



Effects of Repeated Administration of Extracts from *Arachis hypogaea* Hulls on Blood Parameters and Histological Organization of Heart, Liver and Kidneys of Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors DBN and DMAK designed the study. Author DBN performed the statistical analysis, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Authors DMAK and OMD managed the histological study. Authors TBIO and GGL helped for the animal experimentation. Author KAKM managed the proofreading of the manuscript. Author DAJ assisted in the design of the study and helped in hematological and biochemical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Arachis hypogaea* (peanuts) is widely used in food worldwide. Therapeutic use of various parts of this plant has been mentioned in many traditional medicinal systems. The aim of the present study was to evaluate subacute toxicity of methanolic and aqueous extracts of peanuts hulls.

Place and Duration: Laboratory of Biochemical Pharmacodynamics, Faculty of Biosciences, University Félix Houphouët-Boigny, from March to June 2018.

Methodology: Serial extraction was done using methanol and water as solvents. The study was evaluated by orally daily doses of extracts 300, 600 and 1200 mg/kg. The treatment for 28 days concerned seven groups of animals, the control group and six treated groups. Each group included six animals, 3 males and 3 females. Animals of control group didn't have any treatment. Animals were observed for general behavioural and signs of abnormalities during the experiment. After all treatments, blood was collected for haematological and biochemical analysis. Liver, kidney, and heart were removed, weighed for histological study.

Results: The results showed that, there were not any significant ($p > 0.05$) changes in both the absolute and relative organ weights between the control and the test groups. Biochemical parameters were statistically equal in all groups. In addition, both extracts did not induce any significant effect on RBC and indices relating to it (HGB, PCV, MCV, MCH and MCHC) throughout the experimental period. But, there was a decrease (16.33 ± 1.68) on WBC with methanolic extract compared to control (13.79 ± 2.73). Histological examination of the liver, kidneys, and the heart showed normal organisation and structure of heart, kidneys and liver.

Conclusion: It appears that the methanolic and aqueous extracts of hull of *Arachis hypogaea* did not produce any toxicity in oral subacute toxicity study. However, further studies are needed to confirm long term toxicities.

Keywords: Subacute; *Arachis hypogaea* hulls; haematological; blood parameters; histological study.

1. INTRODUCTION

Arachis hypogaea or peanut is a widely consumed food plant in the world. Its great nutritional value comes from the richness of its seeds in fat, proteins and vitamins. According to some authors, this plant equally possesses therapeutic virtues. *Arachis hypogaea* is active against some diseases, among which those concerning the liver [1,2,3]. Unroasted and dried seeds, rich in vitamins A, B1 and B2 are nutritious, and are recommended for the overworked or suffering from asthenia.

Moreover, this plant is recognized for its astringent intestinal value during diarrhoea. In addition to eating disorders, it is recommended for heart regulation. The frequent consumption of peanut is associated with a lower risk of incident hypertension, as mentioned by US male physicians [4,5]. The peanut pod consists of the seeds wrapped in hulls. The Hulls are by-products of peanut culture and account for about 1/3 of the pod by weight. Considering the great world production of groundnuts (more than 46 million tons in 2019 [6], hulls of this legume represent an important source of vegetable matter increasingly used in many fields: animal feed, fertilizers [7], biological filter holders [8], cosmetics ect.

Chemically, the hulls of *Arachis hypogaea* contain proteins, sugars, mineral salts, lipids, but mainly (more than 70%) lignocellulosic compounds [9,10]. In addition, these hulls contain many secondary metabolites including polyphenols [11] safe for human consumption [12]. Yu et al. [13] have isolated from hulls luteolin, carotene, isosaponarene. Polyphenols give peanut shells many therapeutic properties due to their strong antioxidant capacities [14]. However, the presence of other metabolites, particularly alkaloids, in addition to probable pharmacological activities, indicates a toxicological risk.

Traditionally, hulls are prescribed in decoction or maceration against high blood pressure by tradipratitians in Côte d'Ivoire [15] and in Benin [16]. In addition, urinary schistosomiasis is treated with an extract from the maceration of hulls of roasted seeds [17]. These hulls are often used and their level of toxicity is unknown. It is then imperative to study their toxicity in order to inform users (prescribers and consumers) about an eventual involved risk.

Therefore, the aim of this work is to determine the effects of repeated administration of aqueous and methanolic extracts from hulls of *Arachis hypogaea* on haematological and biochemical parameters and its incidence on the

structure of the heart, liver and kidneys of rats by the histological study.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant material

The plant material consists of hulls of *Arachis hypogaea*. The pods of *Arachis hypogaea* were harvested at Aboudé-Mandéké (Agboville, Côte d'Ivoire). The plant was identified at the National Floristic Center of Felix Houphouët Boigny University (Côte d'Ivoire). The harvested pods were washed, dried and shelled manually to obtain hulls.

2.1.2 Animal material

The animals used were 8- to 12-week-old white Wistar rats. Animals were raised and maintained in hard plastic cages containing wood chips renewed every 3 days. They were fed ad libitum with commercial animal foods. The rats were acclimatized to the laboratory conditions (25 to 27°C, with a 12 hours light-dark cycle) for 7 days. Then all the animals were divided into seven groups. Each group is constituted of three males and three females. They were fasted for 18 hours before administration of the extract by gavage. The animals were deprived of food but not water.

2.2 Methods

2.2.1 Preparation of aqueous and methanolic extracts of *Arachis hypogaea* hulls

✓ Aqueous extract

One hundred grams of hulls were boiled for 30 minutes in 1.5 L distilled water. After boiling, hydrophilic cotton filtration provided a decoction which was concentrated under reduced pressure using a Büchi-type rotary evaporator at 60°C and then oven dried at 50°C for 48 hours. The resulting powder designed aqueous extract (AHAq) was weighed and the yield calculated. This extract was used for the different tests.

✓ Methanolic extract

One hundred grams of *Arachis hypogaea* hulls were macerated in 1L of methanol with magnetic

stirring for 24 hours. The macerate was filtered on hydrophilic cotton and then on Whatman 3 mm paper. The filtrate was evaporated under reduced pressure using a Büchi-type rotary evaporator at 35°C. The resulting powder identified as methanolic extract (AHMe) was weighed. This extract was used for the different tests. The extraction yield in both cases is determined by the formula:

$$\text{Extraction yield} = \frac{\text{Weight of extract} \times 100}{\text{Weight of hulls}}$$

2.2.2 Subacute study

This study was carried out according to OECD guideline 407, intituled "Repeated Dose 28-day Oral Toxicity Study in Rodents" [18]. Forty-two albinos Wistar healthy rats were divided into seven groups of 6 animals each (3:3; males:females). They were grouped according to the dose rates (300, 600 and 1200 mg/kg body weight) of methanolic (AHMe) and aqueous (AHAq) extracts of peanut hulls and treated orally with a single daily dose for 28 days.

The groups of treated animals were designed as follows: group I served as control and received distilled water, group II, group III and group IV were administered AHAq extract at a doses of 300, 600 and 1200 mg/kg body weight (b.w.) respectively. Groups V, VI and VII received AHMe extract at doses of 300, 600 and 1200 mg/kg.bw, respectively. Treatments have been done 28 consecutive days and the body weight was determined once before starting dosing, once weekly during the dosing period and once sacrifice day.

The animals were observed closely for signs of toxicity. Appearance and behaviour pattern were assessed daily and any abnormalities in food and water intake were registered. After 28 days of treatment, animals were fasted overnight but allowed access to water ad libitum.

At the end of the study (on day 29), rats were then anesthetized with ether and blood samples were obtained into sterile tubes with anticoagulant EDTA (ethylene diamine tetra acetic acid) for haematological tests and tubes without anticoagulant for biochemical tests. Blood was centrifuged (2500 rpm for 15 min) to obtain serum and stored at -20°C for biochemical analysis.

2.2.3 Organ weight

Immediately after blood collection the animals were sacrificed. The organs of rats in the various groups were removed and weighed: liver, heart and kidney (paired organs were weighed together). The organ weight ratio (relative organ weights) was calculated.

Relative organ weights =

$$\frac{\text{Absolute organ weight (g)} \times 100}{\text{Body weight of rat on sacrifice day (g)}}$$

2.2.4 Biochemical assay

Biochemical parameters were measured with a COBAS INTEGRAS automated (Abbott ®), with Fortress Diagnostics biochemical kits, which assessed levels of alkaline phosphatase (ALP), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), lactate dehydrogenase (LDH), creatine kinase myoglobine (CK-MB), total protein, total bilirubin (T-Bil), direct bilirubin (D-Bil), indirect bilirubin (I-Bil), creatinine, urea, triglycerides (TG), cholesterol (CT), high-density lipoproteins (HDL).

2.2.5 Haematological assay

Haematological analysis was performed with a haematological analyser. The parameters examined included blood cell (RBC), haemoglobin (HGB), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), white blood cell (WBC) and percentage of total lymphocytes (LYMP).

2.2.6 Histological technique

Works of reference are those of Martoja and Martoja-Pierson [19], Humason [20], Gabe [21], Nezelof et al. [22], Locquin and Langeron [23]. To conduct histological studies, samples of liver, heart and kidney were fixed by immersion in aqueous Bouin and dehydrated in ascending series of ethanol (70°, 95° and 100°). Afterwards samples were pre-impregnated in butanol. The impregnation and the embedding were carried out in paraplast (Paraplast MONOJECT scientific, Division of Sherwood Medical, Athy, CO. Kildare, Ireland). Sections of 7 µm were realized on a microtome MICROM, and stained with hemalun and eosin. Observations and photographs were carried

out on a light ZEISS microscope (ZEISS-Primo Star).

2.3 Statistical Analysis

Data were expressed as means ± standard error of the mean (SEM). Between-group differences were evaluated by one-way analysis of variance followed by the Tukey post hoc test. Statistical analysis was performed using the statistical package Graph Pad Prism 5.0 (2007) (Microsoft, USA). *P*-values < 0.05 were considered significant.

3. RESULTS AND DISCUSSION

3.1 Results

The extraction yield of aqueous extract of the hulls was 2.70 ± 0.65%. For the methanolic extract, the extraction yield was 2.08 ± 0.45%. After investigation, all the animals which received subacute doses 300, 600 and 1200 mg/kg body weight, orally for 28 days remained active and healthy throughout the period of study. The animals did not show any changes in general behaviour or other physiological activities and no symptoms of adverse effects were observed during the study. There was no significant difference in food and water consumption between treatment and control groups.

3.1.1 Weights analysis

Parameters such as *body weights*, *organ weights* and *organ weight/body weight* of rats are confined in Table 1. These results showed that there were no significant differences (*P*>.05) in the *body weights evolution* and *organ weights* between control and treated animals. All vital organs as kidney, liver and heart didn't show any significant changes in the *organ weight/body weight ratios* in treated groups, compared to controls.

3.1.2 Haematological and biochemical parameters

Concerning haematological parameters confined in Table 2, no significant changes were observed for Haemoglobin (HBG), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC), Mean Cell Volume (MCV), Red Blood Cell Count (RBC) with extracts treated groups when compared to control: all *P*-values were above 0,05. But at doses of 1200 mg/kg body

weight, aqueous extract induced significant increase in White Blood Cell Count (WBC). However, methanolic extract at dose of 300 mg/kg body weight showed a significant increase in White Blood Cell (WBC) compared with the control group ($P = .003 < .05$).

In addition, the Table 3 shows that treatment with the extracts at various doses during 28 days did not alter most of the serum biochemical parameters ($p > .05$). However, ASAT ($P = .002 < .05$) and ALAT ($P < .0001$) decreased for both extracts. The methanolic extract at 1200 mg / kg BW produced a significant increase ($P < .0001$) of HDL-cholesterol concentration.

3.1.3 Histological observations

Histological sections of livers from control rats showed the typical organization of liver tissue. This tissue possessed portal areas, each containing a triad formed by a branch of hepatic artery with thick wall, a branch of portal vein with less thick wall and an interlobular bile duct. Further on, there is the central vein whose lumen is lined with a simple squamous epithelium in continuity with the endothelial lining of sinusoids (Fig. 1A, C). Between central vein and triad, the hepatocytes, as polygonal cells, are arranged in cell plates. The histological organization of rat liver treated with methanolic extract (Fig. 1B, D, F) and aqueous extract (not shown here) of *Arachis hypogaea* showed no disruption of liver structures.

As for kidneys, the views of control rats (Fig. 2A, C, E, G) indicated a normal organization of renal tissue: the cortex has glomeruli with normal shape and size, surrounded by longitudinal and transverse sections of convoluted tubes. In the medulla, sections of collecting tubes and blood vessels are clearly visible (Fig. 2G). The animals treated with extracts of *Arachis hypogaea* have regular glomeruli and convoluted tubes and well-organized collecting tubes.

The heart sections obtained from the control rats indicate normal presentation of the bundles of cardiac muscle fibers in cross sections (Fig. 3E) and longitudinal (Fig. 3G). The presence of transverse striations and intercalated discs confirm the typical structure of the cardiac tissue. Cardiocytes with visible nuclei are well delineated by loose connective tissue containing blood capillaries in places. The same organization was observed for the animals treated with the methanolic extract of *Arachis*

hypogaea (Fig. 3B, D, F, H). No tissue or cell damages were observed.

3.2 Discussion

Study of toxicological profile for any substance requires a toxicological evaluation after repeated exposure as recommended by regulatory agencies [18,24].

The purpose of this study was to investigate the effects of repeated administration of extracts from *Arachis hypogaea* hulls (Ah) for 28 days in rats. The doses used were 300, 600 and 1200 mg / kg BW.

The mortality, a specific sign of toxicity, was not observed in the current study. However, other variables may be indicative of more subtle adverse effects, such as loss of body mass during the treatment and clinical signs of toxicity (those are diarrhoea, piloerection, and changes in behaviour) [18]. All animals exposed to the extracts of *Arachis hypogaea* hulls did not exhibit clinical signals of toxicity at all doses tested. Treated rats during the 28 days did not show any significant differences in body weight evolution, suggesting that *Arachis hypogaea* does not affect normal growth of animals [18]. Similar results were observed for many plants whose extracts do not influence the weight growth of experimental rats [25,26].

The study of the haematopoietic system is heavily important in evaluation of physiological and pathological state in humans and animals [27]; it is one of the most sensitive targets for toxics. After the haematological analysis, it was observed that both extracts of *Arachis hypogaea* at various doses did not cause any significant effect on RBC and indices relating to them (HBG, HCT, MCV, MCH, MCHC and LYMP). This is an indication that there was no destruction of mature RBCs and no change in the rate of their production during the erythropoiesis [27]. RBC and HBG are very important in transferring respiratory gases; and the non-significant effect of extracts on the RBC and HBG indicates that there has been no change in the oxygen-carrying capacity and amount of oxygen delivered to the tissues. A decrease in White Blood Cells (WBC) was observed. White Blood Cells are the cells of the immune system and thus protect the body from infections [28]. Decreased White Blood Cells in the present study may therefore suggest a low infection resistance, since the cells of the immune system might be affected.

Table 1. Organ weights of rats in subacute toxicity study in control groups and treated with different doses of methanolic and aqueous extracts of *Arachis hypogaea* hulls: A. Absolute organ weight (g) / B. Relative organ weight (%)

Organs	A. Absolute organ weight (g)						
	Control	Aqueous extract			Methanolic extract		
	0 mg/kg bw	300 mg/kg bw	600 mg/kg bw	1200 mg/kg bw	300 mg/kg bw	600 mg/kg bw	1200 mg/kg bw
Liver	5.93 ± 1.24	6.12 ± 1.06	4.53 ± 1.1	5.17 ± 1.36	4.95 ± 1.72	5.12 ± 1.09	4.53 ± 1.24
Kidney	1.01 ± 0.09	1.12 ± 0.13	0.97 ± 0.1	0.9 ± 0.10	1.01 ± 0.16	0.99 ± 0.12	1.49 ± 0.16
Heart	0.58 ± 0.04	0.65 ± 0.05	0.59 ± 0.03	0.57 ± 0.05	0.59 ± 0.05	0.64 ± 0.05	0.66 ± 0.04

Data are expressed as mean±SEM, n=6 for each group. P > 0.05 for all: No statistical difference was found between the control and extracts of *Arachis hypogaea* treated groups

Organs	B. Relative organ weight (%)						
	Control	Aqueous extract			Methanolic extract		
	0 mg/kg bw	300 mg/kg bw	600 mg/kg bw	1200 mg/kg bw	300 mg/kg bw	600 mg/kg bw	1200 mg/kg bw
Liver	3.97 ± 0.908	4.16 ± 0.915	3.1 ± 1.065	3.52 ± 1.035	3.37 ± 1.05	3.49 ± 1.043	3.08 ± 0.915
Kidney	0.68 ± 0.090	0.76 ± 0.089	0.66 ± 0.083	0.61 ± 0.084	0.69 ± 0.090	0.67 ± 0.083	1.01 ± 0.083
Heart	0.39 ± 0.091	0.44 ± 0.080	0.4 ± 0.089	0.39 ± 0.090	0.4 ± 0.087	0.44 ± 0.090	0.45 ± 0.090

Data are expressed as mean±SEM, n=6 for each group. P > 0.05 for all: No statistical difference was found between the control and extracts of *Arachis hypogaea* treated groups

Table 2. Effect of 28-day treatment with extracts of *Arachis hypogaea* on haematological parameters in rats

	WBC (10 ³ /mm ³)	RBC (10 ⁶ /mm ³)	HBG (g/dL)	HCT (%)	MCV (µm ³)	MCH (pg)	MCHC (g/dL)	%LYMPH
Control	13.29±1.13	8.33±2.45	14.18±2.11	44.68±2.59	53.84±3.74	16.93±1.06	32.27±1.88	57.96±4.23
Aqueous extract (mg/kg bw)								
300	15.02±2.12	8.34±2.49	14.02±1.6	46.24±4.27	53.61±3.95	16.82±1.9	33.34±2.24	57.92±1.94
600	13.25±1.4	8.69±2.47	13.89±1.12	44.88±2.49	53.14±3.03	16.28±1.89	31.22±3.4	58.66±3.29
1200	16.33±1.68*	9.09±1.12	13.76±1.62	45.29±3.14	55.76±3.14	17.46±1.28	32.69±3.48	59.38±2.7
Methanolic extract (mg/kg bw)								
300	16.59±1.81*	7.51±0.92	14.38±1.28	46.69±4.19	54.14±3.2	16.03±2.12	32.03±3.06	57.74±4.13
600	15.25±1.21	8.19±1.43	13.36±1.65	45.89±2.46	53.19±3.51	15.57±1.61	31.56±2.71	58.62±1.55
1200	13.86±1.92	9.11±2.21	14.57±1.65	45.37±4.37	53.46±4.03	16.02±0.91	33.26±1.59	58.57±1.8

Data are expressed as mean±SEM, n=6 for each group. (*) Values in the same column significantly different as compared with control, with (P < 0.05)

Table 3. Effect of 28-day treatment with extracts of *Arachis hypogaea* on biochemical parameters in rats

Parameters	Control	Aqueous extract (mg/kg bw)			Methanolic extract (mg/kg bw)		
		300	600	1200	300	600	1200
UREA (g/L)	0.44 ± 0.16	0.6 ± 0.16	0.54 ± 0.12	0.45 ± 0.17	0.66 ± 0.11	0.66 ± 0.18	0.48 ± 0.14
CREAT. (g/ L)	4.9 ± 0.65	5.2 ± 0.63	5.5 ± 0.48	4.7 ± 0.67	4.8 ± 0.42	4.6 ± 0.65	4.8 ± 0.56
ALAT (UI/ L)	48 ± 3.62	46 ± 4.09	40 ± 2.69*	55 ± 3.19	54 ± 3.46	39 ± 2.52*	41 ± 3.56*
ASAT (UI/ L)	163 ± 19.97	179 ± 17.57	148 ± 20.37	155 ± 13.69	174 ± 14.65	143 ± 17.05*	142 ± 12.37*
LDH (UI/ L)	1713 ± 95	1590 ± 72	1601 ± 79	1639 ± 66	1780 ± 63	1683 ± 95	1768 ± 97
CK-MB (UI/ L)	1459 ± 63	1488 ± 54	1391 ± 47	1383 ± 49	1355 ± 63	1415 ± 43	1439 ± 44
ALP (UI/L)	278.8 ± 36.5	289.5 ± 29.6	293.9 ± 29.7	269.1 ± 15.3	290.5 ± 39	291.49 ± 31.9	282.91 ± 25.5
Glucose (g/L)	0.89 ± 0.068	0.90 ± 0.030	0.89 ± 0.040	0.86 ± 0.020	0.88 ± 0.040	0.89 ± 0.055	0.87 ± 0.035
Total Protein (g/ L)	72 ± 2.4	72 ± 2.52	69 ± 2.1	69 ± 2.75	69 ± 2.24	70 ± 1.53	72 ± 2.85
T-Bil. (µmol/L)	0.97 ± 0.114	0.97 ± 0.142	1.07 ± 0.12	0.93 ± 0.151	1.02 ± 0.077	0.95 ± 0.171	0.94 ± 0.089
D-Bil (µmol/L)	0.29 ± 0.042	0.31 ± 0.026	0.32 ± 0.02	0.3 ± 0.035	0.32 ± 0.038	0.33 ± 0.035	0.30 ± 0.031
I-Bil (µmol/L)	0.67 ± 0.13	0.65 ± 0.153	0.75 ± 0.125	0.62 ± 0.138	0.70 ± 0.053	0.62 ± 0.144	0.63 ± 0.06
Triglycerids (g/L)	0.42 ± 0.025	0.43 ± 0.011	0.40 ± 0.021	0.40 ± 0.023	0.41 ± 0.01	0.42 ± 0.039	0.40 ± 0.012
Total Cholesterol (g/L)	0.72 ± 0.06	0.72 ± 0.03	0.76 ± 0.05	0.69 ± 0.03	0.74 ± 0.02	0.76 ± 0.04	0.73 ± 0.04
HDL-Cholesterol (g/L)	0.41 ± 0.03	0.39 ± 0.02	0.37 ± 0.03	0.38 ± 0.02	0.38 ± 0.05	0.38 ± 0.06	0.50 ± 0.02*
Sodium (meq/L)	142.3 ± 1.26	141.7 ± 2.01	142.1 ± 1.90	141.1 ± 1.40	142.7 ± 2.67	143.1 ± 1.75	140.1 ± 2.30
Potassium (meq/L)	7.64 ± 0.24	8.38 ± 0.36	7.90 ± 0.49	7.95 ± 0.37	7.97 ± 0.46	7.81 ± 0.55	8.17 ± 0.59
Chlore (meq/L)	95.48 ± 1.54	93.77 ± 1.37	93.91 ± 1.24	94.97 ± 1.29	96.01 ± 2.01	94.91 ± 1.16	95.87 ± 1.89
Calcium (mg/L)	103.4 ± 3.0	105.6 ± 2.4	107.1 ± 2.1	104.2 ± 3.1	102.16 ± 1.89	105.2 ± 2.6	103.2 ± 2.1

Data are expressed as mean±SEM, n=6 for each group. (*) Values in the same raw significantly different as compared with control. (P > 0.05)

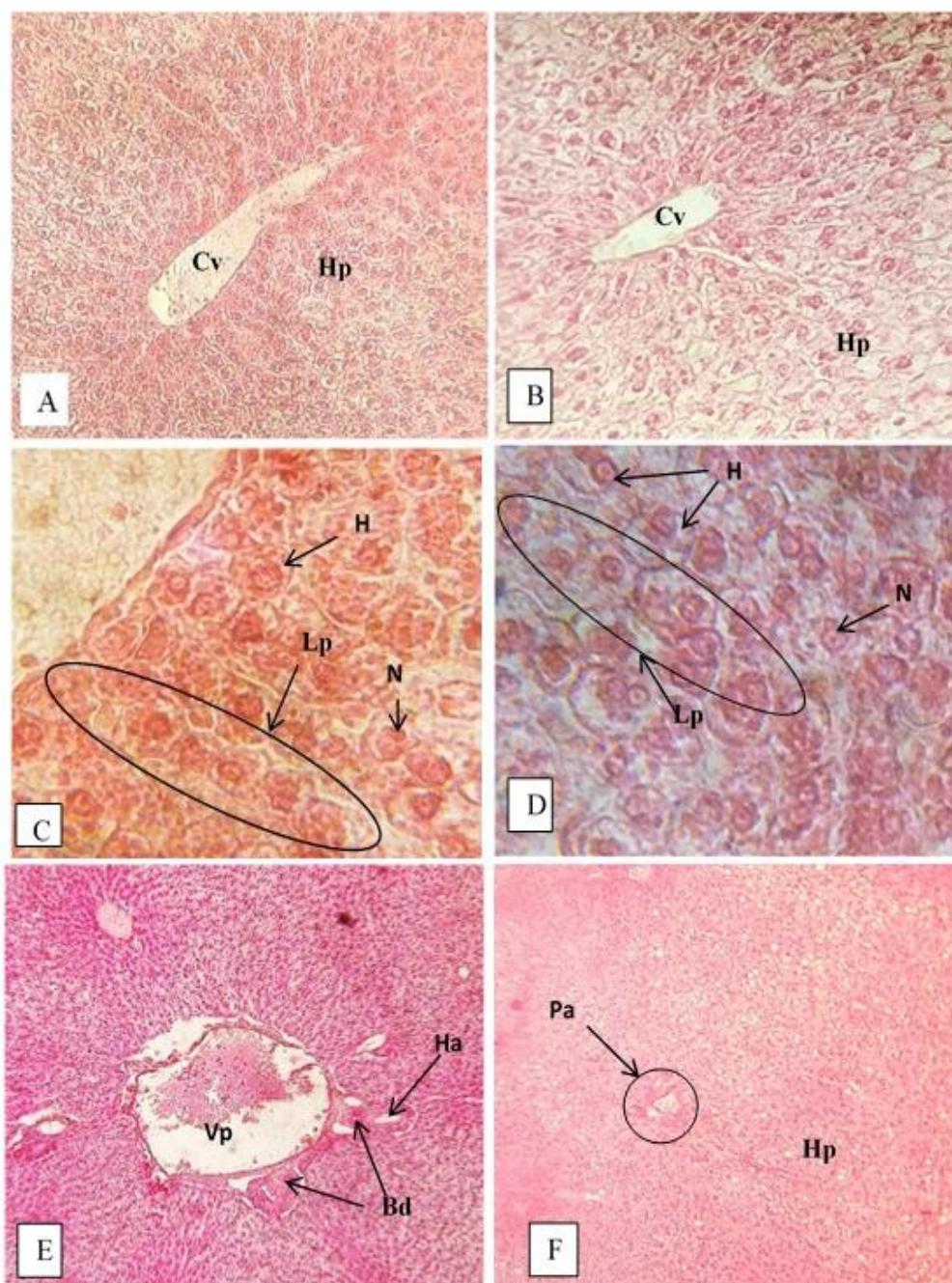


Fig. 1. Histological observations of liver in control rats and treated rats with methanolic extract of *Arachis hypogaea* (Ah) during 28 days (H&E stain)

A: General view of the centrolobular area of the control rat liver, X100.

B: General view of the centrolobular area of the liver for treated rat with Ah, X100.

C: View of liver plates of the control rat liver, X400.

D: View of liver plates of the liver for treated rat with Ah, X400.

E: View of the portal area of the control rat liver, X40.

F: View of the portal area of the liver for treated rat with Ah, X40.

Cv: Central vein; Vp: Portal vein; Hp: Hepatic parenchyma; H: Hepatocyte; Lp: Liver plate; N: Nucleus; Bd: Bile duct; Ha: Hepatic artery; Pa: Portal area

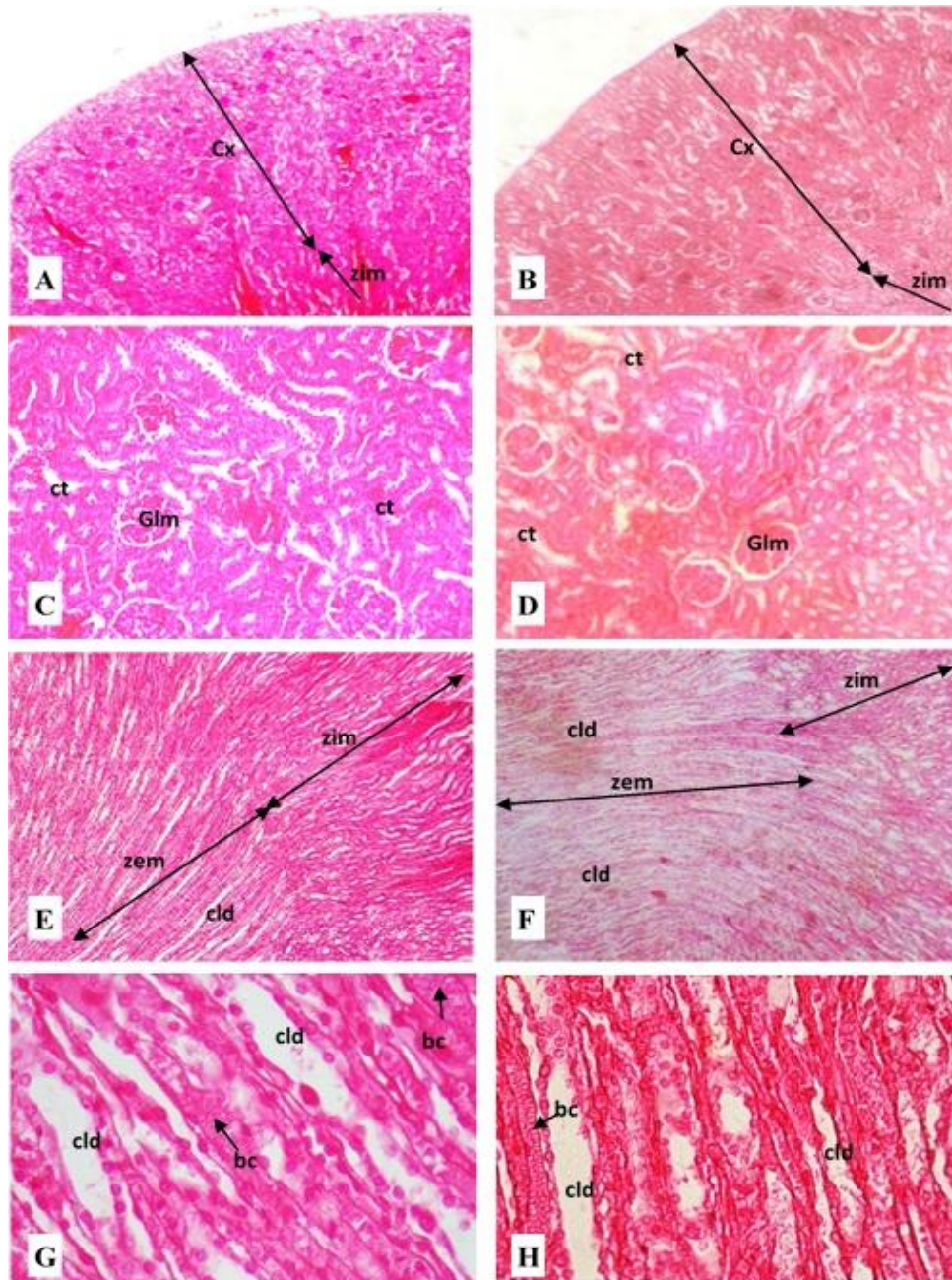


Fig. 2. Histological observations of kidney in control rats and treated rats with methanolic extract of *Arachis hypogaea* (Ah) during 28 days (H&E stain)

A: General view of the cortex of control rat, X100.

B: General view of the cortex for treated rat with Ah, X100.

C: Detailed view of the cortex of control rat, X400.

D: Detailed view of the cortex for treated rat with Ah, X400.

E: General view of the medulla of control rat, X100.

F: General view of the medulla for treated rat with Ah, X100.

G: Detailed view of the medulla of control rat, X600.

H: Detailed view of the medulla for treated rat with Ah, X600

Cx: Cortex; zim: internal area of medulla; zem: external area of medulla; ct: convoluted tubule; cld: collecting duct; bc: blood capillary; Glm: glomerulus

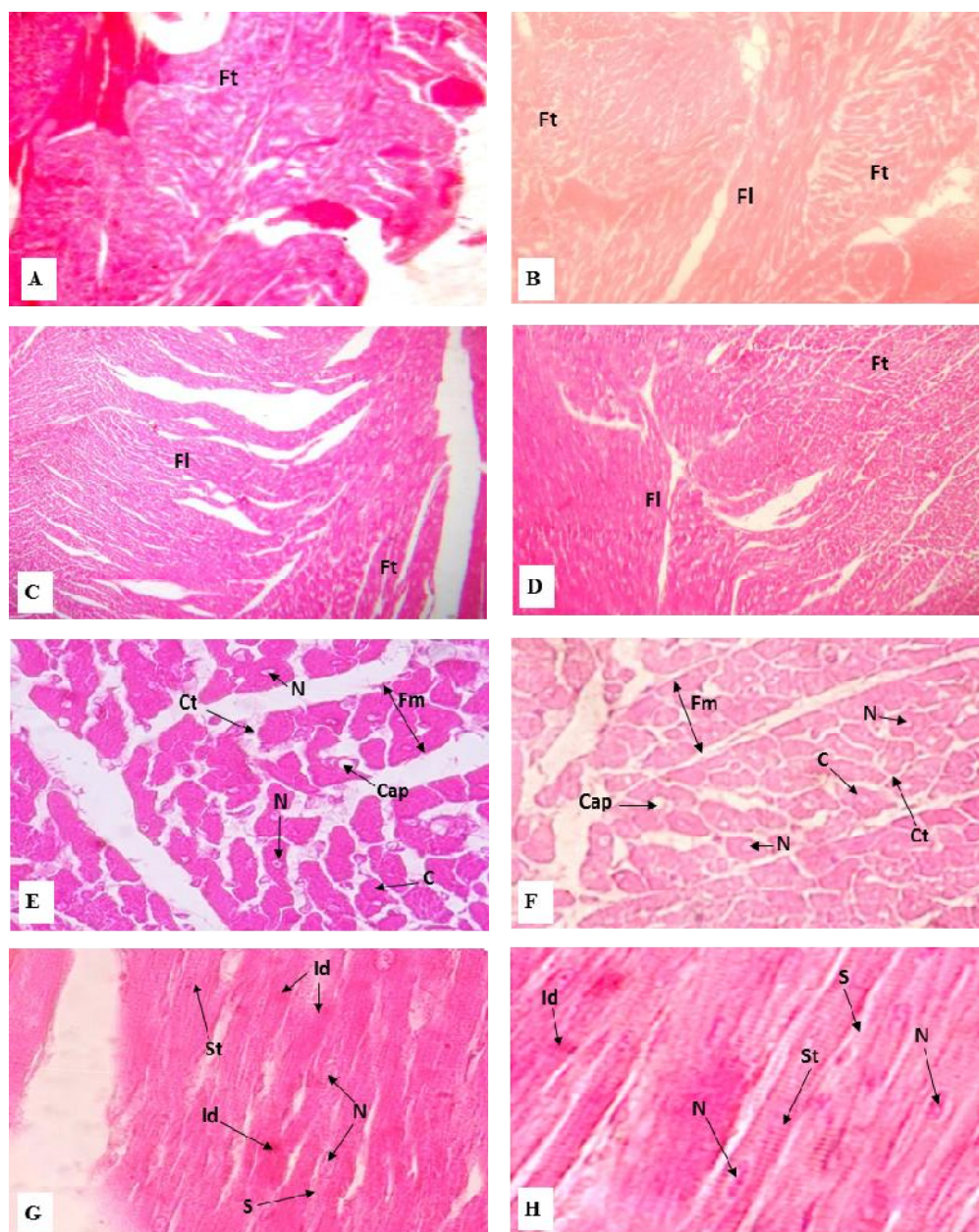


Fig. 3. Histological observations of heart in control rats and treated rats with methanolic extract of *Arachis hypogaea* (Ah) during 28 days (H&E stain)

- A: General view of the atrium of control rat, X40.
 B: General view of the atrium for treated rat with Ah, X40.
 C: General view of the ventricle of control rat, X40.
 D: General view of the ventricle for treated rat with Ah, X40.
 E: Detailed view of the transverse fiber bundles of the control rat, X400.
 F: Detailed view of the transverse fiber bundles for treated rat with Ah, X400.
 G: Detailed view of the longitudinal fiber bundles of the control rat, X400.
 H: Detailed view of the longitudinal fiber bundles for treated rat with Ah, X400.
 Ft: Transverse muscle fiber; Fl: Longitudinal muscle fiber; N: Nucleus; C: Cardiocyte; Fm: Muscle fiber bundle;
 Cap: Capillary; Ct: Connective tissue; St: Transverse Striation; Id: Intercalated discs; S: Sarcoplasm

Many reports of toxicity studies have showed a link between substances found in the blood serum and damages induced by toxins on organs such as heart, liver, kidneys, etc. Thus, enzymes, metabolites and ions vary during intoxications by phytomedicines. Indeed, many enzymes are present only in cells where they exert their catalytic activities: these are the so-called cellular enzymes. They are found in the blood when the cells and tissues supposed to contain them are affected or degraded following normal physiological processes (apoptosis, aging, etc.) or accidental (necrosis, cellular stress, various pathologies, etc.). The normal serum values observed for the cellular enzymes are linked to normal physiological processes. An increase in normal values indicates a deleterious action on a tissue. In the present study, the enzymatic activities did not show any significant increase compared to the controls. The assayed enzymes (ALP, ASAT, ALAT, LDH, CK-MB) are hepatic, renal and cardiac markers. The results therefore indicate that the extracts from *Arachis hypogaea* hulls don't have any deleterious effect on the kidney, heart and liver tissues, the target organs of this study. However, a significant decrease of transaminases (ASAT and ALAT) activities was observed at doses of 600 and 1200 mg / kg BW with the methanolic extract and at a dose of 600 mg / kg BW with the aqueous extract. In clinical enzymology, a decrease in serum activity of enzyme markers does not have any interest, as mentioned by European Document for Ecotoxicology and Toxicology. This guideline indicates that the biological significance of the ALT enzyme decrease was not specific; it was typically dismissed as being of no toxicological importance [29,30]. But for experimental evaluation, this decrease in activity could suggest a protection of the integrity of target organs.

Impaired hepatocellular function may lead to a reduction in serum concentrations of total protein and bilirubin. The insignificant change in serum concentrations of total protein and bilirubin in the treated and control groups further suggests that the synthesis functions of the liver is not altered at any of the test doses of the aqueous and methanolic extracts.

The lipid balance is one of the elements of cardiovascular disease prevention strategy according to Saïle Rachid and Taki Hassan [31]. Therefore, it is used to assess the risks of cardiovascular disease related to the consumption of herbal substances. The

exploration of a possible lipid abnormality consists of determining the concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides. In the current study, only the methanolic extract at 1200mg / kg BW produces a significant increase in HDL-cholesterol, which militates in favour of protection against cardiovascular risk.

Urea is a nitrogenous waste product derived from the breakdown of proteins by the liver, filtered by the kidneys and eliminated in the urine. Creatinine is a waste of the muscular energy metabolism, eliminated essentially by renal filtration. These two substances therefore constitute serum markers of renal function. In the current study, serum creatinine and urea concentrations in treated animals were statistically similar to control animals. Renal function therefore is not affected by the different extracts of *Arachis hypogaea*.

In addition to all these blood markers of integrity and functioning of organs, direct analysis, including macroscopic examination, histological evaluation and organ relative weight were carried out on the isolated organs of both the treated and control groups.

Organ relative weight is used to evaluate organ injury [32], which either atrophy or hypertrophy or swelling [33,34]. The heart, liver and kidney are among the primary organs affected by metabolic reaction caused by toxicant [34]. The organs obtained from both control and treated animals showed normal macroscopic architecture and no difference was observed for the organ relative weights. More, the histological evaluation did not show any difference for the primary structures of the renal, hepatic and cardiac tissues, when animals are treated. This suggest that there were no detrimental changes or morphological disturbances caused by daily oral administration of the *Arachis hypogaea* hulls. The present results have been observed in several studies about toxicity of medicinal plants: Hydroethanolic leaf extract of *Pseudospondias microcarpa* [35], ethanolic extract of *Pericampylus glaucus* [36] and more others, did not produce any significant toxic effects.

4. CONCLUSION

No mortality occurred in this subacute oral toxicity study. The overall data suggest that oral administration of aqueous and methanolic extracts from *Arachis hypogaea* hulls does not

induce any toxic effects. This could stand as an assurance for the use of *Arachis hypogaea* hulls in traditional medicine. Further investigation is needed to evaluate the long-term safety of these plant extracts.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard written ethical permission has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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