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Original Research Article

In vitro immobilizing and spermicidal effects of methanol leaf extract of *Euphorbia hirta* Linn. (Euphorbiaceae) on caprine spermatozoa

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Abstract

Purpose: *To investigate the in vitro effects of methanol leaf extract of E. hirta (MLEEH) on the motility, viability and morphology of caprine spermatozoa.*
Methods: *The effect of MLEEH treatment (1.25, 2.5, 5, 10 and 20 mg per mL) on caprine sperm percentage total and progressive motility, viability and total abnormalities were evaluated at 1, 5 and 10 min post-treatment. Sperm revival test was used to evaluate the reversibility of sperm incapacitation following MLEEH treatment.*
Results: *There were significant interactions ($p < 0.001$) between the effects of MLEEH concentration and the duration of treatment on sperm total motility, progressive motility and viability. Increase in MLEEH concentration and the duration of treatment caused significant decreases ($p < 0.05$) in sperm total motility, progressive motility and viability, whereas sperm morphology was not altered. Washing and supplementation of MLEEH-treated sperm failed to revive sperm motility.*
Conclusion: *E. hirta treatment causes concentration-dependent and time-dependent decreases in total and progressive sperm motility and sperm viability, as well as irreversible immobilization of spermatozoa. These findings suggest possible adverse effects of E. hirta on the fertility of males. Thus, the extract can be potentially developed as an antifertility or contraceptive agent.*

Keywords: *Caprine spermatozoa, Euphorbia hirta, Sperm morphology, Sperm motility, Sperm viability, Spermicidal*

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INTRODUCTION

Phytochemicals from certain plants and plant derivatives may constitute xenobiotics that can impact fertility and reproduction in males, by affecting the testis, sperm cells or the endocrine organs and hormones that control the reproductive cycle [1]. The spermatozoon is a highly specialized cell that has the critical function of fertilizing an oocyte in the process of reproduction [2]. To guarantee this function, the sperm has to possess normal structural integrity and physiological or functional characteristics including motility. Abnormalities in these sperm characteristics can damage the functional

capacity of sperm, thereby decreasing male fertility [3].

Plants and plant products constitute inexpensive and readily available sources of herbal medication for both human and animals in many parts of the world [4]. In addition, growing interests in the enhancement or control of fertility in males have driven research in reproductive toxicology and the application of plant-derived pharmacologic agents in reproduction [5]. As a result, several studies have reported adverse effects of plant-derived extracts on spermatozoa [5].

Euphorbia hirta Linn. (*E. hirta*) is a small, slender-stemmed, branched and hairy annual herb which belongs to the family Euphorbiaceae and genus Euphorbia [6]. It is a very common herb that is distributed worldwide particularly in the pan-tropic and sub-tropic regions [6]. Different parts of *E. hirta* are widely used in traditional medicine worldwide for the treatment of a variety of human and animal conditions, including digestive disorders, skin diseases, inflammation, poor lactation and disorders of the respiratory system [6].

A significant number of studies have reported diverse biological properties of *E. hirta* extracts, including its antimicrobial, antioxidant, anti-inflammatory, antidiarrheal and anticancer potential [6]. In contrast, there is limited information on the effects of *E. hirta* extracts on male reproduction [7,8].

To the best of our knowledge, there is no available information on the *in vitro* effects of *E. hirta* on spermatozoa. Therefore, the aim of the study was to investigate the *in vitro* effects of methanol leaf extract of *E. hirta* on caprine spermatozoal motility, viability and morphology.

EXPERIMENTAL

Plant collection and extraction

Fresh plants of *E. hirta* were collected in May 2019 from the University of Nigeria Nsukka Campus, Enugu State, South-East Nigeria, located on latitude 6° 52′ 02.3″ N and longitude 7° 24′ 30.6″ E within the tropical rain forest belt. The plant specimen was properly identified by Mr Felix Nwafor, a plant taxonomist of the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka, and the voucher specimen (no. PCG/UNN/0216) was deposited in the Herbarium of the same institution. The leaves were dried in the shade for several days, and 200 g of it weighed out and pulverized using hammer mill. Cold extractions were performed with analytical grade of 80 % methanol (JHD, Guangdong Guanghua Sci-Tech Co. Ltd, Guangdong, China). The methanol extract was filtered out, dried using a rotary evaporator, and then kept at 4 °C as methanol leaf extract of *E. hirta* (MLEEH).

Brine shrimp lethality bioassay

The brine shrimp (*Artemia salina*) lethality bioassay was used to evaluate the lethal concentration 50 (LC_{50}) of MLEEH according to the method described previously [9]. Brine shrimp eggs (Sanders Great Salt Lake, Brine Shrimp Company L.C., U.S.A.) were hatched in artificial seawater (Sigma, St. Louis, MO, USA) under light for 48 h at room temperature (25-29 °C). The biotoxicity of MLEEH in 1 % dimethyl sulfoxide (DMSO; Sigma, St. Louis, MO, USA) was tested at 0.1–10000 µg/mL in triplicate, after 24 h incubation. Each well contained ten live nauplii (larvae). Negative controls containing only sea water and 1 % DMSO (v/v) in sea water, and a positive control containing potassium dichromate (0.1–1000 µg/mL) in sea water were included in the assay. After 24 h incubation at room temperature, the culture plate was examined using a stereo microscope to determine the number of dead (non-motile) nauplii in each well. The LC_{50} of MLEEH was derived by probit analysis using SPSS, version 20 (IBM Corp, Armonk, NY, USA).

Ethical approval

The *in vitro* studies utilized semen routinely collected from donor bucks in the Veterinary Teaching Farm of the University of Nigeria Nsukka. The study was approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, University of Nigeria, Nigeria (approval no. 2019-13/189419), and was in accordance the National Research Council's Guide for the Care and Use of Laboratory Animals [10].

Semen collection

Semen samples used for the *in vitro* studies were routinely collected from three sexually mature (13 – 15 months old) West African Dwarf (WAD) bucks. The donor bucks were clinically examined and determined to be healthy with no obvious signs of systemic or reproductive pathologies. Semen was routinely collected using an electroejaculator as previously described [3]. The time of ejaculation was recorded and the collected semen was maintained between 32–37

°C and taken to the laboratory for further evaluation.

Semen evaluation and sperm preparation

The semen was evaluated grossly to determine the colour, volume and pH. Microscopic semen evaluation was performed as previously described [11,12]. Sperm total motility (%) and progressive motility (%) were determined at 37 °C using a phase-contrast microscope (Motic B3; Motic, Carlsbad, CA, USA) equipped with a stage slide warmer (TCS-100; Amscope, Ivrine, CA, USA). Sperm viability (%) was evaluated using eosin-nigrosin vital staining method, and sperm were identified as live (unstained head) or dead (marked pink-stained head) using light microscopy. Sperm morphology and sperm abnormalities were evaluated using phase-contrast microscopy and light microscopy (with eosin-nigrosin staining). Fixed smears of semen samples stained with Papanicolaou method were examined for acrosomal morphology under light microscopy. All values in percentage were determined by examining 200 sperm cells in duplicates. Sperm concentration (per mL of semen) and the total sperm count (per ejaculate) were determined using the haemocytometric method. Micrographs were captured using Moticam 2.0 image system (Motic, Carlsbad, CA, USA). Following the preliminary semen evaluation, the viable and motile sperm cells were harvested using the Direct Swim-up technique in phosphate-buffered saline (PBS, pH 7.4), as previously described [12]. Sperm aspirates were used to prepare suspensions of 10 million sperm cells per mL, which were then used for the *in vitro* experiment.

Sperm *in vitro* treatment with MLEEH

The experiment was replicated three times (n = 3) with semen collected from three bucks. All tests were performed in duplicates. The treatment concentrations of MLEEH were selected based on the minimum concentration required to immobilize 100 % of sperm within 1 min *in vitro*. Measured quantities of MLEEH were dissolved in DMSO and then made up with phosphate-buffered saline (PBS, pH 7.4). Dilutions of these suspensions were then made using 100 µL of sperm sample (10 million sperm/mL) to a final volume of 200 µl in each well, to yield five treatment groups with 1.25, 2.5, 5, 10 and 20 mg/mL of MLEEH in 1 % DMSO, respectively. Two control groups were included in the evaluation: Group 1 (G1 or control A) comprised sperm sample only while Group 2 (G2 or control B) comprised sperm sample in PBS and 1 % DMSO only. Groups 3 to 7 comprised treatments with 1.25, 2.5, 5, 10 and 20 mg/mL MLEEH in 1 % DMSO, respectively. All the groups were incubated at 37 °C, and samples were evaluated for sperm total motility, progressive motility, viability and total abnormalities at 1, 5 and 10 min post-treatment. Sperm revival tests were performed to investigate the extent of the immobilizing capacity of MLEEH treatment, and any reversal of sperm incapacitation. Following 10 min of treatment with MLEEH, the samples were washed twice by diluting to 10 mL with PBS, mixing gently and centrifuging at 800 × g for 10 min. The supernatant was removed and the sperm pellet re-suspended in fresh PBS. Washed sperm samples were then incubated at 37 °C for 1 h in PBS (pH 7.4) supplemented with 4 mg/mL bovine serum albumin, 0.36 mM sodium pyruvate, 23.8 mM sodium lactate and 5.5 mM glucose. Following incubation, sperm total motility was assessed as evidence of sperm revival.

Statistical analysis

Data were analysed using mixed ANOVA with repeated measures via a general linear model built in SPSS, version 20 (IBM Corp, Armonk, NY, USA). The effect of treatment concentration and duration of treatment, and their interaction, were determined for each of the studied sperm parameters. The Greenhouse-Geisser correction was used where Mauchley's test of sphericity was violated. Results are reported as main effects of treatment concentration and duration of treatment. Where ANOVA showed significant difference, pairwise differences were confirmed using Tukey's HSD post hoc test. Values are presented as mean ± standard deviation (SD) and the differences were considered significant when $p < 0.05$.

RESULTS

Brine shrimp lethality

The LC_{50} of MLEEH on *A. salina* was 92.87 µg/mL (95% CI: 50.38 – 182.69) whereas the positive control (potassium dichromate) had an LC_{50} of 8.51 µg/mL (95% CI: 5.37–17.02).

Preliminary donor semen

Grossly, semen samples collected from the donor bucks had normal creamy colour, volume (0.48 ± 0.07 mL), specific gravity (1.03 ± 0.004), viscosity (3.67 ± 0.58 on a scale of 1 – 4) and pH (7.3 ± 0.2). Microscopic analysis of the semen samples revealed the following: sperm total motility (86.3 ± 4.5%), sperm progressive motility

(71.7 ± 3.1%), sperm viability (91.7 ± 3.5%), sperm total abnormalities (9.0 ± 2.0%), sperm concentration ($2.75 \times 10^9 \pm 0.26 \times 10^9$ spermatozoa per mL) and total sperm count ($1.33 \times 10^9 \pm 0.23 \times 10^9$ spermatozoa per ejaculate).

Effect of MLEEH treatment on sperm total motility

There was a significant interaction between the effects of MLEEH concentration and duration of treatment on sperm total motility (F[12, 28] = 181.4, p < 0.001, partial eta-squared [$\eta p2$] = 0.987). There was a significant main effect for MLEEH treatment on sperm total motility (F[6, 14] = 1163.8, p < 0.001, $\eta p2$ = 0.998). Pairwise analysis (Table 1) showed that total motility decreased (p < 0.001) with an increase in MLEEH concentration. The duration of treatment with MLEEH also had a significant main effect on sperm total motility (F[2,28] = 1053.2, p < 0.001, $\eta p2$ = 0.987). Total motility decreased with longer durations of treatment.

Effect of MLEEH treatment on sperm progressive motility

There was a significant interaction between the effects of MLEEH concentration and duration of treatment on sperm progressive motility (F[12,28] = 241.3, p < 0.001, $\eta p2$ = 0.990). Main effects analysis showed a significant effect for treatment with MLEEH on sperm progressive motility (F[6,14] = 1607.6, p < 0.001, $\eta p2$ = 0.999). Pairwise comparison (Table 2) showed a significant decrease (p < 0.001) in progressive motility as MLEEH concentration increased. The duration of treatment with MLEEH also had a significant main effect on sperm progressive motility (F[2,28] = 1109.8, p < 0.001, $\eta p2$ = 0.988). Progressive motility also decreased with longer durations of treatment.

Effect of MLEEH treatment on sperm viability

The effects of MLEEH treatment on caprine sperm viability are shown in Table 3 and Figure 1A-B**.** Analysis of variance with Greenhouse-Geisser correction showed a significant interaction between the effects of MLEEH concentration and duration of treatment on sperm viability (F[8.1, 18.8] = 21.9, p < 0.001, $\eta p2$ = 0.904). A significant main effect was also observed for MLEEH treatment on sperm viability (F[6, 14] = 33.3, p < 0.001, $\eta p2$ = 0.934). Pairwise comparison showed a significant decrease (p < 0.05) in viability as the MLEEH concentration increased.

Table 1: *In vitro* effects of MLEEH treatment on caprine sperm total motility

Group	MLEEH conc. (mg/mL)	Duration of treatment (min)		
		1	***5***	***10***
1 (control A)	0	$93.7\pm1.53^{(a)}$	$93.0\pm1.00^{(a)}$	$93.0\pm1.00^{(a)}$
2 (control B)	0	$93.0\pm1.00^{(a)}$	$92.7\pm1.53^{(a)}$	$93.0\pm1.00^{(a)}$
3	1.25	$90.7\pm2.31^{(a)(x)}$	$85.0\pm2.65^{(b)(y)}$	$72.3\pm4.16^{(b)(z)}$
4	2.5	$70.0\pm2.65^{(b)(x)}$	$62.3\pm4.51^{(c)(y)}$	$39.0\pm3.00^{(c)(z)}$
5	5	$46.7\pm3.79^{(c)(x)}$	$31.0\pm2.00^{(d)(y)}$	$11.3\pm2.52^{(d)(z)}$
6	10	$16.0\pm1.73^{(d)(x)}$	$0.0\pm0.0^{(e)(y)}$	$0.0\pm0.0^{(e)(y)}$
7	20	$0.0\pm0.0^{(e)}$	$0.0\pm0.0^{(e)}$	$0.0\pm0.0^{(e)}$

Control A (sperm sample); control B (sperm sample in 1 % DMSO). Values represent mean ± SD (n = 3). Columns with different superscripts (a–e) represent significant differences (p < 0.001) in groups within each time point. Rows with different superscripts (x–z) represent significant differences (p < 0.001) in time points within each group

Table 2: *In vitro* effects of MLEEH treatment on caprine sperm progressive motility

Group	MLEEH conc. (mg/mL)	Duration of treatment (min)		
		1	***5***	***10***
1 (control A)	0	$86.0\pm1.00^{(a)}$	$86.3\pm1.53^{(a)}$	$85.7\pm2.08^{(a)}$
2 (control B)	0	$86.7\pm1.53^{(a)}$	$87.0\pm1.00^{(a)}$	$86.0\pm2.00^{(a)}$
3	1.25	$76.0\pm2.65^{(b)(x)}$	$65.3\pm3.21^{(b)(y)}$	$41.0\pm2.00^{(b)(z)}$
4	2.5	$47.7\pm3.51^{(c)(x)}$	$30.0\pm2.65^{(c)(y)}$	$16.3\pm2.08^{(c)(z)}$
5	5	$19.0\pm2.00^{(d)(x)}$	$8.7\pm1.53^{(d)(y)}$	$0.0\pm0.0^{(d)(z)}$
6	10	$3.3\pm1.53^{(e)(x)}$	$0.0\pm0.0^{(e)(y)}$	$0.0\pm0.0^{(d)(y)}$
7	20	$0.0\pm0.0^{(e)}$	$0.0\pm0.0^{(e)}$	$0.0\pm0.0^{(d)}$

Control A (sperm sample); control B (sperm sample in 1% dimethyl sulfoxide). Values represent mean ± SD, n = 3. Columns with different superscripts (a–e) represent significant differences (p < 0.001) in groups within each time point. Rows with different superscripts (x–z) represent significant differences (p < 0.001) in time points within each group

There was also a significant main effect for duration of treatment with MLEEH on sperm viability (F[1.3, 18.8] = 237.0, p < 0.001, $\eta p2$ = 0.944). As the duration of treatment increased, sperm viability declined significantly (p < 0.01).

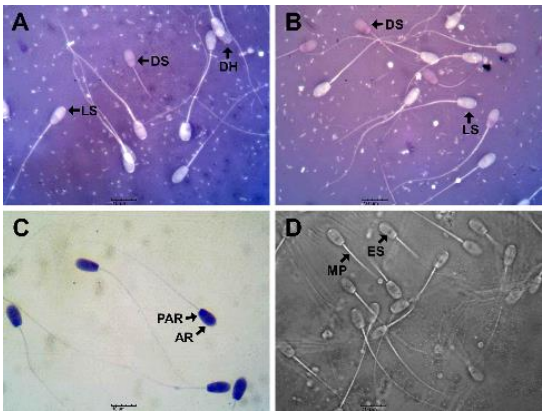

Figure 1: Micrographs of caprine sperm characteristics following *in vitro* treatment with *E. hirta* methanol leaf extract (scale bar = 10 µm). A and B: sperm viability and morphology, shown using eosin-nigrosin staining. Live sperm (LS), dead sperm (DS), and sperm with detached head (DH). C: sperm morphology, shown using Papanicolaou staining. Note the normal dark-bluish post-acrosomal region (PAR) and the pale-bluish acrosomal region (AR) of the sperm head. D: sperm morphology using phase-contrast microscopy. Midpiece (MP) region of the tail, and the equatorial segment (ES) of the sperm head separating the acrosomal and the post-acrosomal regions

Effect of MLEEH treatment on sperm total abnormalities

There were very low percentages of sperm abnormalities that were mainly sperm with detached head (Table 4 and Figure 1). There were no significant differences in individual sperm abnormalities (data not shown). There was no significant interaction between the effects of MLEEH concentration and duration of treatment on sperm total abnormalities (F[12, 28] = 0.35, p = 0.97, $\eta p2$ = 0.131). Analysis for main effects showed no significant effect for both MLEEH treatment (F[6, 14] = 0.34, p = 0.90, $\eta p2$ = 0.128) and duration of treatment (F[2, 28] = 0.58, p = 0.57, $\eta p2$ = 0.039) on sperm total abnormalities.

Effect of MLEEH treatment on sperm revival

Sperm revival assay showed that sperm in the control groups had no significant differences in total motility: Group 1 (61.7 ± 3.06%); Group 2 (63.0 ± 4.0%). All the MLEEH-treated groups (G3 – G7) recorded zero values for percentage total sperm motility.

Table 3: *In vitro* effects of MLEEH treatment on caprine sperm viability

Group	MLEEH conc. (mg/mL)	Duration of treatment (min)		
		1	*5*	*10*
1 (control A)	0	99.0±1.00$^{(a)}$	98.7±0.58$^{(a)}$	99.0±1.00$^{(a)}$
2 (control B)	0	98.7±1.15$^{(a)}$	99.0±1.00$^{(a)}$	99.0±1.00$^{(a)}$
3	1.25	98.0±1.73$^{(a)(x)}$	97.0±1.00$^{(ab)(xy)}$	91.3±2.31$^{(bc)(z)}$
4	2.5	96.7±1.53$^{(ab)(x)}$	95.0±2.00$^{(bc)(y)}$	89.7±3.06$^{(c)(z)}$
5	5	94.3±2.08$^{(bc)(x)}$	92.0±2.65$^{(c)(y)}$	86.0±2.00$^{(d)(z)}$
6	10	91.7±2.52$^{(cd)(x)}$	87.0±2.00$^{(d)(y)}$	83.0±1.73$^{(d)(z)}$
7	20	90.0±2.00$^{(d)(x)}$	84.7±2.89$^{(d)(y)}$	76.3±2.08$^{(e)(z)}$

Control A (sperm sample); control B (sperm sample in 1% dimethyl sulfoxide). Values represent mean ± SD, n = 3. Columns with different superscripts (a-e) represent significant differences (p < 0.05) in groups within each time point. Rows with different superscripts (x-z) represent significant differences (p < 0.01) in time points within each group

Table 4: *In vitro* effects of MLEEH treatment on caprine sperm total abnormalities

Group	MLEEH conc. (mg/mL)	Duration of treatment (min)		
		1	*5*	*10*
1 (control A)	0	2.00±1.00	2.33±1.15	2.67±1.15
2 (control B)	0	2.67±0.58	2.33±1.53	2.67±0.58
3	1.25	2.33±1.15	3.00±1.00	2.33±0.58
4	2.5	2.67±0.58	3.00±1.00	2.67±1.15
5	5	3.00±1.00	2.67±1.15	3.00±1.00
6	10	2.67±1.15	3.00±1.00	3.00±2.00
7	20	3.00±1.00	3.33±0.58	3.67±2.08

Control A (sperm sample); control B (sperm sample in 1% dimethyl sulfoxide). Columns showed no significant differences in groups within each time point. Rows showed no significant differences in time points within each group

DISCUSSION

This study has revealed that treatment with methanol leaf extract of *E. hirta* significantly decreased caprine sperm motility and viability. The brine shrimp lethality assay of MLEEH revealed an LC_{50} of 93 µg/mL, which provided evidence of an extract with very potent biotoxicity. Generally, extracts with LC_{50} < 1000 µg/mL are considered to have bioactivity in toxicological assessment of medicinal plants [13,14]. This high biotoxicity may be responsible for the significant immobilizing and spermicidal effects of MLEEH on sperm. The semen and sperm parameters of the donor samples were within the reference ranges observed in normal WAD bucks [15-17]. Therefore, sperm obtained from these donor samples were valid for further *in vitro* studies. In addition, the use of Direct Swim-up technique enabled the harvest of only viable and motile sperm cells for the study. The inclusion of a control group containing DMSO was used to rule out any confounding effects of the solvent on the treated spermatozoa. This was confirmed by the absence of significant differences in all the sperm parameters between the two control groups.

Sperm total and progressive motility were significantly lowered by MLEEH treatment in a concentration-dependent and time-dependent manner. There were increased proportions of sperm with abnormal motility including sluggish, circular, rolling and wobbling movements. Sperm motility was totally lost at higher concentrations (10 – 20 mg/mL) with longer durations of treatment. This immobilizing effect was consistent with a previous report from an *in vivo* study, where oral dosing of WAD rams with *E. hirta* aqueous leaf extract for 14 days caused a reduction in sperm motility from 83 to 48 % [8]. Normal sperm motility involves a rapid, progressive movement which is crucial for sperm transport and fertilization. Abnormal and low sperm motilities are known to significantly lower fertility in males [18,19].

Sperm viability was also significantly lowered by MLEEH treatment in a concentration-dependent and time-dependent manner. When compared with the effect on sperm motility, this spermicidal effect of MLEEH treatment was more delayed. Thus, even at lower concentrations, MLEEH treatment was potentially spermicidal with prolonged exposure to sperm. At 10 min post-treatment with the highest concentration of 20 mg/mL, 100% of sperm were immobilized whereas only 25 % of sperm were dead. However, the significant loss of motility indicates that fertilization potential of treated sperm is likely to be low. The observed spermicidal effect was also consistent with the *in vivo* observation by Oyeyemi *et al* [8], where treatment with *E. hirta* aqueous leaf extract caused a reduction in sperm viability from 91 to 33 %. A high proportion of dead spermatozoa can negatively impact fertility [20]. In this *in vitro* study, the immobilizing and spermicidal effects on sperm were observed following a direct contact between sperm and MLEEH. Although the precise mechanisms are not clear, the spermicidal effect involved a disruption of the sperm membrane integrity which allowed the passage of eosin to stain the dead sperm. Normal sperm motility is known to be positively correlated with the sperm plasma membrane integrity and normal morphology [21]. Therefore, the loss of sperm motility may be related to the spermicidal and membrane disruptive effect of MLEEH treatment. Although these immobilizing and spermicidal effects were similar to the *in vivo* observations, the mechanisms of bioavailability and reproductive toxicity of MLEEH may need to be further investigated.

Sperm morphology was not altered by MLEEH treatment. The low prevalence of morphological abnormalities was mainly due to the harvesting and utilization of only viable and highly motile sperm for the study. A previous *in vivo* study observed testicular and seminiferous tubular degeneration in rats treated with *E. hirta* aqueous leaf extract [7]. However, Oyeyemi *et al* [8] did not report any sperm abnormalities in the semen of treated rams. An increase in sperm morphological abnormalities can adversely affect sperm function and fertility [20,22]. These findings suggest that the antifertility effect of MLEEH is most likely related to its immobilizing and spermicidal effects, rather than an alteration in sperm morphology.

The washing and supplementation of MLEEH-treated sperm failed to revive sperm motility. Therefore, MLEEH induced a permanent and irreversible immobilization of spermatozoa *in vitro*, suggesting potential as a spermicidal agent for application in fertility control and contraception. A number of phytochemicals are present in *E. hirta* extracts including alkaloids, phenols, flavonoids, glycosides, tannins, triterpenoids and sterols [6], and further studies will investigate the active principles responsible for the immobilizing and spermicidal effects of the plant.

CONCLUSION

The *in vitro* treatment of caprine spermatozoa with methanol leaf extract of *E. hirta* caused

concentration-dependent and time-dependent decreases in sperm total and progressive motility and sperm viability, but had no effect on sperm morphology. The sperm immobilizing effect of the extract was irreversible. These findings may suggest possible adverse effects on fertility in males treated with *E. hirta*. The extract may also have potential as an antifertility or contraceptive agent.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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