IJPSR (2023), Volume 14, Issue 10 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 21 February 2023; received in revised form, 27 April 2023; accepted, 31 May 2023; published 01 October 2023

SAFETY USE OF *PHASEOLUS VULGARIS* L. IMMATURE PODS DECOCTION AND CARDIOVASCULAR EFFECT IN RAT WITHOUT HYPERTENSION

Windingoudi Rimwagna Christian Ouedraogo [1, 2], Lazare Belemnaba [* 1], Mathieu Nitiema [1], Boukaré Kaboré [3], Souleymane Compaoré [1], Moumouni Koala [1], Rasmané Semde [2] and Sylvin Ouedraogo [1]

Laboratory of Research-Development of Phytomedicines and Medicines [1], Department of Medicine, Traditional Pharmacopoeia and Pharmacy, Research Institute for Health Sciences (MEPHATRA-PH /IRSS), 03 BP 7047 Ouagadougou, Burkina Faso.
Laboratory of Drug Development [2], Doctoral School of Sciences and Health, University Joseph KI-ZERBO, 03 BP 7021 Ouagadougou 03, Burkina Faso.
Laboratory of Organic Chemistry and Physic [3], Doctoral School of Sciences and Technology, University Joseph Ki Zerbo, 03 BP 7021 Ouagadougou 03, Burkina Faso.

Keywords:

Sub-acute toxicity, Cardiovascular effect, *Phaseolus vulgaris*, Hematology, Biochemestry

Correspondence to Author:
Belemnaba Lazare

Research Scholar,
Laboratory of Research-Development of Phytomedicines and Medicines, Department of Medicine, Traditional Pharmacopoeia and Pharmacy, Research Institute for Health Sciences 03 BP 7047 Ouagadougou, Burkina Faso.

E-mail: lbelemnaba2@gmail.com

ABSTRACT: This investigation evaluates the oral toxicity of *Phaseolus vulgaris* immature pods decoction a the biochemical, hematological, and cardiac profile of Wistar rats. **Methods:** The Economic Cooperation and Development Organization (OECD) guidelines 423 and 407 were applied. A 2000 mg/kg dose of PAD was administered to six female mice. Mortality, body weight, clinical signs of toxicity, food and water intake were recorded for 14 days. In the subacute oral toxicity test, daily doses of 100, 500, and 1000 mg/kg of PAD were administered to groups of five rats of both sexes for 28 days. Mortalities, clinical signs of toxicity, weight growth, water, and food intake were recorded throughout the period. **Results:** PAD did not cause mortality or significant clinical signs of toxicity at 2000 mg/kg bw. The LD_{50} of this extract was estimated to be 5000 mg/kg bw. All animals survived daily administration of 100, 500, and 1000 mg/kg of PAD decoction. The weight gain was comparable to that of the control groups in male and female-treated rats. Furthermore, no statistically significant hydration was observed in rats given 500 and 1000 mg/kg PAD decoction compared to the rule. No alterations in the appearance, shape, size, and color of vital organs were recognized with the naked eye, magnifying glass. Furthermore, the hematologic and biochemical profiles, systolic and diastolic blood pressure and heart rate, didn't show statistically significant variations between the control and treated groups. **Conclusion:** These toxicological experiments contribute to the determination of the effective preclinical dose and therapeutic range of PAD.

INTRODUCTION: *Phaseolus vulgaris* L., native to Mexico and the Isthmus of Central America, is an annual plant with stems that grow in bushy and climbing forms [1]. Commonly called "green bean," this plant is dextrorotary and carries alternate trimeric leaves and white papilionaceous flowers. This plant of the Fabaceae family has diuretic, anorexigenic, tonicardiac, rheumatic, anti-infectious, nutritional, energetic, and nervous system-repairing properties. Also, *P. vulgaris* is a plant rich in dietary fiber, protein, unsaturated fatty acids, and vitamins [2, 3]. The immature pods, long and narrow with variable color, green, yellow or purple, are used in Burkina Faso to treat metabolic pathologies such as gout, diabetes, obesity, kidney and heart diseases,

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(10).5050-61

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.14(10).5050-61

including hypertension (HTA) [2]. HTA, a significant risk factor for cardiovascular accidents, is the cause of many families' mourning. The WHO estimates its mortality at 9.4 million deaths per year worldwide [4]. These immature green pods are rich in carbohydrates, chlorophyll, and mineral salts (P, Si, and Ca). In addition, they are a source of vitamins A, B_1, B_2, B_3, B_9, C, E, and proteins necessary in the growth process [2, 5]. Despite many nutritional uses, cases of toxicity have been reported when the pods are consumed raw. Phytohemagglutinin (PHA), a phytoalexin specific to *P. vulgaris* L., is a lectin that is antinutritional and toxic to humans [6].

Thus, a long cooking period is mandatory to destroy PHA to take advantage of the medicinal virtues of the pods and origins of this herbaceous plant. Many people have long used the biodiversity of plants through medicinal plants to feed and heal himself. The African pharmacopeia is a cultural and economic heritage whose importance will longer be demonstrated. Transmitted from generation to generation, ancestral knowledge still practiced in Africa enjoys a good reputation thanks to the renewed confidence of the populations. Indeed, the isolation of cities, the high cost, and the lack of access to modern treatments associated with precarious economic conditions push people to return to their sources, i.e., to nature. Furthermore, the multiple adverse effects of so-called conventional drugs make them remarkably depreciated. This strategic reorientation is strongly encouraged by the WHO through establishing centers or structures for the evaluation and scientific validation of traditional medicine products and their commercial regulation [7, 8].

Existing modern treatments that have shown their limits during the coronavirus pandemic have plunged the world population into doubt and concern. Phytotherapy by exploring medicinal plants constitutes a promising alternative for researching drug candidates and new active molecules [9, 10]. The preparations of medicinal plants contain hundreds of compounds that can have antioxidant, antihypertensive, vasorelaxant, immunostimulant, immunosuppressive, anti-inflammatory, antibacterial, antiviral, and antiparasitic properties [11–13]. Although considered safe, some natural compounds can be toxic to the body because any biologically active compound can be toxic depending on the dose [14, 15]. Numerous studies have reported cases of toxicity due to the abuse of plant-based preparations. Thus, in a dynamic of research and development of phytomedicines or drugs, studies are essential to evaluate the safety of use and the harmlessness of plant extracts. Expanding standardized techniques is a real asset in this scientific validation process of endogenous knowledge's quality and therapeutic potential. The present paper aims to evaluate rats hematological, biochemical, and blood pressure parameters during the acute and subacute oral toxicity study of *P. vulgaris* immatures pods decoction.

MATERIALS AND METHODS:

Materials:

Collection of Plant Material: The immature pods of *P. vulgaris* were collected in Loumbila, located 15 km from Ouagadougou, Burkina Faso (North 12°52'88.1' and West 14°35'07.1'). An herbarium was made and registered at the herbarium of the department of plant biology of Université Joseph KI-ZERBO under the number 18018. The collected plant material was processed, dried in an enclosure, and protected from sunlight and dust with a humidity of 51.33±6.65%. The dried green immature pods were crushed with a mechanical grinder to obtain a dry powder.

Laboratory Animals: The animal facility of the Institut de Recherche en Sciences de la Santé / Centre National de la Recherche Scientifique et Technologique (IRSS/CNRST) provided the animals for the study. They consisted mainly of female NMRI mice (mean weight =23.15±3.09 g) and male and female WISTAR rats (indicate weight =183.56±8.16 g). The animals were placed in an enclosure at temperature of 25±2°C with a relative humidity of 50-70% with a cycle of 12 h of light and 12 h of darkness following the rearing conditions of this species. They had free access to water and food. The experimental steps were carried out strictly with the Guide of Good Practices in Animal Experiments procedures, according to the Declaration of Helsinki [16]. Furthermore, all experimental animal procedures have been performed according to the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health and the EU Directive 2010/63/EU for animal experiments. The study

protocol was approved by the local ethics committee of Université Joseph KI-ZERBO (Protocol number: CE-UOI/2019-04) [17].

Methods:

Preparation of the Aqueous Decoction of *P. vulgaris:* First, 500 mL of distilled water was added to a flask containing 50 g of the dry powder of *P. vulgaris* pods. The mixture was then boiled for 30 min according to the traditional preparation method. After the mixture filtration, a centrifugation step (5000 rpm for 5 min) was performed. The clear aqueous decoction was freeze-dried with a CHRIST® Type ALPHA 1-2 freeze dryer (BIO Block Scientific) equipped with a pump (Rotary Vane Vacuum Pump Type RZ2 series 21525419). The PAD is the resulting lyophilisate stored in an antiadsorbent package against humidity.

Determination of Acute oral Toxicity: The acute oral toxicity study was conducted following Economic Cooperation and Development Organization (OECD) guideline 423 on the sharp sensitive class method [18]. The PAD extract was tested using a sequential process with two (02) steps using three female mice each. Briefly, after fasting for three (03) h, mice were divided into two (02) groups of three (03) mice per cage. Group (I), serving as a negative control, received physiological water (0.9% NaCl) while a single dose of 2000 mg/kg body weight (bw) of PAD was administered to group (II) parenterally. Individual observation of the mice indicated the presence or absence of clinical signs of toxicity and mortality related to the administration of PAD throughout the study. At the end of the trial, the mice were sacrificed with dignity. Their vital organs were removed, cleaned, weighed, and macroscopically observed for lesions or morphological changes. Blood collected by cardiac puncture was used for a blood count.

Determination of Subacute oral Toxicity: The evaluation of subacute or repeated dose oral toxicity was conducted over 28 days, according to OECD Guideline 407 [19]. Twenty (20) male and twenty (20) female rats were selected for the study. Four (04) homogeneous groups of five (05) rats per sex were formed. The control groups (I) male and female components were administered physiological water (NaCl 0.9%). One hundred (100), 500, and 1000 mg/kg bw of PAD were administered daily orally to the other three groups identified as groups II, III, and IV, respectively. Furthermore, toxicity signs and symptoms were routinely recorded according to their frequency of occurrence throughout the study. In addition, the animal's daily water consumption, feed consumption, and body weight were recorded. Specifically, changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) of each rat were recorded weekly.

Recording of Blood Pressure Parameters: Blood pressure training sessions were conducted for one week before the 28-day repeat dose toxicity study started. Blood pressure recordings were made with a non-invasive cuffed plethysmograph (Blood Pressure Recorder System 58500, Ugo Basile) [17]. Each rat was placed in a compact thermal chamber (37 °C) using an individual holder for 30 min. After this thermal regulation phase, the plethysmography cuff-tail method was applied for blood pressure measurement. The average blood pressure value was calculated after at least three correct sizes, excluding noise or animal agitation outliers.

Biochemical and Hematological Analyses: After fasting for 12 h, the animals were anesthetized with ketamine (50 mg/kg bw) and then sacrificed. For each animal sacrificed, blood was collected by cardiac puncture and then introduced into two (02) tubes: a dry line with a coagulation activator and a tube containing K_3 EDTA as an anticoagulant. A biochemical and hematological assessment was performed for each blood sample.

The biochemical test involved parameters such as blood glucose (GLU), blood urea (Urea), blood uricemia (AU), blood creatinine (CREAT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), chloraemia (Cl^-), natremia (Na^+), kalaemia (K^+), calcaemia (Ca^{2+}), cholesterol (CHO), High-Density Lipoprotein-cholesterol (HDL-C), Low-Density Lipoprotein-cholesterol (LDL-C), triglycerides (TG), and total protein (TP). These different parameters were analyzed with the ROCHE COBAS 411E automaton in serum obtained after centrifugation of dry tubes at 3000 rpm for 5 min. The hematological study consisted

mainly of blood cell count (CBC) parameters. Red bloodline parameters were red blood cell count (RBC), hemoglobin (Hb), mean corpuscular volume (MCV), hematocrit (HCT), mean corpuscular hemoglobin content (MCHC), and mean corpuscular hemoglobin concentration (MCHC). For the white blood cell line, leukocytes or white blood cells (WBC), neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosinophils (EOS), and basophils (BAS) were counted. As for the platelet lineage, the number of platelets or thrombocytes (PLT) was determined. The XN-1000^{TM} hematology analyzer of the SYSMEX brand allowed counting the different symbolic elements of the blood collected in K_3 - EDTA tubes.

Necropsy and Relative Weight of Noble Organs: After dissection in the ulnar position and blood collection, the liver, kidneys, lungs, spleen, heart, and gonads were isolated, cleaned, and macroscopically observed for abnormalities in shape, size, or color. Each organ was weighed on a Sartorius balance (accuracy = 0.1 mg) to determine the relative weight.

Statistical Analysis: The data recorded during the tests were organized and processed using the Microsoft Excel spreadsheet. Results were expressed as mean ± standard deviation. Statistical analyzes were conducted by one-way ANOVA followed by the Bonferroni *post hoc* test to determine the significance of the observed variations between the experimental groups. For this purpose, the Graph Pad Prism 8.0.1 (244) software for Windows was used. A p-value of less than 0.05 was considered statistically significant.

RESULTS:

Oral Acute Toxicity: The results of acute oral toxicity showed no mortality from the administration of the PAD decoction at a single dose of 2000 mg/kg bw during the 14-day study period. No toxicity or unusual ethological changes were observed during the study period. **Table 1** provides information on the observations of mortality and symptoms of toxicity observed during this short-term toxicity. According to the guidelines prescribed by OECD line 423, the 50% lethal dose (LD_{50}) of the PAD extract could be estimated at 5000 mg/kg bw.

TABLE 1: MORTALITY AND CLINICAL SIGNS DURING THE ACUTE ORAL TOXICITY STUDY OF PAD

Recording		
	1st step	2nd step
NaCl (0.9%)	0/3	0/3
PAD (2000 mg/kg bw)	0/3	0/3
Excitement	-	-
Drowsiness	-	-
Hairstraightening	-	-
Lack of appetite	-	-
Diarrhea	-	-
Vomiting	-	-
Hyperventilation	-	-

Evolution of Weight Growth: Fig. 1 shows the growth curves of body weight on the mice included in the acute oral toxicity study (14 days). A graphical analysis reports a weight gain in mice treated with a single dose of 2000 mg/kg bw of PAD extract. This result was evidenced by an almost vertical rise (during the first three days) in the body weight curves. This positive variation in body weight of the treated group was not statistically significant compared to the control group that received 0.9% NaCl ($p>0.05$).

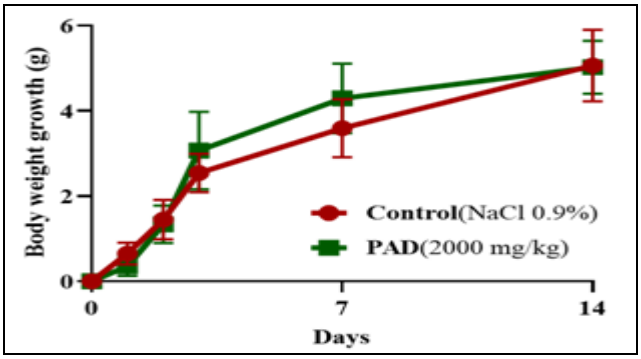


FIG. 1: BODY WEIGHT GROWTH CURVES OF MICE DURING ACUTE TOXICITY. PAD = *PAD= Phaseolus vulgaris* aqueous decoction

Daily Water and Food Consumption during the Acute Oral Study: The histograms in **Fig. 2A** and **2B** represent the average daily food and water consumption of the mice in the study, respectively. On the one hand, mice who received the single 2000 mg/kg dose of PAD orally consumed 8.27±0.92 g of food, while those in the NaCl-fed group ate 7.39±0.40 g daily **Fig. 2A**. Analysis of the daily feed intake of the study animals did not show statistically significant changes after 14 days of study ($p>0.05$). On the other hand, the graphical analysis in **Fig. 2B** shows that the histograms representing the average daily water consumption

of mice given the PAD extract at a single dose of 2000 mg/kg bw are higher than those of mice in the control group. Statistical analysis reports that this difference is significant ($p<0.05$).

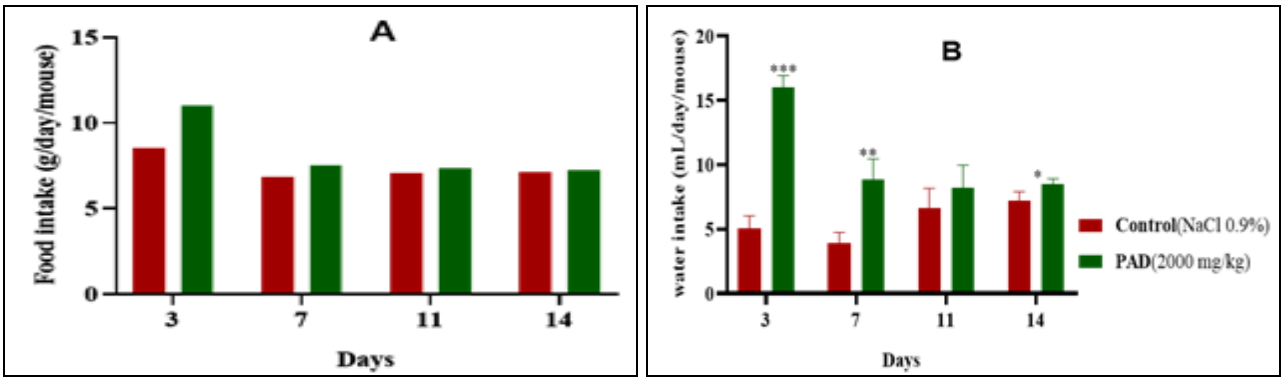


FIG. 2: HISTOGRAM OF THE AVERAGE FOOD AND WATER CONSUMPTION OF MICE DURING THE ACUTE ORAL TOXICITY STUDY. PAD = *Phaseolus vulgaris* aqueous decoction. Note: $p<0.01$; *$p<0.05$ (PAD versus control).

Relative Vital Organ Weights of Animals in the Acute Oral Study: The autopsy performed on vital organs such as lungs, liver, heart, spleen, and kidneys did not reveal any changes in shape, color, or suspicious inclusions to the naked eye or with a magnifying glass. The analysis of **Table 2** below, representing the values of the average relative weight of the vital organs, showed no statistically significant difference between the two groups ($p>0.05$).

TABLE 2: RESUME OF THE VITAL ORGAN RELATIVE MEAN WEIGHT OF MICE AT ACUTE TOXICITY

	Organ relative mean weight (g/100 g) (mean±SD)				
	Lungs	**Liver**	**Heart**	**Rate**	**Kidneys**
Control (NaCl 0.9%)	0.51±0.10	5.74±1.40	0.46±0.09	0.49±0.09	0.98±0.16
PAD (2000 mg/kg bw)	0.62±0.16	4.90±0.16	0.45±0.06	0.54±0.13	1.14±0.11

Hematological Parameters of Animals at the end of the Acute Oral Study: The mean values of the blood counts are given in **Table 3** below. The table shows that oral administration of PAD extract at a single dose of 2000 mg/kg bw was not responsible for any abnormalities in peripheral blood cells. Thus, data analysis did not report a statistically significant decrease or increase in red blood cell parameters, leukocytes, and their subpopulations, and platelets were written by the data analysis ($p>0.05$).

TABLE 3: HEMATOLOGICAL PARAMETERS OF MICE IN THE ACUTE STUDY

Hematological parameters	NaCl 0.9% (0.5 mL/kg)	PAD (2000 mg/kg)
RBC (10^6 /µL)	8.85±0.57	9.55±0.11
Hb (g/dL)	13.66±1.36	15.33±0.51
VGM (fL)	56.00±2.68	54.00±2.68
HCT (%)	50.00±5.36	51.66±1.36
WBC(10^3 /µL)	3.60±0.30	3.33±1.11
NEU(10^3 /µL)	1.45±0.17	1.33±0.44
LYM(10^3 /µL)	1.72±0.14	1.59±0.53
MON(10^3 /µL)	0.33±0.02	0.30±0.10
EOS(10^3 /µL)	0.08±0.01	0.06±0.02
BAS(10^3 /µL)	0.03±0.00	0.03±0.01
PLT (10^3 /µL)	413.66±81.39	444.66±20.21

RBC: Red Blood Cell; Hb: Hemoglobin; MGV: Mean Blood Cell Volume; WBC: White Blood Cell; PLT: Platelet; HCT: Hematocrit; NEU: Neutrophil; LYM: Lymphocyte; MON: Monocytes; EOS: Eosinophil; BAS: Basophil.

Sub-acute Toxicity: All male and female rats survived for 28 days in the repeat-dose toxicity study. Daily oral administration of PAD decoction at 100, 500, and 1000 mg/kg bw to male and female rats in Groups II, III, and IV, respectively, did not adversely affect their habitual behavior. No clinical signs of toxicity were recorded during the observation periods. Doses of 100, 500, and 1000

mg/kg were determined based on the LD_{50} of PAD which was estimated to be 5000 mg/kg.

Weight Growth of Rats: Fig. 3A and **3B** show the weight gain curves for male and female rats during the sub-acute oral toxicity study. Positive changes in body weight were observed during the 28 days of the study in both the control group (0.9% NaCl) and rats treated with the PAD decoction at 100, 500, and 1000 mg/kg, respectively, groups II, III, and IV. Specifically, in male rats, at 28 days, the average gain in body weight was 55.80±5.12 g for the control group compared to 48.40±4.22 g, 54.20±2.77g, and 49.40±2.30 g for the treated groups II (100 mg/kg), III (500 mg/kg) and IV (1000 mg/kg) respectively. For rats, an average weight growth of 30.80±5.89 g was observed in the control group, followed by 35.20±3.96 g, 31.00±4.36 g, and 33.20±3.11 g, respectively, for groups II, III, and IV.

Analysis of the figures shows that there was no statistically significant difference in weight gain in the treated groups (II, III, and IV) compared to the control for animals of both sexes ($p>0.05$).

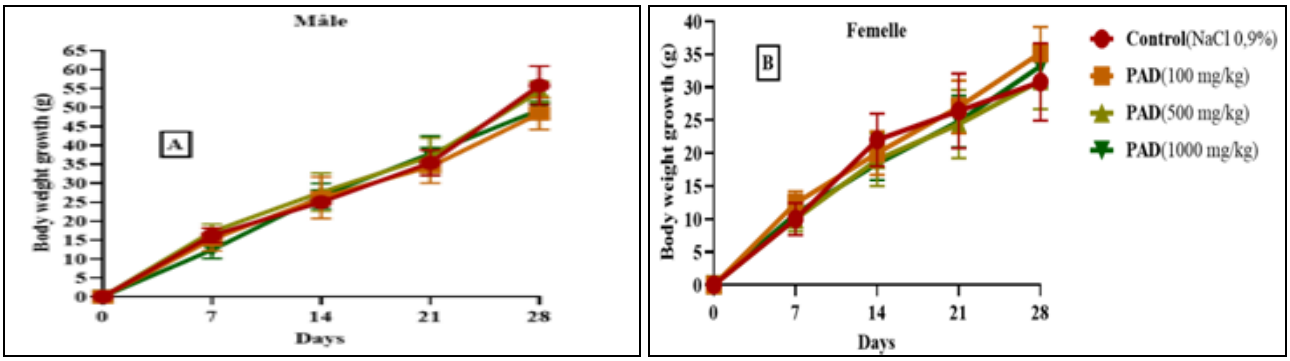
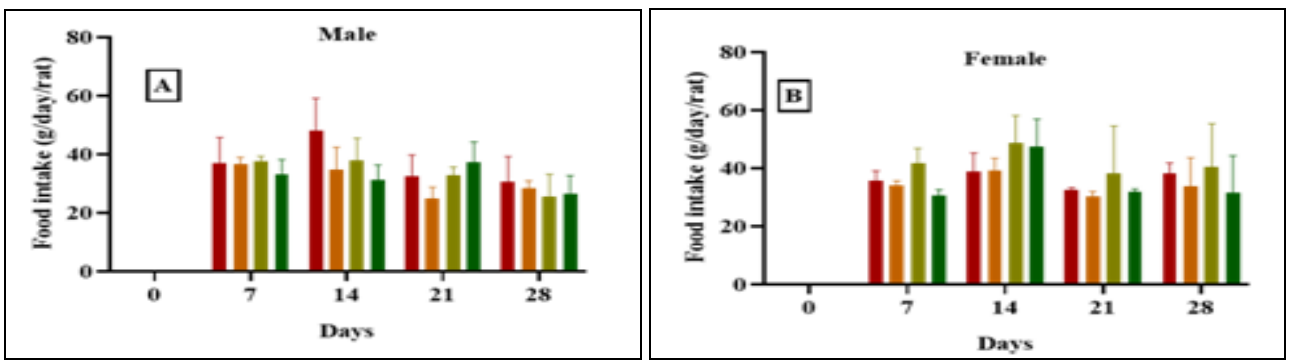


FIG. 3: GROWTH CURVE OF BODY WEIGHT OF THE STUDY ANIMALS

Food and Water Consumption: During the study, rats took various amounts of water and food. Regarding food intake, no unusual changes in appetite were observed in the rats. The quantities of food consumed by the animals given the PAD decoction, particularly groups II, III, and IV, Did not varied significantly compared to the control group **Fig. 4A** and **4B**.

No statistically significant differences were found in the data analysis ($p>0.05$). During the 28 days of the acute oral toxicity test, the average weekly water intake was recorded for all randomized rats. No changes in water intake were observed in male rats in the treated groups compared to the control ($p>0.05$). On the contrary, there was a notable change in water intake in female rats. Significant difference was observed until day 21 of the study in all rats in the treated and control groups. Interestingly, an increase in drinking water consumption was observed in rats gavaged with PAD decoction at 500 and 1000 mg/kg bw compared to the control group during the last week.

The results showed that this change in water intake was statistically significant for both groups involved. The p-value was less than 0.05 for group II treated with 500 mg/kg of PAD and less than 0.01 for rats given 1000 mg/kg bw.



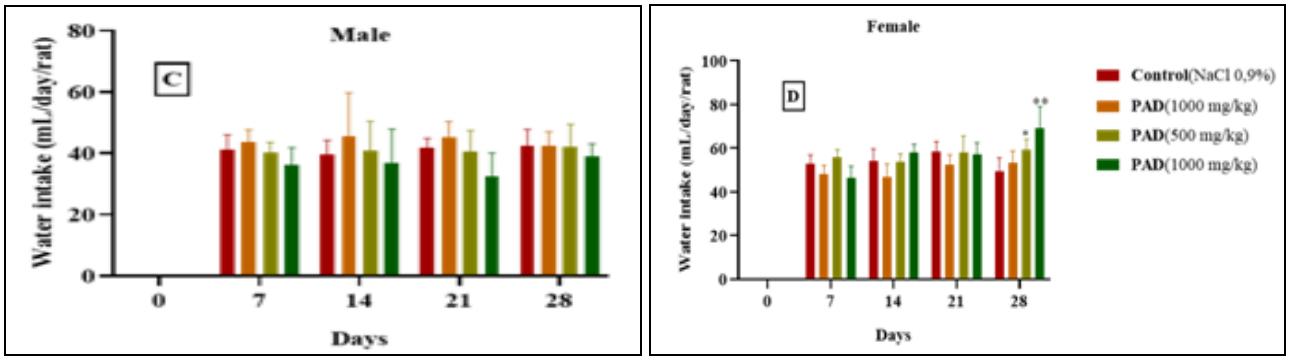


FIG. 4: HISTOGRAM OF FOOD AND WATER CONSUMPTION OF RATS IN THE STUDY. PAD = *Phaseolus vulgaris* aqueous decoction. Note: ** p<0.01; (PAD 100 versus control); *p<0.05 (PAD 500 versus control);

Impact of PAD Extract on Blood Pressure Parameters: Recording of blood pressure parameters such as systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) allowed monitoring of the heart pool of each animal throughout the study. This investigation found that daily administration of PAD at doses of 100, 500, and 1000 mg/kg did not significantly influence the changes in rats' SBP, DBP, and HR. The plots in **Fig. 5A**, **5B, 5C, 5D, 5E**, and **5F** show the impact of the PAD extract on SBP, DBP, and HR of male and female rats during the study of subacute oral toxicity. Statistical analysis did not report a significant difference between the PAD-treated and control groups (p>0.05).

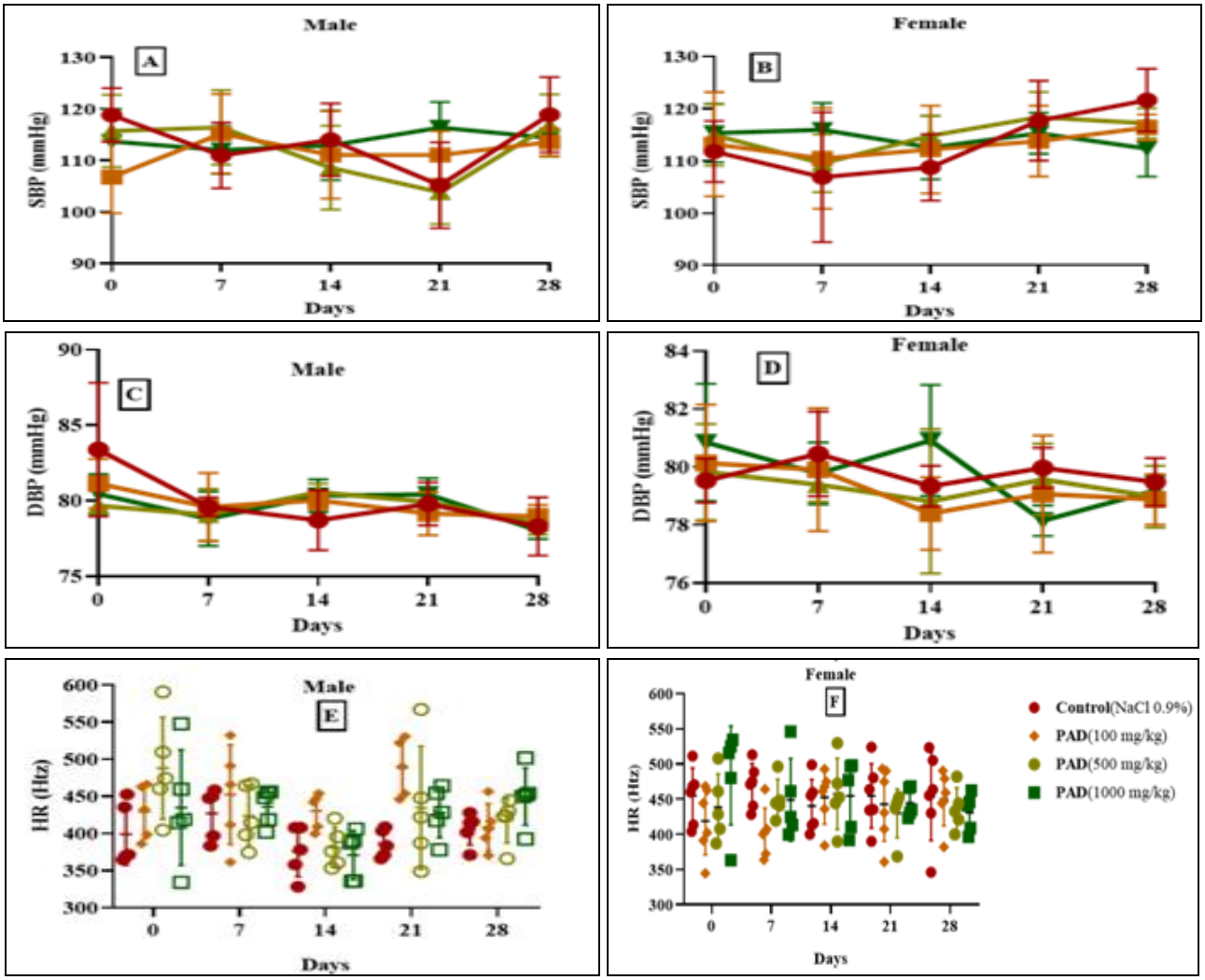


FIG. 5: IMPACT OF *PHASEOLUS VULGARIS* AQUEOUS DECOCTION EXTRACT OF BLOOD PRESSURE PARAMETERS. SBP = Systolic blood pressure, DBP = Diastolic blood pressure, HR= Heart rate.

Evolution of Biochemical and Hematological Parameters: Table 4 shows the biochemical balance parameters performed in male and female rats at the end of the subacute toxicity study of PAD decoction. The results indicate that PAD did not induce significant changes in the biochemical parameters analyzed from the treated rats compared to the respective controls. Specifically, the transaminases of the groups treated with 100, 500, and 1000 mg/kg of PAD. ALT, AST, and GGT did not show significant variations compared to the control group. Similarly, the lipid balance composed of CHO, HDL, LDL, and TG showed a similar evolution for all animals in the study.

The values of the CREA, URE, AU, PT, and GLU parameters of the treated rats did not show pathological changes compared to the respective controls. Furthermore, the ionic values of the treated groups, such as Ca^{2+}, Cl^{-}, Na^{+}, and K^{+}, showed almost similar changes compared to the control groups. Statistical analysis of the results in the table indicates no significant difference between the groups treated with PAD 100, 500, and 1000 mg/kg and the controls.

Hematological Parameters: As shown in **Table 5**, the absolute values of blood cells, red blood cells, white blood cells, and platelets in the treated groups did not show suspicious variations that could cause a disturbance in the normal function of these cells. More importantly, PAD treatment contributed to a slight improvement in the values of the blood's red, white, and platelet people. No statistically significant differences were found between treated rats and their respective control in hematological parameters during the 28-day intervention period.

The Relative Weight of Vital Organs: Table 6 presents the results of the average relative weights of the noble organs of the animals in the different study groups. Analysis indicates that the PAD extract was not responsible for any pathological variation in the macroscopic observation of the vital organs with the naked eye and magnifying glass. No changes in the appearance, texture, size, or color of the liver, lungs, heart, spleen, kidneys, and gonads were observed in any of the animals used in this 28-day repeated dose oral toxicity study.

TABLE 4: BIOCHEMICAL PARAMETERS OF RATS AFTER THE SUBACUTE ORAL TOXICITY TEST OF PAD

	Substances							
	NaCl 0.9% (0.5mL/kg)		PAD 100 mg/kg bw		PAD 500 mg/kg bw		PAD 1000 mg/kg bw	
	Male	Female	Male	Female	Male	Female	Male	Female
ALT (IU/L)	38.34±6.19	33.46±3.01	20.77±0.78	20.97±1.52	29.44±3.35	38.81±0.79	31.46±2.45	29.24±0.55
AST (IU/L)	161.62±12.60	173.71±24.23	143.37±10.86	189.95±2.90	128.63±8.02	183.35±14.48	129.53±6.03	197.74±4.35
Ca^{2+} (mmol/L)	2.72±0.09	2.64±0.05	2.76±0.06	2.54±0.08	2.76±0.09	2.64±0.03	2.70±0.08	2.56±0.04
CHO(mmol/L)	1.02±0.13	1.63±0.21	1.29±0.07	1.34±0.09	1.37±0.08	1.30±0.08	1.15±0.05	1.91±0.03
Cl^{-} (mmol/L)	105.83±1.51	105.50±1.48	104.39±0.58	107.22±1.22	102.83±1.49	106.24±3.23	103.99±1.91	109.30±2.09
CREA(µmol/L)	65.08±1.71	73.96±3.52	63.63±3.85	68.52±4.70	58.23±2.09	74.21±1.76	67.04±1.67	65.27±1.24
GLU (mmol/L)	8.42±0.90	5.61±0.67	7.82±0.40	5.35±0.23	7.76±0.11	8.06±0.75	8.36±0.28	5.18±0.09
HDL (mmol/L)	0.52±0.09	0.90±0.15	0.70±0.05	0.68±0.03	0.73±0.03	1.03±0.17	0.52±0.01	0.95±0.01
K^{+} (mmol/L)	8.55±1.37	6.81±0.67	5.59±0.57	7.18±0.91	5.76±0.61	7.44±0.65	6.39±0.46	5.75±0.11
Na^{+} (mmol/L)	145.27±3.86	135.30±0.82	145.22±0.96	135.22±1.56	140.72±1.78	134.83±2.75	135.68±2.44	137.55±2.63
TG (mmol/L)	0.25±0.04	0.34±0.03	0.24±0.01	0.27±0.02	0.26±0.03	0.22±0.02	0.23±0.02	0.38±0.00
AU (mmol/L)	161.27±34.14	104.31±11.57	99.03±11.53	143.41±37.45	68.61±6.18	134.55±24.53	100.79±18.68	114.85±2.19
UREA(mmol/L)	10.07±0.72	10.39±0.44	10.31±0.96	8.92±0.68	8.97±0.08	9.05±0.71	8.34±0.29	8.56±0.16
TP (g/L)	57.53±2.98	53.75±1.73	54.34±2.77	65.72±1.84	53.84±2.51	59.25±1.11	58.95±1.21	59.90±1.14
GGT (IU/L)	7.35±0.92	6.89±0.66	4.72±0.63	8.21±0.57	6.99±0.84	7.78±0.42	6.77±0.46	8.31±0.15
LDL (mmol/L)	0.38±0.05	0.57±0.07	0.48±0.04	0.53±0.05	0.52±0.09	0.54±0.04	0.52±0.05	0.78±0.01

ALT: alanine aminotransferase; AST: aspartate aminotransferase; CA: calcium; CHO: cholesterol; CL: chloride; CREA: creatinine; GLU: glucose; HDL-C: high-densitylipoprotein-cholesterol; K: potassium; NA: sodium; TG: triglycerides; AU: uricacid; UREA: urea; TP: total protein; GGT: gamma-glutamyl transferase; LDL-C: low-densitylipoprotein-cholesterol.

TABLE 5: ABSOLUTE VALUES OF HEMATOLOGICAL PARAMETERS OF RATS AFTER THE STUDY PERIOD

	Substances							
	NaCl 0.9%(0.5mL/kg)		PAD 100 mg/kg bw		PAD 500 mg/kg bw		PAD 1000 mg/kg bw	
	Male	Female	Male	Female	Male	Female	Male	Female
RBC (10^{6} /µL)	8.53±0.32	8.27±0.18	8.91±0.18	8.49±0.16	9.04±0.16	8.08±0.15	9.32±0.20	8.86±0.35
Hb(g/dL)	15.38±0.51	14.55±0.39	15.66±0.51	15.06±0.28	15.95±0.25	14.68±0.28	16.28±0.38	16.14±0.46
HCT (%)	49.96±1.50	48.84±1.57	48.46±1.43	46.78±0.87	49.07±0.69	45.60±0.76	50.14±1.23	49.06±1.32

VGM (fL)	58.68±1.00	58.74±1.58	54.34±0.62	55.08±0.64	53.77±1.24	56.44±0.18	53.22±0.64	55.56±1.63
MCHT (µg)	18.04±0.31	17.49±0.24	17.56±0.22	17.74±0.22	17.46±0.40	18.16±0.05	17.26±0.20	18.26±0.36
CCMH (%)	30.80±0.38	29.65±0.43	32.28±0.25	32.20±0.09	32.19±0.31	32.20±0.10	32.15±0.33	32.90±0.30
GB (10^3 /µL)	3.86±0.87	2.01±0.38	4.59±0.85	3.68±0.95	3.52±0.54	3.62±0.36	4.68±0.84	2.98±0.32
NEU (10^3 /µL)	1.22±0.42	1.61±0.06	1.65±0.31	1.01±0.33	1.03±0.12	1.25±0.14	1.05±0.31	1.68±0.13
LYM (10^3 /µL)	2.04±0.44	1.70±0.29	2.33±0.51	2.24±0.5	1.91±0.37	2.91±0.23	2.78±0.43	1.80±0.37
MON (10^3 /µL)	0.39±0.08	0.10±0.00	0.29±0.05	0.32±0.17	0.22±0.02	0.20±0.03	0.54±0.18	0.39±0.10
BAS(10^3 /µL)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
EOS (10^3 /µL)	0.20±0.06	0.10±0.03	0.42±0.08	0.10±0.02	0.36±0.07	0.26±0.02	0.30±0.18	0.11±0.02
PLT (10^5 /µL)	6.44±0.32	6.09±0.86	7.68±0.32	8.27±0.43	7.34±0.27	7.14±0.53	7.24±0.37	7.82±0.40

RBC: Red Blood Cell; Hb: Hemoglobin; MGV: Mean Blood Cell Volume; WBC: White Blood Cell; PLT: Platelet; HCT: Hematocrit; NEU: Neutrophil; LYM: Lymphocyte; MON: Monocytes; EOS: Eosinophil; BAS: Basophil.

TABLE 6: RELATIVE VITAL ORGAN WEIGHTS OF RATS IN THE SUBACUTE ORAL STUDY

Organs	Relative organs mean weight (g/100 g) (mean ±SD)							
	NaCl 0.9% (0.5 mL/kg)		PAD 100 mg/kg bw		PAD 500 mg/kg bw		PAD 1000 mg/kg bw	
	Male	Female	Male	Female	Male	Female	Male	Female
Liver	2.68±0.11	2.31±0.57	2.59±0.10	2.89±0.22	2.34±0.07	2.93±0.12	2.36±0.05	2.64±0.23
Heart	0.35±0.01	0.34±0.09	0.41±0.03	0.40±0.02	0.43±0.03	0.38±0.02	0.41±0.03	0.40±0.04
Lungs	0.61±0.01	0.59±0.15	0.63±0.01	0.72±0.05	0.61±0.03	0.64±0.04	0.60±0.02	0.69±0.05
Rate	0.31±0.03	0.22±0.05	0.29±0.03	0.32±0.02	0.25±0.01	0.32±0.03	0.25±0.01	0.23±0.01
Kidneys	0.75±0.02	0.64±0.16	0.75±0.02	0.62±0.09	0.71±0.01	0.77±0.08	0.68±0.01	0.74±0.24
TEST/Ovaries	1.39±0.23	0.05±0.01	1.42±0.02	0.06±0.00	1.44±0.12	0.06±0.00	1.33±0.11	0.05±0.00

DISCUSSION: In recent years, increasing attention has been paid to natural products and herbal medicines. This use is partly due to the adverse effects associated with allopathic treatments [20–22]. There is a strong demand for natural products considered non-toxic and culturally used by the population. With the aim of standardization, several lines of research are being developed to provide tools for the scientific validation of these products of natural origin to improve the treatment of infectious diseases and metabolic disorders [23, 24]. Many medicinal plants and natural products, although having therapeutic properties, have shown pronounced toxicity at a certain dose [14]. Therefore, determining lethal doses or concentrations and toxicological parameters is essential to guarantee the safety of using these natural products [25]. The study of acute oral toxicity makes it possible to check a chemical product's harmful and dangerous effects on a living organism at the time of exposure with a strong dose for a short time [26]. In the acute oral toxicity experiments of PAD decoction, the single dose of 2000 mg/kg bw provided to mice did not result in mortality or unusual ethological behaviors. The absence of lethal effects and mortality in mice in the acute oral toxicity test at 2000 mg/kg bw of PAD. This result indicates that the LD_{50} of this extract could be estimated at 5000 mg/kg bw regarding the OECD guideline 423 [18]. This record suggests that PAD could be considered a low acute hazard under the United Nations Globally Harmonized System of Chemical Classification and Labelling [18]. Furthermore, administration of the PAD extract did not induce weight loss in treated mice. Interestingly, a positive weight growth similar to that of the control group was observed throughout the test. No significant differences were found in food consumption, relative organ weight, and hematological parameters of the mice randomized to the study. Additionally, mice treated with PAD decoction observed a conspicuous water intake. This difference in water consumption was statistically significant compared to the control. This hyperhydration would probably be due to the aspect of the extract that requires a certain amount of water for the digestion of plant fibers. Thus, this observation does not constitute evidence of toxicity since the intake of large amounts of water helps to reduce the formation of kidney stones, bladder infections, and urolithiasis [27, 28]. The determination of LD_{50}, although essential for the scientific validation process and the determination of the preclinical therapeutic doses, must be completed by a repeated dose toxicity test to decrease probable functional disorders in the medium. With this in mind, daily oral administration of PAD extract was performed at 100, 500, and 1000 mg/kg bw to healthy normotensive male and female rats for 28 days. Rats of both sexes divided into groups I

(NaCl), II (100 mg/kg), III (500 mg/kg), and IV (1000 mg/kg) all survived the 28-day daily treatment without clinical signs of local or systemic toxicity. No significant effects on body weight were observed during the treatment period. At the same time, no significant differences in food intake or gross aberrations in the morphology, size, color, and appearance of vital organs observable with the eye and magnifying glass during necropsy were reported. Previous studies have shown that the hematopoietic system is one of the best targets for toxic chemicals. These hematopoietic parameters determine the physiological and pathophysiological state in humans and animals [29]. Thus, the blood cell count could be a crucial indicator for detecting probable intrusion of toxic substances. The absolute values of blood cells, such as red blood cells, white blood cells, and platelets of rats after treatment with different doses of 100, 500, and 1000 mg/kg, showed no aberrant variations. This investigation indicates that the PAD extract did not induce any pathological condition such as anemia or dehydration for hemoglobin, mean corpuscular volume, and hematocrit values.

Furthermore, no abnormalities similar to leukopenia, infection, or inflammatory reaction were diagnosed by the values of white blood cells and their subpopulations. The absolute platelet count showed the absence of thrombocytopenia. No hematological abnormalities were analyzed from the results of the blood count. The liver's indispensable role in the body's metabolic processes ensures detoxification and elimination of dangerous compounds to maintain homeostasis [30]. ALT, AST, and GGT values did not show any pathological variation that could suggest dysfunction or a liver alteration. Likewise, lipid parameters such as CHO, HDL, LDL, and TG were within the standard range. These parameters indicate the proper functioning of lipid metabolism. High CHOL, LDL, and TG concentrations are frequently associated with atherosclerotic plaque formation and atherosclerosis, contributing to increased cardiovascular events [31, 32]. Our results corroborate those of Ganesan *et al.* (2017), who showed that *P. vulgaris* contains phytosterols that could reduce LDL cholesterol [33]. The ions Na^+, K^+, Cl^-, and Ca^{2+} ensure in the organism the transmission of the nervous impulse, the frequency of cardiac beats, the levels of systole and diastole in the heart, and the vascular tonus. These ions ensure the exchanges between the blood and the kidney while assuring hydrous homeostasis and the solutes [34]. Elevation or decrease of these ions generates spasmogenic anomalies, vasoconstrictors of the smooth muscles, convulsive nervous disorders, an elevated severe nervous disease, a formal explosive jumpy disorder, and especially a high tumultuous nervous condition, and incredibly elevated blood pressure [35, 36]. This situation would significantly increase the concentration of CREA, URE, AU, and PT, contributing to kidney dysfunction. The chronicity of such a phenomenon would lead to the appearance of renal failure and heart congestion [37]. In this study, the PAD extract did not contribute to an aberrant variation of Na^+, K^+, Cl^-, and Ca^{2+}. No significant increase in CREA, URE, and AU was also detected. This extract would be safe for the liver, heart, and kidneys. Glucose concentrations are commonly used for the early diagnosis of diabetes [38]. Numerous studies have shown the use of medicinal plants to prevent and manage diabetes by stimulating insulin production [39].

PAD decoction did not cause an increase in blood glucose levels in the treated groups of rats of both sexes. Our results corroborate those of Moreno-Garcia et al. who showed that *P. vulgaris* seeds could prevent the occurrence of hyperglycemia [40]. Blood pressure measurement is a common practice to monitor blood pressure changes and establish the presumptive diagnosis of the development of hypertension [41]. PAD extract did not negatively influence blood pressure in male and female rats treated compared to the control. No significant systolic, diastolic, or heart rate abnormalities were diagnosed during the study. These results could be explained by maintaining the ionic balance Na+, K+, Ca2+, Cl- and the absence of cardiomegaly during the study [42, 43]. These observations suggest that the PAD extract would not disturb blood pressure in normotensive subjects. Furthermore, phenolic compounds, plant fibers, and phytosterols in immature pods could play a cardioprotective role. These compounds reduce the risk of developing cardiovascular diseases, arterial hypertension, atherosclerosis, and myocardial infarction.

CONCLUSION: The present toxicological investigation reports that the PAD extract could be

classified as a natural product unlikely to cause a dangerous toxic effect at a dose of 2000 mg/kg bw in the WISTAR rat. Oral ingestion of this extract would not cause pathological changes in blood cells, biochemical balance, systolic and diastolic blood pressure, and heart rate in healthy normotensive subjects. Additionally, cytotoxicity and anatomopathological studies are necessary to confirm the tolerance of this extract at the cellular level.

ACKNOWLEDGMENTS: The authors thank the West African Researcher Association (WARA) for the granting of a scientific mobility scholarship and the Department of Physiology of the Institute de la Jeunesse et de l' Education Physique et Sportive of Benin.

Funding: Not applicable

CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest.

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How to cite this article:
Ouedraogo WRC, Belemnaba L, Nitiema M, Kaboré B, Compaoré S, Koala M, Semde R and Ouedraogo S: Safety use of *Phaseolus vulgaris* L. immature pods decoction and cardiovascular effect in rat without hypertension. Int J Pharm Sci & Res 2023; 14(10): 5050-61. doi: 10.13040/IJPSR.0975-8232.14(10).5050-61.