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# ACUTE AND SUB-ACUTE ORAL TOXICITY STUDIES OF *ARTABOTRYS THOMSONII* OLIV (ANNONACEAE) LEAVES AQUEOUS EXTRACT IN WISTAR RAT

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**Keywords:**

*Artabotrys thomsonii*, Aqueous extract, Toxicological profile, Rat

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**ABSTRACT:** *Artabotrys thomsonii* is a medicinal plant used in the African pharmacopoeia for the treatment of various diseases. It was therefore important to assess its potential risk to human and animal health. This study highlights the acute and sub-acute effects of the toxicological profile of *A. thomsonii* leaves aqueous extract. The extract was prepared using the traditional protocol, then administered orally to Wistar rats at a single dose of 2000mg/kg for acute test, and of 100, 200 and 400mg/kg once a day for 28 days for sub-acute test. In acute toxicity, no behavioral disturbances were observed in treated animals compared with the control. The lethal dose 50 was estimated to be greater than 2000mg/kg and the extract was noted to possess low toxicity. Similarly, no significant changes in body mass, relative organ mass, hematological or biochemical parameters were observed after 28 days of repeated administration of the extract. Furthermore, administration of the extract did not alter motor coordination or develop anxiety in sub-acute treatment. Histological sections revealed that *A. thomsonnii* extract tall tested doses did not induce any damage in the liver, kidneys and heart compared with control group. These results indicate that the use of *Artabotrys thomsonii* leaves aqueous extract at all the doses tested would not be associated with any notable toxic effects, at least at the limit of exposure time observed in the present study.

**INTRODUCTION:** For decades, people in developing countries, and more to days in developed countries, use medicinal plants to treat themselves [1]. In many developing countries, a large proportion of the population relies heavily on traditional practitioners using medicinal plants to meet primary health care needs [2].

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Historical and cultural reasons can justify the large use of medicinal-based plant materials, and they are also considered to be low costed, efficient, healthy, pure and safe as it is obtained from natural resources [2, 3].

Although, it should be emphasized that the use of any plant material for therapeutic purposes not always guarantees its safety [3, 4], since the majority of medicinal plant material is harvested from wild stocks, where the quality of produced phytochemical constituents are often compromised by the presence of contaminants from either natural or anthropogenic sources, which may result in

adverse effects on human health [3]. Thus, while the pharmacological effects of many plants have been scientifically proven, the toxicity of some of them belongs unknown.

Toxicity is the measure of a toxic substance's capacity to cause harmful and adverse effects on the health or survival of any life form, whether the vitality of the entity or one of its parts is involved [5]. It also makes it possible to determine the degree or harmfulness of a substance and to regulate its use. The toxicity of a substance can be assessed on the basis of several parameters as: the method or route of administration, the dose to be administered, the weight variation, the organ histopathology, anxiety, muscle tone, changes in biochemical parameters and mortality rate [6]. Guidelines for the assessment of herbal medicines were prepared by WHO and adopted by the sixth ICDRA in Ottawa, Canada, in 1991 [7, 8].

*Artabotrys thomsonii* is a vine found in Nigeria, Angola and the Democratic Republic of Congo. Traditional uses of *Artabotrys* species include the treatment of various diseases [9]. In Democratic Republic of Congo, stem water of *Artabotrys thomsonii* is used as abeverage, and its water or sap is used for liver dysfunction and pregnancy, as genital stimulants, depressants and antiaborifacients [10]. In Cameroon, *Artabotrys thomsonii* is found in the Central, East, Littoral, South, South-West and West regions [9]. The sap, stem and leaf of *Artabotrys thomsonii* are used as an aphrodisiac, as an aid to get pregnant and as a treatment for enlarged spleens [11]. In addition, isolated compounds of crude extracts from other *Artabotrys* plant species showed cytotoxic, antimicrobial, anticancer, antioxidative, antidiabetic, antifungal, antiviral, antiplasmodial, antileishmanial, hepatoprotective, genotoxic, and mosquito repellency activities [12-16]. However, scientific studies to assess the *in-vivo* toxicity of *Artabotrys thomsonii* have not yet been done. This study aims to assess the acute and subacute toxicity of the leaves aqueous extract of *Artabotrys thomsonii* in rats.

## MATERIAL AND METHODS:

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

**Animals:** Two months-old male and non-pregnant female Wistar rats weighing 150-170g were procured from the animal breeding facility of the Faculty of Science at the University of Douala. They were housed in colony cages (5 rats/cage) at controlled room temperature (24°C±2°C) and humidity (80 – 85%), under light and dark cycles (12h/12h). They had free access to a standard food diet and tap water *ad libitum* throughout the study [17]. The experimental procedure was conducted in accordance with All Guidelines for Care and Use of Laboratory Animals as described in the European Community Guidelines (EEC Directive 2010 / 63 / EU of September 22, 2010) and after the approval for Animal Experimentation N°2459 CEI-UDO/10/2020/M by the Institutional Ethics Committee of the University of Douala.

**Plant Material and Extraction:** *Artabotrys thomsonii* (*A. thomsonii*) leaves were collected in ObakMount, Cameroon Center Region in March 2022, and a sample was sent to the Cameroon National Herbarium for identification by comparison with specimen number 52237HNC. The leaves were dried at ambient temperature in a shadow room for four weeks and reduced into powder using a grinder. Five hundred grams (500g) of powdered leaves were introduced into the pot containing 5L 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 filtrate was evaporated at 40°C using an oven, and the obtained crude extract (38.7g) stored at 4°C was used as *A. thomsonii* leaves aqueous extract (At) throughout the study.

The choice of the dose to be studied during acute toxicity was made based on OECD guideline n° 423 [18]. In sub-acute toxicity, it was based on the traditional human dose (32.46mg/kg) determined by following the traditional instructions for preparing the plant extract and translated to the rat dose as:

Rat dose (mg/kg) = [Human dose (mg/kg)] × k (with k=6.2) [19].

Thus, the obtained rat dose adjusted to the fixed upper/lower value, its half and double were used for the study. For administration to rats in each experiment, plants aqueous extract concentrated solutions were prepared by dissolving weighed quantities of crude dried plant aqueous extract in distilled water to obtain 200mg/mL stock solution once for acute test and 40mg/mL stock solutions every 3 days for prolonged test.

**Acute Toxicity Assessment:** For the current study, female's rats were used as recommended by the OECD guidelines for testing of chemical N°423 [18] because of their high sensitivity to natural substances. Six overnight fasted female rats were randomly divided into 2 groups of 3 animals each as: A control group (DW) receiving distilled water (10mL/kg; *p.o*) and a plant extract-treated group (At) receiving *A. thomsonii* extract at a single dose of 2000mg/kg (*p.o*).

Animals were observed continuously for 4 hours, then at time intervals of 4 hours for 12 hours and at the 24th hour following treatment to detect mortalities and any change in behavioral (tremor, twitches, respiration, sleeps, aggressivity) and neurological (mobility) profile. Further, the animals were observed once every 12 hours for the next 14 days and weighed on days 1, 7 and 14. At the end of the study, rats were sacrificed, and vital and detoxification organs (liver, lung, kidney, heart and spleen) were removed, weighed and macroscopically analyzed.

**Sub-acute Toxicity Assessment:** In this study, male and female animals were used with the aim of highlighting the influence of sexual hormones on the prolonged administration of natural substances. Thus, following the modified OECD guideline for the testing of chemicals, line N°407 [20], sixty rats of both sex were randomly divided into 6 groups of 10 animals each (5 males and 5 females) as follows: Group I: control, receiving distilled water, 10mL/kg (*p.o*); Groups II, III, IV: treated groups, receiving *A. thomsonii* at doses of 100, 200 and 400mg/kg/day respectively (*p.o*); Group V: satellite control group, receiving distilled water, 10mL/kg (*p.o*) and Group VI: treated satellite group, receiving *A. thomsonii* at the dose of 400mg/kg/day (*p.o*). The treatments were administered during 28 days. The control and treated satellite groups were observed for an additional 14 days without treatment in order to check the reversibility, persistence or late appearance of toxic effects. Body weight, mortality and behavior signs (including change in the fur, eyes, mucus membranes, the occurrence of secretions and excretions, autonomic activity) were registered weekly throughout the experimental period (28 days for groups 1-4, and 42 days for satellite groups). Anxiety (open field test) and motor coordination (grid hanging test) were assessed before and at the end of experimentation (day 28 for groups 1-4 and day 42 for satellite groups). On the $29^{th}$ or $43^{rd}$ day of the experimentation, animals were anesthetized using ketamine (50mg/kg) then diazepam (10mg/kg) (*im*), then sacrificed by behead. The blood was collected into EDTA tubes for hematological analyses. The blood collected into dry tubes was centrifuged at 3000rpm for 10 minutes, and the serum obtained was stored at -20°C for serum biochemical parameters determination. The organs (liver, spleen, heart, kidney, thymus and brain) were isolated, cleaned and weighted for the evaluation of relative masses. The liver, kidney and heart were fixed in formalin buffer at 10% for histological analyses.

**Open Field test for Anxiety Assessment:** The open field test, assessing exploratory behavior of animals in an enclosed space was used for appreciating the rat anxiety level following the method described by Nakagawa and collaborators [21]. Briefly, the principle is to place the animal in the center of the experimental device (opaque plywood box ($40 \times 40 \times 40cm^3$) with a bottom divided into 16 equal squares of $10 \times 10cm^2$), and observe its behavior for 5minutes using a camera. Parameters such as crossing (number of times the animal crosses a square), grooming (number of licks on the body coat), time spent in the central zone, and time spent in the peripheral zone, were recorded.

**Grid Hanging Test for Motor Coordination Study:** The grid suspension test was carried out to assess grip strength, agility and muscular coordination requirements following Ruhl and collaborators method [22]. A rectangular metallic grille (55cm long and 45cm wide), with diamond-shaped meshes measuring 1.5cm on each side, was mounted on a wooden frame. The grid was kept horizontal at a height of 100cm from the ground

where a basin containing shavings were placed. The maximum gripping time were 60 seconds by animal, with a rest interval of 3minutes between three passes. The gripping time of each animal was recorded for each pass using a stopwatch.

**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 leukocyte formula (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 were established [23]. For each sample, the results were printed and interpreted.

**Biochemical Analysis:** The serum was analyzed using a spectrophotometer (GENESYS 10S UV-Vis, Madison WI 53711, USA) and commercial spectrophotometric diagnostic kits from SGM ITALIA laboratory (Rome, ITALY) for Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) activities (kinetic), total proteins, creatinine, total bilirubin, direct bilirubin, urea, triglyceride, total cholesterol, HDL-cholesterol and LDL-Cholesterol contents (colometric) [24].

**Histopathological Analysis:** The procedure described by Cheng *et al*. [25] was followed for histological analysis by Hematoxylin-Eosin staining (H-E). Briefly, each organ (liver, kidney and heart) previously fixed in formalin buffer 10% was embedded in paraffin, cut into sections of 5µm using a Microtome (Reichert-Jung 2030) and mounted on slides. Slides of each organ were stained with hematoxylin and - eosin (H-E) after deparaffinization, then photographed using an Olympus brand optical microscope (Leitzwetzlar Germany 513) to detect any alterations comparing with the control.

**Statistical Analysis:** Results are expressed as mean ± SEM. The difference between the treated and control groups was determined using a one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Post-test.

The analysis was performed using PRISM Software (Graph Pad Software, Inc., San Diego, CA, version 5.03). A p-value less than 0.05 were considered significant [26].

## RESULTS:

**Effects of Acute Oral Administration of *Artabotrys thomsonii* Aqueous Extract in Rat:**

**Acute Effects on Mortality and Animal Behavior: Table 1** shows that oral administration of *A. thomsonii* leaves aqueous extract at a single dose of 2000mg/kg induced no significant change in rat behavior as compared to control group.

**TABLE 1: ANIMAL'S BEHAVIORAL CHANGE AFTER *A. THOMSONII* AQUEOUS EXTRACT SINGLE ADMINISTRATION**

| Behavioral parameters | Treatments (n = 3/group) | |
|---|---|---|
| | DW (10mL/kg) | At (2000mg/kg) |
| Tremor | N | N |
| Twitches | N | N |
| Respiration | N | N |
| Sleep | A | A |
| Mobility | N | N |
| Mortality | A | A |

DW = Distilled water; At = *Artabotrys thomsonii* aqueous extract; A= absent; N = normal.

**Acute Effects on Body Weight, Relative Weights and Macroscopic Analysis of Organs:** The single dose of *A. thomsonii* aqueous extract (2000mg/kg) did not show any significant change in rats' body weights during the 14 days following the administration as compared to control group **Table 2**. Visual observation of the liver, lungs, heart and kidneys of rats treated with aqueous extract of *A. thomsonii* showed no difference from the control group. Furthermore, as shown in **Table 2**, no significant changes in organ weights were noted when comparing treated rats to control rats after 14 days.

**TABLE 2: BODY WEIGHT EVOLUTION AND VITAL ORGANS RELATIVE WEIGHTS OF RATS TREATED WITH A SINGLE DOSE OF *A. THOMSONII* IN ACUTE TOXICITY TEST**

| Treatments (n = 3/group) | Body weight (%) | | | Relative vital organs weight | | | | |
|---|---|---|---|---|---|---|---|---|
| | D1 | D7 | D14 | Liver | Kidney | Lung | Heart | Spleen |
| DW (10mL/kg) | 100.00±0.00 | 105.23±0.67 | 121.10±3.49 | 3.62±0.14 | 0.35±0.01 | 0.96±0.05 | 0.37±0.02 | 0.39±0.02 |
| At (2000mg/kg) | 100.00±0.00 | 107.19±1.59 | 116.23±2.86 | 3.85±0.11 | 0.34±0.02 | 0.82±0.9 | 0.39±0.03 | 0.38±0.01 |

Data represent the mean ± ESM; DW = Distilled water; At = *Artabotrys thomsonii* aqueous extract.

**Effects of Sub-acute Oral Administration of *Artabotrys thomsonii* Aqueous Extract in Rat:**

**Sub-Acute Effects on Mortality and Animal Behavior:** Repeated administration of *A. thomsomii* aqueous extract did not cause any significant alteration in behavior compared to control, and there was no death recorded during the 28 days of treatment in treated and untreated rats **Table 3**.

**TABLE 3: BEHAVIORAL PATTERN OF ANIMALS FOLLOWING 28 DAYS REPEATED ADMINISTRATION OF *ARTABOTRYS THOMSONII***

| Behavioral parameters | Treatments(n = 10/group) | | | | | |
|---|---|---|---|---|---|---|
| | C | At 100 | At 200 | At 400 | SC | S At 400 |
| 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 |
| Occur. of secret. and excret. | A | A | A | A | A | A |
| Autonomic activity | A | A | A | A | A | A |
| Mortality | A | A | A | A | A | A |

n=10; C = Control; At (100, 200 and 400) = *Artabotrys thomsonii* leaves aqueous extract respectively at doses of 100, 200 and 400mg/kg; SC = Satellite control; SAt 400 = satellite *Artabotrys thomsonii* group of rats treated with the plant extract dose of 400mg/kg; A=Absent; N=Normal; Occur of secret and excret. = Occurence of secretion and excretion.

**Sub-acute Effects on Anxiety and Motor Coordination:** The effects of *A. thomsomii* leaves aqueous extract on anxiety parameters (time spent at the periphery or the center; number of crossings and groomings) in male and female rats are shown respectively in **Fig. 1** and **Fig. 2**.

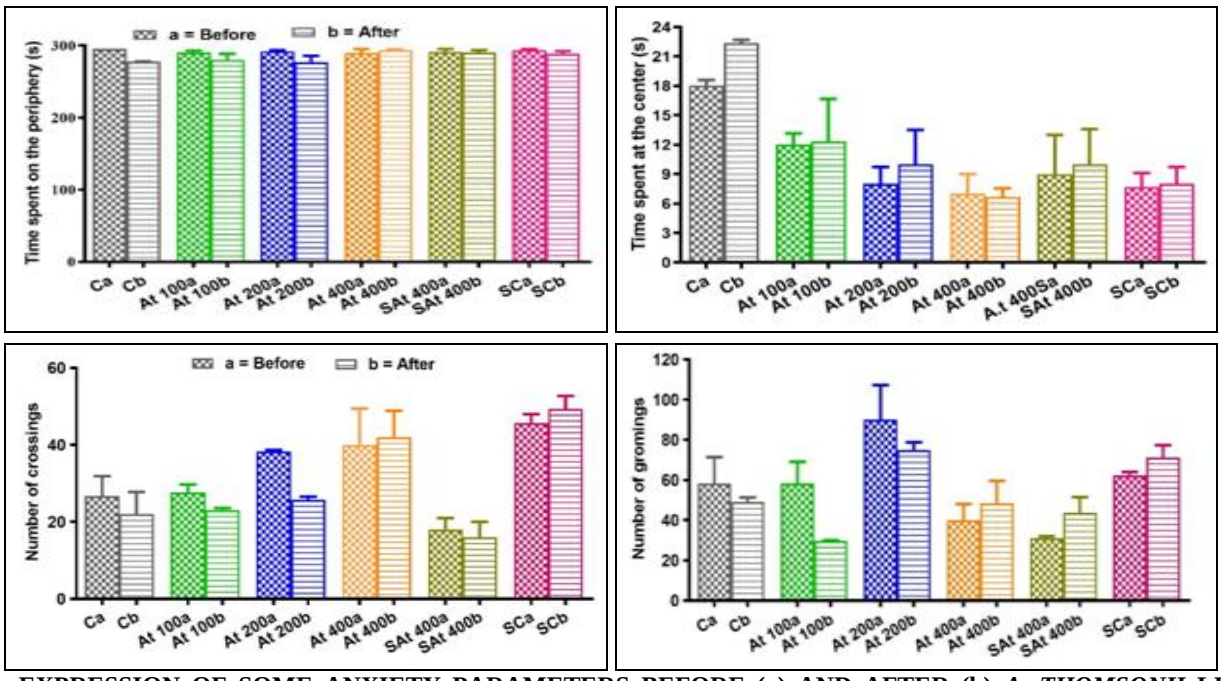



**FIG. 1: EXPRESSION OF SOME ANXIETY PARAMETERS BEFORE (a) AND AFTER (b) *A. THOMSONII* LEAVES AQUEOUS EXTRACT ADMINISTRATION IN MALE RATS.** Each bar represents respectively the time spent on the periphery, the time spent in the center, the number of crossing and number of grooming; n=3; C = control; At (100, 200 and 400) = *Artabotrys thomsonii* aqueous extract at doses of 100, 200 and 400mg/kg respectively; SC = Satellite control; SAt 400 = Satellite *Artabotrys thomsonii* extract group of rats treated receiving the plant dose of 400mg/kg; a = Before treatment; b = After treatment.

The results show that the plant extract did not induce anxiety in treated rats after 28 days of administration and the additional 14 days compared to controls; moreover, there is not any alteration in motor coordination in treated rats after 28 days of administration and the additional 14 days compared to controls **Fig. 3**.

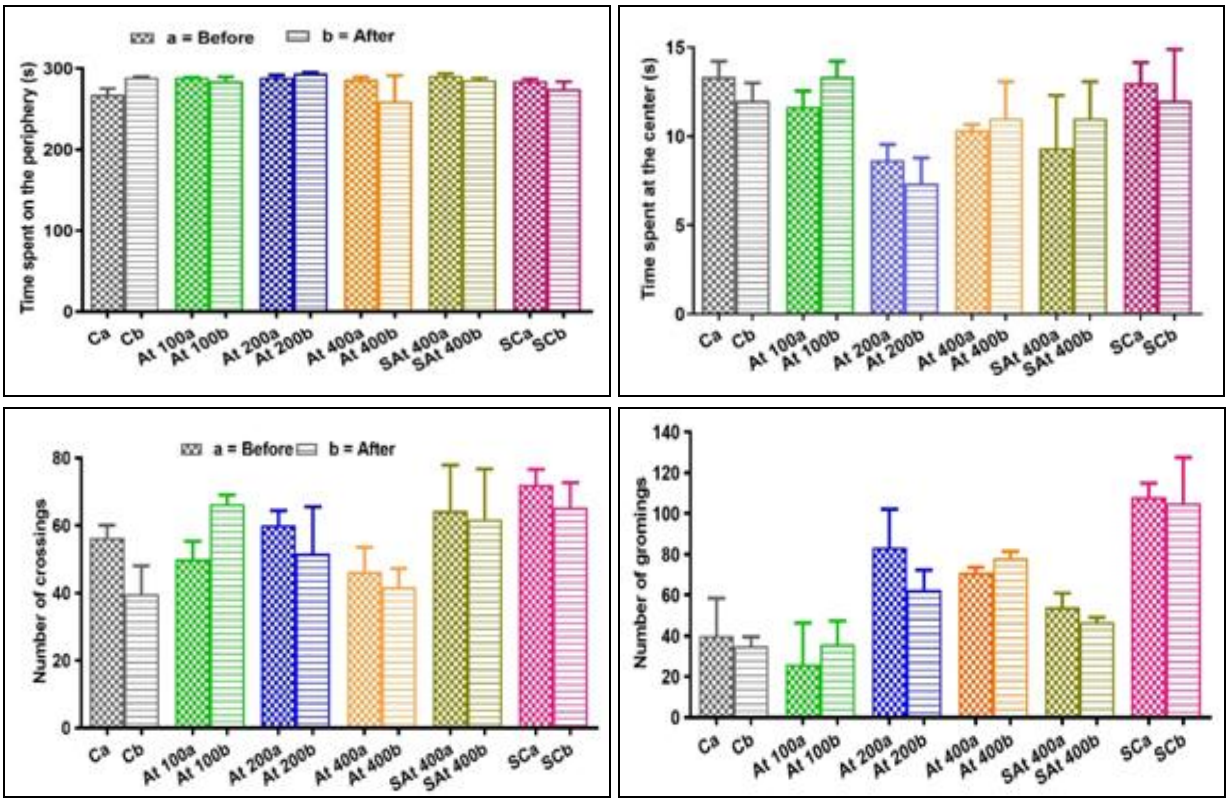



**FIG. 2: EXPRESSION OF SOME ANXITETY PARAMETERS BEFORE (a) AND AFTER (b) *A. THOMSONII*LEAVES AQUEOUS EXTRACT ADMINISTRATION IN FEMALE RATS.** Each bar represents respectively the time spent on the periphery, the time spent in the center, the number of crossing and number of grooming; n=3; C = Control; At = *Artabotrys thomsonii* aqueous extract (100, 200, 400mg/kg); SC = Satellite control; SAt 400 = *Artabotrys thomsonii* aqueous extract satellite; a = Period before administration; b = Period after administration.

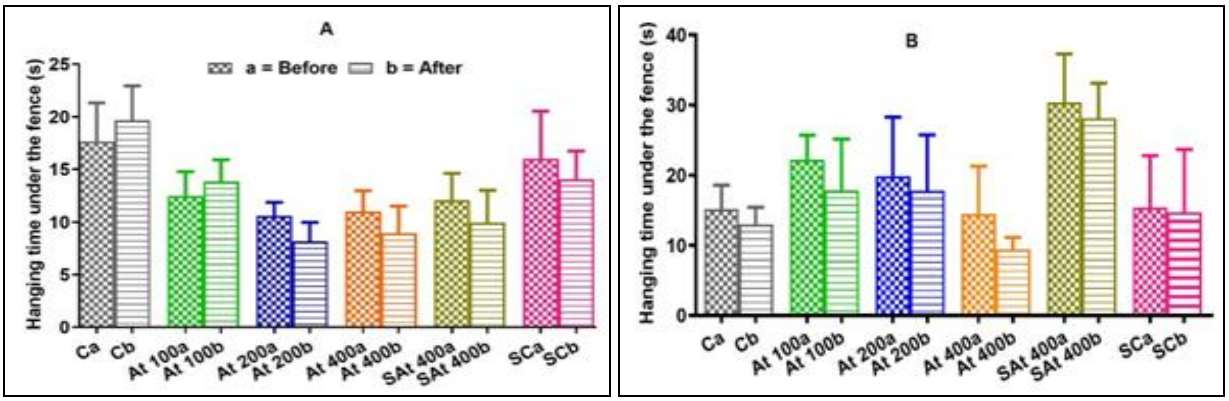



**FIG. 3: MOTOR COORDINATION IN MALE (A) AND FEMALE (B) RATS BEFORE (a) AND AFTER (b) THE ADMINISTRATION OF *A. THOMSONII* LEAVES AQUEOUS EXTRACT.** Each bar represents the average time spent gripping the mesh; n=3; C = Control; At = *Artabotrys thomsonii* aqueous extract (100, 200, 400mg/kg); SC = Satellite control; SAt 400 = *Artabotrys thomsonii* aqueous extract satellite; a = Period before administration; b = Period after administration.

**Effect of Sub-acute Administration of *Artabotrys thomsonii* Aqueous Extract on body Weight and Absolute Organ Weight:** Effects of sub-acute administration of *Artabotrys thomsonii* (*A. thomsonii*) aqueous extract on body weight and absolute organ weights are shown in **Tables 4** and **5** respectively. Repeated oral dosing of *A. thomsonii* aqueous extract did not induce any

significant change in animals' body weight throughout the experimental period as compared to controls **Table 4**, nor any difference in the organ weight between groups in both sexes compared to controls **Table 5**.

**TABLE 4: BODY WEIGHT EVOLUTION OF MALE AND FEMALE RATS DURING SUB-ACUTE TOXICITY TEST**

| Treatments | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
|---|---|---|---|---|---|---|
| | | | **Male Rats** | | | |
| C | 125±8.44 | 148.66±9.88 | 176.44±13.54 | 186.33±18.88 | - | - |
| At 100 | 135±6.49 | 153.8±6.27 | 189.8±7.38 | 205.3±9.58 | - | - |
| At 200 | 134.33±7.99 | 150.60±5.67 | 177.86±5.47 | 195.60±7.01 | - | - |
| At 400 | 131.86±6.03 | 142.90±5.15 | 176.60±5,15 | 196.8±4.45 | - | - |
| SC | 132.16±3.91 | 142.75±6.14 | 170.33±7.82 | 187.37±11.93 | 189.77±20.95 | 214±25.14 |
| SAt 400 | 131.5±4.99 | 144.75±4.24 | 176.83±5.03 | 192.87±5.89 | 198.08±7.02 | 212.58±8.47 |
| | | | **Female Rats** | | | |
| C | 129.7±3.41 | 145.83±5.16 | 158.11±3.21 | 161.83±4.22 | - | - |
| At 100 | 126.46±2.61 | 147.9±2.80 | 160.06±2.12 | 168.9±2.00 | - | - |
| At 200 | 139.46±6.86 | 148.1±4.59 | 165.66±4.35 | 172.8±4.54 | - | - |
| At 400 | 131,00±3.99 | 143.6±4.23 | 164.06±3.42 | 171.6±2.11 | - | - |
| SC | 131.83±2.60 | 143.5±3.68 | 162.41±3.83 | 171.62±3.88 | 173.88±6.71 | 183.44±7.52 |
| SAt 400 | 137±7.45 | 144,37±4.97 | 158.5±4.62 | 167.12±4.44 | 170,30±4.66 | 177±4.33 |

Data represent the mean ± ESM, n=5, C = Control; At = *Artabotrys thomsonii* aqueous extract (100, 200, 400mg/kg); SC = Satellite control; SA. t 400 = *Artabotrys thomsonii* aqueous extract satellite.

**TABLE 5: SUBACUTE EFFECTS OF *ARTABOTRYS THOMSONII* ON ABSOLUTE ORGAN WEIGHT**

| Organs | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | C | At 100 | At 200 | At 400 | SC | SA t 400 |
| | **Male Rats** | | | | | |
| Liver | 3.67 ± 0.06 | 3.80 ± 0.09 | 3.55 ± 0.14 | 3.39 ± 0.13 | 3.27 ± 0.45 | 3.37 ± 0.11 |
| Kidney | 0.34 ± 0.02 | 0.28 ± 0.01 | 0.30 ± 0.01 | 0.28 ± 0.01 | 0.31 ± 0.02 | 0.26 ± 0.01 |
| Heart | 0.30 ± 0.01 | 0.31 ± 0.01 | 0.34 ± 0.01 | 0.34 ± 0.01 | 0.29 ± 0.03 | 0.30 ± 0.01 |
| Spleen | 0.47 ± 0.01 | 0.42 ± 0.01 | 0.57 ± 0.12 | 0.54 ± 0.08 | 0.37 ± 0.02 | 0.37 ± 0.03 |
| Thymus | 0.16 ± 0.01 | 0.13 ± 0.03 | 0.15 ± 0.02 | 0.17 ± 0.02 | 0.13 ± 0.02 | 0.15 ± 0.01 |
| Brain | 0.94 ± 0.17 | 0.79 ± 0.02 | 0.88 ± 0.02 | 0.86 ± 0.02 | 0.77 ± 0.05 | 0.78 ± 0.01 |
| | **Female Rats** | | | | | |
| Liver | 3.60 ± 0.13 | 3.12 ± 0.08 | 3.27 ± 0.09 | 3.23 ± 0.10 | 3.83 ± 0.14 | 3.21 ± 0.12 |
| Kidney | 0.28 ± 0.02 | 0.29 ± 0.01 | 0.29 ± 0.01 | 0.30 ± 0.01 | 0.29 ± 0.01 | 0.27 ± 0.01 |
| Heart | 0.37 ± 0.01 | 0.34 ± 0.01 | 0.35 ± 0.01 | 0.35 ± 0.01 | 0.35 ± 0.01 | 0.34 ± 0.01 |
| Spleen | 0.58 ± 0.04 | 0.42 ± 0.01 | 0.47 ± 0.04 | 0.38 ± 0.03 | 0.43 ± 0.03 | 0.48 ± 0.05 |
| Thymus | 0.20 ± 0.01 | 0.18 ± 0.01 | 0.23 ± 0.02 | 0.25 ± 0.03 | 0.19 ± 0.01 | 0.21 ± 0.01 |
| Brain | 1.00 ± 0.02 | 0.92 ± 0.02 | 0.95 ± 0.01 | 0.92 ± 0.01 | 0.99 ± 0.02 | 0.87 ± 0.04 |

Data represent the mean±SEM, n=5, C = Control; At = *Artabotrys thomsonii* aqueous extract (100, 200, 400mg/kg); SC = Satellite control; SAt 400 = *Artabotrys thomsonii* aqueous extract satellite.

**Effect of Sub-acute Administration of *Artabotrys thomsonii* Aqueous Extract on Hematological Parameters:** The hematological pattern of both male and female rats presented in **Table 6** was not modified at the end of the experiment with the tested plant extract doses as compared to the controls.

**TABLE 6: HEMATOLOGICAL PROFILE OF RATSAFTER 28 DAYS OF *ARTABOTRYS THOMSONII* ADMINISTRATION**

| Treatments | WBC ($10^3$/l) | LYM ($10^3$/l) | MI ($10^3$/l) | GRAN ($10^3$/l) | RBC ($10^2$/l) | HT (g/dl) | MCV (fl) | MCCH (pg) | MCHC (pg) | DWRBC (pg) | Platelets (l) | MPV (fl) | WDW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Male Rats** | | | | | | | | | | | | |
| C | 7.8± 1.19 | 3.26± 0.56 | 0.76± 0.14 | 4.1± 0.92 | 4.03± 0.52 | 15.3± 0.21 | 85.8± 1.47 | 35.5± 30.44 | 34.3± 0.61 | 44.83± 7.74 | 274.6± 10.26 | 13.4± 1.05 | 11.3± 0.92 |
| At 100 | 8.46± 0.66 | 3.83± 0.05 | 0.96± 0.21 | 6.26± 0.17 | 4.93± 0.33 | 17.6± 0.65 | 83.6± 2.54 | 34.8± 0.44 | 35.9± 0.76 | 54.43± 0.95 | 224± 44.26 | 13.0± 1.09 | 13.6± 0.26 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| At 200 | 10.4± 0.27 | 3.14± 0.20 | 1.32± 0.09 | 5.16± 0.50 | 3.97± 0.25 | 14.3± 0.70 | 92.5± 2.09 | 35.4± 0.31 | 33.14± 0.94 | 48.46± 3.59 | 219.2± 28.01 | 13.4± 1.74 | 12.3± 0.39 |
| At400 | 8.02±0. 88 | 3.18±0. 42 | 1.58±0. 09 | 4.90±0. 59 | 4.21± 0.27 | 15.1± 0.40 | 94.3± 2.50 | 33.8± 0.73 | 33.74± 1.15 | 55.9± 0.01 | 246.2± 20.10 | 14.2± 1.18 | 12.1± 0.64 |
| SC | 9.5±0.8 4 | 3.06±0. 48 | 0.76±0. 28 | 4.26±0. 59 | 4.43± 0.52 | 14± 0.80 | 83.6± 2.54 | 35.53 ±0.24 | 35.53± 0.66 | 54.5± 0.61 | 279.33± 12.06 | 11.76± 0.85 | 11.6± 1.02 |
| SAt400 | 8.02±0. 88 | 3.42±0. 23 | 1.7±0.3 | 6.0±0.2 5 | 4.49± 0.29 | 14.4± 0.75 | 93.95 ±2.97 | 34.92 ±0.72 | 33.95± 0.77 | 50.4± 3.62 | 288.5± 2.75 | 13.65± 1.76 | 13.4± 0.75 |
| **Female Rats** | | | | | | | | | | | | | |
| C | 7.33±0. 60 | 3.63±0. 28 | 1.36±0. 02 | 4.60±1 8.26 | 4.63± 0.23 | 13.5± 0.51 | 78.9± 6.13 | 35.9± 0.43 | 35.96± 0.43 | 45.16± 7.87 | 231.00± 11.93 | 6.83± 0.74 | 11.9± 0.7 |
| At 100 | 8.52±0. 88 | 2.27±0. 49 | 1.30±0. 32 | 4.48±0. 52 | 4.85± 0.25 | 14.10 ±0.46 | 90.1± 1.56 | 36.0± 0.17 | 34.70± 0.46 | 48,26± 2,66 | 227.00± 26.33 | 11.8± 1.67 | 13.7± 0.52 |
| At 200 | 9.18±0. 74 | 2.86±0. 54 | 1.04±0. 09 | 5.80±0. 28 | 4.8± 0.23 | 14.5± 0.50 | 96.8± 1.41 | 34.5± 0.44 | 34.5± 0.57 | 53.70± 1.39 | 267.00± 9.74 | 9.88± 1.22 | 13.3± 0.31 |
| At400 | 9.74±0. 68 | 2.80±0. 33 | 1.26±0. 06 | 5.30±0. 73 | 5.07± 0.23 | 13.9± 0.71 | 90.8± 2.98 | 33.8± 0.68 | 34.14± 0.76 | 55.70± 0.14 | 244.20± 24.75 | 12.9± 1.26 | 11.9± 0.55 |
| SC | 9.53±1. 24 | 3.40±0. 19 | 1.46±0. 24 | 4.00±0. 76 | 4.26± 0.48 | 14.56 ±1.02 | 88.36 ±4.13 | 32.63 ±1.01 | 33.96± 1.48 | 55.90± 0.01 | 252.67± 20.27 | 16.0 ± 0.76 | 12.32± 1.02 |
| SAt400 | 8.87±0. 91 | 3.35±0. 32 | 1.07±0. 18 | 5.37±0. 89 | 4.55± 0.43 | 15.12 ±0.05 | 90.27 ±1.95 | 35.47 ±0.23 | 31.55± 1.72 | 48.05± 5.83 | 214.25± 19.68 | 15.35± 1.16 | 13.04± 0.51 |

Data represent the mean ± ESM, n=5; no significant difference compared to control (distilled water); C = Control; At = *Artabotrys thomsonii* aqueous extract (100, 200, 400mg/kg); SC = Satellite control; SAt 400 = *Artabotrys thomsonii* aqueous extract satellite. WBC=White Blood Cell, LYM=Lymphocyte, MI=Monocyte, GRAN = Granulocyte, RBC=Red Blood Cell, HT=Hematocrit, MCV=Mean corpuscular volume, MCCH=Mean Corpuscular Concentration in Hematocrit, MCHC=Mean corpuscular hemoglobin concentration, DWRBC=Distribution Width of Red Blood Cells, MPV=Mean Plasma Volume, WDW=Wafer Distribution Wid.

**Sub-Acute Effect of *Artabotrys thomsonii* Aqueous Extract on Biochemical Parameters:** The effect of *A. thomsonii* aqueous extract 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 groups, nor between sexes.

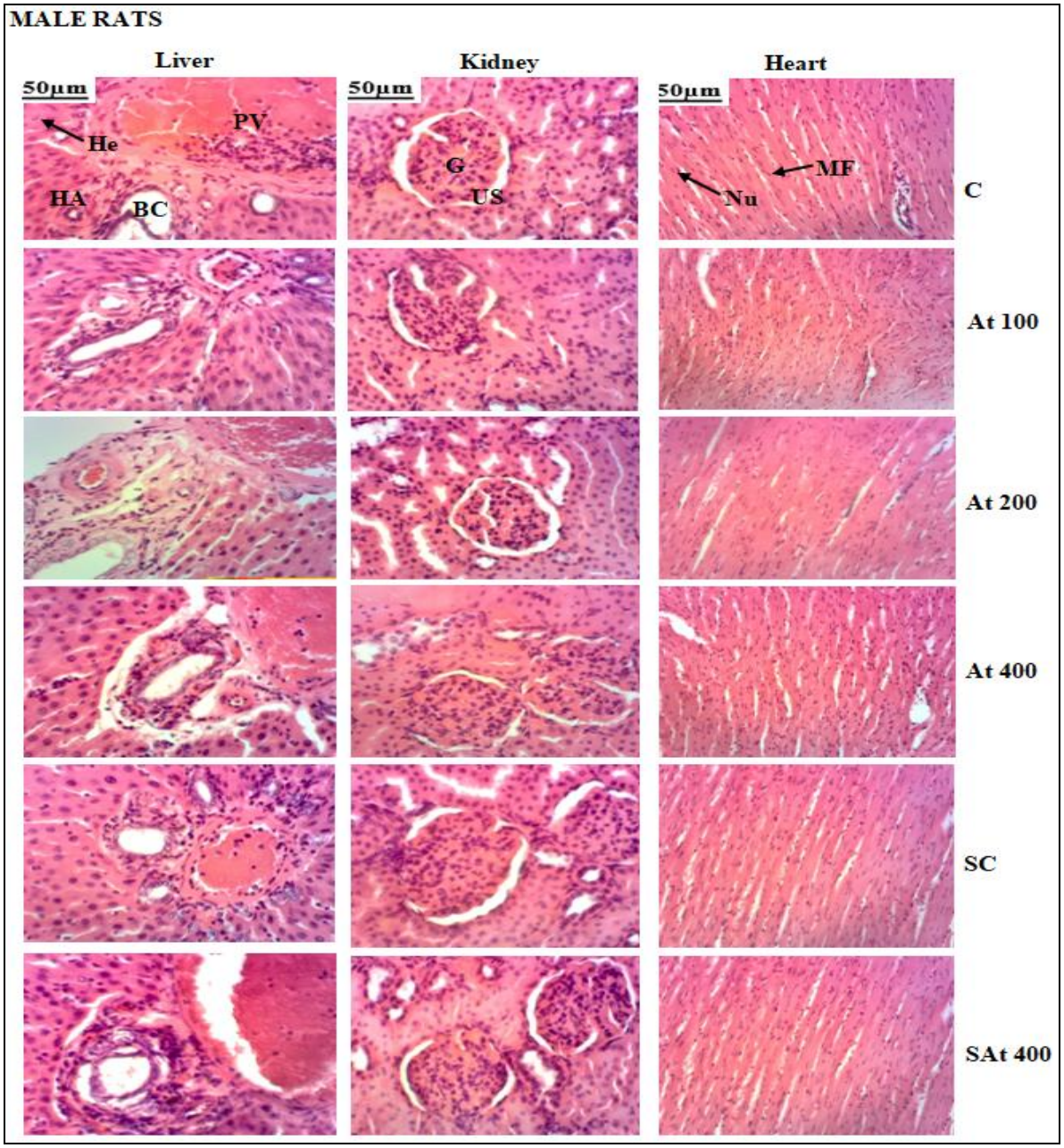
**TABLE 7: BIOCHEMICAL PARAMETERS VALUES IN RATS TREATED WITH *ARTABOTRYS THOMSONII***

| | C | At 100 | At 200 | At 400 | SC | SAt 400 |
|---|---|---|---|---|---|---|
| **Males** | | | | | | |
| Total Chol. (g/L) | 1.05 ± 0.04 | 1.07 ± 0.07 | 1.05 ± 0.03 | 0.95 ± 0.01 | 0.90 ± 0.03 | 0.85 ± 0.03 |
| Trigly. (g/L) | 0.43 ± 0.04 | 0.39 ± 0.06 | 0.31 ± 0.03 | 0.49 ± 0.02 | 0.46 ± 0.01 | 0.36 ± 0.03 |
| HDL-C (g/L) | 0.19 ± 0.04 | 0.17 ± 0.01 | 0.17 ± 0.01 | 0.20 ± 0.01 | 0.20 ± 0.02 | 0.24 ± 0.03 |
| LDL-C (g/L) | 0.87 ± 0.01 | 0.82 ± 0.05 | 0.83 ± 0.03 | 0.91 ± 0.04 | 0.69 ± 0.10 | 0.70 ± 0.13 |
| Creatinine (mg/L) | 4.76 ± 0.45 | 6.56 ± 0.56 | 5.05 ± 0.51 | 4.00 ± 0.33 | 6.18 ± 0.43 | 4.94 ± 0.13 |
| Urea (g/L) | 0.24 ± 0.04 | 0.34 ± 0.03 | 0.29 ± 0.03 | 0.20 ± 0.02 | 0.25 ± 0.02 | 0.20 ± 0.03 |
| ALT (IU/L) | 132.25±13.86 | 138.86±5.46 | 135.55±6.50 | 111.55±6.80 | 117.50±2.31 | 117.50±1.00 |
| AST (IU/L) | 241.64±17.71 | 299.60±22.92 | 341.95±13.09 | 283.40±23.66 | 243.67±21.43 | 215.00±15.00 |
| ALP (IU/L) | 109.00±13.88 | 149.75±10.63 | 142.40±2.90 | 120.20±18.72 | 122.33±25.92 | 147.25±2.88 |
| Total Bili. (mg/dL) | 6.77±0.27 | 6.63±0.88 | 6.18±1.07 | 4.97±0.61 | 4.95±0.22 | 7.10±1.35 |
| Direct Bili. (mg/dL) | 3.21±0.36 | 3.69±0.57 | 3.53±0.71 | 2.36±0.22 | 3.10±0.04 | 3.39±0.50 |
| Total Proteins (g/L) | 65.57±0.30 | 59.26±1.27 | 58.08±0.57 | 66.64±0.98 | 65.07±0.82 | 67.40±1.75 |
| **Females** | | | | | | |
| Total Chol. (g/L) | 0.82 ± 0.02 | 0.90 ± 0.18 | 0.87 ± 0.01 | 1.00 ± 0.03 | 0.67 ± 0.08 | 0.69 ± 0.06 |
| Trigly. (g/L) | 0.55 ± 0.02 | 0.43 ± 0.09 | 0.44 ± 0.06 | 0.39 ± 0.02 | 0.48 ± 0.04 | 0.47 ± 0.08 |
| HDL-C (g/L) | 0.15 ± 0.01 | 0.12 ± 0.02 | 0.15 ± 0.01 | 0.14 ± 0.01 | 0.15 ± 0.01 | 0.15± 0.01 |
| LDL-C (g/L) | 0.62 ± 0.03 | 0.70 ± 0.14 | 0.67 ± 0.02 | 0.73 ± 0.02 | 0.64 ± 0.10 | 0.60 ± 0.03 |
| Creatinine (mg/L) | 8.43 ± 0.22 | 7.39 ± 1.48 | 7.64 ± 0.33 | 5.80 ± 0.29 | 7.38 ± 0.64 | 5.92 ± 0.28 |
| Urea (g/L) | 0.65 ± 0.04 | 0.49 ± 0.10 | 0.49 ± 0.03 | 0.58 ± 0.04 | 0.61 ± 0.02 | 0,63 ± 0,04 |
| ALT (IU/L) | 130.91±13.60 | 104.87±20.97 | 118.40±7.82 | 118.37±4.35 | 115.67±2,05 | 115.00±2.50 |
| AST (IU/L) | 272.16 ±7.09 | 283.56±1988 | 299.62±20.44 | 320.01± 6.18 | 283.32±11.80 | 288.60±3.08 |
| ALP (IU/L) | 133.33±17.58 | 136.40±27.28 | 118.80 ± 4.22 | 91.40±2.36 | 103.27 ± 1.27 | 104.40±3.79 |
| Total Bil. (mg/dL) | 4.61 ± 0.51 | 6.01 ± 1.20 | 5.79 ± 0.80 | 6.75 ± 0.70 | 5.32 ± 0.73 | 4.71 ± 0.21 |

| | | | | | | |
|---|---|---|---|---|---|---|
| DirectBili. (mg/dL) | 1.76 ± 0.24 | 2.39 ± 0.48 | 2.47 ± 0.17 | 2.64 ± 0.24 | 2.20 ± 0.17 | 1.96 ± 0.09 |
| Total Proteins (g/L) | 67.90 ± 0.65 | 61.96 ± 12.39 | 63.24 ± 1.17 | 62.54 ± 0.83 | 68.53 ± 1.22 | 70.71 ± 2.31 |

Data represent the mean ± ESM, n=5; no significant difference compared to control (distilled water); C = Control; At = *Artabotrys thomsonii* aqueous extract (100, 200, 400mg/kg); SC = Satellite control; SAt 400 = *Artabotrys thomsonii* aqueous extract satellite. Total Chol: total cholesterol; Trigly: triglyceride; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; ALT: alanine amino transferase; AST: aspartate amino transferase; ALP: alkaline phosphatase; Total Bil: total bilirubin; D Bil: bilirubin direct.

**Histopathological Analysis of Organs After Sub-Acute Oral Administration of *A. Thomsonii* Leaves Aqueous Extract in Rats:** Comparisons of histological sections of the liver **Fig. 4** from normal control, plant aqueous extract treated (100, 200 and 400mg/kg) and satellite rats groups showed normal architecture of the liver parenchyma, with a centrilobular vein and well-distinct hepatocytes. Histology of the kidney also showed normal parenchyma with a well-distinct glomerulus and urinary space in all groups. Likewise, the histology of the heart showed a normal myocardial structure with well-distinct muscle fibers and myocytenucleiin all groups **Fig. 4.**



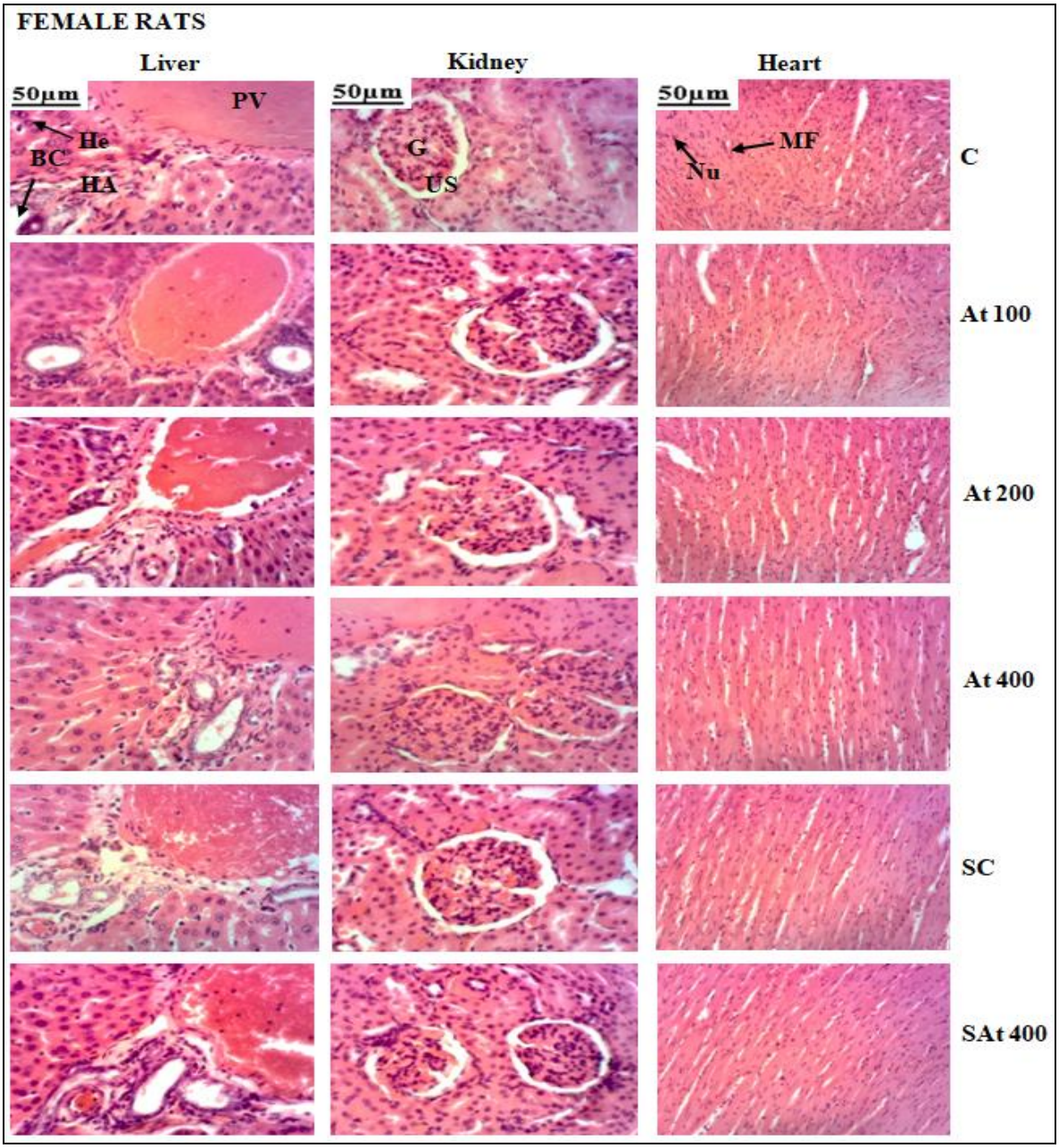



**FIG. 4: HISTOLOGY OF THE ORGANS (LIVER, KIDNEY AND HEART) OF RATS TREATEDWITH *A. THOMSONII* AQUEOUS EXTRACT.** G: glomerula; US: urinaryspace; PV: portal vein; BC: biliary canaliculus; HA: hepaticartery; He: hepatocytes; MF: muscular fiber; Nu: nucleus; C = Control; At = *Artabotrys thomsonii* aqueous extract (100, 200, 400mg/kg); SC = Satellite control; SAt 400 = *Artabotrys thomsonii* aqueous extract satellite.

**DISCUSSION:** The present study aimed to evaluate the toxicological profile of *Artabotrys thomsonii* leaves aqueous extract in rats. *Artabotrys thomsonii* leaves aqueous extract did not induce any death nor behavioral changes when administered acutely (2000mg/kg) or sub-chronically (100, 200 and 400mg/kg) for 28 days in rats. However, the absence of deaths observed after the acute administration of *A. thomsonii* extract makes it possible to estimate its lethal dose 50 ($LD_{50}$) greater than 2000mg/kg and to classify it in the category of slightly toxic substances according to the OECD [18]. It has been reported that prolonged administration of chemical and natural substances could cause anxiety and alter muscle tone. Additionally, stress and anxiety can cause or worsen muscle tone disorders (hypotonicitis/hypertonicitis) [27, 28]. An anxiogenic effect of the tetrahydrocannabinol (phytocannabinoid compound from cannabis plant) has been reported in animals [28]. The absence of alteration of crossing number, grooming number, periphery time spent, and center time spent observed after prolonged administration (28 days) of *A. thomsonii*

extract in rats would suggest that *A. thomsonii* would not induce anxiogenic effects at the limit of exposure time. Furthermore, *A. thomsonii* extract would not alter nervous system function in view of the non-altered muscle tone observed in treated rats compared with controls. Arika *et al.* (2019) [29] also showed that oral administration of *Gnidia glanca* aqueous extract in rats did not alter crossing number, grooming number, periphery time spent and center time spent.

Anxiety disorders can be triggers for weight loss [30]. In most cases, prolonged administration of substances can induce variation in body mass (increase or decrease) [31]. The body mass increase is generally explained by an increase in protein synthesis. Acute and sub-acute administration of *A. thomsonii* extract neither decreased nor increased body mass, proteinemia and organs masses, both in male and female normal rats. This suggests that the plant extract would not affect protein synthesis in normal condition. Leaves aqueous extracts of *Artabotrys aurantiacus*, a plant species of the same genus [17], and of other plants from Center Africa such as *Reissantia indica* [32], *Thymus schimperi* [33], *Moroccan mentha* [34] and *Mangifera indica* [35], also have been reported to not showing deleterious effects on body and organs masses in acute and sub-acute administration in rats.

Toxic substances can alter hematological profile parameters [35]. In general, analysis of blood parameters is relevant in pharmacological study, because it provides information on hematopoietic function (assessment of myeloid lineage cells), on the occurrence of allergies (study of white blood cells) and on intravascular effects such as hemolysis [36]. Therefore, the extent of toxic effect of drugs and/or plant extracts can be determined by assessment of hematological parameters [36, 37]. In most animals, diagnosis of anemia is based on assessment of specific blood indicators such as red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) [38]. These parameters (RBC, MCV, MCH and MCHC) did not change following the *Artabotrys thomsonii* extract administration for 28 days in either sex compared with controls, suggesting that the plant extract would not induce anemia. Leaves aqueous extract of *Thymus schimperi* also did not induce any difference in red blood cells counts of treated rats when compared to control [33]. Likewise, Mezui *et al*. [39] showed that the administration of the aqueous extract of the stem bark of *Anthocleista schweinfurthii* for 28 days does not modify the number of red blood cells in rats. Furthermore, prolonged administration of a substance can cause an immune response characterized by an increase or a decrease in the number of white blood cells [40].

The absence of variation in white blood cell count after 28 days of administration of *A. thomsonii* extract in both rats' sexes compared with controls suggests that the plant extract would not induce leukocytosis nor leukopenia [40]. Moreover, platelet count may also increase or decrease following prolonged administration of substances [41]. *A. thomsonii* extract did not change the number of platelets after 28 days of administration in rats compared to controls, suggesting that the plant extract would not induce thrombocytosis nor thrombocytopenia. The leaves aqueous extract of *Thymus schimperi* also did not induce any difference in platelets counts of treated rats when compared to control [33]. The liver is the crossroad of the metabolism of all substances entering the body, and as such, the perfect place for nutrient metabolism (carbohydrate, protein and lipid) [42].

Among many factors, abnormal lipid levels (too high (dyslipidemia) or too low (hypolipidemia) probably due to some substance's intake altering lipid metabolism can also cause and reflect hepatic dysfunction [43]. Moreover, dyslipidemia is a lipid metabolism affection highly correlated to cardiovascular diseases [44]. Serum levels of triglyceride, total cholesterol, LDL cholesterol, and HDL cholesterol did not change after *A. thomsonii* extract administration for 28 days in rats compared to controls, suggesting that the plant extract would not induce dyslipidemia or hypolipidemia and probably may protect against hepatic steatosis and cardiovascular diseases as reflected by the histological sections of the liver and heart. Interestingly, Emambo *et al.* [17] also observed that *Artabotrys aurantiacus* leaves aqueous extract did not alter the lipid profile parameters after 45 days of administration in rats. Furthermore, levels of transaminases, alkaline phosphatase and bilirubin are serum biomarkers of liver function and

integrity. Thus, high serum levels of transaminases provide information on the degree of hepatocyte damage, while the increase in phosphatasemia is a consequence of cholestasis (obstruction of the bile ducts) or hepatitis [45]. Moreover, elevated total bilirubinemia associated with the direct fraction higher than the indirect fraction can be observed in hepatitis, side reactions to several drugs or alcoholic liver disease [46]. Serum levels of transaminases, alkaline phosphatase, total bilirubin and direct bilirubin did not vary between groups in the present study, suggesting an absence of hepatocyte injury and impairment of hepatobiliary function in rats treated with the plant extract [47]. These results were confirmed by the histological sections of the liver which presented a normal architecture in the treated rats compared to the controls.

During toxicities due to xenobiotics, renal damage has also often been recorded given its involvement in the detoxification function. Thus, the glomerular filtration function can be altered causing an increase in serum creatinine and urea, predictive biomarkers of renal function. Creatinine comes from the breakdown of muscle creatine in the kidneys. Its blood or urine level also provides information on the rate of breakdown of muscle creatine. Urea is a nitrogen waste resulting from the hepatic degradation of proteins and eliminated by the kidneys. Its level is thus a reflection of the functioning of the kidneys, the liver and the dietary protein intake [48, 49]. In the present study, after 28 days of *A. thomsonii* administration, serum creatinine and urea levels did not change in either sexes compared with controls, suggesting that the plant extract did not alter glomerular filtration, hepatic function and intestinal absorption of proteins. These results were reinforced by the histological sections of the kidneys as suggested by Murray *et al*. [50] that showed no alteration in *A. thomsonii* extract-treated rats compared to controls, meaning that the plant extract has non-deleterious effect on organs. Similarly, the aqueous extracts of *Hibiscus sabdariffa* [51] and *Thymus schimperi* [33] also do not present deleterious effects on kidneys histology during sub-acute administration in rats.

**CONCLUSION:** The leaves aqueous extract of *Artabotrys thomsonii* is safe at the limit of doses tested in acute and sub-acute administrations in rats, since it did not cause death or alter behavioral and haematological parameters, serum biochemical profile, and the vital organs.

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**CONFLICTS OF INTEREST:** None

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