

ORIGINAL PAPER

Physiological efficiency of Purslane (*Portulaca oleracea*) Administration on the quality and quantity of spermatozoa in adult male albino mice

Sattar Ibrahim Kareem¹, Chro Gafoor Raouf², Yousf Latif Ahmad¹, Ayman M. Mustafa¹,
Hiwa Faraidun Muhammed¹, Shwana Othman Rahman¹, Ibrahim Saman Sleman¹,
Awyar Ramzi Mohammed¹

¹ Department of Medical Laboratory Science, College of Health Sciences, University of Human Development, Kurdistan Regional Government, Sulaimani, Iraq;

² Nursing Department, College of Nursing, University of Human Development, Kurdistan Regional Government, Sulaimani, Iraq.

Summary

Background: *Portulaca oleracea* (purslane) is a widely distributed succulent that is rich in flavonoids, omega-3 fatty acids, and antioxidant vitamins and minerals, and has attracted growing interest as a phytotherapeutic candidate for male reproductive health. Oxidative stress is a well-recognized contributor to defective spermatogenesis, and antioxidant-rich plant preparations may plausibly protect the germinal epithelium. This study aims to evaluate the effect of an aqueous purslane preparation, administered at three dosing frequencies, on epididymal sperm concentration and sperm morphology in adult male albino mice.

Methods: Twenty adult male albino mice (*Mus musculus*; 12 weeks; 25-36 g) were randomly allocated to four groups (n = 5): an untreated control (C) and three treated groups receiving 1 mL/kg body weight of aqueous purslane preparation by oral gavage once every two days (T1), once every three days (T2), or once every four days (T3). After the treatment period, epididymal sperm concentration was determined with an improved Neubauer hemocytometer, and sperm morphology was assessed on eosin-stained smears (100 spermatozoa per animal). Data were analyzed by one-way ANOVA with Tukey's post-hoc test (sperm count) and by the chi-square test with pairwise Fisher's exact tests (morphology); $p < 0.05$ was considered significant.

Results: Sperm concentration differed significantly among groups ($F(3,12) = 17.47$, $p < 0.001$, $\eta^2 = 0.81$). T2 produced the highest concentration ($166.75 \pm 17.65 \times 10^6/\text{mL}$), significantly exceeding the control (101.50 ± 20.16), T1 (72.50 ± 14.15) and T3 (98.25 ± 23.63) groups (Tukey, all $p \leq 0.002$). The proportion of morphologically normal spermatozoa was 85% in controls versus 99%, 97% and 84% in T1, T2 and T3, respectively; the group difference was highly significant ($\chi^2(3) = 94.63$, $p < 0.001$). Relative to control, abnormal forms were markedly reduced by T1 (1%; Fisher $p < 0.001$) and T2 (3%; $p < 0.001$) but not by T3 (16%; $p = 0.77$). Folded midpiece/tail was the predominant defect across groups.

Conclusions: Aqueous *Portulaca oleracea* shows promise as a natural agent for enhancing male reproductive function, with treatment efficacy strongly influenced by dosing frequency. Further studies are warranted to elucidate the underlying mechanisms and evaluate their translational potential for managing male infertility.

KEY WORDS: *Portulaca oleracea*; Purslane; Spermatogenesis; Sperm morphology; Sperm count; Male fertility; Oxidative stress; Albino mice.

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INTRODUCTION

Over the past two decades there has been a marked resurgence of interest in complementary and plant-based approaches to health, and reproductive medicine has been no exception. *Portulaca oleracea* L. (purslane) is among the medicinal plants that have emerged as particularly promising sources of bioactive natural compounds, owing to a phytochemical profile that combines flavonoids, terpenoids, alkaloids, phenolic acids, essential fatty acids, and antioxidant vitamins and minerals (1, 2). Infertility remains a substantial global health concern, affecting an estimated 15% of couples of reproductive age, with a male factor implicated in roughly half of cases (3, 4). Although assisted reproductive technologies and pharmacological therapies have advanced considerably, many conventional treatments are costly, are not universally accessible, and may carry adverse effects. This has driven interest in safer, more sustainable, and more affordable adjuncts, among which herbal preparations occupy a prominent position (5, 6).

The pharmacological repertoire of purslane is broad. Its extracts exhibit anti-inflammatory, antioxidant, immunomodulatory, antidiabetic, and cytoprotective activities that have been documented across numerous experimental models (7-9). Of particular relevance to reproduction, spermatogenesis is exquisitely sensitive to oxidative stress: reactive oxygen species can damage sperm membrane lipids, fragment sperm DNA, and disrupt the morphology and motility of spermatozoa. Antioxidant-rich preparations may therefore protect the germinal epithelium and improve seminal quality (10). A growing body of preclinical evidence supports a beneficial reproductive role for purslane. Supplementation has

been reported to alleviate testicular dysfunction and oxidative injury and to improve steroidogenesis and sperm parameters in rodent models of chemically and metabolically induced testicular damage (11-13).

Purslane, alone or combined with chicory, has been shown to attenuate glucocorticoid- and diabetes-associated testicular toxicity by suppressing oxidative reactions (13, 14). Despite these encouraging observations, few studies have systematically examined how the frequency of purslane administration influences reproductive outcomes in healthy (non-diseased) animals.

The present study was therefore designed to evaluate the physiological efficiency of an aqueous purslane preparation on the quality (morphology) and quantity (epididymal concentration) of spermatozoa in adult male albino mice, and to determine whether these outcomes depend on the dosing interval.

MATERIALS AND METHODS

Plant material and preparation of the aqueous extract

Two kilograms of fresh *Portulaca oleracea* leaves were purchased from a local market in Sulaimani, Kurdistan Region of Iraq.

The plant material was authenticated as *Portulaca oleracea* L. by a plant specialist, and a voucher specimen was retained at the College of Health Sciences, University of Human Development.

The leaves were rinsed, air-dried indoors at room temperature away from direct sunlight, and reduced to a fine powder using an electric grinder. An aqueous stock preparation was obtained by dissolving two tablespoons (≈ 10 g) of the powder in 200 mL of distilled water. The mixture was kept at 20 °C for one hour and then filtered through filter paper to separate the clear aqueous phase from the residual solids (15). The filtrate was used as the working solution for oral administration.

Experimental animals and housing

Twenty young adult male albino mice (*Mus musculus*), aged approximately 12 weeks and weighing 25-36 g, were used. All animals were bred and maintained in the Animal House of the University of Human Development. Mice were housed in clean plastic cages under standard conditions (23 ± 2 °C; 12 h light/12 h dark cycle) and had ad libitum access to drinking water. Cages were cleaned and bedding was changed twice weekly. Animals received a standard rodent diet composed of 24.5% barley, 30% wheat meal, 22.5% yellow maize, 15.2% soya, 7.5% animal protein, 0.45% salt, and 0.2% limestone.

Experimental design and treatment groups

Mice were randomly allocated to four groups of five animals each ($n = 5$): an untreated control (C) and three treated groups (T1, T2, T3). The three treated groups received an identical dose of 1 mL/kg body weight of the aqueous purslane preparation by oral gavage, differing only in dosing frequency, as summarized in Table 1.

The 2-, 3-, and 4-day intervals were selected a priori to span a physiologically relevant range of dosing frequencies while holding the per-dose volume constant, so that any between-group differences could be attributed to

Table 1.

*Experimental design showing the treatment groups and oral dosing schedule of aqueous *Portulaca oleracea* (purslane) extract administered to male mice.*

Group	Treatment	Dosing frequency
C	Standard diet only (untreated control)	–
T1	Purslane 1 mL/kg BW, oral gavage	Once every 2 days
T2	Purslane 1 mL/kg BW, oral gavage	Once every 3 days
T3	Purslane 1 mL/kg BW, oral gavage	Once every 4 days

BW: body weight. All treated groups received the same per-dose volume; the groups differed only in the interval between doses.

dosing interval rather than dose magnitude; the chosen intervals fall within the timescale of the murine spermatogenic cycle (~ 35 days), over which cumulative exposure could plausibly influence developing spermatozoa.

Sperm morphology assessment

At the end of the treatment period, animals were euthanized humanely and the reproductive tract was dissected. The vas deferens was excised from the testis and placed in a small Petri dish containing normal saline. Using fine scissors and forceps, spermatozoa were expressed and a thin smear was prepared on a clean glass slide for each animal. Smears were stained with eosin for 10 minutes, gently rinsed with normal saline, and air-dried. For each animal, approximately 100 spermatozoa were examined (≈ 400 spermatozoa per group), and each was classified as morphologically normal or abnormal. Abnormal forms were further categorized as hook-less head, head-less, folded (bent midpiece/tail), short-tailed, or two-tailed.

All smears were coded and scored by a single trained observer who was blinded to treatment-group allocation.

Epididymal sperm count

The epididymis was excised from the caudal region of each testis. Each epididymis was transferred to an Eppendorf tube containing 1 mL of normal saline and minced finely with scissors, then left to stand for 5 minutes to allow spermatozoa to disperse. A 20 μ L aliquot of the sperm suspension was mixed with 20 μ L of normal saline and 10% formalin and left for a further 5 minutes. Five microlitres of the final suspension were loaded onto an improved Neubauer hemocytometer that had been placed in a humidified Petri dish (16, 17), and spermatozoa were counted under the microscope.

Evaluable concentration data were obtained from four of the five animals in each group; in each group one sample could not be evaluated owing to clotting/loss during processing, yielding a balanced design of four animals per group (total $n = 16$) for the concentration analysis.

Microscopic examination

Slides were examined with an Olympus light microscope. Sperm morphology was evaluated at 40 $\times$ and 100 $\times$ magnification. For counting, the four corner squares and the central square of both chambers of the hemocytometer were enumerated. The counting field was located with the 4 $\times$ objective, the grid squares were identified with the

10× objective, and individual cells were counted with the 40× objective. Sperm concentration is reported as $\times 10^6$ cells/mL.

Statistical analysis

Sperm concentration data are expressed as mean $\pm$ standard deviation (SD), with the standard error of the mean (SEM) also reported. Differences among the four groups were assessed by one-way analysis of variance (ANOVA); when the overall test was significant, pairwise comparisons were performed using Tukey's honestly significant difference (HSD) test, and the effect size was expressed as eta-squared (η^2). Sperm morphology (normal vs abnormal) was analyzed as count data using the Pearson chi-square test for the overall 4×2 contingency table, followed by pairwise two-tailed Fisher's exact tests versus the control group with odds ratios (OR). A probability of $p < 0.05$ was considered statistically significant. Analyses were performed in GraphPad Prism (v9.3.0) and cross-checked in Python (SciPy/statsmodels).

No a priori power calculation was performed; the group size ($n = 5$) reflects the exploratory, pilot-scale nature of the study, and the findings should be interpreted in light of this modest sample size.

Ethical approval

All procedures were carried out in accordance with institutional ethical guidelines and were approved by the University of Human Development Ethics Committee (approval code UHD-CHS A22). Animals were housed under controlled temperature, humidity, and a 12 h light/dark cycle, and every effort was made to minimize pain, stress, and discomfort throughout the study.

RESULTS

Epididymal sperm concentration

Epididymal sperm concentration differed significantly among the four groups (one-way ANOVA, $F(3,12) = 17.47$, $p < 0.001$), with the treatment factor accounting for the large majority of the observed variance ($\eta^2 = 0.81$). Group means ($\times 10^6$ /mL) were 101.50 ± 20.16 for the control, 72.50 ± 14.15 for T1, 166.75 ± 17.65 for T2, and 98.25 ± 23.63 for T3 (Table 2, Figure 1).

Post-hoc Tukey comparisons localized the effect to the T2 group: mice dosed once every three days had a significantly higher sperm concentration than controls (mean difference $+65.25$; $p = 0.002$), than T1 ($+94.25$; $p < 0.001$), and than T3 ($+68.50$; $p = 0.001$). By contrast, neither T1 nor T3 differed significantly from the control ($p = 0.20$ and $p = 0.99$, respectively). Notably, the most frequently dosed group (T1) showed the numerically lowest concentration

Table 2.

Effect of different purslane dosing intervals on epididymal sperm concentration in male mice.

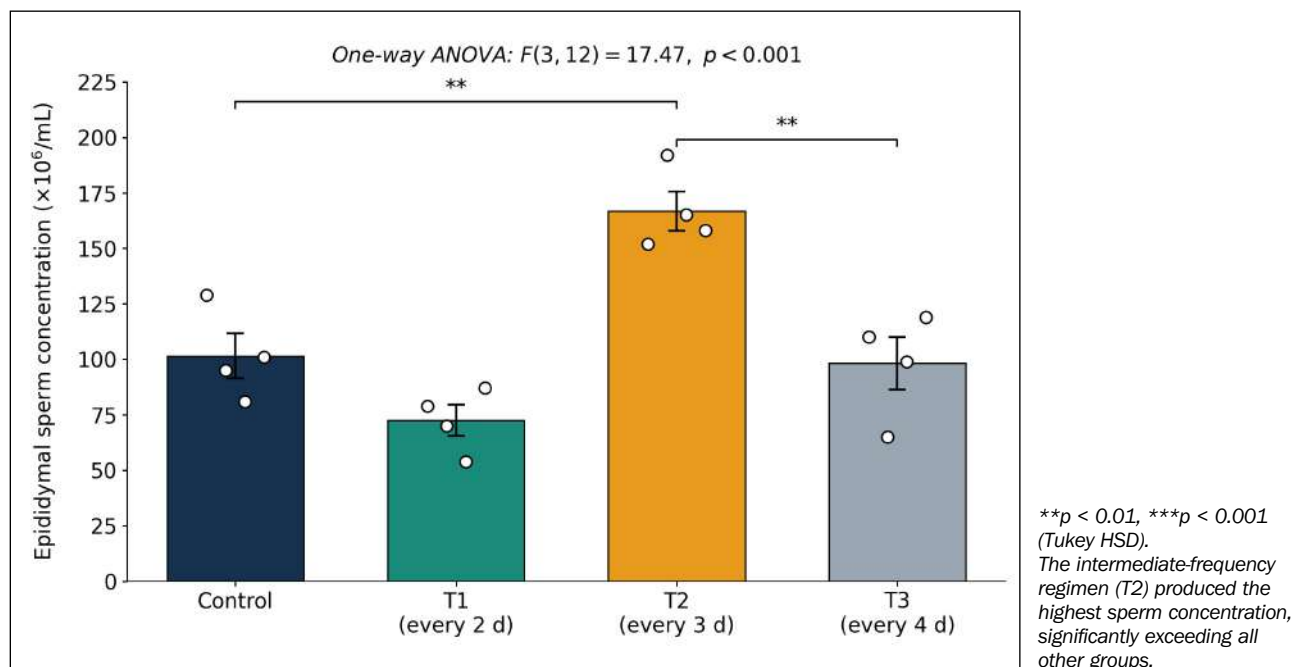
Group	Mean	SD	SEM	Range
Control	101.50	20.16	10.08	81-129
T1 (every 2 d)	72.50	14.15	7.08	54-87
T2 (every 3 d)	166.75	17.65	8.83	152-192
T3 (every 4 d)	98.25	23.63	11.81	65-119

SD: standard deviation; SEM: standard error of the mean. One-way ANOVA: $F(3,12) = 17.47$, $p < 0.001$, $\eta^2 = 0.81$. Tukey HSD: $T2 > \text{Control}$ ($p = 0.002$), $T2 > T1$ ($p < 0.001$), $T2 > T3$ ($p = 0.001$); all other pairwise comparisons non-significant. Values were computed from four evaluable samples per group.

Figure 1.

Epididymal sperm concentration across treatment groups.

Bars show group means and error bars the SEM; overlaid points are individual samples.



of all groups, although this reduction relative to control did not reach statistical significance.

Sperm morphology: normal versus abnormal forms

The distribution of morphologically normal and abnormal spermatozoa differed markedly among groups (Pearson $\chi^2(3) = 94.63$, $p < 0.001$; Table 3, Figure 2). The proportion of normal forms was 85% in the control

group and rose to 99% and 97% in the T1 and T2 groups, respectively, but remained essentially unchanged at 84% in T3.

Pairwise Fisher's exact tests confirmed that, relative to control, abnormal forms were significantly reduced by both T1 (1% abnormal; OR = 0.06, $p < 0.001$) and T2 (3% abnormal; OR = 0.16; $p < 0.001$). In contrast, the T3 group (16% abnormal) was statistically indistinguishable from control (OR = 1.08, $p = 0.77$). Thus, more frequent dosing (T1, T2) improved morphological quality, whereas the least frequent regimen (T3) did not.

Distribution of morphological defect subtypes

Among abnormal spermatozoa, the folded (bent mid-piece/tail) defect was the most common form across groups. In the control group, abnormalities comprised folded (52%), hook-less (33%), short-tailed (8%), head-less (5%), and two-tailed (2%) forms. In T1, the small number of abnormal cells ($n = 4$) were exclusively folded (100%). In T2, abnormalities were distributed between hook-less ($\approx 45\%$), folded ($\approx 45\%$), and two-tailed ($\approx 10\%$) forms.

In T3, folded (52%), hook-less (36%), and two-tailed (12%) forms predominated (Table 4, Figure 3).

Table 3.

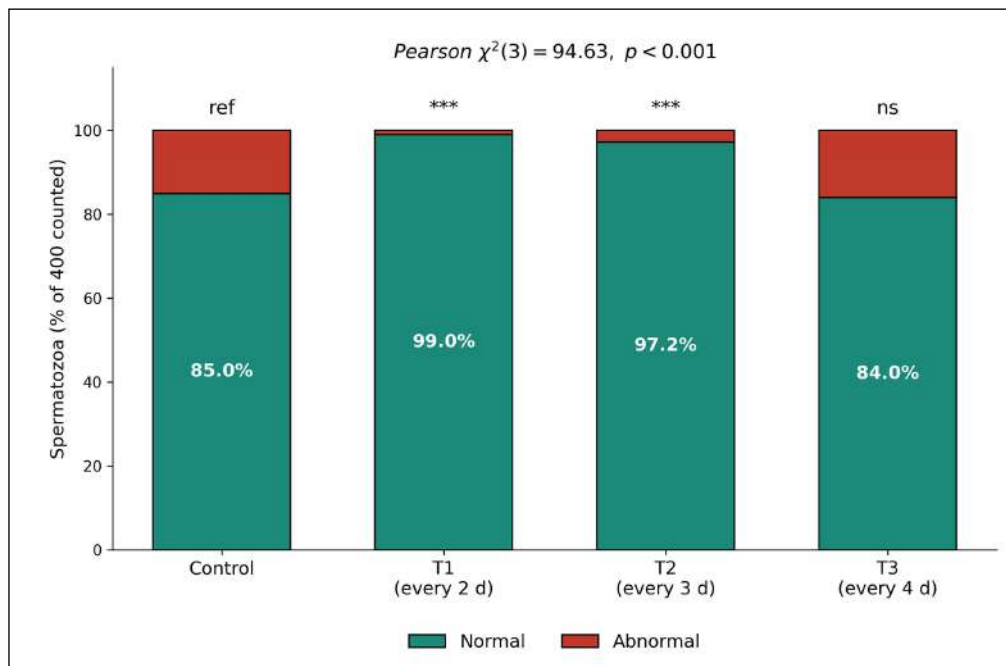
Effect of different purslane dosing intervals on sperm morphology, showing the numbers and percentages of normal and abnormal spermatozoa in each experimental group.

Group	Normal (n)	Abnormal (n)	Abnormal (%)	p vs C
Control	340	60	15.0	– (ref)
T1 (every 2 d)	396	4	1.0	< 0.001
T2 (every 3 d)	389	11	2.8	< 0.001
T3 (every 4 d)	336	64	16.0	0.77 (ns)

Overall Pearson $\chi^2(3) = 94.63$, $p < 0.001$. p vs C, two-tailed Fisher's exact test versus the control group; ns: not significant. Counts derive from the reported group percentages applied to 400 spermatozoa counted per group.

Figure 2.

Proportion of morphologically normal (teal) and abnormal (red) spermatozoa by group.



*** $p < 0.001$ versus control (Fisher's exact test); ns, not significant. T1 and T2 significantly reduced abnormal forms, whereas T3 was comparable to control.

Group	Hook-less	Folded	Two-tailed	Short-tailed	Head-less
Control	20 (33%)	31 (52%)	1 (2%)	5 (8%)	3 (5%)
T1 (every 2 d)	0	4 (100%)	0	0	0
T2 (every 3 d)	5 (45%)	5 (45%)	1 (10%)	0	0
T3 (every 4 d)	23 (36%)	33 (52%)	8 (12%)	0	0

Percentages are expressed relative to the abnormal spermatozoa within each group; counts are derived from those percentages and may be affected by rounding. The folded (bent midpiece/tail) form was the predominant defect in all groups except T2.

Table 4.

Distribution of abnormal sperm morphological subtypes among the experimental groups following different purslane dosing intervals.

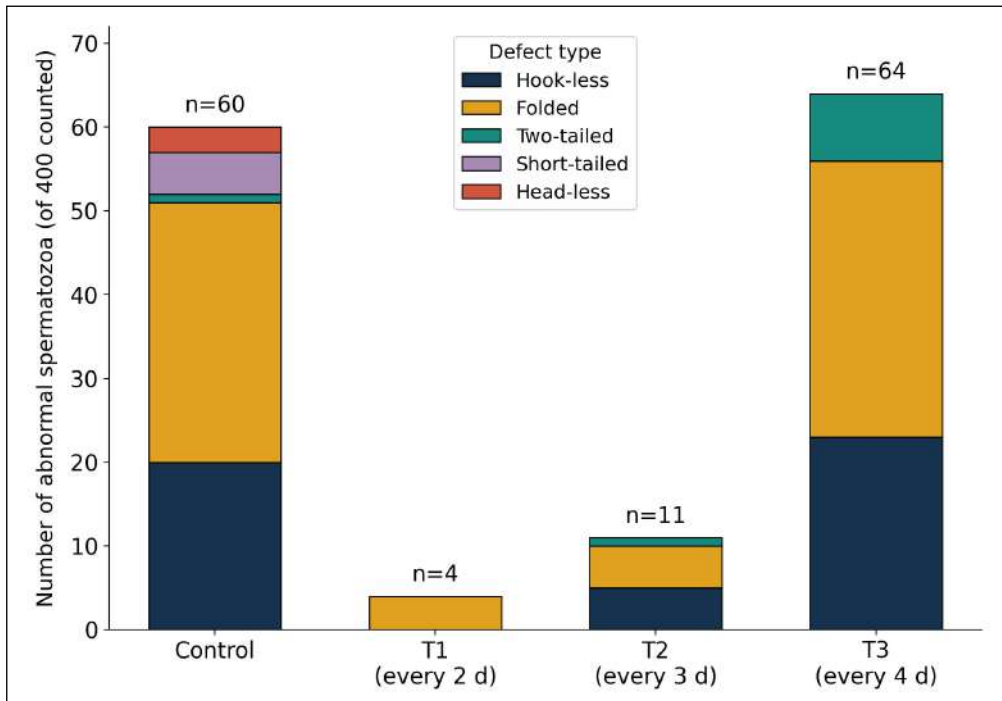


Figure 3. Composition of abnormal spermatozoa by defect type across groups. The height of each stacked bar corresponds to the total number of abnormal cells per group (of 400 counted). Folded forms dominated the abnormal fraction in the control, T1 and T3 groups.

Representative sperm micrographs

Representative eosin-stained micrographs illustrating each morphological category are shown in Figure 4.

Integrated summary of outcomes

Taken together, the two endpoints reveal a dose-frequency-dependent, non-linear response. The intermediate regimen (T2, once every three days) was optimal, simultaneously producing the highest sperm concentration and

near-normal morphology. The most frequent regimen (T1, once every two days) produced the best morphology but did not increase – and numerically reduced – sperm concentration.

The least frequent regimen (T3, once every four days) conferred no measurable benefit over control on either endpoint. This inverted-U pattern indicates that the reproductive benefit of purslane depends critically on the exposure interval.

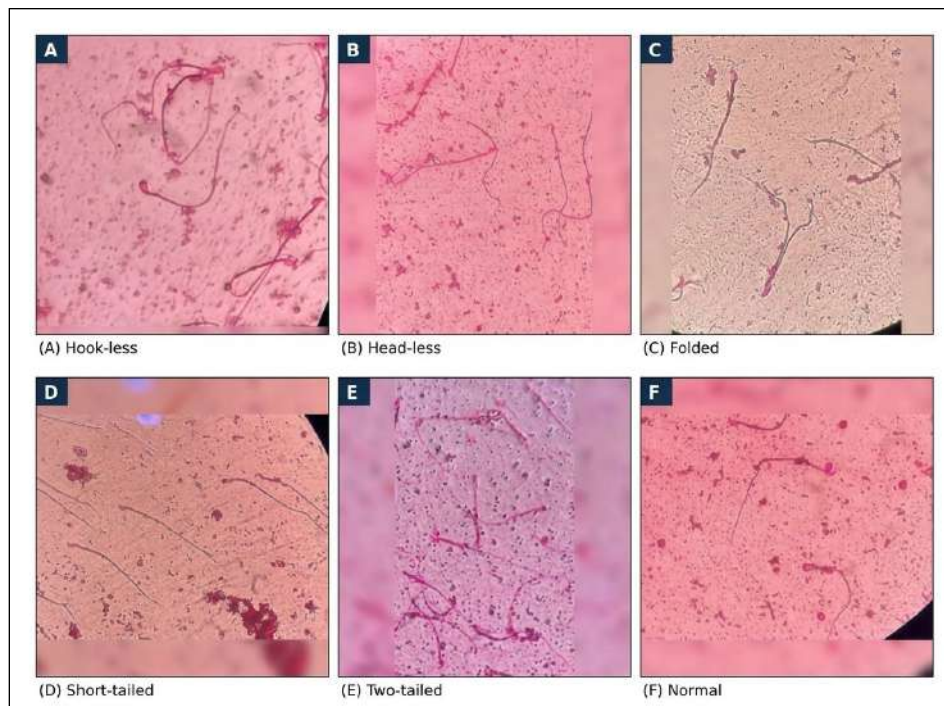


Figure 4. Representative eosin-stained micrographs of murine spermatozoa. (A) hook-less head; (B) head-less; (C) folded (bent midpiece/tail); (D) short-tailed; (E) two-tailed; (F) normal spermatozoon.

DISCUSSION

This study evaluated how the frequency of aqueous purslane administration affects sperm quantity and quality in healthy adult male mice. Three findings stand out. First, purslane influenced both endpoints, but the two responded differently to dosing frequency. Second, an intermediate dosing interval (once every three days) was clearly optimal, delivering the highest sperm concentration together with excellent morphology. Third, the relationship between dosing frequency and outcome was non-linear, consistent with a hormetic (inverted-U) dose-response.

The improvement in sperm morphology observed with more frequent dosing (T1 and T2) is biologically plausible in light of the antioxidant capacity of purslane. The plant is a rich source of β -carotene, ascorbic acid, α -tocopherol, glutathione, and flavonoids, which scavenge reactive oxygen species and reduce oxidative stress (7). Because oxidative stress is a principal driver of sperm DNA fragmentation, membrane lipid peroxidation, and morphological defects, a reduction in oxidative load would be expected to preserve the structural integrity of developing spermatozoa (10, 14, 18). In the T1 group, abnormal forms fell to just 1%, and the few remaining defects were exclusively of the folded type, suggesting that antioxidant protection was most complete under frequent exposure.

The concentration data add an important nuance. Whereas morphology improved monotonically with dosing frequency, sperm concentration peaked at the intermediate interval (T2) and was, if anything, slightly depressed under the most frequent regimen (T1). Several mechanisms could account for this dissociation. Purslane contains bioactive constituents – including polysaccharides and phytosterols – that can modulate steroidogenesis and the hypothalamic-pituitary-gonadal axis (11, 12, 19); an intermediate exposure may optimize testosterone-driven spermatogenesis, whereas very frequent exposure may exceed the beneficial window. This interpretation is consistent with hormetic behavior, in which moderate doses of a bioactive agent are stimulatory while higher cumulative exposure is neutral or mildly inhibitory.

The absence of any benefit in the least frequent group (T3), whose sperm concentration and morphology were indistinguishable from control, indicates that a minimum exposure frequency is required to engage these protective pathways. Together, the T1-T3 comparison delineates an optimal dosing window centered on the once-every-three-days schedule.

These observations align with a growing preclinical literature. Purslane, administered alone or in combination with chicory, has been reported to alleviate diabetes- and glucocorticoid-induced testicular dysfunction and to improve sperm parameters, largely through suppression of oxidative stress and support of steroidogenesis (13, 14). Purslane polysaccharides have likewise been shown to mitigate chemically induced male reproductive damage by improving gut-microbiota and metabolic homeostasis (12). Our results extend this body of work to healthy animals and, importantly, identify dosing frequency as a decisive determinant of outcome—an issue that has received little prior attention.

Some limitations should be acknowledged. The sample size was modest, and complete sperm-concentration data were available for four animals per group, which limits statistical power despite the large observed effect sizes. Sperm morphology was quantified as pooled counts per group rather than as per-animal proportions, so the morphology tests treat individual spermatozoa as the unit of analysis; this may overstate statistical precision and should be interpreted with corresponding caution.

Because per-animal morphology counts were not recorded, a cluster-adjusted analysis (e.g., a mixed-effects logistic model or generalized estimating equations with animal as a random effect) could not be performed retrospectively; future studies should record per-animal proportions to enable such analyses.

Motility, viability, testicular histopathology, hormonal assays (e.g., testosterone, FSH, LH), and direct markers of oxidative stress were not measured, and the phytochemical content of the specific preparation was not quantified. Finally, only a single per-dose volume was tested, so the effects of dose magnitude (as distinct from frequency) remain to be characterized.

Future work should incorporate larger, randomized cohorts with per-animal replication for every endpoint; a full seminal profile (count, motility, viability, and morphology); testicular histology and steroidogenic enzyme expression; measurement of seminal and testicular oxidative-stress and antioxidant markers; and standardized phytochemical characterization of the preparation. A factorial design varying both dose magnitude and frequency would allow the optimal exposure window suggested here to be defined precisely.

DECLARATIONS

Ethical approval and consent to participate: All experimental procedures involving mice were conducted in accordance with institutional ethical guidelines and were approved by the University of Human Development Ethics Committee (approval code UHD-CHS A22).

Availability of data and material: The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Clinical trial number: Not applicable.

Authors' contributions: All authors contributed equally to the study conception, experimental work, data collection, and manuscript preparation, and approved the final version.

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CONCLUSIONS

Aqueous *Portulaca oleracea* shows promise as a natural agent for enhancing male reproductive function, with treatment efficacy strongly influenced by dosing frequency. Further studies are warranted to elucidate the underlying mechanisms and evaluate their translational potential for managing male infertility.

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Correspondence

Sattar Ibrahim Kareem
sattarkarim@gmail.com
Yousf Latif Ahmad
yusiflatif@gmail.com
Ayman M. Mustafa (Corresponding Author)
Aymanmajid75@gmail.com
Hiwa Faraidun Muhammed
hiwafaraidun@gmail.com
Shwana Othman Rahman
shwanaosman@gmail.com
Ibrahim Saman Sleman
lbrahimsleman@gmail.com

Awyar Ramzi Mohammed
awyarramzi@gmail.com
Department of Medical Laboratory Science, College of Health Sciences,
University of Human Development, Kurdistan Regional Government,
Sulaimani, Iraq

Chro Gafoor Raouf
chrorauf@gmail.com
Nursing Department, College of Nursing, University of Human
Development, Kurdistan Regional Government, Sulaimani, Iraq