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Evaluation of Acute and Sub-acute Toxicity of the Aqueous Extract from the Fruit of *Solanum indicum* Linn. (Solanaceae) in Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors PBT and FMT designed then supervised the study which protocol was written by author NJE and the experiments performed by authors NJE, HTT and OLMD. Authors NJE, HTT and PBT wrote the paper, did the literature search and the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The fruit of *Solanum indicum Linn* have been reported traditionally to have anti-hypertensive and diuretic properties. This study was undertaken to evaluate the toxicological potential of *S. indicum* fruits aqueous extract through the acute and sub-acute toxicity tests in rats.
Methodology: For acute toxicity evaluation, a single oral dose of 5000 mg/kg of the plant extract was administrated in 60 days old female albino *Wistar* rats. Then, the animals were observed for 14 days. Sub-acute toxicity studies were conducted with 50 adult rats of both gender that orally

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received during 28 days, increasing doses of the plant extract. Their body weight and food intake were weekly collected. At the end of the experiment, biochemical and hematological parameters as well as histological analysis of organs (liver, kidneys and spleen) were undertaken.
Results: Single oral administration of 5000 mg/kg dose of the fruit plant aqueous extract produced no mortality or signs of toxicity. During sub-acute test, no variations in body weight and food intake of both animals gender were observed. An important decrease in male's rat liver weight were obtained at the dose 25 mg/kg; serum urea, total cholesterol, TAG, ALP and AST levels were significantly lowered in male especially at the dose 50 mg/kg, but this decrease was noticed only in serum urea, ALP and ALT in female rats. Furthermore, a significant decrease in platelets number, serum PCT, MPV and PDW levels were recorded in all treated male rats except those receiving the highest extract dose. No structural changes in treated animal organs section histology were observed when compared to controls.
Conclusion: The fruits aqueous extracts of *S. indicum* is safe when administered acutely and for 28 days in rats. However, alterations on their hematological and biochemical parameters were not closely related with the dose, implying caution on its use.

Keywords: Solanum indicum; acute toxicity; sub-acute toxicity; Wistar rats.

1. INTRODUCTION

Medicinal plants have long been used for the prevention and treatment of various diseases. They are well known throughout history and have always been part of human culture [1,2]. The use of herbal remedies is increasing as a result of their accessibility, efficacy and social acceptability by patients [3, 4]. The adverse side effects of synthetic drugs may also explain the popular utilization of these herbal remedies [5]. Herbal drug like *Tetrapleura tetraptera* which have the capacity to reduce body weight gain and lipids as compared to rats fed HFD alone has been demonstrate to be safe for acute and subacute toxicity [6]. However, a recent survey undertaken by Mayur et al. [7] has indicated that, despite the increasing use of medicinal plants, they could present some adverse effects which might result from their chronic utilization. Hence, it becomes necessary to evaluate the toxicity of medicinal plant extracts in order to establish their safety for longer period of administration.

Solanum indicum Linn belonging to the Solanaceae family, is a bushy herb, up to 1.8 m high containing prickly spikes in the stem and found throughout warmer parts of India, Asia and Africa [8]. The different parts (fruits, leaves, roots) of this plant are used by traditional healers in the treatment of blood disorders, abdominal pain, inflammation, insomnia, urinary complications, cardiac weakness [9]. The plant roots are used as diaphoretic, diuretic, expectorant, stimulant and for the treatment of bronchitis, itches and body aches. The leaves are placed in the cradles of infants to promote sleep [8]. The fruits are used as nutritious vegetables given their high content in starch, calcium, vitamin A, ascorbic acid and phosphate. The fruits have also been claimed in folk medicine to have anti-hypertensive properties, analgesic, antipyretic and Central Nervous System depressant activities on established animal models [8,10]. This large spectrum of action of *Solanum indicum* Linn could be due to the presence in this plant the chemical constituents like flavonoids, glycosides, sugars, alkaloids and tannins which have low toxicological potential as observed by Abdel-Aziz et al. [11]. Although, toxicological studies undertaken by Abdel-Aziz et al. [11] on a standardized product of the whole plant's fruit of *Solanum indicum* ssp. Distichum have proven no signs of toxicity, this result must be ascertain in the present study during which acute and sub-acute toxicological evaluation of a closer species (*Solanum indicum* Linn) fruit aqueous extract would be investigated.

2. MATERIALS AND METHODS

2.1 Plant Materials Source

The fresh fruits of *Solanum indicum Linn* were collected in Foumban, Noun Division of the Western region of Cameroon during the month of May 2016. The plant was identified and authenticated at the National Herbarium, Yaounde, Cameroon, in comparison with the reference voucher specimen number N° 60814/HNC. Fruits sample were then dried at room temperature and crushed into powder.

2.2 Preparation of Plant Extract

Five hundred grams (500 g) of *Solanum indicum* Linn. powder were infused in 1 L of distilled

water. Then, the mixture was filtered by whatman paper (N°1) and the infusion obtained was evaporated in a regulated drying oven at 45°C. After evaporation, the obtained dried mass of the aqueous extract was 71 g corresponding to an extraction yield of 14.2%. A small part was used for the calculation of the concentration which allowed us through several data collected in traditional healers, to calculate the therapeutic dose (25 mg/kg). The dried mass was then stored at 4ºC for subsequent experiments.

2.3 Animals

Fifty six (56) albino *Wistar* rats of both gender, aged 2-2.5 months and having an average weight of 140 g were used for acute and sub-acute toxicity studies. They were grown up in the animal house of the Department of Biochemistry of the University of Dschang in an environmentally controlled room (23±3°C and 12 hour light/dark photocycle). The animals received food and water *ad libitum*. Experimental protocols used in this study were accepted by the local ethical committee of our Faculty (Faculty of sciences, University of Dschang, Cameroon) and were designed in strict concordance with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of the 24th November 1986.

2.4 Acute Oral Toxicity Test

The acute toxicity test was conducted in accordance with the Organization for Economic Co-operation and Development, with minor modifications [12]. Six female rats were divided into 2 groups of 3 animals each and allowed for overnight fasting. The animals were weighed and the H_2O-control group received distilled water while the second group was administered a unique dose of 5000 mg/kg of AESI. The animals were kept under ambient laboratory conditions and neither food nor water was administered until 4 hours after the administration of the extract. The animals were observed every day for 14 days during which signs of toxicity such as behavior changes and physical appearance were observed.

2.5 Sub-acute Oral Toxicity Test

This assay was conducted in adult rats according to OECD Test Guideline No. 407 [13]. The animals of both gender were divided into five experimental groups of 10 animals each (5 males and 5 females). Group 1 used as control received distilled water, while four different doses of AESI (12.5, 25, 50 and 100 mg/kg) were orally and daily administered, during 28 days, to the four remaining groups. These doses were obtained according to traditional healers measures (25 mg/kg). From that traditional healing dose we used lower (x ½) and upper (x 2) and (x 4) to obtain the doses of 12.5, 50 and 100 mg/kg respectively. During the treatment period, animals body weight were registered every two days while food intake was noticed daily through measurement of the difference between the amount of initial food supplied and the next day remaining food. Possible signs of toxicity like general behavior, abnormal clinical signs and mortality were observed and recorded. At the end of the experimental period, all the animals were fasted overnight (except water that was given *ad libitum*) and they were anesthetized on the 29th day using intraperitoneal injection of Ketamine/diazepam (80/5 mg/kg). Blood samples were collected by cardiac puncture in dried tubes for biochemical analysis and into those containing ethylene diamine tetraacetic acids (EDTA) for hematological test. Then, vital organs such as liver, kidney, spleen, lungs, heart, testes and ovary were collected, washed in 0.9% NaCl solution and weighed. The liver, spleen and kidney were cleaved and conserved in 10% formaldehyde solution for histological analysis.

2.5.1 Hematological analysis

Five minutes following the gentle mixture of 1 mL blood samples into EDTA tubes, the complete blood count was registered using an automated hematology counter (ERMA). Hematological evaluations included: red blood cell count (RBC), white blood cell count (WBC), differential leukocyte count (lymphocyte, monocyte, granulocyte), platelets count, red cell distribution width (RDW), mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT). Hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV).

2.5.2 Biochemical analysis

Blood samples were collected into dried test tubes and allowed to stand for 45 min at room temperature before being centrifuged at 4000 rpm for 1 min. The serum separated was used for analysis. Urea, creatinin, total protein, albumin, glycemia, enzymes like alanine amino transaminase (ALT) and aspartate transaminase

(AST), alkalin phosphatase (ALP), and lipids parameters (triglycerides, total cholesterol, HDL and LDL-cholesterol) were assayed using standard analytical kits (purchased from INMESCO/ Cameroon).

2.5.3 Histopathology

For histological analysis, cleaved tissue samples of liver, spleen and kidneys; conserved in 10% formaldehyde solution were dehydrated using increasing concentration of ethanol (70%, 90% and 100%) and finally embedded in paraffin. The different tissue microtome sections (6 µm thick) were fixed on slide initially de-waxed with Xylene and stained using hematoxylin and eosin (HE). Analyses of tissues general structure, degenerative changes and necrosis evidence were done trough optical microscope. Finally, microphotographs of interesting sections were recorded.

2.6 Statistical Analysis

The software SPSS 13 was used for analysis. The data were registered as Mean ± sem (standard error of the mean). The statistical differences between the values were shown by ANOVA (Analysis of Variance) test. The Fisher PPDS test was used for the comparison of means; significance of the differences was established at 5% ($P<0.05$).

3. RESULTS

3.1 Acute Toxicity Study

The results obtained from the acute toxicity study revealed that oral administration of a single 5000 mg/kg dose of the AESI to female rats did not cause death or any toxic signs in treated animals during the 14 days observation period. Thus, the LD_{50} of the fruit aqueous extract of *Solanum indicum* Linn is considered to be higher than 5000 mg/kg. Also, no change in feces consistency, appearance and morphological characteristics (fur, skin, eyes and nose) was observed. There was no convulsion, no salivation nor lethargy or sleep during the first four hours of extract administration. Body and organ weights were not affected by the treatments (Table 1). Macroscopic examination of liver, kidneys, heart, lung, ovary and spleen did not reveal any changes.

3.2 Sub-acute Toxicity Study

3.2.1 AESI effect on body weight and food intake

During the sub-acute toxicity tests, administration of 12.5, 25, 50 and 100 mg/kg doses of AESI did not cause death nor show any visual symptoms of toxicity or mortality in animals, whatever their gender, following 28 days of treatment. Independently of the administered doses and relatively to the beginning of the treatment (week 0), a gradual and significant increase ($p<0.01$) in body weight gain was recorded in all animals during the remaining treatment (weeks 1 to 4) (Fig. 1). Whatever the treatment period, no significant differences of body weight gain and food intake of AESI of treated animals was noticed compared to their respective controls (Fig. 2).

3.2.2 AESI effect on organ weight

The organ weight of animals submitted to sub-acute toxicity test is shown in Fig. 3. A significant ($P < 0.05$ and $P < 0.01$) reduction in kidney and ovary weight were observed in female rats treated at 25 and 100 mg/kg AESI doses when compared to control values. The same trend were notice in male's liver weight at the doses 12.5, 25 and 100 mg/kg and also for lung weight at 12.5 mg/kg when compare to control.

Table 1. Effects of single administration of AESI on body and organs weights of female rats

Female	Control (H_2O)	AESI 5000mg/kg
Initial body weight	127±2.49	131±2.35
Final body weight	172±2.30	185±2.30
Liver	7.87±0.40	7.75±1.15
Kidney	1.28±0.16	1.34±0.39
Heart	0.57±0.31	0.54±0.27
Spleen	0.75±0.40	0.74±0.26
Lung	1.34±0.36	1.26±0.47
Ovary	0.12±0.13	0.13±0.12

All data are reported as the mean ± S.E.M. for n = 3 per group. (ANOVA and LSD). [a]p <0.05:significant differences compared with control. AESI= aqueous extract of Solanum indicum

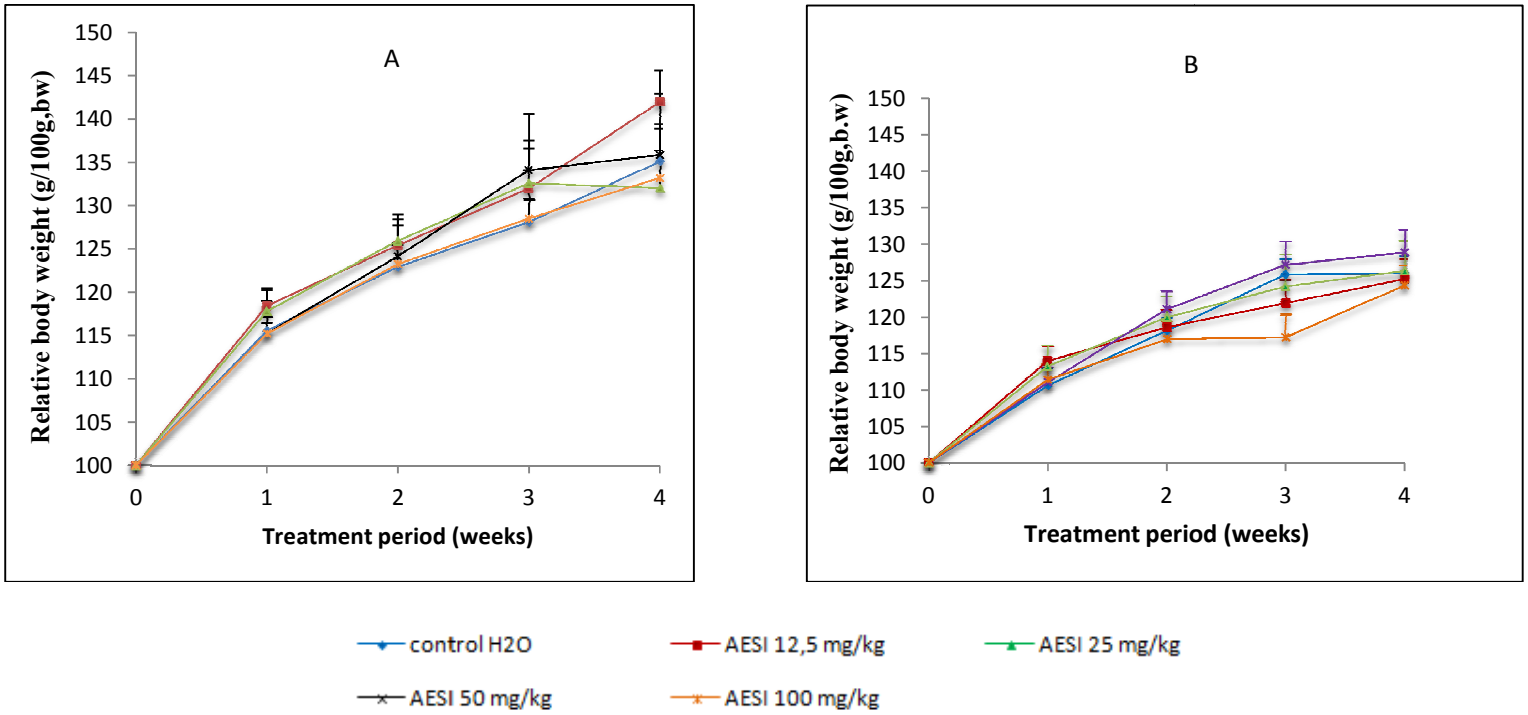

Fig. 1. Effect of daily oral administration of AESI on relative body weight (g) in male (A) and female (B) rats. The animals received AESI (12.5, 25, 50 or 100 mg/kg, b.w.) and vehicle (distilled water)

All data are reported as the mean ± sem for n = 5 per group. (ANOVA and LSD). AESI= Aqueous Extract Solanum indicum

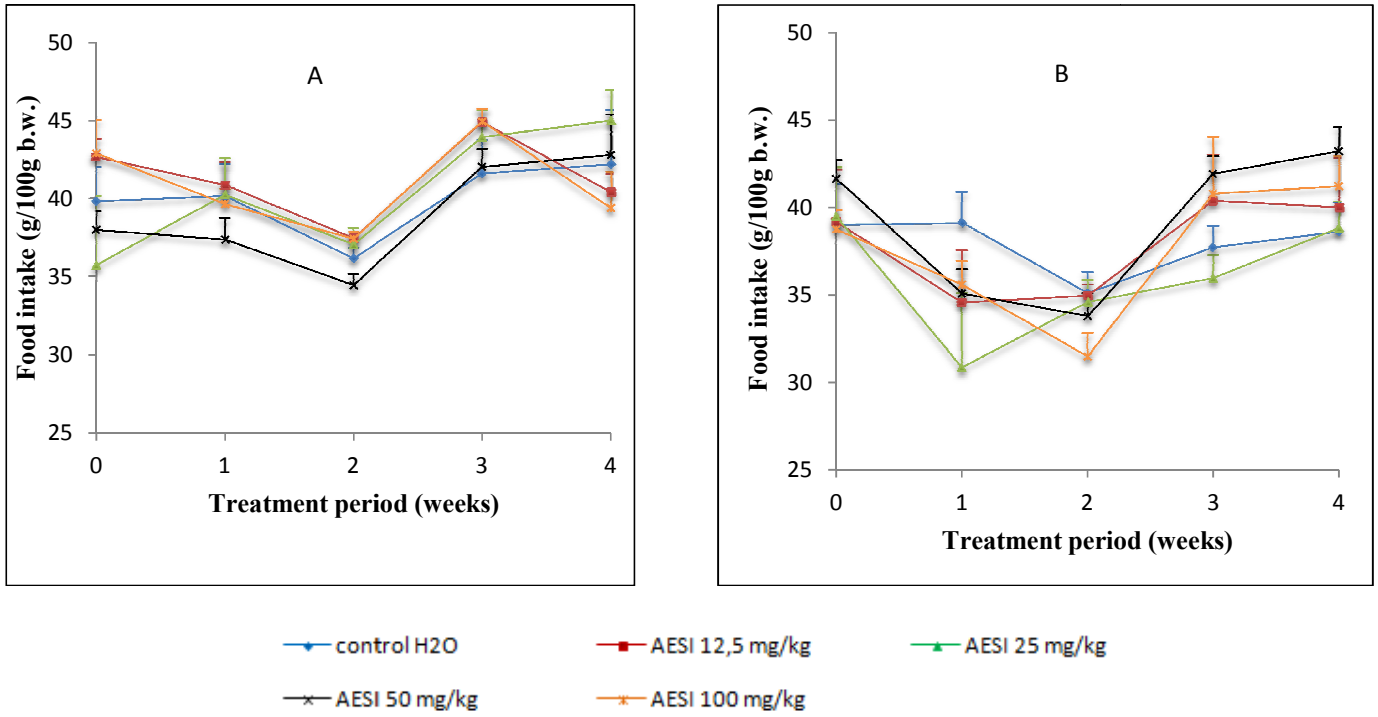

Fig. 2. Effect of daily oral administration of AESI on food intake (g) in male (A) and female (B) rats. The animals received AESI (12.5, 25, 50 or 100 mg/kg, b.w.) and vehicle (distilled water)

All data are reported as the mean ± S.E.M. for n = 5 per group. (ANOVA and LSD). AESI= Aqueous Extract Solanum indicum

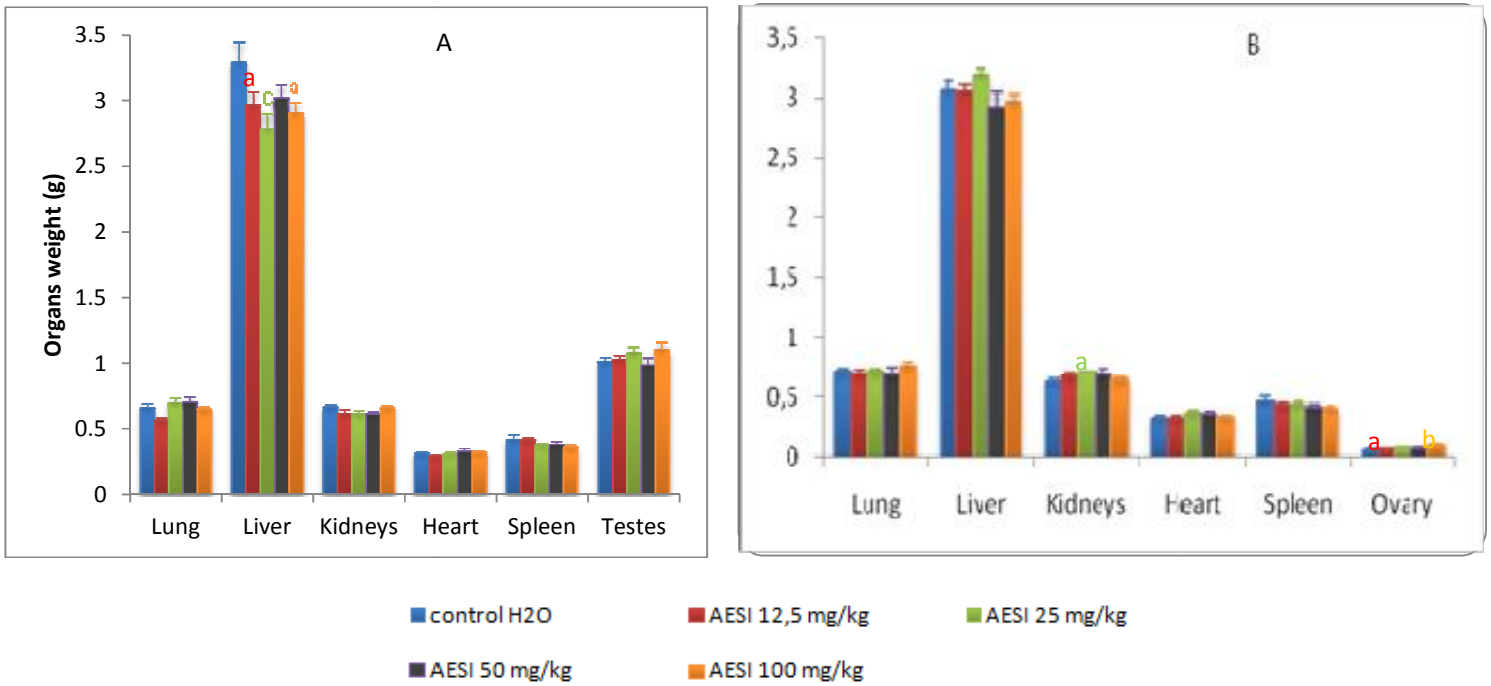

Fig. 3. Effects of daily oral administration of AESI on the organ weight (g) in male (A) and female (B) rats. The animals received AESI (12.5, 25, 50 or 100 mg/kg, b.w.) and vehicle (distilled water)

All data are reported as the mean ± S.E.M. for n = 5 per group. [a]p <0.05; [b]p <0.01 and [c]p <0.001represent the significant differences compared with control H2O. (ANOVA and LSD). AESI= Aqueous Extract Solanum indicum

Table 2. Effect of administration of AESI on some hematological parameters of sub-acute toxicity in male (A) and female (B) rats

	Control (H_2O)	AESI 12.5 mg/kg	AESI 25 mg/kg	AESI 50 mg/kg	AESI 100 mg/kg
Male					
RBC (million/µl)	7.25±0.11	7.19±0.45	7.50±0.06	7.73±0.17	7.53±0.04
Haemoglobin (g/dl)	15.07±0.13	14.57±0.70	15.35±0.34	15.80±0.30	15.37±0.11
Hematocrit (%)	36.97±0.41	34.56±2.20	36.67±0.95	37.57±0.67	37.37±0.72
MCV (fl)	50.97±0.67	44.38±2.95	48.7750±0.86	50.06±0.37	49.57±0.72
MCH (pg)	20.72±0.17	23.84±3.08	20.40±0.30	20.40±0.30	20.35±0.09
MCHC (g/dl)	40.72±0.37	38.89±1.87	41.82±0.26	42.02±0.45	41.15±0.74
RDW	16.70±0.15	17.70±0.35	17.52±0.19	16.15±1.00	17.40±0.30
WBC (10^9)	6.47±0.39	7.70±0.59	4.62±0.32a	7.15±0.63	9.09±0.87b
Lymphocytes	4.27±0.20	5.72±0.47a	3.28±0.37	4.97±0.41	4.26±0.32
Monocytes	0.84±0.06	0.82±0.05	0.72±0.07	1.08±0.05	0.76±0.11
Granulocytes	1.19±0.09	1.12±0.09	1.10±0.10	1.32±0.11	1.13±0.17
Lymphocytes (%)	66.22±1.31	74.05±0.99	67.10±1.71	70.45±1.32	68.20±5.23
Monocytes (%)	13.62±1.32	10.72±0.26a	12.92±0.83	11.75±0.88	16.05±1.29
Granulocytes (%)	20.15±1.54	15.22±1.25a	19.97±1.48	17.80±1.03	13.97±0.77b
PLT	821.00±40.46	652.25±41.64b	608.75±39.66b	591.50±44.37c	691.50±47.19a
PCT	0.56±0.04	0.41±0.02b	0.38±0.02c	0.37±0.03c	0.45±0.03a
MPV	6.80±0.17	6.40±0.04a	6.40±0.09a	6.37±0.03a	6.65±0.14
PDW	9.22±0.55	8.02±0.01a	8.12±0.29a	8.25±0.10a	8.42±0.32
Female					
RBC (million/µl)	7.20±0.13	7.29±0.07	7.31±0.26	7.51±0.12	7.88±0.36
Haemoglobin (g/dl)	17.15±1.30	15.30±0.09	15.30±0.45	15.55±0.22	17.00±0.75
Hematocrit (%)	38.00±0.66	37.67±0.21	38.02±0.88	37.60±0.59	40.27±1.35a
MCV (fl)	52.77±1.04	51.62±0.86a	52.15±1.10	50.05±1.18	51.25±0.89
MCH (pg)	21.88±0.76	20.95±0.32	20.92±0.30	20.65±0.28	21.55±0.39
MCHC (g/dl)	45.32±4.01	40.57±0.13a	40.15±0.30a	41.32±0.44a	42.10±0.49
RDW	15.95±0.24	16.55±0.18	17.25±0.05b	16.35±0.26	16.40±0.21
WBC (10^9)	5.57±0.39	3.87±0.21b	3.33±0.39b	6.46±0.24	5.30±0.44
Lymphocytes	4.10±0.29	3.10±0.23a	2.61±0.27b	4.62±0.20	3.70±0.32
Monocytes	0.72±0.05	0.53±0.03a	0.37±0.05c	0.72±0.05	0.63±0.04
Granulocytes	0.73±0.04	0.67±0.06	0.69±0.11	1.00±0.04a	0.97±0.08a
Lymphocytes (%)	73.95±0.27	74.57±2.32	75.95±3.32	69.35±2.39	70.77±1.75

	Control (H_2O)	AESI 12.5 mg/kg	AESI 25 mg/kg	AESI 50 mg/kg	AESI 100 mg/kg
Monocytes (%)	12.40±0.41	13.65±0.76	11.22±1.36	12.80±0.57	12.80±0.39
Granulocytes (%)	13.65±0.53	11.75±0.60	15.22±0.99	16.39±0.93	15.38±0..98
PLT	650.50±22.01	672.75±37.52	627.75±26.12	619.00±33.06	583.25±41.14
PCT	0.44±0.02	0.44±0.02	0.40±0.01	0.42±0.02	0.38±0.02a
MPV	6.85±0.18	6.55±0.10	6.50±0.13a	6.82±0.12	6.55±0.08
PDW	8.80±0.37	8.30±0.27	8.07±0.32	8.85±0.44	8.57±0.29

All data are reported as the mean ± S.E.M. for n = 5 per group. (ANOVA and LSD). [a]p <0.05, [b]p <0.01and [c]p <0.001: significant differences compared with control. RBC = Red blood cell count, WBC = white blood cell count, differential leukocyte count (lymphocyte, monocyte, granulocyte), HGB = hemoglobin, HCT = hematocrit, MCHC = mean corpuscular hemoglobin concentration, MCH = mean corpuscular hemoglobin, MCV= mean corpuscular volume, RDW = red cell distribution width, MPV=mean platelet volume, PDW = platelet distribution width, PCT = plateletcrit. AESI = aqueous extract of Solanum indicum

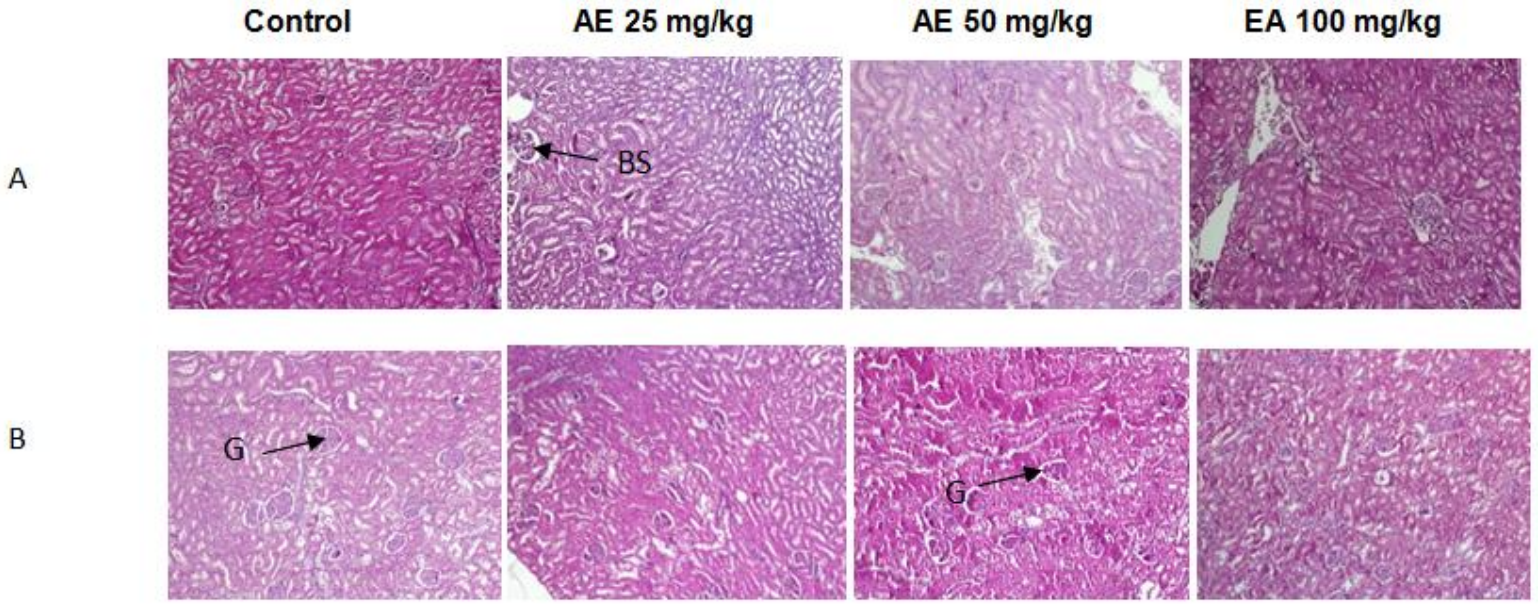

Fig. 4. Effects of AESI on male (A) and female (B) rats kidneys histology (H&E × 200)
G= Glomerular, BWS = Bowman space

Table 3. Effect of administration of AESI on some biochemical parameters of sub-acute toxicity in male (A) and female (B) rats

	Control (H_2O)	AESI 12.5 mg/kg	AESI 25 mg/kg	AESI 50 mg/kg	AESI 100 mg/kg
Male					
Urée	48.85±2.8	45.66±4.9	25.82±2.7c	32.47±2.04c	29.61±3.07c
Creatinine (mg/dl)	1.06±0.05	1.03±0.11	1.13±0.07	1.08±0.11	0.96±0.18
TAG	106.08±3.74	72.56±5.50c	84.65±4.11c	73.15±6.95c	93.91±7.3
C-Total	26.59±1.52	10.05±0.74c	27.93±2.93	13.79±1.90c	20.57±1.24a
C-HDL	10.63±1.27	7.39±0.71a	18.94±1.80c	6.41±1.06	12.16±0.67
C-LDL	3.37±0.56	2.63±0.52a	2.19±0.94	1.82±1.45a	3.59±1.05
ALP (U/L)	142.79±14.67	170.24±11.67	121.28±13.49	84.25±7.94b	97.03±7.39a
ALT (U/L)	16.79±0.94	29.48±1.80c	18.82±2.20	16.08±1.74	11.63±1.74b
AST (U/L)	51.49±4.48	30.19±2.28c	29.14±1.64c	30.53±3.06c	29.87±3.67c
Albumine	3.81±0.15	4.42±0.33	3.93±0.27	3.91±0.15	4.27±0.23
Total protein (g/dl)	6.77±0.18	7.12±0.44	7.19±0.41	7.46±0.30	7.40±0.35
Glycemie	32.72±3.42	30.01±2.65	39.79±3.75	48.05±4.69b	47.37±3.42b
Female					
Urée	59.50±3.86	30.62±2.35c	25.22±1.60c	31.92±1.49c	26.99±1.79c
Creatinine (mg/dl)	1.08±0.08	0.94±0.07	0.79±0.05	1.44±0.20a	1.02±1.79
TAG	78.74±2.82	86.56±6.10a	58.76±6.26	69.15±5.02	75.85±3.82
C-Total	13.76±1.16	23.05±3.01b	24.75±1.65b	13.24±0.72	14.29±1.14
C-HDL	10.30±1.26	7.74±1.23	4.59±0.56b	6.27±0.45a	9.39±0.81
C-LDL	1.49±0.18	6.10±1.14c	9.90±1.86c	1.08±0.32	0.87±0.22
ALP (U/L)	82.02±7.04	64.31±4.08	69.92±9.63	58.79±3.85b	69.86±7.97
ALT (U/L)	25.09±1.17	15.12±1.39b	21.40±2.43	17.29±1.19b	21.04±1.90
AST (U/L)	42.75±2.01	33.50±2.05a	36.57±2.51	45.65±2.14	26.21±2.90c
Albumine	4.98±0.35	4.19±0.17a	4.71±0.25	4.26±0.11a	4.47±0.24
Total protein (g/dl)	6.50±0.42	8.43±0.53b	7.57±0.54	9.47±0.18c	6.43±0.41
Glycemie	50.61±6.30	40.63±4.53a	33.23±1.35b	36.38±2.86b	41.00±4.20a

All data are reported as the mean ± S.E.M. for n = 5 per group. (ANOVA and LSD). [a]p <0.05, [b]p <0.01and [c]p <0.001: significant differences compared with control. TAG = triglycérides, ALT = Alanine amino Transferase, AST = Aspartate amino Transferase, ALP = Alkaline Phosphatase, AESI = Aqueous extract of Solanum indicum

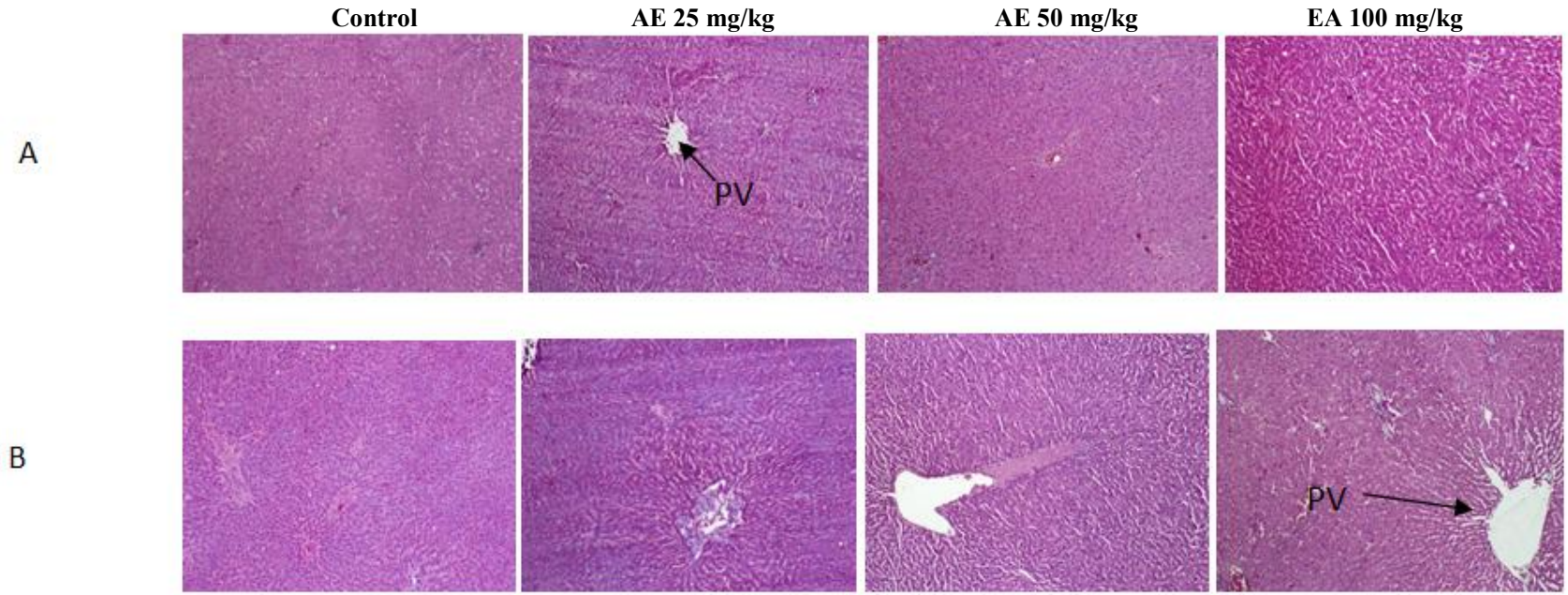

Fig. 5. Effects of AESI on male (A) and female (B) rats liver histology (H&E × 200)
PV = Portal vein

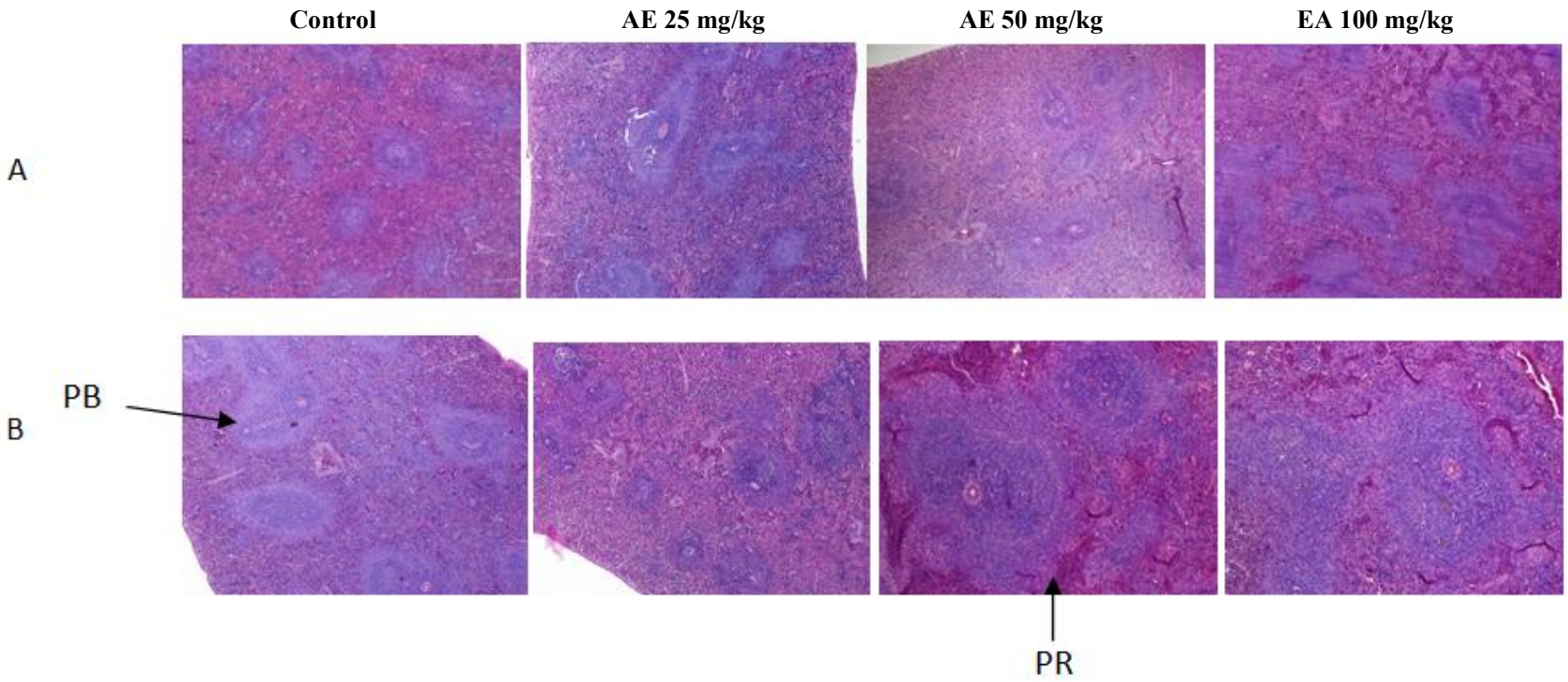

Fig. 6. Effects of AESl on male (A) and female (B) rats spleen histology (H&E × 200)
PB = Pulpe blanche, PR = Pulpe rouge

3.2.3 AESI effect on hematological parameters

The effects of sub-acute administration of AESI on hematological parameters are summarized in Table 2. In male rats, at 12.5, 25 and 50 mg/kg, the results revealed a significant ($p<0.05$ and $p<0.001$) increase in platelet count, plateletcrit, MPV and PDW as compared to their respective control values. In female rats, only the WBC count increased at the doses of 100 mg/kg while the other parameters plateletcrit, MCHC and lymphocytes decreased ($p<0.05$ and $p<0.01$) at 12.5 and 25 mg/kg after treatment with AESI for 28 days.

3.2.4 AESI effect on biochemical parameters

The effects of AESI on biochemical parameters of treated rats showed in both gender, a significant decrease ($P < 0.05$ and $P < 0.001$) in seric level of urea, TAG, total-C, LDL-C, ALP, ALT and AST at all doses when compared to the respective control values. We observed also a significant ($P < 0.01$) increase in seric level of glucose and total protein with 50 mg/kg AESI dose in male and female rats respectively when compared to their respective controls. However, in all treated female's rats a significant ($P < 0.01$ and $P < 0.001$) decrease was observed not only at previous mention parameters but also in seric level of glucose at the doses 50 and 100 mg/kg (Table 3).

3.2.5 Histological analysis of liver, spleen and kidneys

Histology of the liver, kidneys and spleen section in all treated groups did not revealed any structural changes after 28 days of treatment (Figs. 4, 5 and 6).

4. DISCUSSION

Herbal products represent the main source of treatment for people throughout the world. Generally, acute and sub-acute toxicity tests are usually used in laboratory to perform the safety studies on herbal medicines [14]. In the present study, we performed the acute and sub-acute oral toxicity of fruits of *Solanum indicum Linn* in *Wistar* albino rats.

In the acute toxicity study, administration of a single 5000 mg/kg dose of the aqueous extract of *Solanum indicum* Linn to 3 female rats did not show any death nor abnormal clinical signs of toxicity during the 14 days of observation. Therefore, the LD_{50} values of AESI may be considered greater than 5000 mg/kg. Furthermore, OECD (2008a) mentioned that, orally ingested substances with a LD_{50} value greater than 5000 mg/kg should be considered as relatively safe.

The administration of AESI for 28 days revealed no clinical signs of toxicity or mortality in both gender. Along the treatment period, animals exhibited a gradual increase in their body weight gain without significant changes in their consumption. This result could be justified by the fact that animal felt comfortable with the diet, suggesting that the extracts did not affect appetite and or consequently did not interfere with animal growth [15,16]. This result also corroborates the data of Abdel-Aziz et al. [11] during which a standardized extract of *Solanum indicum* ssp. Distichum did not affect food intake or rate of growth of the animals. Another important indicator of toxicity is the change in organ weight that is usually determined in subchronic and chronic toxicity tests [17]. Indeed, plant-derived products, when ingested, can be toxic to organs such as kidneys, liver, spleen, stomach and lungs due to their various function in the body [18]. The variations observed in liver, lung, kidneys and ovary were not gender nor dose responsive dependent; given that, macroscopic observation of these treated rat organs showed no abnormality in their morphology during the 28 days of experiment.

Hematological parameters represent an important clinical response to toxic products due to its components which are highly sensitive to toxins [19]. In this study, significant variations in hematological parameters were observed relatively to the dose administered and also to gender. Gender variation observed in these parameters can be due to sexual hormones produced. Indeed, estrogens present a mark effect not only on B and T lymphocyte cells equilibrium but also on cytochrome P450 enzymes metabolism [20]. The main hematological change in both gender was the decrease in MCHC, WBC, PLT, PCT, MPV, PDW, percentage of Granulocytes (%) and Monocytes. Platelets play an important role in the coagulation of blood that occurs in plasma following rupture of the blood vessel or injury of their epithelium [21]. It is also known their formation is controlled by thrombopoietin [22]. Thus, the significant decrease in platelet count obtained in all AESI treated animals could be due

to the fact that the extract inhibited the production of thrombopoietin. This decrease may also be attributed to the presence in the extract of metabolites which pharmacological effect are similar to diuretics, ibuprofen, aspirin and chloramphenicol [18,23].

Serum biochemical analyses were carried out to evaluate the effect of the extract on hepatic and renal functions and also on lipid profile. It is known that several toxic compounds accumulate in liver where their detoxification occurs. Liver damages are usually assessed by the determination of seric alanine amino transaminase (ALT) and aspartate amino transaminase (AST) [24]. The increase in AST and ALT activity of these enzymes indicates liver injury [25]. In this study, the decrease in AST and ALT activities for both gender treated groups suggest that AESI could present some hepatoprotective properties [19]. In AESI treated female rats, a reduction observed in seric level of glucose compared with the control group was in line with the work reported by Benmehdi et al. [26]. According to Shafaei et al. [27], the observed hypoglycemic effects of the pulp extract of *Citrullus colocynthis* may be due to wounded intestine and injured renal proximal tubules and their subsequent reduced ability to regulate glucose transportation. In addition, impaired hepatic function and glucose metabolism may be a contributing mechanism for induced hypoglycemia [28]. Many others studies reported similar findings regarding hepatoprotective effect of plants [29,30].

The level of tissue protein is one of the most widely used ways to measure hepatocellular damage. Indeed, measurement of these proteins can reflect nutritional status and be used to screen or help diagnose kidney and liver diseases as well as many other conditions. High levels of total protein can be observed with chronic inflammation or liver infections [18]. In this study, the significant increase in serum concentration of total protein in the treated animals further suggests the alteration the synthetic functions of the liver.

The metabolism of cholesterol and triglycerides are largely regulated via hepatic synthesis. Changes in the levels of these lipids could give information on the predisposition of the heart to cardiovascular diseases and some liver dysfunctions [31]. The administration of extract at the dose 12.5 and 50 mg/kg reveals a significant decrease in TAG and total cholesterol in male rats. This result suggests that the extract may not predispose the animals to heart diseases.

Urea and creatinine are considered to be effective markers of kidney function and the increase of these parameters may be associated with renal damage [32]. In renal disease, serum urea accumulates because the rate of serum urea production exceeds the rate of its clearance [33,34]. Creatinine is mainly derived from endogenous sources through the breakdown of tissue creatine. Plasma creatinine level in normal individuals is usually affected by body muscle mass. It increases in case of functional nephron damages [35,36]. There were no significant changes in the levels of serum creatinine in the AESI treated groups compared with the control. These results suggest that the kidney functions are not altered in animals treated with the extract. The decrease in serum urea concentration in the treated rats confirms that functioning of the kidney is normal. This result was similar to the study of Kokou et al. [2].

The histopathological findings in the liver, kidney and spleen did not reveal any damage or abnormalities of these organs, suggesting no morphological alterations. Our data are in agreement with those found by Affy et al. [1] and Kwan et al. [37]. All the biochemical, physiological and histological findings of the present study indicate the safety of *Solanum indicum Linn* chemical constituents of its fruit aqueous extract. Similar observations have been made by Abdel-Aziz et al. [11] while working on the fruit ethanolic extract of the same medicinal plant. As demonstrated by Rizwan et al. [9] who completed the phytochemical analysis of the medicinal plant, the harmless nature of both aqueous and ethanolic extracts could be directly related to the low toxicicological potential of their polar constituents such as flavonoids, glycosides, sugars, alkaloids and tannins.

5. CONCLUSION

The present study reveals the harmless nature potential of *Solanum indicum linn.* fruit extract since it does not cause death or any evident symptoms in the acute and subacute oral toxicity studies. However, changes observed on some hematological and biochemical parameters calls us to a little more caution in its use. So, only complementary studies including the Long-term toxicity will help to confirm its harmless nature.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Experimental protocols used in this study were accepted by the local ethical committee of our Faculty (Faculty of sciences, University of Dschang, Cameroon).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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