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Chemical Characterization and Effects of Oral Administration of *Pelargonium graveolens* Essential Oil on Testicular Histomorphometry of White Rabbits

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ABSTRACT

Pelargonium graveolens is a species of pelargonium known for its aromatic leaves, which are used to produce essential oil. This essential oil is highly valued in aromatherapy for its anti-inflammatory, antibacterial, and skin-healing properties. This study was undertaken to assess the impacts of oral administration of *Pelargonium graveolens* essential oil (PEO) on fertility enhancement of rabbits' testicles and Gas chromatography coupled with mass spectrometry (GC-MS) examination. The chemical elements of PEO oil were characterized in the physico-chemical center (TPPCA), Algeria. Thirty-one white prepubescent male rabbits belonging to the local population aged 2 months, divided into three groups including one control batch and two experimental batches, administered orally with PEO at 0.4 mL/kg and 0.6 mL/kg /body weight doses. After 21 days of treatment, the rabbits were sacrificed and dissected, the testes were excised, defatted, weighed and preserved in Bouin Holland's solution. Phytochemical screening of *P. graveolens* essential oil shows the presence of 26 and 18 bioactive molecules of the plants collected in May and November 2023, respectively. In contrast to the reference group, the macroscopic parameters were higher in the treated groups and the histological analysis demonstrated notable microscopic variations between the treated rabbits and the baseline batch, such as the appearance of the first elongated spermatids in some seminiferous tubules. These results suggest that the *P.graveolens* essential oil could improve the development and the reproductive process of prepubescent male rabbits.

***Keywords*:** Essential oil, GC-MS, Histomorphometry, *Pelargonium graveolens*, Testicles.

Introduction

A feasible approach to drug development is to use natural ingredients sourced from plants[1].Studies have shown that the active metabolites generated by plants are the key compounds that determine their therapeutic properties[2].A key step in evaluating the healing potential of medicinal plants is the extraction of phytochemicals via procedures for example decoction, infusion, maceration and others.[3] The identification of chemical elements is essential to understand their roles in various chemical compounds.[4] The use of essential oils as dietary additives has gained increased attention due to these positive effects on promoting growth.[5] Dietary supplementation with antioxidant properties may improve sperm quality, motility and increase hormone synthesis.[6] *P.graveolens* (geranium) indigenous to Southern Africa and part of the Geraniaceae family. Geranium essential oil is a preferred ingredient in the perfumery and cosmetic industries[7,8], exhibits high antioxidant and antimicrobial activities,[9] is used as an antidepressant and antiseptic remedy,[10] it possesses the ability to inhibit free radicals.[11] The target of the study was to scrutinize the impacts of PEO administration on body weight gain, testicular weight, and reproductive function in prepubescent white rabbits and to determine its bioactive chemical components.

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Materials and Methods

Identification of plant and extraction process

P. graveolens leaves were harvested in the spring and autumn (during May and November 2023) in the Yakouren region (Algeria), located at an elevation of 800m.The collected plant material was preserved in a herbarium, department of Plant Biology, Mouloud Mammeri University of Tizi Ouzou, Algeria, and identified by a taxonomy specialist (Voucher number: 2024/UMMTO/31). Geranium essential oil was acquired via steam distillation using a still made of stainless steel. Two oils were obtained from spring and autumn samples. The yields were estimated and computed from the following formula.[12]

$$\text{Yield (\%)} = \frac{Oil\ mass\ (g)}{dry\ matter(g)} \times 100.$$

Chemical screening of Pelargonium graveolens essential oil.

Examination of secondary metabolites samples from *P. graveolens* was conducted at the Scientific and Technical Research Center for Physico-Chemical analysis (CRAPC)-TPPCA- Algeria, utilizing a SHIMADZU GCMS QP2020 instruments.

Ethical approval

Each procedure of experimentation was conducted in compliance with Algerian legislation. Article 58 of Law 08-88 governs the veterinary profession and the preservation of animal health and law 95-322/195 concerning the protection of animals for experimentation in Algeria. The members of the working group who participated in the realization of this study ensured the welfare of rabbits used for experimentation (housing and feeding). All animals were treated following the ARRIVE guidelines and all applicable national and international laws and approved by the Faculty's scientific committee.

Experimental animals

The current study utilized 31 prepubertal male white rabbits, all 2 months of age (57 days), procured from a private farm located in Tigzirt (Algeria). They were placed in cages designed for rabbits farming in the faculty of Biological Sciences, and housed in the same controlled climatic conditions of temperature, humidity and light. The rabbits are fed a dry diet, and watering is provided by a nipple system during the three-weeks period. Each rabbit was treated humanely and monitored daily to note any changes in general health. The 31 rabbits were randomly separated into 3 groups. Batch I serves as the reference group, received 0.5 mL of distilled water, batch II and batch III were orally administered with *P. graveolens* essential oil at doses of 0.4 mL/kg and 0.6 mL/kg/body weight, respectively for 21 days. After the duration of treatment, the rabbits were sacrificed under chloroform anesthesia, and the male reproductive organs were immediately excised, degreased, and subdivided into testicles and epididymis.

Macroscopic parameters study

Before sacrificing, each rabbit weighed regularly and record the measurements for analysis. Following collection, the testicular samples were observed (colour, shape and consistency), and an electronic scale was used for the measurements of testicular weights.

Histological analysis

The right testicles were preserved for five days in a fixation solution, after that the tissues were immersed in increasing concentrations of ethanol for dehydration and subsequently embedded in paraffin, the Leica microtome was used to section the samples into 5 µm thick sections. Masson's trichome staining was used to colour the sections.[13] The prepared tissues are then examined under an optical microscope using the Optica Vision Lite software.

Histomorphometric analysis

Using a microscope with Optica Vision Lite software, photomicrographs of the various structures that make up the seminiferous tubule were taken, histomorphometric parameters were assessed from photos. The surface and volume of seminiferous tubules (STS, VTS) were obtained using two formulas, STS = π. (d2/4) and VTS: π.h (d2/4), where d is the tubule diameter (µm) and h is the section width (5 µm).[14]

Statistical analysis

Results analysis was conducted utilizing the R software version 4.2.0. The values were expressed as average ± SEM. The obtained variables were analysed executing variance assessment (ANOVA) and Paired t-test. Statistical significance of the evaluated parameters was defined as $p<0.05$.

Results and Discussion

The soil composition, altitude, and light exposure are important environmental factors that significantly impact the quantity and composition of secondary metabolite.[15] The isolating of PEO was performed by complete distillation, the highest yield obtained during the flowering period was 0.12%, lower than that given by,[15] who reported that the yields of distillation were 0.20%. In our study, two oils obtained from *P. graveolens* were screened using GC-MS. The spring sample contained 26 distinct components, while the autumn sample contained 18 distinct compounds. All the constituents of the spring and autumn essential oils were identified (100%), with changes in the quantitative of the major constituents. Table 1 shows the percentage of chemical components in *Pelargonium graveolens* essential oil as well as the retention times. Also, the impact of PEO on the weight of the test animals was compared with that of the batch I. The treated groups which received geranium essential oil at the doses of 0.4 mL /Kg (GII) and 0.6 mL /Kg (GIII) indicated a notable increase in the body weight (before and after treatment) at ($p<0.001$) as demonstrated in Figure 1 and Table 2, from paired t-test analysis. However, no difference was noted in the baseline body weights measurements as illustrated in Table 2. From the analysis of variance between the groups, there was a considerable rise ($p<0.001$) in the final values of the body weights as indicated in Table 2. Similarly, the effect of *P. graveolens* essential oil on relative testis weight showed statistically significant results from the analysis of variance. The mean relative testicular weight was greater ($p<0.001$) in treated rabbits at doses of 0.6 mL/kg than in the group I. The impact of PEO on the relative testis weight of the male rabbits is illustrated in Figure 2. The histological study's results indicate that while round spermatids were present in the groups treated with both doses, these germ cells were absent in the reference group, and some seminiferous tubules from the group treated with 0.6 mL/kg dose showed the emergence of the first elongated spermatids (Table 3).Statistical analysis revealed significant increases ($p<0.05$) in the histomorphometrical parameters of the groups treated with the EO of *P. graveolens* in comparison with the group I. Geranium essential has no noticeable impact on the lumen diameter. The mean±SEM and *p*-values of histometrical parameters are presented in Table 4. Photomicrographs of the testes from both the control and the treated groups are presented in Figure 3. According to the literature, the changes in the makeup of essential oil of the identical botanical species are probably due to their distinct method of extraction, as well as other environmental and biological influences (region and origin of the plant, photoperiod and climatic parameters.)[14] .The essential oil extracted in May contains more chemical molecules (26 components) than that extracted in November (18 components), and both PEO samples showed similar compounds with quantitative changes related to the major components. The essential oil extracted in the spring and autumn represents 100% of the total essential oil contained. The spring sample had the highest concentrations of 2-Octen-1-ol 3,7 dimethyl (27.52%), D-limonene (12.64%), geraniol (14.84%), and geranyl acetate (12.41%), Phenethyl alcohol (8.30%), Linalool (6.22%), while the major components of *P. graveolens* collected in November were 2-Octen-1-ol,3,7-1-ol-dimethyl-(29.08%), D-Limonene (15.57%), Geraniol (15%), Geranyl acetate (11.09 %), Phenethyl alcohol (8.64%), Linalool (6.73%). The study of [16] found that the major chemical constituents of *P. graveolens* were *β*-citronellol, geraniol, and citronellyl formate. Our findings are consistent with the study of[17], which reported that the major molecules of geranium essential oil were Geraniol and Linalool. Citronellol, geraniol and citronellol ester are the most common chemical components of geranium essential oil, according to.[9] The use of essential oils may have a positive impact on intestinal morphological characteristics that improve nutrient absorption, assimilation and digestion.[5] Thus, the growth performance of the rabbits was positively affected by the oral administration of *P. graveolens* essential oil. In this study, after receiving treatments for 21 days, a notable difference was observed in BW and RTW of the rabbits between the treated rabbits and control group ($p<0.05$). Comparable findings were noted, showing that adding *P. graveolens* essential oil enhanced the growth performances of the rabbits.[5] Thyme essential oil plays a vital role in body weight gain in rabbits.[12] Our results aligned with the findings of[18] which mentioned that the essential oil of Sativa seeds increases the weight of rabbit reproductive organs. Our results were consistent with those of[19] witch found an increase in weight of rats treated with *M. piperita* extract. According to[20] *Mentha piperita*, *Salvia officinalis* and *Rosmarinus officinalis* essential oils induce an increase in the average values of body weight as well as the weight and volume of male gonads, including the testes and epididymis. Findings from the investigation of the reproductive performances of *P. graveolens* essential oil disclosed that PEO may offer protective benefits against TiO2 NP-induced damage to testicular tissues. The results also indicate that the antioxidant properties of PEO bioactive components could be the source of geranium essential oil activity.[10] A study conducted by[16] indicated that *P. graveolens* essential oil enhanced overall sperm motility, viability and morphology in mouse spermatozoa while preventing testicular oxidative damage. The conclusion of another study, suggested that geranium oil might affect GnRH secretion via estrogen upregulation.[4] Estrogen is known to improve and maintain reproductive and neurological system functions.

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Table 1: GC-MS analysis, volatile components of PEO extracted from *Pelargonium graveolens* harvested during the spring and autumn seasons 2023

Peak	R.T spring season	R.T autumn season	Components	Area (%) spring season	Area (%) autumn season
1	8.542	-	L-α-Pinene	0.13	-
2	12.268	-	o-Cymene	0.40	-
3	-	12.307	p-Cymenene	-	0.28
4	12.427	12.454	D-Limonene	12.64	15.57
5	15.655	15.685	Linalool	6.22	6.73
6	16.197	16.230	Phenethyl alcohol	8.30	8.64
7	17.726	-	Dihydrocarveol	0.88	-
8	-	17.765	dl-Isopulegol	-	0.89
9	18.155	-	L-Menthone	0.06	-
10	-	18.195	Cyclopentanone, 2,5-dimethyl-	-	0.07
11	18.238	18.276	Isoborneol	1.51	4.73
12	18.654	-	Endo-Borneol	3.20	-
13	19.842	-	Néobornyl alcohol	2.86	-
14	-	19.877	(IS)-1,3,3-trimethyl-6-methylnorbornan-2-ol	-	2.62
15	20.176	-	γ-terpineol	0.92	-
16	-	20.209	1,5,5-Trimethyl-6-methylen-cyclohexene	-	0.93
17	21.159	21.197	Citronellol	0.23	0.19
18	21.635	21.652	2-Octen-1-ol,3,7-dimethyl-	27.52	29.08
19	22.225	-	Cis-Citral	0.28	-
20	22.811	22.833	Geraniol	14.84	15.00
21	23.005	-	Formic acide,2-(2-methoxyethyl) hexyl ester	0.01	-
22	23.557	-	Citral	0.57	-
23	23.765	23.794	Citronellyl formate	2.41	2.00
24	24.046	-	Cis-Geraniol	0.24	-
25	24.588	24.615	Menthyl acetate	0.98	0.93
26	24.988	-	Neryl formate	0.77	-
27	-	25.031	Geranyl formate	-	0.38
28	28.501	28.518	Geranyl acetate	12.41	11.09
29	30.020	-	Aromandendrene	0.13	-
30	31.014	31.034	Guaia-6,9-Diene	0.60	0.55
31	35.705	-	Neryl butyrate	1.57	-

32	-	35.742	Geranyl butyrate	-	0.31
33	37.022	-	Diethyl Phthalate	0.31	-
			Total components	100%	100%

R. T: Retention Time

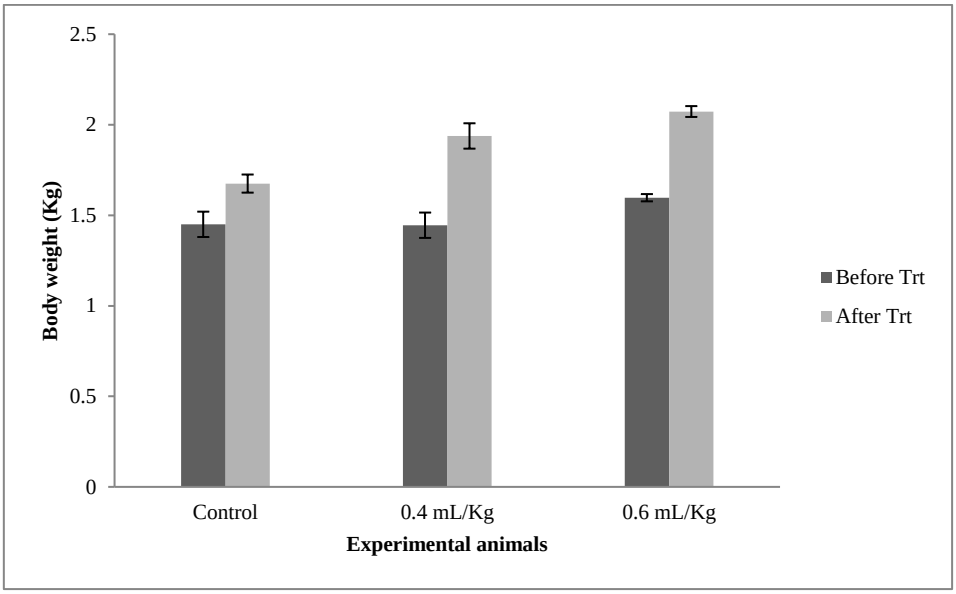

Figure 1: Graphical representation of the evolution of body weight (before and after treatment) in both the control and treated rabbits.

Table 2: The statistical test and *p*-values of body weight and relative testis weight in rabbits

Body and relative testis weights	**Statistical test**	**Control group**	**GII**	**GIII**
B.W (before, after treatment)	Paired t-test	0.0020**	0.000059***	0.000024***
B.W before treatment	ANOVA	/	N.S	N.S
B.W after treatment	ANOVA	/	0.00648**	<0.001***
Relative testis weight	ANOVA	/	0.0265*	<0.001***

N.S: non-significant, B.W: body weight, *: *p* less than 0.05, **: *p* less than 0.01, ***: *p* less than 0.001.

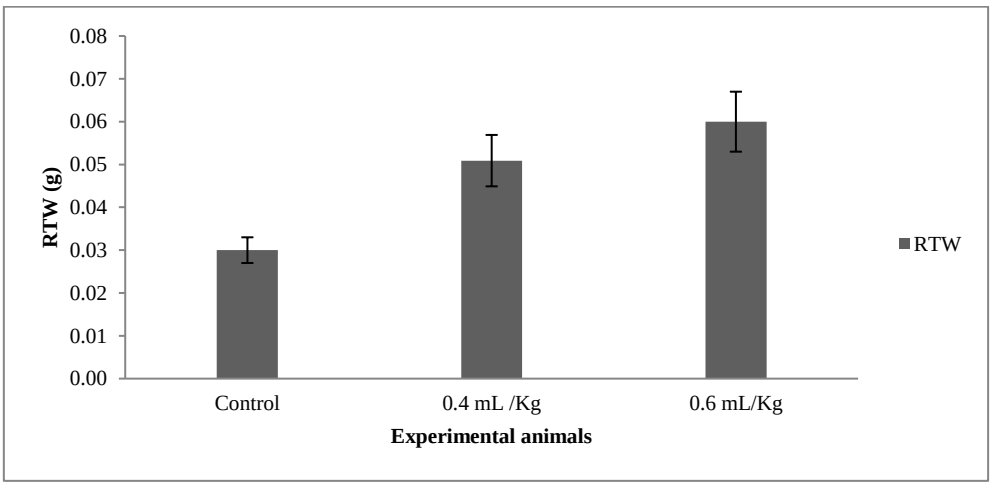

Figure 2: Graphical representation of the evolution of relative testicle weight in both the control and treated rabbits with *P. graveolens* essential oil. Relative TW: Relative testis weight, Before T: Before treatment, After T: After treatment

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Table 3: Some microscopic parameters of testicles obtained from treated and control rabbits

Microscopic Parameters	Control group	Group II	Group III
Round spermatids	Absence	Presence	Presence
Elongated spermatids	Absence	Absence	Presence

Table 4: The means ± SEM, *p*-values of some histometrical parameters of the rabbit testis

Histomorphometric parameters (µm)	Control group	Group II Mean ± SEM	Group II *P-value*	Group III Mean ± SEM	Group III *P-value*
ST Diameter (µm)	75.85 ± 1.84 [b]	92.83 ± 6.35*[a]	0.04742	100.30 ± 4.55**[a]	0.00274
Area of ST (µm)	4605.17 ± 475.51[b]	7021.85 ± 1010.33[ab]	0.07276	8046.04 ± 735.97**[a]	0.00645
Height Epithelium (µm)	29.3 ± 1.28[b]	37.61 ± 1.25[ab]	0.0769	39.31 ± 1.96*[a]	0.0248
Diameter lumen (µm)	24.02 ± 1.71[a]	34.9 ± 1.91[a]	0.200	28.95 ± 3.23[a]	0.657

ST: seminiferous tubules, [a, b, ab] the means that lack a common letter are significantly different between the batch.

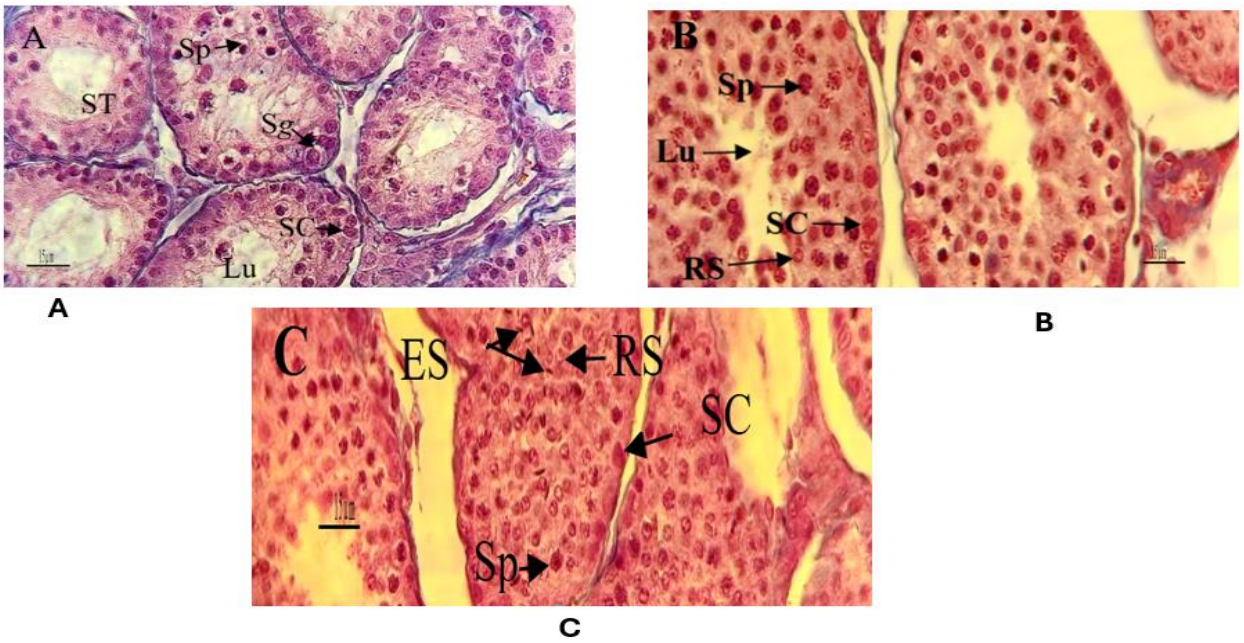

Figure 3: Photomicrographs of the histological study, testis samples of control and treated rabbits with PEO. A, B and C testicular structures of the control group, treated groups at dose I and dose II respectively. ST: Seminiferous Tubule, Sg: Spermatogonia, Sp: Spermatocyte, SC: Sertoli Cells, RS: Round Spermatids, ES: Elongated Spermatids, Lu: lumen.

Conclusion

The study's findings indicate that oral administration of *Pelargonium graveolens* essential oil had a positive impact on the body weight and both macroscopic and microscopic parameters of the testes in white local rabbits. This essential oil can be used as an alternative to growth-stimulating agents in animals and as a stimulator of testicular functions in humans and animals. Our findings may help in the development of combinatorial infertility treatments.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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