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# The Ameliorative Effects of *Phyllanthus gomphocarpus* Hook. F. on Sperm Parameters of Rats Induced with Bisphenol A

**Evana Kamarudin[1], Razif Dasiman[1,5*], Ebby Anuar Bahari[2], Kamalru Azman Daud[3], Nur Rohmah Suwandi[4]**

[1] Faculty of Health Sciences, Universiti Teknologi MARA, Selangor Branch, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia
[2] Hospital Universiti Teknologi MARA (HUiTM), 42300 Bandar Puncak Alam, Selangor, Malaysia
[3] Department of Pathology, Hospital Tunku Azizah Kuala Lumpur (HKL), Jalan Pahang, 50586 Kuala Lumpur, Malaysia
[4] Faculty of Health Sciences, Universitas Aisyiyah Bandung, Jl. Banteng Dalam No.6, kel Turangga, Kec. Lengkong, Kota Bandung,40264 West Java, Indonesia
[5] Maternofetal and Embryo Research Group (MatE), Faculty of Medicine, Universiti Teknologi MARA, Selangor Branch, Sungai Buloh Campus, 47000 Sungai Buloh, Selangor, Malaysia

## ABSTRACT

Infertility due to poor sperm quality is a common problem nowadays, particularly in developed countries. Herbs are one of the natural treatment options that have been proposed. *Phyllanthus gomphocarpus* Hook. F is a tropical plant that has traditionally been used in Malaysia to treat male infertility. Antioxidant characterization of its root aqueous extraction was performed using ferric reducing antioxidant power (FRAP), 1,1-diphenyl-2-picrylhydrazyl (DPPH), total phenolic content (TPC), and total flavonoid content (TFC). Male rats were administered two different concentrations of the extract orally along with BPA for 21 days, and their sperm was evaluated using Computer-Assisted-Sperm-Analysis (CASA). The results showed that this plant has a high antioxidant capacity, with 78.6 ± 0.65 %, 0.14 ± 0.01 mg/ml, 28.52 ± 0.13 mg GAE/g and 164.73 ± 23.13 mg QE/g, for DPPH, FRAP, TPC and TFC respectively. The ability of this plant to enhance sperm concentration, motility, velocity, progression ratio, elongation, and percentages of normal sperm morphology significantly with $p<0.05$, appears to be due to its high antioxidant capacity. Furthermore, this plant was found to have sperm-protective effects against the endocrine disruptor BPA. A thorough investigation is required to determine the full mechanism of this plant's beneficial effects on male fertility and human health.

**Keywords:** Infertility; sperm parameters, *Phyllanthus gomphocarpus*, antioxidant, Computer-Assisted-Sperm-Analysis (CASA)

## INTRODUCTION

Infertility is defined as the inability to conceive a child after one year of regular sexual intercourse without the use of contraception. 1 in 6 people globally are affected by infertility and about 17 % of adults worldwide experience infertility, with male factors accounting for nearly half of all cases (Agarwal et al., 2021). Approximately 76% of male infertility problems are caused by sperm production and function disorders, the effective treatments for which are not well defined. Recently, there has been widespread concern about a decline in sperm quality among young men, particularly in some industrialized areas around the world (Kumar & Singh, 2022). This is extremely concerning because studies have shown that when men's sperm quality is poor, the rate of pregnancies drops dramatically (Chapuis et al., 2017). Low sperm quality was defined as a decline in several sperm parameters, including sperm count, motility, and normal sperm morphology, necessitating a variety of approaches to address the issue (Andersen et al., 2000; Jensen et al., 2002; Jørgensen et al., 2001; Milachich & Dyulgerova-Nikolova, 2020). Several factors, including chemical exposure in industries, genetic disorders, neurological defects, poor lifestyle choices, pesticide exposure, and others, have all contributed to this problem. (Chiu et al., 2015; Fode et al., 2012; Massart et al., 2012; Mocarelli et al., 2011; Kumar & Singh, 2022).

Numerous studies have been conducted to elucidate therapeutic interventions, and some of them have yielded positive outcomes by implementing significant advances in diagnosis and treatment as a means of resolving the problem (Behnaz et al., 2013; Assidi, 2022). To address this issue, surgical treatments such as microsurgery, which was introduced, require a high level of

***Corresponding author : Razif Dasiman**
**Email : razifdasiman@uitm.edu.my**


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expertise and technology at a high cost (Rimar et al., 2013). Intracytoplasmic injection of testicular spermatozoa was also introduced; however, this technique was highly recommended and appears to be more effective for those with normal spermatogenesis rates, despite the presence of patients with spermatogenesis problems (Vernaeve et al., 2003; Vloeberghs et al., 2013). Treatments such as hormonal replacement therapy and the use of synthetic drugs such as clomiphene citrate and bromocriptine were also introduced, but the results revealed that they have some limitations in terms of negative side effects and their effectiveness in treating this problem (Behnaz Khani, Soroor Rabbani Bidgoli, Fariborz Moattar, 2013; Sengupta et al., 2021).

Herbs are one of the alternative methods for treating male infertility by improving sperm quality. It has been known since ancient times and applies to all countries and races around the world. Herbal plants appear to be more beneficial in the treatment of male infertility than other methods because they are less invasive and less expensive in terms of physical and emotional treatment (Mohammadi &, Hossein Nikzad, Aliakbar Taherian, Javad Amini Mahabadi, 2013; Roozbeh et al., 2021). A large number of studies have recently shown that herbs can naturally improve sperm quality and treat male infertility (Malviya et al., 2011; Nantia et al., 2009; Pratap & Rajender, 2012; State et al., 2013; Umadevi et al., 2013). Most herbs with anti-infertility potential have also been shown to be more effective in treating male infertility, with little to no adverse effects on human health (Malviya et al., 2011). Furthermore, some research suggests that antioxidant supplements in subfertile men may improve the outcomes of live birth and pregnancy rates with no negative side effects (de Ligny et al., 2022). As a result, to effectively treat this male infertility problem, extensive research on the effectiveness of herbal therapies against sperm quality must be conducted.

Malaysia is a rainforest country rich in a thousand species of herbs that are beneficial to human health when consumed as a food supplement (Othman et al., 2014). *Phyllantus gomphocarpus Hook. F.* (Cermela hutan) is a Phyllanthus species that has traditionally been claimed to have the potential in treating male infertility among Malaysian traditional practitioners and the Orang Asli community. This plant is commonly found in Malaysia's eastern and southern regions. Folklore holds that a man suffering from infertility should drink a decoction made from the boiled roots of this plant. There is currently little scientific evidence that this plant has anti-infertility or other medicinal properties. As a result, the purpose of this study is to determine the antioxidant capacity and anti-infertility potential of *Phyllanthus gomphocarpus* Hook. F. root aqueous extract using rats as experimental animal models.

## METHODOLOGY

### Collection and Identification of Plant

Three kilograms of fresh *Phyllanthus gomphocarpus* Hook. F. (PGR) roots were collected from Felda Keratong in Pahang Darul Makmur, Malaysia. The plant was also collected for identification and verification at the Rimba Ilmu Botanical Garden at the University of Malaya, Malaysia. The plant has been identified as *Phyllanthus gomphocarpus* Hook. F., also known as Carmela Hutan in Malaysia. This plant material was given the voucher number KLU 47925 when it was deposited in the herbarium.

### Preparation of PGR Extract

The roots of *Phyllanthus gomphocarpus* Hook. F. (PGR) were cleaned, chopped into small pieces, and dried for approximately 1 week in a hot air oven at 40° C. The dried materials were ground into a powder before being collected in tightly sealed universal bottles. A ten percent (10%) PGR aqueous extraction was prepared and warmed at a temperature of 40° C for 12 hours. The PGR extract powder was then filtered and freeze-dried before being stored in the refrigerator.

### Antioxidant Activities

#### -1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity

The radical scavenging activity of PGR aqueous extracts was determined using Kumaran's method (2007) and Atanassova et al. (2011). For the control, 0.5 ml absolute ethanol was mixed with 1 ml of 0.45 mM DPPH, and for the PGR sample, 0.5 ml of extract (5 mg/ml) was mixed with 1 ml of 0.45 mM DPPH. The procedure was then repeated, but this time BHT was used as the standard antioxidant. The reaction mixture was thoroughly mixed with a vortex mixture before being incubated at room temperature in the dark. After 30 minutes, the absorbance at 517 nm wavelength was measured with a spectrophotometer, and the percentages of inhibition were calculated using the following equation:

DPPH Radical Scavenging Activity (%) = [(A0 – A1) / A0] × 100
where A0 was the absorbance of the control which was DPPH radical + absolute ethanol and A1 was the absorbance of the DPPH radical + extract/standard.

**Ferric Reducing Antioxidant Power (FRAP)**

The FRAP assays were carried out exactly as Baghat et al. described (2014)(Bhagat et al., 2014). The changes in absorbance at 593 nm were used to track the reductions of the ferric tripyridyl triazine (FeIII TPTZ) complex to the ferrous form. 2.5 ml of 10 nm TPTZ was mixed with 2.5 ml of 20 mM ferric chloride and 25 ml of 0.3 mM acetate buffer to make the FRAP reagent. The plant extracts were then combined with 300 µL of distilled water and 3 ml of FRAP reagent in a volume of 100 µL. The mixture was then incubated at room temperature for 4 minutes before the absorbance at 593 nm was measured. FRAP values were calculated using a standard curve of ferric sulfate as the standard in the concentration range of 0 mg/ml to 1 mg/ml.

**Polyphenolic Content**

**Total Flavonoid Content (TFC)**

The total flavonoid content of root sample extracts was determined using the aluminum chloride method. Aliquots of extract solutions were prepared by adding methanol to a volume of 3 ml. Then 0.1 mL AlCl3 (10%), 0.1 mL Na-K tartrate, and 2.8 mL distilled water were added in that order. After 30 minutes of incubation, the test solutions were vigorously shaken, and the absorbance at 415 nm was measured. Using known concentrations of quercetin, a standard calibration plot was generated at 415 nm (Khatiwora et al., 2010). The flavonoid concentrations in the test samples were calculated using the calibration plot and expressed as mg quercetin equivalent /g extract powder.

**Total Phenolic Content (TPC)**

The Folin-Ciocalteu (FC) method was used to determine the total soluble phenols content: 500 L of Folin-Ciocalteu reagent: water (1:1) reacted for 6 minutes in assay tubes with 100 L aliquots of the aqueous extract. Following neutralization with 1.25 mL of 20% Na2CO3, the volume was adjusted to 10 mL with distilled water. After shaking the mixture on a vortex and leaving it in the dark for 2 hours to stabilize it, the absorbance was measured at 765 nm. A gallic acid standard curve was created to express the total soluble phenols content in mg of gallic acid equivalents (GAE)/g of extract powder (Khatiwora et al., 2010).

**Animals and experimental protocol**

Twenty-four male Wistar albino rats were purchased from Laboratory Animal and Facilities Management (LAFAM), Universiti Teknologi Mara (UiTM), Malaysia, and were kept under standard laboratory conditions, including temperature (24 - 27°C), 12 hours of light and 12 hours of darkness, open ventilation, and ad libitum. The experiments were approved by the UiTM Committee on Animal Research and Ethics (UiTM CARE: 71/2015). Rats were randomly divided into four groups (n=6) and given BPA and different PGR aqueous extract concentrations orally for 21 days. The negative control (NOC) group received 2 ml of distilled water as a placebo, while the positive control (POC) group received 1 ml of 200 mg/kg of BPA along with 1 ml of distilled water as a placebo. Rats in the PGR 50 and PGR 100 experimental groups were given 1 ml of 200 mg/kg BPA along with 50 mg/kg and 100 mg/kg PGR aqueous extract, respectively.

**Sperm analysis**

Rats were anesthetized and sacrificed using the cervical dislocation technique after 21 days of treatment. The cauda epididymis was removed and placed in a petri dish with pre-warmed M16 medium. Gentle tissue mincing and tearing were used to allow spermatozoa to swim out into the medium. Before analysis, sperm samples were incubated for 15 minutes at 37°C. The Computer-Assisted Sperm Analyzer (CASA) system (TOX IVOS, Hamilton Thorne Research) was used to measure sperm concentration, elongation, motility, velocity, and progression ratio. The CASA system included a Zeiss phase contrast microscope and a COHU camera system for capturing images at 100x magnification. Several fields of images were captured, ranging in size from 2.4 mm to 29.8 mm, with a minimum of 300 spermatozoa counted for each analysis. This analyzer measures the following sperm parameters:

**Sperm Concentration**

The total concentration of sperm from the sample is indicated in million per milliliter of sperm volume (M/ml).

**Sperm Elongation**

The ratio is reported in percentages (%) of head width to head length of the sperm.

**Sperm Motility**

**Percentages of Sperm Motility (MOT)**

The percentage of sperm that show any movement or motility.

**Percentages of Progressive Sperm Motility (PRG)**
The percentages of sperm with VAP ≥ 25 µ/s and STR ≥ 80%.

**Sperm Velocity**
**Path Velocity - µm/s (VAP)**
The average velocity of the smoothed cell path was measured in microns per second (µ/s).

**Track Speed Velocity - µm/s (VCL)**
The average velocity measured over the actual point-to-point track followed by the sperm cell.

**Progressive Velocity (VSL)**
The average velocity was measured in a straight line from the beginning to the end of the sperm movement track.

**Sperm Progression ratio**
**Straightness (STR)**
The average value of the ratio VSL/VAP. Straightness measures the departure of the cell path from a straight line.

**Linearity (LIN)**
The average value of the ratio VSL/VCL. Linearity measures the departure of the cell track from a straight line.

The eosin Y stain was used to assess normal sperm morphology. Two drops of Eosin Y (1:10) were dropped into a tube containing sperm suspension and incubated for 45 – 60 minutes to allow the sperm to completely absorb the stain's color. Slides were prepared, and a minimum of 200 sperm were examined for morphology. The number of normal and abnormal sperms was counted, and the percentage of each was calculated. The outcome was expressed as a percentage of normal sperm morphology.

**Statistical analysis**
The Statistical Package for Social Science (SPSS) program version 21 was used to analyze all data. The significance of the differences between means for antioxidant activity was determined using t–test analysis of the results. In the case of sperm parameters, analysis of variance (ANOVA) was used, followed by a post hoc Tukey test. All information is presented as an average standard deviation (SD). Statistical significance was denoted by $p<0.05$ values.

## RESULT AND DISCUSSION

Bisphenol A (BPA) is an endocrine disruptor that is commonly found in the environment and is widely used in the production of polycarbonate and plastic materials (Chouhan et al., 2014). With a large number of studies conducted, it is impossible to deny that BPA has a significant impact on male fertility. However, the results were unexpected and have sparked debate about the various concentrations and routes of BPA exposure that can impair the system (Vom Saal & Vandenberg, 2021).

Antioxidant testing with DPPH and FRAP methods was carried out to determine the effect of PGR. Table I shows the free radical scavenging activity and reducing ability of PGR aqueous extract and BHT against 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power of PGR aqueous extract and BHT (FRAP). BHT, as a standard antioxidant, inhibited DPPH with a significantly higher percentage of inhibition (89.87 ± 0.91 %) than PGR extract, which inhibited DPPH with only 78.6 ± 0.65 % percent. Furthermore, BHT had a significantly higher reducing ability value of 0.35 ± 0.02mg/ml when compared to PGR extract, which had a value of 0.14 ± 0.01 mg/ml.

The antioxidant and nutritional compounds that are rich in may help to improve sperm quality and protect against BPA in the PGR. Numerous studies have revealed and discussed the effectiveness of herbs against male fertility, which is strongly linked to their nutritional compounds and antioxidant activities (Chauhan et al., 2014;; Suleiman et al., 2014; Taid et al., 2014; Vr et al., 2013; Alahmadi, 2020; Martins et al., 2021). Constant exposure to hazardous chemicals such as BPA, as well as the various stages of the spermatogenesis process, have the potential to generate excessive ROS and impair male fertility (Agarwal & Sekhon, 2010; Ansoumane et al., 2014; Darbandi et al., 2018). Antioxidant supplementation may reduce excess ROS while also preventing ROS from causing male infertility. Analysis of the TPC and TFC of PGR tabulated in Table II showed that PGR aqueous extract was positive for the presence of phenolic and flavonoid content, with 28.52 ± 0.13 mg GAE/g and 164.73 ± 23.13 mg QE/g of sample. PGR has a high level of polyphenolic content and antioxidant activities, which may be responsible for the protective and ameliorative effects on sperm quality as well as male fertility.

**Table I.** ***Antioxidant activities of PGR via DPPH and FRAP analysis. Samples with (*) are significantly different (p<0.05). Values expressed as mean ± SD.***

| SAMPLE | DPPH (%) | FRAP (mg/ml) |
|---|---|---|
| PGR | 78.6 ± 0.65 | 0.14 ± 0.01 |
| BHT | 89.87 ± 0.91 | 0.35 ± 0.02 |

**Table II.** ***Polyphenolic content of PGR via TPC and TFC analysis. Results expressed as mean ± SD in mg GAE/g for TPC and mg QE/g for TFC.***

| POLYPHENOLIC CONTENT | PGR |
|---|---|
| TPC (mg GAE/g) | 28.52 ± 0.13 |
| TFC (mg QE/g) | 164.73 ± 23.13 |

**Table III.** ***Mean ± SD of sperm parameters of rats after 21 days of PGR and BPA supplementation. Different superscript letters indicate significant difference (p<0.05) based on analysis of variance (ANOVA) statistical analysis. [*Significantly different to NOC group (p<0.05), [a]Significantly different to POC group (p<0.05), [b]Significantly different to PGR 50 group (p<0.05)].***

| SPERM PARAMETERS | RESULT | | | |
|---|---|---|---|---|
| | NOC | POC | PGR 50 | PGR 100 |
| Sperm Concentration (M/ml) | 9.50 ± 1.15 | 8.93 ± 1.10 | 13.28 ± 1.28*a | 23.02 ± 2.95*ab |
| Sperm Elongation (%) | 23.67 ± 2.50 | 22.83 ± 1.47 | 34.00 ± 3.69*a | 33.50 ± 1.76*a |
| Normal morphology (%) | 68.88 ± 9.52 | 30.29 ± 4.69* | 68.92 ± 4.54a | 77.17 ± 2.99a |
| Sperm Motility : | | | | |
| - MOT (%) | 32.00 ± 4.24 % | 23.25 ± 2.50 % | 36.40 ± 4.88a | 54.00 ± 8.76 *ab |
| - PRG (%) | 12.5 ± 1.05 | 8.31 ± 2.31 % | 16.25 ± 1.71 a | 16.67 ± 3.20*a |
| Sperm Velocity : | | | | |
| - VAP (μm/s) | 285.28 ± 19.27 | 217.03 ± 14.31* | 347.08 ± 20.09*a | 294.53 ± 7.98ab |
| - VCL (μm/s) | 428.98 ± 16.65a | 366.8 ± 12.38* | 441.12 ± 13.53a | 542.12 ± 7.61*ab |
| - VSL (μm/s) | 165.65 ± 14.14 | 150.90 ± 10.01 | 226.25 ± 10.06*a | 230.40 ± 10.55*a |
| Sperm Progression ratio: | | | | |
| - STR (%) | 39.50 ± 2.53b | 41.15 ± 2.52b | 50.07 ± 2.69 | 42.95 ± 2.22b |
| - LIN (%) | 58.29 ± 6.36 | 69.65 ± 4.43 * | 66.51 ± 3.86 | 78.22 ± 2.73 *ab |

In this study, the experimental male rats were given 200 mg/kg of BPA orally for 21 days to induce infertility. The positive control (POC) group received only BPA and distilled water, with no PGR supplementation. Based on normal and abnormal sperm morphology as depicted in Figure 1 analysis of sperm concentration and elongation have been examined.

As tabulated in Table III, sperm concentration and elongation were significantly higher in the PGR 50 (13.28 ± 1.28 M/ml and 34 ± 3.69 %) and PGR 100 (23.02 ± 2.95 M/ml and 33.5 ± 1.76 %) groups than in the NOC (9.50 ± 1.15 M/ml and 23.67 ±2.50 %) and POC (8.93 ± 1.10 M/ml and 22.83 ± 1.47 %) groups. The morphology analysis revealed the difference in percentages of normal sperm morphology, PGR 50 with 68.92 ± 4.54 %, PGR 100 with 77.17 ± 2.99 %, and NOC group with 68.88 ± 9.52 % were not statistically significant when compared to the POC group, 30.29 ±4.69 %.

Most of the sperm parameters showed a declining pattern when supplemented with BPA, according to the findings. Path Velocity (VAP), Track Speed Velocity (VCL), and Progressive Velocity (VSL) were used to calculate sperm velocity, which is shown in Table 3. For VAP, the POC group had the lowest velocity with 217.03 ± 14.31 μm/s, which was significantly lower than the other treatment groups with 285.28 ± 19.27 μm/s, 347.08 ± 20.09 μm/s, and 294.53 ± 7.98 μm/s for NOC, PGR 50, and PGR 100, respectively. The highest VCL was recorded by the PGR 100 group at 542.12 ± 7.61 μm/s, followed by the PGR 50 group at 441.12 ± 13.53 μm/s, the NOC group at 428.98 ± 16.65 μm/s, and the POC group at 366.8 ± 12.38

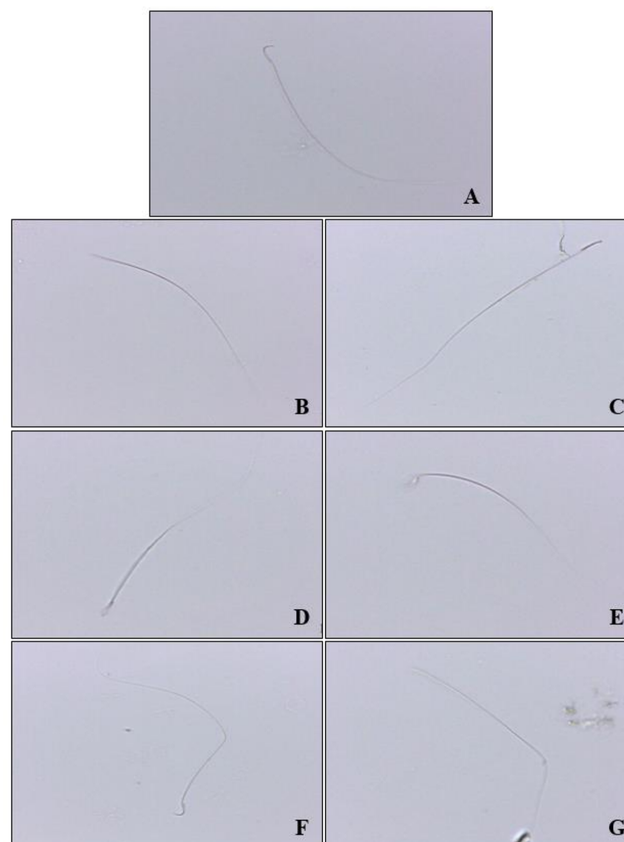


**Figure 1. Microscopic description which shows examples of sperm morphology. A –Normal sperm; B, C, D, E, F, G – Abnormal sperm; B - Headless; C - Flattened head; D - Pinhead; E - Bent Neck; F – Bent Tail; G – Multiple Abnormalities (Headless and Bent Tail).**

μm/s. ANOVA revealed that the PGR 100 group was significantly higher than the other treatment groups. The POC group, which had the lowest result, also showed significant differences from the other treatment groups. PGR 100 group had the highest VSL result of 230.40 ± 10.55 μm/s, followed by the PGR 50 group with 226.25 ± 10.06 μm/s, NOC group with 165.65 ± 14.14 μm/s, and POC group with 150.90 ± 10.01 μm/s. ANOVA revealed that the PGR 100 and PGR 50 groups were significantly higher than the control, POC, and NOC groups, but there was no significant difference between them. The analysis of variance (ANOVA) revealed that only the Path velocity (VAP), track speed velocity (VCL), and percentages of normal sperm morphology were significantly lower when compared to the NEC group. Sperm motility was divided into two categories: sperm motility (MOT) and progressive motile sperm (PRG). The results clearly showed that the PGR 100 group had the highest percentage of MOT and PRG, with 54 ± 8.76 % and 16.67 ± 3.20 %, respectively. The PGR 50 group came in second, with 36.4 ± 4.88 % for MOT and 16.25 ± 1.71 % for PRG. Both MOT and PRG analyses from these two groups yielded significantly higher results than the POC group, with 23.25 ± 2.50 % for MOT and 8.31 ± 2.31 % for PRG. The NOC group had 32± 4.24 % MOT and 12.5 ± 1.05 % PRG, with a significant difference only with the PGR 100 group and not with the other groups. Table 3 shows the results of sperm motility assessments. The results for sperm concentration, motility, elongation, progressive motility, and progressive velocity were lower, but not statistically different from the NEC group. Ansoumane et al. (2014) discovered a similar result when rats treated with three different concentrations of BPA did not significantly affect all sperm parameters, but only sperm concentration and motility when compared to the control group. Declining patterns for most sperm parameters suggest that BPA may affect sperm quality if the treatment is extended for a longer period of supplementation. According to a study conducted by Tamilselvan et al. (2013), 200 mg/kg of BPA results in significantly lower sperm quality

compared to the normal control group after 30 days of treatment. When compared to the findings of this study, it can be concluded that prolonged treatment may produce better results in terms of induced infertility in rats. PGR supplements appear to be effective in reducing the effects of BPA on sperm quality.

The linearity (LIN) and straightness (STR) of sperm movements were used to calculate the sperm progression ratio, which is shown in Table 3. The PGR 50 group had the highest percentage of LIN at 50.07 ± 2.69 %, followed by the PGR 100 group at 42.95 ± 2.22 %, the POC group at 41.15 ± 2.52 %, and the NOC group at 39.50 ± 2.53 %. Only the PGR 50 group was found to be significantly higher than the other treatment groups. In terms of STR, the PGR 100 group had the highest percentage with 58.29 ± 6.36 %, followed by the POC group with 69.65 ± 4.43 %, the PGR 50 group with 66.51 ± 3.86 %, and the NOC group with 58.29 ± 6.36 %. When compared to the other treatment groups, the PGR 100 group performed significantly better. The POC group was significantly higher than the NOC group, but there was no significant difference in the PGR 50 group. It was significantly lower in the NOC group compared to the POC and PGR 100 groups, but there was no significant difference in the PGR 50 group. The results showed that rats supplemented with PGR, specifically PGR 50 and PGR 100, in conjunction with BPA significantly increased the VAP, VCL, and percentages of normal sperm morphology that were affected by BPA. It is clearly suggested that PGR supplementation has BPA-protective effects. Lower sperm quality caused by BPA supplementation was attributed to DNA strand breaks in germ cells of testis tissues caused by increased apoptotic activity (Eshak & Osman, 2014). The protective effects of PGR against BPA on sperm quality corresponded with a study conducted by Eshak and Osman. (2014), which revealed that chitosan, a polycationic biopolymer, provides protective effects against BPA when supplemented together with rats. In another study, Tamilselvan et al. (2013), discovered that when rats were given lycopene, it had the same effects on BPA. In both studies, sperm quality was significantly improved in rats supplemented with chitosan and lycopene in addition to BPA when compared to rats supplemented with BPA alone. Similar to the findings of this study, PGR supplementation in that specific rat improved sperm quality in a reversible manner. As a result, it was clear that PGR has protective effects against BPA, which harmed sperm quality.

In addition to protecting against BPA, the findings of this study suggested that PGR has enhancement effects on sperm quality by increasing sperm concentration, elongation, motility, progressive motility, percentages of straightness, and linearity when compared to the NOC group. The NOC group was supplemented with distilled water, which represents the rats' normal control or condition. To assess the effectiveness of PGR on sperm quality, the results were compared to groups of rats supplemented with PGR. In comparison to previous research, Suleiman et al. (2014) discovered that an aqueous extract of *Fadogia andersonii* root significantly increased sperm count and motility when compared to a control group treated with normal saline. Furthermore, Vr et al. (2013) concluded that ethanol extract of *Caryophyllaceae* improves fertility by increasing sperm count, motility, and normal sperm morphology in rats when compared to a normal control group. All of these findings were comparable to PGR, which improves sperm quality by increasing sperm concentration, motility, and normal morphology. As a result, PGR is one of the natural herbs that can be recommended as a male fertility booster.

However, concentration also plays a significant role in determining the effectiveness of the PGR extract in terms of sperm quality. In this study, two different concentrations of PGR aqueous extract were used: low (50 mg/kg) and high (100 mg/kg). Generally, higher concentrations produce better results in terms of improving sperm quality. Previous research found that higher concentrations of Nigella sativa and *Moringa oleifera* extracts produced better results on sperm parameters than lower concentrations (Marbat et al., 2013; Suleiman et al., 2014). On the other hand, Parandin & Ghorbani (2010) discovered that higher doses of *Achilea mellefolium* flowers reduced sperm quality as well as male fertility. All of these findings indicated that a higher concentration of extracts was required to provide the best effects on sperm parameters and male fertility. Similar results were obtained from this experiment, in which the highest concentration of PGR had a significant effect on some sperm parameters by increasing sperm concentration, motility, track speed (VCL), and the straightness (STR) of the sperm movement. However, there was no significant difference between these two groups in sperm elongation, progressive motility (PRG), progressive velocity (VSL), or normal sperm morphology. It clearly demonstrated that, even at low doses, PGR can provide a protective mechanism against BPA while also improving sperm quality in rats. In line with the findings of the present study, a thorough review by George & Liske, (2021) provides information on the benefits

of *Eurycoma longifolia* (tongkat ali), *Lepidium meyenii* (maca), *Withania somnifera* (ashwagandha), and *Trigonella foenum-graecum* (fenugreek) as plant extracts able to improve male general wellbeing and restore reproductive health. The majority of plant types are recognized as potent suppliers of antioxidants. These antioxidants can function as scavengers for reactive oxygen species (ROS), thereby helping to alleviate the harmful impact of oxidative stress on the functionality of sperm (Ros-Santaella & Pintus, 2021).

## CONCLUSION

The findings demonstrated that PGR has a high level of antioxidant capacity and can protect against BPA. Furthermore, PGR is effective in increasing male fertility by improving sperm quality. As a result, we can conclude that this plant has a positive effect on male fertility and has the potential to be studied further to determine other beneficial effects on human health.

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