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Research Article
Acute and Subacute Oral Toxicity Assessment of Gender of Ahaggar's *Aerva javanica* in Animal Models

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Abstract

Background and Objective: The aqueous infusion of *Aerva javanica* (AJ) has been reported to possess several pharmacological properties as antivenom and anti-inflammatory, being widely distributed its use in phytotherapy has increased, however, its safety profile remains unknown. The main purpose of this study is the identification of bioactive compounds to assess the acute and subacute oral toxicity of *Aerva javanica* gender in mice and rats. **Materials and Methods:** The phytochemical screening was carried out using standard colorimetric methods. In LD_{50} assays, the oral doses (500, 750, 1000, 2500 and 5000 mg kg^{-1} b.wt.) of tested plant extract was administered to mice. In subacute toxicity, 250 and 500 mg kg^{-1} b.wt., were tested on rats and changes in weight, biochemical, haematological and histopathological parameters were studied. The data obtained were determined using one-way analysis of variance (ANOVA) and *post hoc* Tukey test. **Results:** Present findings highlight the difference of bioactive component levels between male and female plants, no death was recorded at acute toxicity assessment, the plant is considered as non-toxic at a single dose, the LD_{50} value was above 5000 mg kg^{-1}. Subacute treatment did not significantly alter animal weight growth, organ to body weight ratios, biochemical parameters or any damage of internal organs. The hemoglobin (HB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet levels significantly decreased in the female rats submitted to 500 mg kg^{-1} b.wt., of the female plant extract. **Conclusion:** The dioecy could have a real impact on the chemical composition of plant genders and their medicinal effects. The AJ aqueous extracts does not cause acute toxicity but in subacute toxicity the female (AJ) cause a hypochromic anemia on female rats. However, further toxicological assays would be necessary to confirm the safety of this plant extract.

Key words: *Aerva Javanica*, Amaranthaceae, dioecy, herbal medicine, phytochemical, oral toxicities, safety profile

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In this last decade, the use of alternative remedies by natural drugs has increased tremendously, increased with the surge and acceptance of the public to turn back to natural therapies. In Algeria, as in other countries, herbal remedies are becoming available in drug stores, in food stores and supermarkets; as a result, the population is relying more on traditional natural remedies which display one part of the culture of aboriginal communities in Southern Algeria[1].

The ethnobotanic survey carried out (2010-2015) in "Ahaggar" (Highland area in Southern Algeria) has reported more than 80 natural plants involved in natural medicine[1]. Among the most used plant species, *Aerva javanica*, named locally in Tamahaq (local language spoken in Southern Algeria) as "Timkerkezt, Timkerzite or Timkerzist" which is widely used against pains and various illnesses particularly against snake bites and scorpion stings by the aboriginal population of Tamanrasset, Southern Algeria.

Aerva javanica (Burm. F) Juss ex Schult is a plant species that belong to Amaranthaceae family[2] which is characterized by hybridization and polyploidy of some genus leading to the difficulties in the identification of some of plant species[3]. The field report finding has underlined the dioecy of *Aerva javanica* by the presence of distinct male and female plants grouped in well distributed populations on different soils[1]. Also, it has reported the wide use of its different parts by the native population and healers against pains and ills[1,4].

Previous studies carried out on *Aerva javanica* (independently of the plant gender) in Egypt, Pakistan, India and Australia have reported the presence of many chemical compounds such as flavonoides, steroids, triterpenes, tannins and saponins. Also, other primary components are listed as carbohydrates with glycosides, lipids, fatty acids and essential oils as hentriacontane, squalene, etc.[5-9].

Some of so-called toxic elements produced by plants have no known threshold and may even be useful at low dose[10], thus, it is convenient to study the toxicity of tested plant extracts[11].

This investigation was conducted to distinguish the difference of reaction between male and female plants to ensure the safety of the population related to the ubiquitous self-harvesting of plants by individuals or healers. For this purpose, a preliminary phytochemical screening of the crude aqueous extract was carried to identify the major bioactive compounds in both male and female plants, followed by the determination of the acute and subacute toxicity to evaluate the safety of the herbal preparation.

This study is the first attempt in analyzing a possible reaction between male and female plants response *in vivo* on mammal animals. So far, no comparative study on randomly used male and female Ahaggar's *Aerva javanica* has been published.

MATERIALS AND METHODS

Plant collection and identification: The plants were collected in spring March, 2015, at Oued Amsel village, Tamanrasset, Southern Algeria (Al: 1254 m, Long: 05°30,816E; Lat: 22°40.644N) and identified through their features by the use of flora Ozenda[12] and the book of Sahki and Sahki[13], which gives a summarized description of all Hoggar plants.

The plant species (male and female) were authenticated according to Maire[14] and confirmed by researchers at the National Institute of Forestry Research of Tamanrasset (INRF), Algeria. A voucher specimen was deposited in the Herbarium of the Laboratory of Research on Arid Zones, University of Sciences and Technology Houari Boumediene, Algeria (N°1-2012 Tam, PAM/LRZA/USTHB).

Preparation of aqueous infusion extracts: The aerial parts of male and female plants were dried separately under the shade and grinded into powder. The aqueous infusion extract was prepared according to a traditional method, an aqueous infusion of the powdered plant material in boiled saline water at different doses (250, 500, 750, 1000, 2500 and 5000 mg kg^{-1} b.wt.) was prepared.

The two prepared aqueous extracts were named as follows: MPE: Male plant extract and FPE: Female plant extract.

Animals: Swiss albino mice (20-25 g) and Wistar albino rats (150-200 g) breeding in Pasteur Institute, Algiers, Algeria were tested. The animals were maintained in a monitored temperature environment (20-24°C), under the light 12 h a day, 50-65% of humidity and free access to water and food (croquette scheme from the National Office of Animal feed, Bejaia, ONAB, Algeria). The animals were allowed to adapt to the laboratory for at least 1 week before testing and were used only once.

The rats which showed signs of morbidity attributed to a commonly observed anomaly in animals during rearing (torticollis, turn in circles in continuous manner) were excluded from this study and replaced by healthy rats treated under the same conditions.

Experiments on animals were performed in accordance with guidelines of the Institutional Animal Ethics Committee and were carried out in accordance for the handling and use of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals, as specified by Izmirli *et al.*[15].

Table 1: Colorimetric reagents used in identification of some secondary metabolites

Identified chemicals compounds	Chemicals and reagents added	Obtained reactions
Anthocyanins	HCl	Red coloring
	Ethanol/Metallic zinc/HCl	Red coloring
	Ammonia 1/2	Blue coloring
Leuco anthocyanins	Propanol/HCl	Red coloring
Tannins	$FeCl_3$ (5%)	Blue-black coloring
Gallic tannins	Sodium acetate and $FeCl_3$	Dark blue coloring
Catechic tannins	Stiasny's reagent	Red coloring
Free quinones	HCl, Chloroform and Ammonia ½	Red coloring
Saponins	HCl (0.1 N)	Foam formation
	NaOH (0.1N)	Foam formation
	Pb $(C_2H_3O_2)_2$	Precipitation
Carotenoids	HCl and H_2SO_4	Blue-green coloring
Sennosids	HCl, ethyl ether and ammonia 1/2	Purple-red coloring
Alkaloids	Mayer's reagents	White-yellowish precipitation
	Dragendorff's reagents	Red precipitation
Coumarins	Ethyl alcohol, KOH and HCl	Cloudy solution
Flavonoids	HCl, Mg chips and Isoamyl alcohol	Release of heat then an orange-red or purplish coloring
Steroids	Chloroform, acetic anhydride and H_2SO_4	Blue-green coloring
Terpenoids	Methanol, H_2SO_4 and acetic anhydride	Purple to pink coloring
Phenols	$FeCl_3$ and $K_3Fe(CN)_6$	Purplish-blue coloring

Phytochemical screening: The phytochemical examinations were carried out for the extracts as per the standard methods using the following chemicals and reagents[16-20] as shown in Table 1.

Experimental

Acute oral toxicity assessment: One hundred and ten Swiss albino mice were divided into 11 groups of 10 animals each (5 males and 5 females). The acute oral toxicity was evaluated as per OECD guideline[21], with slight modifications. The control group received orally 1 mL/100 g b.wt., of 0.9% NaCl. The experimental groups received respectively 500, 750, 1000, 2500 and 5000 mg kg^{-1} b.wt., of MPE and FPE of *Aerva javanica*.

After drug administration, food and water were withheld for 4 h, animals were observed during 14 days[22] for obvious toxic symptoms: Irritation, corrosion, dermatitis, vomiting, dizziness, incoordination, shortness of breath, diarrhea, blood in the urine, hyperactivity, sleep, coma and mortality. The median lethal dose of the extract (LD_{50}) was estimated using probit[23,24].

Subacute oral toxicity study: Thirty six rats were randomly divided into 5 groups of 6 rats each (3 males and 3 females). The subacute oral toxicity was evaluated as per OECD guideline[25] with slight modifications.

Aqueous extracts were administered orally once a day for 4 weeks as follows: Control groups received 0.9% NaCl (10 mL kg^{-1} b.wt.), experimental groups I, II received 250 and 500 mg kg^{-1} b.wt., of MPE and the groups III, IV received 250 and 500 mg kg^{-1} b.wt., of FPE. Clinical signs were observed at least once a day during the treatment. The rats were weighted every 4 days[10]. The weight body variation of animals was expressed in percentage and calculated according to the following equation:

$$\text{Variation in body weight (\%)} = \frac{\text{BWD-BWD1}}{\text{BWD1}} \times 100$$

Where:

BWD = Body weight on day D
BWD1 = Body weight on day 1 of treatment

For the haematological and biochemical analysis the rats were fasted overnight prior to blood collection. On day 29, the blood samples were collected separately by retro-orbital technique in two tubes: One with EDTA for immediate analysis of haematological parameters, another dry tube containing heparin to separate plasma for biochemical estimations.

Haematological analysis: The haematological analysis was performed using an automatic haematology analyzer (SysMex K21), the parameters included: Red blood cells (RBC), white blood cells (WBC), hemoglobin (HB), hematocrit (HT), mean corpuscular volume (MCV), mean corpuscular haemoglobine (MCH), mean corpuscular haemoglobine concentration (MCHC) and plateles (PLT)[26,27].

Biochemical analysis: The blood was centrifuged at 3000 rpm for 5 min, the serum was separated and tested for evaluating the following parameters: Glucose, creatinine, total triglyceride (TT), total cholesterol (TC) and catalytic activities of enzymes such as alanine transaminase (ALT) and aspartate transaminase (AST)[28-31].

Condition of internal organs: At the end of the treatment, all rats were sacrificed, carefully dissected and internal organs (liver, kidneys, hearts and lungs) were removed for weight and macroscopic observation. The estimation of weight evolution of all these organs was determined according to Ahmadi[32]:

$$\text{Relative weight of the organ (RWO) (\%)} = \frac{\text{Absolute weight of organ (g)}}{\text{Body weight of the animal (g)}} \times 100$$

Histopathological examination: The histological and immunological examination was performed according to the protocol of Anatomical Pathology Laboratory of Pierre and Marie Curie Center (Mustapha Pacha University Hospital Center, Algiers, Algeria).

The liver, kidney and stomach were removed from any adherent tissue and fixed in neutral buffered 10% formalin for 6 h so as to immobilize the cellular and tissue constituents in a state so close to the living state and allow the organs to withstand all manipulations.

Paraffin wax was used as an embedding medium for this natural tissue analysis to obtain enough numbers of satisfactory sectioned slices, parts of organs were dipped in several ethanol and xylene baths at increasing concentrations, then, immersed into molten paraffin in the cassettes[33-36].

One micrometer-thick sections were made using an autocut-microtom and put on regular slides[35]. Dewaxing and hydration steps were realized to remove the paraffin from the tissues so that the dyes can penetrate them (several xylem and ethanol baths with decreasing concentrations)[33].

For each organ, sections were transferred for hemalum-eosin (HE) staining and then mounted between blade and lamella using Eukitt[35].

Statistical analysis: Data of experiments obtained were presented as Mean±SD. Significance between control and extract treated groups were determined using one-way analysis of variance ANOVA, followed by Tukey test. The $p<0.05$ was considered significant. Each test was repeated trice.

RESULTS

Phytochemical composition: Phytochemical screening revealed the same composition of bioactive constituents in both male and female tested plants, except a difference in the amount of some constituents such as anthocyanins, leucoanthocyanins, alkaloids, terpenoids, flavonoids and phenols which seemed to be more abundant in female plants than in males. On the other hand, tannins, gallic tannins and sennosids showed a high abundance in male plants as compared to females. However, both of them showed the presence of the same amount of free quinones, saponins, carotenoids and steroids and the absence of catechic tannins (Table 2).

Table 2: Bioactive compounds composition of aerial parts of male plants "MPE" and females ones "FPE" of *Aerva javanica*

	Chemical reactions	
Bioactive compounds	MPE	FPE
Anthocyanins	++	+++
Leucoanthocyanins	++	+++
Tannins	+++	++
Gallic tannins	+++	++
Catechic tannins	-	-
Free quinones	++	++
Saponins	++	++
Carotenoids	+++	+++
Sennosids	+++	+
Alkaloids	++	+++
Coumarins	+++	+++
Flavonoids	++	+++
Steroids	+++	+++
Terpenoids	+	+++
Phenol	++	+++

-: Total absence, +: Presence, ++: Moderate amount, +++: High amount

Acute oral toxicity study: The oral administration of given doses (500, 750, 1000, 2500 and 5000 mg kg^{-1} b.wt.) from crude extracts of MPE and FPE of *Aerva javanica* have not caused any visible sign of acute toxicity or instant death for all mice tested during the first 4 h after treatment, after that, a hyperactivity of animals which fall asleep was observed, throughout the 14 days observation period there was no mortality in both sexes of mice.

Subacute toxicity study: During the treatment period, neither abnormal behavior or physiological activities, nor intoxication signs, nor death were noticed on male and female rats among of the treated groups.

The data obtained from the mean body weights of the control and experimental groups I, II, III and IV crude aqueous treated rats are given in Fig. 1, using one-way ANOVA and Tukey test ($p<0.05$).

Control rats showed no significant variation throughout the experimental period, the mean body weight values of male rats increased with the average of 155.23±2.3 g at the beginning of the experiment up to 201±8.2 g at the end of the 28 days of study, the body variation is of 29.48% with a weight gain at a rate of almost 4.21% of body weight every 4 days. In female rats, mean body weight values increased from the average of 190±2.5 to 208±10.7 g with a body variation of 9.54%, thereby the weight gain is at a rate of

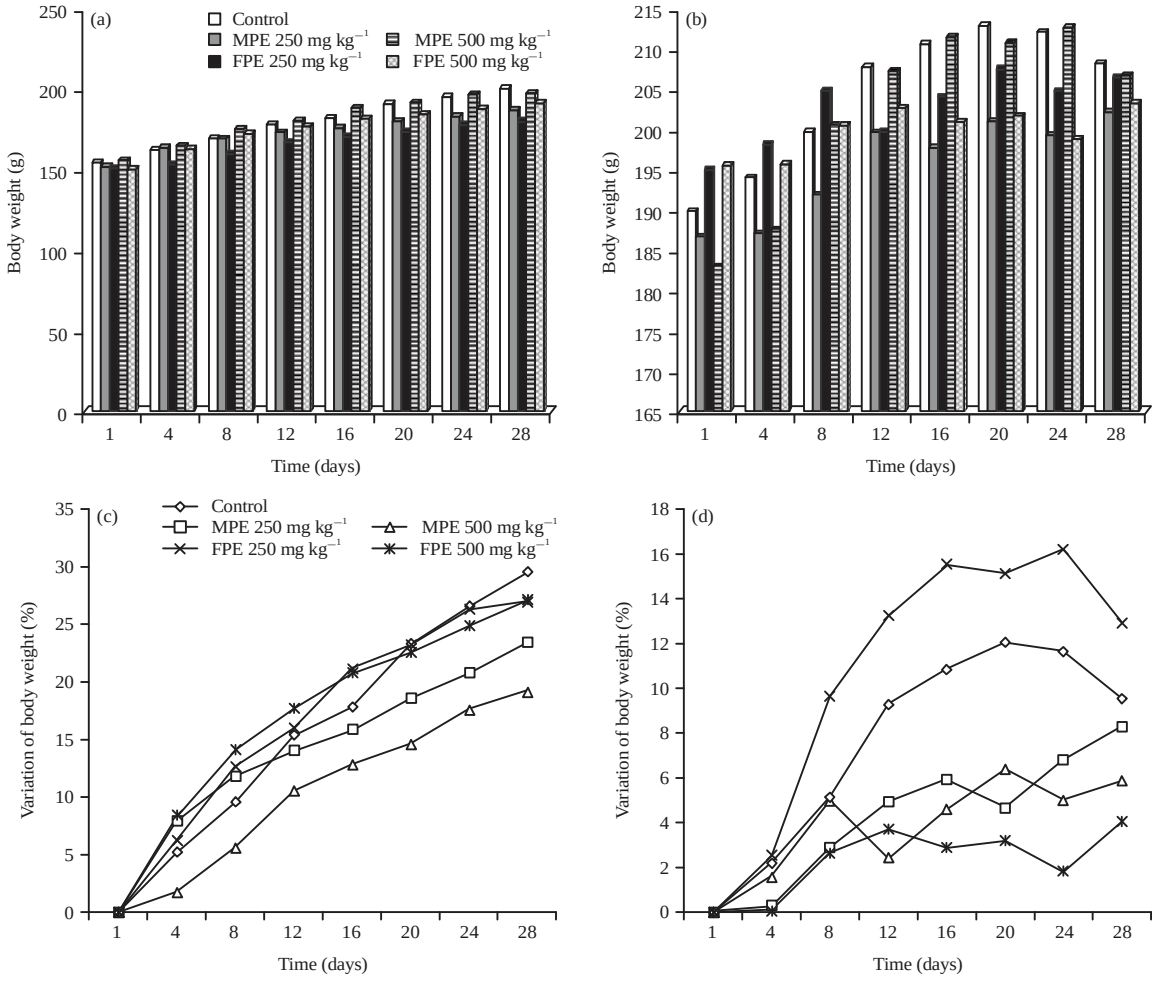

Fig. 1(a-c): (a, b) Body weight daily changes in gram and (c, d) Their variation with time (%) in both sexes of rats (A: Male, B: Female) after treatment in subacute toxicity (Mean±SD, n = 3)

almost 2.41% of body weight every 4 days until the 20th day and then decreased by 1.25% every 4 day until the 28th day of treatment.

Concerning the body weight evolution of experimental groups, no significant difference was noted between male or female rats with their respective controls, while a general gain of body weight of all rats tested was observed after the 28 days of treatment.

Compared to male rats, the 4 treated groups were homogeneous, their mean body weight value increased with the average of 152.85±2.4 g at the beginning up to 189.75±7.4 g at the end of the experiment, the body variation is of 24.12%, thereby, the weight gain is almost 3.44±0.5% every 4 days.

However, in female rats, groups I and II treated with MPE at the dose of respectively 250 and 500 mg kg^{-1} b.wt., formed a homogeneous group, their mean body weight value increased with the average of 198.87±5.9 g at the beginning up to 204.33±3.1 g at the end of the experiment, the body variation is of 7.08±1.7% thereby the weight gain is almost 1.01±0.2% every 4 days. Unlike groups III and IV treated with the FPE extract at the dose of 250 and 500 mg kg^{-1} of b.wt., respectively, which show a significant difference on the 24th day of treatment at $p<0.05$. The mean body weight value of group III increased with the average of 183.03±2.3 g at the beginning up to 206.67±8.5 g at the end of the experiment, the body variation is of 12.91±1.8% thereby the weight gain is almost 1.84% every 4 days. The mean body weight value of group IV increased with the average of 195.47±4 g at the beginning up to 203.33±4.5 g at the end of the experiment, the body variation is of 4.02% thereby the weight gain is almost 0.5% every 4 days. This difference in variation of body weight between Groups III and IV is due to the difference in food consumption which means that the FPE extracts influences the appetite of female rats, at low-dose of FPE (250 mg kg^{-1} of b.wt.) the rats eat more than at high-dose of FPE (500 mg kg^{-1} b.wt.) where the appetite decreases.

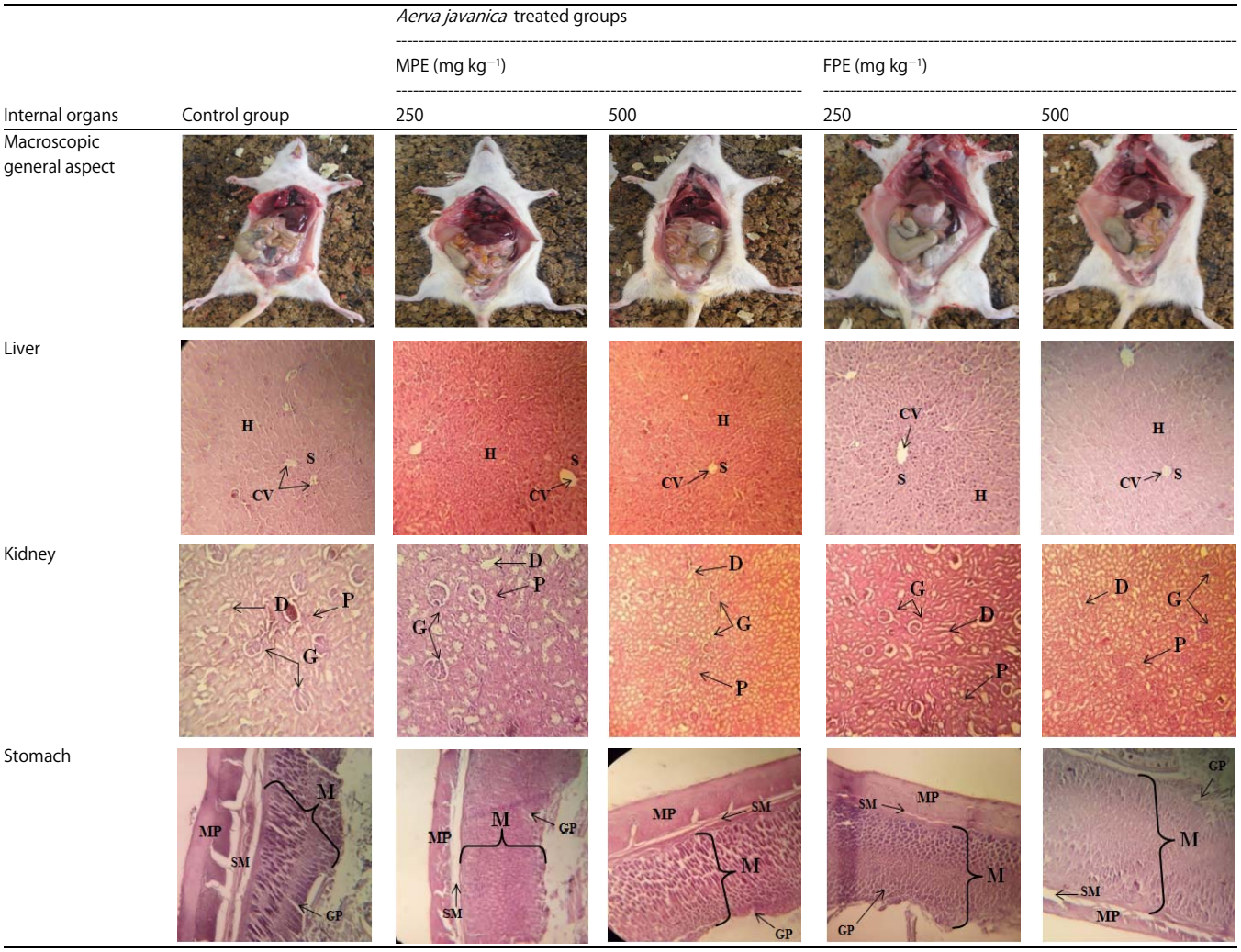

Fig. 2: Macroscopic examination and histopathological pictures of liver, kidney and stomach of control and experimental groups of rats in subacute toxicity taken under optical microscope (45 and 100X) (Mean±SD, n = 3). The macroscopic examination pictures highlighting the state of the internal organs of the control and treated rats after the biopsy showing no internal bleeding or cysts. The liver of control and experimental groups of rats show normal histological structure of central vein (CV), surrounding hepatocytes (H) and sinusoids (S). The kidney of control and experimental groups show normal histological structure of glomerulus (G), proximal convoluted tubules (P) and distal convoluted tubules (D). The stomach of control and experimental groups of rats show muscular layer (M), submucosa layer (SM), muscularis propria (MP) and gastric pits (GP) in good condition

Furthermore, haematological studies revealed no significant effect of plant extracts (MPE and FPE) on haematological parameters of male rats, alike, female rats seemed to be affected by the extract of female plants at 500 mg kg^{-1} b.wt.

This efficiency was underlined by the available high significant decrease of the percentage of HB (5.9±0.1 g dL^{-1}), MCH (8.93±0.2 pg) and MCHC (26.6±0.3%) at ($p<0.001$) and a significant effect at ($p<0.05$) on the lowering of PLT (760.33±67.5×10^3 µL) as seen in Table 3.

As summarized in Table 4 the MPE and the FPE of *Aerva javanica* in 250 and 500 mg kg^{-1} b.wt., did not significantly change the level of glucose, creatinine, TT, TC, AST and ALT in males and females rats according to the respective controls.

Moreover, the morphological features examination of internal organs (heart, kidney, liver and lung) has not shown any significant histopathological change as well as in internal organs of experimental groups treated by either male or female plants as in those of control groups (Table 5, Fig. 2). These observations suggest that

Table 3: Effect of aerials infusions extracts of *Aerva javanica* on haematological parameters of male and female rats in subacute toxicity (Mean±SD, n = 3)

		Aerva javanica treated groups								
		Male rats					Female rats			
		MPE (mg kg^{-1})		FPE (mg kg^{-1})			MPE (mg kg^{-1})		FPE (mg kg^{-1})	
Parameters	Control group	250	500	250	500	Control group	250	500	250	500
WBC (×10^3 µL^{-1})	7.96±0.9	7.97±0.8^{ns}	7.30±0.4^{ns}	7.72±0.8^{ns}	7.42±0.2^{ns}	6.07±0.3	6.07±0.5^{ns}	7.39±0.7^{ns}	6.32±0.7^{ns}	5.35±0.7^{ns}
RBC (×10^6 µL^{-1})	6.30±0.4	6.74±0.9^{ns}	6.83±0.8^{ns}	5.47±0.1^{ns}	6.48±0.3^{ns}	7.03±0.5	7.24±0.2^{ns}	7.12±0.4^{ns}	7.86±0.3^{ns}	6.80±0.1^{ns}
HB (g dL^{-1})	13.07±1.4	13.30±0.9^{ns}	13.63±1^{ns}	12.77±1.3^{ns}	12.63±1^{ns}	12.57±0.5	12.63±0.6^{ns}	12.74±0.6^{ns}	13.8±0.1^{ns}	5.90±0.1***
HT (%)	39.07±4.3	41.85±3.6^{ns}	40.17±1.5^{ns}	38.00±5.9^{ns}	40.37±0.5^{ns}	38.83±2	39.47±0.8^{ns}	38.89±1.4^{ns}	41.33±2.9^{ns}	36.57±0.7^{ns}
MCV (fL)	60.67±1.6	59.73±1.4^{ns}	62.10±2.3^{ns}	62.90±0.6^{ns}	62.93±2.6^{ns}	56.63±0.9	54.45±1.2^{ns}	54.69±0.9^{ns}	54.4±0.8^{ns}	54.20±1.1^{ns}
MCH (pg)	20.30±0.4	19.63±0.2^{ns}	20.37±1^{ns}	21.27±1.2^{ns}	20.07±1.2^{ns}	18.40±0.1	18.02±0.2^{ns}	17.95±0.1^{ns}	17.36±0.9^{ns}	8.93±0.2***
MCHC (%)	33.50±0.2	32.13±0.8^{ns}	32.77±0.6^{ns}	33.77±1.6^{ns}	30.13±2.3^{ns}	32.40±0.4	33.02±0.5^{ns}	32.83±0.4^{ns}	32.03±0.1^{ns}	26.60±0.3***
PLT (×10^3 µL^{-1})	842.33±47.4	811.00±59^{ns}	788.67±32.1^{ns}	712.00±40.7^{ns}	815.67±63.4^{ns}	964.00±65	822.00±67.8^{ns}	823.33±23.4^{ns}	903.67±26.5^{ns}	760.33±67.5*

ns: Not significant, *p<0.05, ***p<0.001 relative to the control group (one-way ANOVA, *post hoc* Tukey test)

Table 4: Effect of *Aerva javanica* on blood chemistry values of male and female rats in subacute toxicity (Mean±SD, n = 3)

		Aerva javanica treated groups								
		Male rats					Female rats			
		MPE (mg kg^{-1})		FPE (mg kg^{-1})			MPE (mg kg^{-1})		FPE (mg kg^{-1})	
Parameters	Control group	250	500	250	500	Control group	250	500	250	500
Glucose (mg dL^{-1})	1.02±0.3	1.39±0.4^{ns}	0.54±0.1^{ns}	0.93±0.1^{ns}	1.15±0.2^{ns}	1.02±0.2	1.04±0.7^{ns}	1.09±0.7^{ns}	0.99±0.6^{ns}	1.02±0.2^{ns}
Creatinine (mg dL^{-1})	6.61±1.1	5.41±0.9^{ns}	7.57±1.1^{ns}	6.20±1.1^{ns}	6.98±0.2^{ns}	8.18±0.7	6.54±0.2^{ns}	7.50±0.7^{ns}	7.47±1.3^{ns}	8.00^{ns}
TT (mg dL^{-1})	0.83±0.5	0.81±0.5^{ns}	0.83±0.5^{ns}	1.03±0.3^{ns}	0.84±0.2^{ns}	0.73±0.2	0.64±0.2^{ns}	0.48±0.02^{ns}	0.53±0.2^{ns}	0.60±0.1^{ns}
TC (mg dL^{-1})	0.51±001	0.54±0.1^{ns}	0.47±0.1^{ns}	0.56±0.2^{ns}	0.60±0.04^{ns}	0.75±0.1	0.65±0,03^{ns}	0.66±0,1^{ns}	0.62±0.1^{ns}	0.61±0.1^{ns}
AST (U L^{-1})	136.00±28.9	118.33±6.5^{ns}	173.00±32.6^{ns}	144.67±26.5^{ns}	118.33±17.9^{ns}	124.60±23	115.00±4.2^{ns}	156.00±19.8^{ns}	152.50±10.6^{ns}	154.50±21.9^{ns}
ALT (U L^{-1})	75.38±14.8	74.33±7.4^{ns}	73.33±26.7^{ns}	84.33±10.6^{ns}	65.00±7.5^{ns}	81.00±14.7	78.50±2.1^{ns}	66.00±7.1^{ns}	71.66±1.5^{ns}	59.50±9.2^{ns}

ns: Not significant

Table 5: Effect of aerial infusions extracts of *Aerva javanica* on the relative weight of the organs (RWO) of male and female rats in subacute toxicity (Mean±SD, n = 3)

		Aerva javanica treated groups								
		Male rats					Female rats			
		MPE (mg kg^{-1})		FEP (mg kg^{-1})			MPE (mg kg^{-1})		FPE (mg kg^{-1})	
Internal organs RWO (g g^{-1})	Control group	250	500	250	500	Control group	250	500	250	500
Kidney	0.61±0.02	0.63±0.01^{ns}	0.63±0.03^{ns}	0.61±0.03^{ns}	0.63±0.05^{ns}	0.68±0.02	0.59±0.07^{ns}	0.60±0.06^{ns}	0.65±0.01^{ns}	0.73±0.02^{ns}
Liver	4.63±0.5	4.21±0.2^{ns}	3.94±0.4^{ns}	4.10±0.05^{ns}	4.33±0.2^{ns}	4.12±0.2	3.53±0.2^{ns}	3.57±0.1^{ns}	3.98±0.4^{ns}	3.83±0.2^{ns}
Lung	0.65±0.09	0.66±0.09^{ns}	0.73±0.07^{ns}	0.67±0.05^{ns}	0.68±0.09^{ns}	1.06±0.07	1.15±0.2^{ns}	1.15±0.01^{ns}	1.32±0.09^{ns}	0.84±0.02^{ns}
Heart	0.30±0.03	0.30±0.02^{ns}	0.31±0.05^{ns}	0.28±0.02^{ns}	0.29±0.02^{ns}	0.31±0.03	0.36±0.02^{ns}	0.31±0.03^{ns}	0.31±0.02^{ns}	0.33±0.02^{ns}

ns: Not significant

plant extracts (male and female ones) independently of their gender do not affect the internal organ functions.

Obviously, no sign of pathological lesions was neither noted in the liver, the kidneys, nor in the stomach of the experimental groups as compared to the rats control group. There was no histological difference between the internal organs of male and female rats (Fig. 2, only the female rat organs are shown).

There were no pathological changes in the liver of experimental rats groups as compared to healthy control livers which showed normal lobular architecture with central vein, surrounding hepatocytes and sinusoid without any vascular congestion or dilation of the sinusoidal capillaries or infiltration of inflammatory cells or necrosis of bypass or early fibrosis.

The histological appearance of the kidney of control and experimental rats groups showed a normal histological structure of glomerulus, proximal convoluted tubules and distal convoluted tubules, neither degenerate nor necrotic glomerulus nor inflammation or tubular clarification was observed.

The layers of the stomach were well arranged and visible as mucosa layer, submucosa layer and muscularis propria layer. The mucosa layer of the stomach of the control and the experimental rats groups showed normal histology with intact epithelial lining and gastric pits.

DISCUSSION

The richness of *Aerva javanica* of Southern Algeria in polyphenols and phenolic components could be a useful tool in the appreciation of biological properties that influence the human health. In spite of several reports of their abundance in this plant[5-8], in this study the application of colorimetric reactions showed a difference between male and female plants. This difference of composition might be explained by the dioecy which is a feature impacting the phytochemical and pharmacological properties of a plant species[37]. Similar results were also observed by Jose-Chagas *et al.*[38] and Bajpai *et al.*[39], who had shown a significant qualitative and quantitative intersex variation in phenolic classes. These authors had suggested the important use of LC-MS and MS/MS methods to differentiate between male and female plants relative to their chemical profiles and quantities of alkaloid bioactive markers.

This phytochemical difference between male and female *Aerva javanica* supports the random selection of female plants by the Touareg population which might have an impact on the daily health care. Also, it may also justify the abundance of male plants as compared to female ones in Ahaggar, by their higher resistance to desert arid conditions[40].

Secondary constituents identified such as flavonoids, saponins and alkaloids widely reported as having health benefits, say anti-inflammatory, antioxidant, protection against neurodegenerative disorders, vasculo-protective and veinotonous property[41,42], promoting tissue regeneration, decreasing the permeability of the blood capillaries and reinforcing their resistance to haemolysis[43]. All these issues could justify the large traditional use of this plant in the treatment of any ailment.

The data obtained after an oral toxicity test in animals may be used for hazard classification purposes through the LD_{50} and for the assessment of risks to human health[44-46] despite some criticisms[47].

The absence of death and other toxicological exhibition by the oral gavage of 5000 mg kg^{-1} b.wt., advice that the aqueous infusion extract may be safe by this mode[48] and this dose is considered practically non-toxic according to National Research Council (NRC)[44].

To identify risks to human health from repeated exposure to the extract, a subacute toxicity test was performed. Periodic weight monitoring of treated rats and the relative weight of their organs were used as indices in a toxicological evaluation[49]. In this study it was analyzed that the repeated oral administration of the extract at tested doses of 250 and 500 mg kg^{-1} b.wt., did not cause any changes in experimental groups of rats as compared to the control group, indeed, the weight gain of all rats at the end of the experiment suggests that the metabolic disorder were not affected by a toxic substance[50].

This difference in weight between male and female rats was not significant, it could be due to the difference in age between the animals (35 days for male rats and 45 days for female rats) and as reported earlier, the evident knowledge that male rats gain almost twice too much weight with the age than female ones[51].

The comparison of ALT and AST parameters, which are mostly used to displaying cellular injuries[52-54], showed a stable state between the control and experimental groups under our experimental conditions. This situation might explain the absence of damage of some internal organs as liver, lungs and heart morphologically observed after each rat dissection[55,56]. Also, the high amount of coumarins, flavonoids and steroids could ensure a natural antioxidant power that can prevent cellular damage and pathological consequences[57].

The blood chemistry data obtained seems to be correlating with the macroscopic examination and the histopathological analysis of internal organs, no form or stain changes as well as no necrosis or inflammation were detected in all internal organs in the experimental groups compared to the control group. These results suggest that the plant extracts independently of its gender does not affect the internal organ functions.

All these observations could explain the huge traditional treatment of *Aerva javanica* against the inflammation of internal organs[58] as the nephro-protective activity underlined by Movaliya *et al.*[59] and the hepatoprotective activity against hepatotoxicity underlined by Arbab *et al.*[60] and Rajesh *et al.*[61]. The haematopoietic system is one of the most sensitive targets of toxicants, which is a good index of toxicity of a substance. The comparison of haematological parameters has highlighted that the extract of female plants induced a hypochromic anemia (iron deficiency) by causing a significant decrease of HB, MCH and MCHC, within a decrease in platelet levels in the blood, while no significant effect was noted in male rats treated by the same extract.

Advanced hepatopathies may be the cause of anemia[62]. However, the deterioration of the hepatic function has neither been observed in the histological study of the liver or in the ALT and AST parameters, nor in the determination of total cholesterol where the liver is the essential location of its synthesis[63]. Anemia caused by haematologic changes is often accompanied by neoplasms[64] and may result in haemolysis or inhibition of red blood cell production in the bone marrow[65]. In this case, the extract would contain components which at high dose could have a deleterious action over the haematopoietic system[63]. In addition, this anemia could be explained by gynecological losses very known in female living things as rats which are generally benign (abundant menstruation, poorly adapted contraception, intrauterine device, uterine fibroid).

Regarding the traditional use of *Aerva javanica* by Touareg healers, most of them have reported its large efficiency by two different modes of use, the oral uptake to relieve pain of the digestive system, child delivery and the external skin application against scorpion stings, snake bites, burns and skin affections.

Concerning the dosage in the oral uptake, the consumption of about a handful of plant material, the average of 30 g of aerial plant parts[66], infused in 2 L of tap water and administered in glass doses (125 mL) once to twice a day during 1 week which is equivalent to the dose of 27-54 mg kg^{-1} weight/day for a 70 kg adult, enough to treat digestive and child delivery pains.

On the other hand, the external skin application is achieved by rubbing fresh or wet leaves on the bite spot just after scorpion stings to avoid fever and stop the poison movement, or by the application of the crushed roots mixing with "Natron"[13]. Concerning burns cases, the whole dried plant or lonely roots powder are melted into animal fat before application or directly sprinkled onto the injury places to minimize contamination and pus formation.

In comparison to the dose applied in these experiments, the maximum dose tested in the acute toxicity test was 5000 mg kg^{-1} b.wt., which appears 92 times more than that authorized dose cited above. A maximum dose of 500 mg kg^{-1} body weight per day during 28 days was applied in the subacute toxicity determination, these conditions (9 times larger concentration and 4 times longer) are harsher than those authorized by healers. Note that this assay helps to have an idea of the safety margin in toxicological risk assessment of the tested plant extract[67].

The results of these studies explain the traditional frequent use of the plant mostly by women in several cases as pregnancy health against aches and pains and to accelerate child delivery which implies a potential estrogenic effect of the extract[68], knowing that the phyto-oestrogenic effect of the flavonoids class has been highlighted by Gonzales[69].

Nevertheless, the difference of reaction of male and female rats to *Aerva javanica* treatment could be due to bioactive composition difference between male and female *Aerva javanica* extracts as flavonoids and even more to the selective action of female plants specifically over the female animal gender.

However, the haematological results as well as the bioactive composition confirm the presence of a difference between male and female *Aerva javanica*, joining the results of earlier reports on dioecious plants[38-40] where phytochemical constituents may be responsible for many ethnomedicinal uses of the plant by increasing its safety margin.

CONCLUSION

It is concluded that although, the real difference of bioactive component levels between male and female plants, Aerva javanica is considered as non-toxic by oral administration in a single dose. However, given for several weeks at the dose of 500 mg kg^{-1} b.wt., the female plant induces hypochromic anemia on female rats, which implies a potential estrogenic effect of the female plant unlike the male ones. However, this experimented dose remains more than 9 times stronger than the recommended dose by

healers, thus this open the mind to the large use of this plant by Touaregs to cure various diseases. Indeed, the parameters evaluated in this study showed the safety of extracts of aerial parts of *Aerva javanica* independently of its gender. Otherwise, further toxicological assays would be necessary to confirm the safety of this plant extract.

SIGNIFICANCE STATEMENT

This study discovers the presence of a real difference of bioactive component levels between male and female *Aerva javanica* for the first time with the possible selective action of the plant gender on the animal sex that can be beneficial for the traditional healers in their huge usage patterns of medicinal plants and the possibility of using *Aerva javanica* as therapeutic treatment against various inflammatory diseases as it is considered as non-toxic plant at a single dose.

This study will help the researchers to uncover the critical areas of the interactions of natural products with biological systems that many researchers were not able to explore. Thus, a new theory on the impact of dioecy in the chemical composition of plants and their medicinal effect may be arrived at.

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