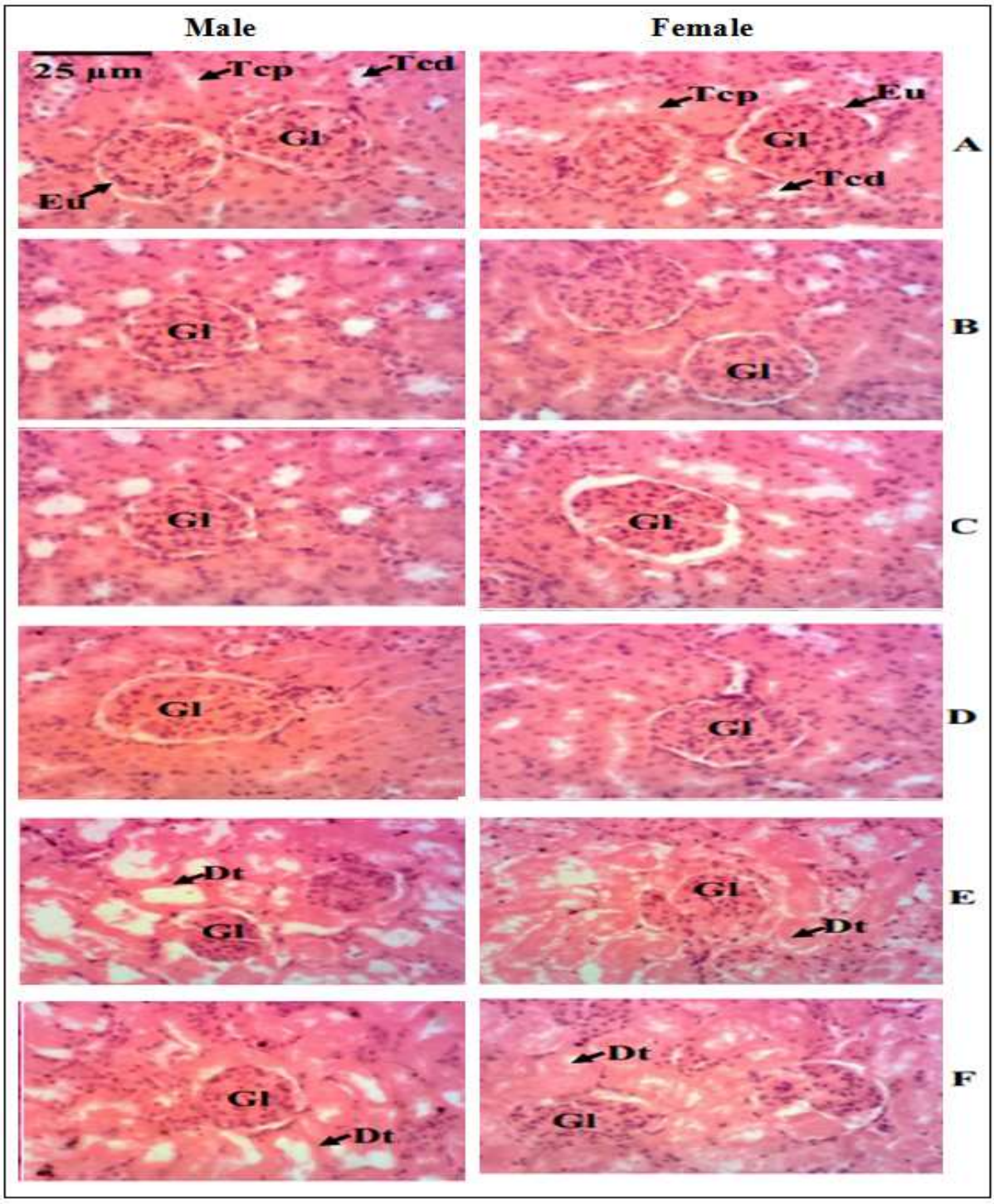

Fig. 4. Microphotography of kidney (HE x 200).

GI: glomerular infiltration; Dt: distal tubular; Tcd: distal convoluted tubular; Cu: urinary chamber; Tcp: proximal convoluted tubular; A= Distilled water, B= Satellite group, C,D,E,F = treated group respectively 50, 100, 200,400 mg/kg).

The oral administration of *Artaboyrys aurantiacus* aqueous extract in the rat for 45 consecutive days has shown that no alteration of the morphology liver was observed at all the doses of 50, 100, 200 and 400 mg/kg in both sexes. However, the presence of tubular degeneracy was observed at the doses of 200 and 400 mg/kg in both sexes. On the other hand, a correction of this tubular degeneracy has been observed in the satellite group of both sexes after 14 days without treatment.

This can be justified with the fact that the kidney takes a bite of time to metabolize the bioactive elements contained in this extract of plant regarding the duration of the treatment.

Conclusion

The aqueous extract of *Artaboyrys aurantiacus* is safe on acute administration since there was no adverse effect on the target organs. However, the prolonged use of this extract of the plant may produce a toxic effect on the kidney with higher doses.

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