

Original Research Article

Effects of polyherbal formulation on the quality of spermatozoa in male Wistar rats

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Sent for review: 17 December 2023

Revised accepted: 28 October 2025

Abstract

Purpose: To determine whether the administration of Formula X (containing *Euricoma longifolia* radix extract, *Pimpinella pruatjan* radix extract, *Tribulus terrestris* fructus extract, *Zingiber officinale* rhizome extract, and *Areca catechu* seed extract) can improve the quality of spermatozoa in male Wistar rats.

Methods: Fourteen (14) male Wistar rats were divided evenly into 2 groups: the treatment group (given 45 mg/kg of Formula X) and normal control (given distilled water) orally for 48 days. Thereafter, the rats were sacrificed and their cauda epididymis was extracted and the motility, morphology, and total sperm count were determined.

Results: The results reveal that the treatment group had a significantly higher mean sperm motility (3.71 ± 0.49) compared to control (3.0 ± 0.0 ; $p < 0.05$). Although both groups had normal average morphology, the treatment group showed a significantly higher value (76.85 ± 0.48 %) in comparison with normal control (63.45 ± 1.26 %; $p < 0.05$). In addition, the treatment group had a significantly higher mean total spermatozoa count (32.86 ± 3.93 million/mL) compared to normal control (16.43 ± 4.76 million/mL; $p < 0.05$).

Conclusion: Formula X has a spermatogenic effect, which implies improved spermatozoa quality. Studies to explore the effect of Formula X on testicular histopathology and spermatozoa viability would be required.

Keywords: Male Wistar rats, Polyherbal, Sexual dysfunction, Spermatogenesis, Spermatozoa

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INTRODUCTION

Sexual dysfunction is a major clinical disruption in the ability to respond sexually or gain sexual pleasure. It is influenced by several factors, including age, hormonal disorders, other diseases (such as diabetes, cardiovascular disorders and cancer) and side effects of drugs (e.g., antihypertensives, antipsychotics, antidepressants, alpha-blockers, anticholinergics

and narcotics) [1]. Male sexual dysfunction is prevalent and becomes more common with age. In Asia, it affects approximately 10 – 15 % of men between the ages of 40 and 49 years, alongside 39 % of men between the ages of 60 and 70 years [2].

In Indonesia, alternative treatment of sexual dysfunction using herbal plants that increase libido (hereinafter referred to as aphrodisiacs)

has been widely explored. As an enhancer of intimacy, aphrodisiacs are in high demand to satisfy couples' needs for a healthy sexual life [3]. Aphrodisiacs have different mechanisms of action, such as increasing nitric oxide (NO), cyclic guanyl monophosphate (cGMP), Luteinizing Hormone (LH), Follicle-stimulating hormone (FSH), testosterone, and sympathetic-parasympathetic nerve mechanisms, as well as decreasing secretion of prolactin [4]. Previous studies have reported three secondary metabolite compounds that exhibit aphrodisiac activity, namely flavonoids, alkaloids, and saponins [5].

Pasak bumi (*Eurycoma longifolia*) contains eurycomanone that increases spermatozoa count by enhancing testosterone levels and spermatozoa motility [6]. Similarly, stigmasterol found in *purwoceng* (*Pimpinella pruatjan*) has also been shown to increase testosterone levels, resulting in higher spermatozoa count [7]. Furthermore, *Tribulus terrestris*, commonly known as *rujak polo*, contains protodioscin that increases libido, testosterone, and dihydrotestosterone levels by stimulating LH [8]. Ginger (*Zingiber officinale*) contains arginine and vitamin E, which have protective effects on spermatozoa morphology, as well as gingerol, which increases LH and antioxidants, thus protecting spermatozoa from free radicals [9]. Another study has also reported that areca nut (*Areca catechu*) contains alkaloids, which increase mounting, intromission, and testosterone levels in male Wistar rats [10]. Due to the benefits of these herbal plants, a polyherbal formulation designated as Formula X was prepared by combining *pasak bumi*, *purwoceng*, *rujak polo*, ginger, and *pinang* in different amounts. This study, therefore, aims to examine this polyherbal formulation for its effectiveness in increasing the quality and quantity of spermatozoa, which has not been tested previously.

EXPERIMENTAL

Animals

Fourteen (14) male Wistar rats (200 – 250 g; 12 – 18 months) were sourced from a certified breeding center in Klaten. The animals were kept in cages maintained at a room temperature below 30 °C, relative humidity of 40 – 70 %, with a daily replenishment of feed and water.

Ethical approval

This study received ethical approval from the Animal Ethics Committee of the School of

Veterinary Medicine and Biomedical Sciences, Institut Pertanian Bogor (IPB) University, Indonesia (approval no. 017/KEH/SKE/VII/2022).

Sample preparation

Formula X capsule powder (PT. Natura Nuswantara Nirmala, South Tangerang, Indonesia) was dispersed separately in distilled water at a concentration of 1 %w/v. Each capsule contains 200 mg *Eurycoma longifolia* radix extract, 100 mg *Pimpinella pruatjan* radix extract, 50 mg *Tribulus terrestris* fructus extract, 100 mg *Zingiber officinale* rhizome extract and 50 mg *Areca catechu* seed extract.

Rat dose treatment and spermatozoa retrieval

Rats were divided into 2 groups consisting of 7 rats/group: Group 1 was normal control (given distilled water), while Group 2 served as the treatment group that received 1% Formula X (45 mg/kg BW). Oral administration was conducted every day for 48 days [11]. On day 49, the rats were sacrificed by cervical dislocation, following animal ethical rules. The cauda epididymis was removed and washed in physiological NaCl solution, and the semen was extracted, added to 1 mL physiological NaCl solution, and homogenized to produce the sperm stock solution [12].

Determination of spermatozoa motility

Solution A was made by combining a drop of sperm stock solution with NaCl solution and homogenizing on a microscope slide. Then, one drop of Solution A was applied to a new hemacytometer, and spermatozoa motility was examined through a microscope at 37 °C for optimal motility with 400x magnification. Spermatozoa motility was evaluated based on the scoring presented in Table 1 [13].

Table 1: Spermatozoa motility scoring

Score	Description
0	Immotile spermatozoa
1	1–25% spermatozoa movement
2	26–50% spermatozoa movement
3	51–75% spermatozoa movement
4	76–100% spermatozoa movement

Determination of spermatozoa morphology

One milliliter of sperm stock solution was added to 1 mL of 0.5 % (w/v) eosin solution in a 1:1 ratio and prepared on an object glass. Then it was examined with a microscope with a magnification of 400x and 10 field repetitions. Normal spermatozoa (NS) consist of a head,

body and tail, while abnormal spermatozoa have either incomplete or deformed parts. Normal spermatozoa morphology was calculated with Eq 1 [12].

$$NS (\%) = \{N/(A+N)\}100 \dots\dots\dots (1)$$

Where N = Total number of normal spermatozoa,
A = Total number of abnormal spermatozoa

Total spermatozoa count

Sperm stock solution was pipetted with an erythrocyte pipette up to the 0.5 mm mark and loaded onto a hemacytometer. Then, eosin coloring was pipetted up to the 0.01 mm mark and added to the sperm stock solution to produce Solution A, which was then homogenized in a pipette and applied to the Neubauer hemacytometer. The spermatozoa were counted on the Neubauer in the upper left, upper right, center, lower left, and lower right rooms (Figure 1A, area L).

Each room consists of 16 small squares. If the spermatozoa head is on the boundary line, it is only counted from 2 sides, namely the left side and bottom, while the right side and top are not counted. Total spermatozoa count (TSC) was calculated using Eq 2 [14].

$$TSC = N * 5 * df * 10,000 \dots\dots\dots (2)$$

Where N = Average number of spermatozoa in the counting chamber; 5 = Number of rooms counted; df = dilution factor; 10,000 = conversion factor

Data analysis

The obtained data are presented as Mean $\pm$ standard deviation (SD) of seven replicates and analyzed using Kruskal-Wallis test and Mann-Whitney test in SPSS for Windows (v 22) to examine the difference ($p < 0.05$) in the effects of formula X on the quality and quantity of spermatozoa.

RESULTS

Spermatozoa motility

Table 2 shows the results of Formula X administration on spermatozoa motility, morphology, and total count. Significant differences were observed between the treatment group with Formula X and normal control on the three parameters tested. With regard to sperm motility, the treatment group had a significantly ($p < 0.05$) higher mean of 3.71 ± 0.49 compared to control (3.0 ± 0.0).

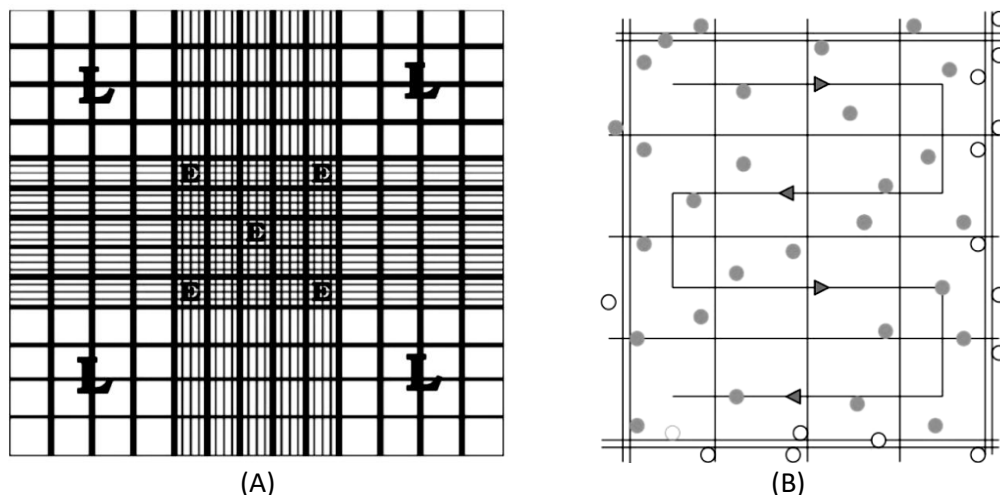


Figure 1: (A) Schematic of the improved Neubauer hemocytometer counting chamber. L: Leukocyte counting grid (used for spermatozoa count). E: Erythrocyte counting grid (not used); (B) Spermatozoa count scheme

Table 2: Comparison of sperm motility, morphology and count between groups

Parameter	Normal Control	Formula X
Weight gain after 48 days (%)	12.49 $\pm$ 0.08	12.63 $\pm$ 0.04
Spermatozoa motility	3.0 $\pm$ 0.0	3.71 $\pm$ 0.49*
Spermatozoa morphology (%)	63.45 $\pm$ 1.26	76.85 $\pm$ 0.48*
Total spermatozoa count (million/mL)	16.43 $\pm$ 4.76	32.86 $\pm$ 3.93*

* $P < 0.05$ vs control

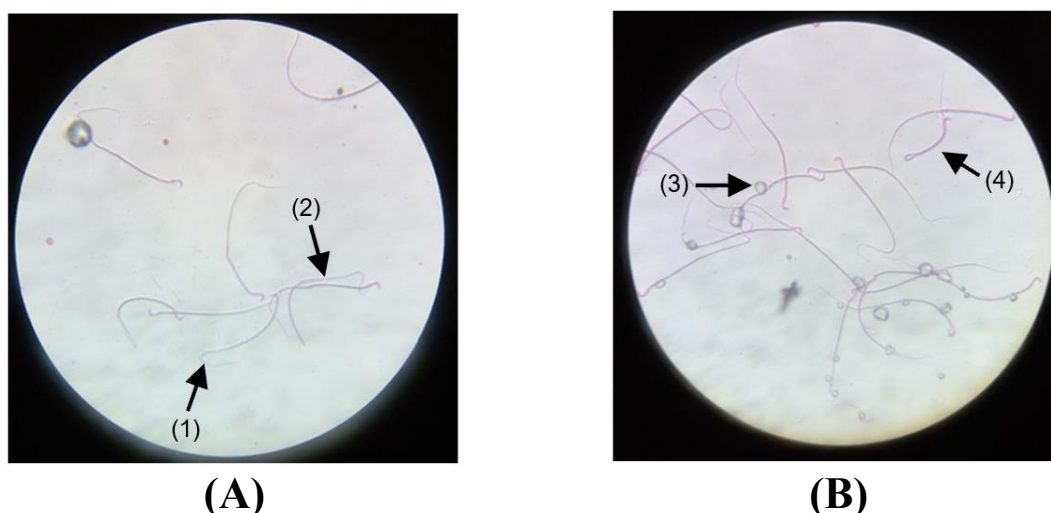


Figure 2: Microscopic comparison of spermatozoa morphology (400x magnification). (A): Spermatozoa from normal control group, showing folded tail (1) and spermatozoa without the head abnormality. (B): Spermatozoa from a rat administered with Formula X, displaying some abnormality without the body (3) and normal spermatozoa (4)

Spermatozoa morphology

Figure 2 presents the morphology of the tested animals' spermatozoa. As displayed in Table 2, both groups had normal average morphology, with the treatment group having a significantly ($p < 0.05$) higher value ($76.85 \pm 0.48 \%$) in comparison with normal control ($63.45 \pm 1.26 \%$).

Total spermatozoa count

As shown in Table 2, the treatment group had a significantly higher mean total spermatozoa count (32.86 ± 3.93 million/mL) compared to normal control (16.43 ± 4.76 million/mL; $p < 0.05$).

DISCUSSION

The nature of spermatozoa movement determines the sterility of an individual, with slow and erratic movement having the possibility to result in fertilization failure. There are primary and secondary abnormalities of spermatozoa. Factors contributing to primary abnormalities include heredity, poor environment and failure in the process of spermatogenesis. Meanwhile, secondary abnormalities occur after spermatozoa leave the seminiferous tubules or at the time of staining in the process of making review preparations. The quality of spermatozoa is determined by their motility, morphology, and total count. Spermatozoa motility is a useful parameter in determining fertility in men, both *in vitro* and *in vivo*. Normal morphology is crucial for the progressive movement of spermatozoa, and the number of spermatozoa depends on the

process of spermatogenesis that occurs in the seminiferous tubules. The administration of Formula X in this study resulted in a significant improvement in key sperm quality parameters. Typically, normal value of spermatozoa morphology in rats is above 50 %, and a rat is considered infertile if it has a value below the standard [15]. Rats' spermatozoa had morphology spermatozoa increased above the norm after being treated with Formula X. The observed morphological abnormalities suggest that Formula X protected the process of spermatogenesis by mitigating free radical damage to α -tubulin protein in spermatids and the membranes of Leydig and Sertoli cells [16].

Rats' spermatozoa were doubled in number after being given Formula X, compared to normal control. This is hypothesized by eurycomanone in *pasak bumi* and stigmasterol in *purwoceng*, which are associated with an increase in the maturation of spermatozoa in the epididymis. *Pasak bumi* is a potential agent that has been shown to reverse the effects of estrogen by increasing spermatogenesis and the sperm count in rats, which is observable after administration for 14 consecutive days [14]. In addition, *pasak bumi* improves the affinity of the receptor membrane to α -reductase enzyme that plays a vital role in the conversion of testosterone into a more potent dihydrotestosterone, which is believed to increase the incidence of spermatogenesis, particularly in the spermatogenesis phase [15]. Besides stigmasterol, *purwoceng* also contains eurikomolactone and amarolinda that enhance the activity of the anterior hypophysis cell membrane towards Gonadotropin-releasing

hormone (GnRH). The hormone-receptor complex bond activates the enzyme adenylate cyclase, converting adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP), which then initiates the release of LH to stimulate the formation and secretion of testosterone in Leydig cells [17].

Rujak polo contains protodioscin (saponin) that increases libido, testosterone, and dihydrotestosterone levels by stimulating LH [8]. Previous studies have reported that *rujak polo* extract works as an aphrodisiac, which, in rats, improves spermatozoa morphology compared to control group. Another study has also stated that the hydroalcoholic extract of *rujak polo* protects testicular tissue and spermatozoa in cisplatin-induced rats [18].

The active ingredients of ginger extract in the form of phenolic compounds that act as antioxidants have also been shown to increase the number of spermatozoa in the treatment group receiving Formula X. Antioxidants are needed to inactivate development of oxidizing compound reactions by donating one or more electrons to pro-oxidant compounds, then converting oxidant compounds into more stable compounds [9]. Formula X also contains areca nut, which is rich in alkaloids (one of which is arecoline) that have been reported to boost testosterone in the reproductive tract of male rats. However, alkaloids interfere with the activity of ATPase enzyme in the spermatozoa cell membrane in the middle of the tail. ATPase maintains internal homeostasis for sodium and potassium ions, and the inhibition of this enzyme may lead to the membrane losing permeability to soluble materials, thus hindering their transport into the cytoplasm. This results in insufficient nutrient transport needed for spermatozoa to move [19].

CONCLUSION

Oral administration of Formula X, which contains *pasak bumi*, *purwoceng*, *Rujak polo*, ginger, and areca nut, has a spermatogenic effect, as seen by increased motility, normal morphology, and count of sperm in rats, compared to control. Nevertheless, further studies remain essential to exploring the effects of Formula X on testicular histopathology and spermatozoa viability.

DECLARATIONS

Acknowledgment/Funding

The authors would like to acknowledge PT Natura Nuswantara Nirmala for funding and providing polyherbal samples for this research.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was carried out by the authors whose names are mentioned 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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