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Effects of Bitter Melon (*Momordica charantia L.*) Fruit Extract on Spermatozoa Concentration through StAR Protein Expression in Mice (*Mus musculus*)

Efectos del extracto de melón amargo (*Momordica charantia L.*) sobre la concentración de espermatozoides a través de la expresión de la proteína StAR en ratones (*Mus musculus*)

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SUMMARY

Background: *Rapid population growth remains a major challenge in many developing countries, including Indonesia. Strengthening family planning programs requires greater male participation and the availability of acceptable male contraceptive options. Bitter melon (Momordica charantia L.), widely distributed in Indonesia, has been traditionally used in several Asian and African communities and may have antifertility potential.* **Objectives:** *To evaluate the effect of bitter melon (Momordica charantia L.) fruit extract on spermatozoa concentration and testicular StAR protein expression in male mice, and to examine whether StAR expression mediates the extract's effect on spermatozoa concentration.* **Methods:** *An experimental, randomized post-test–only control group study was conducted over 36 days. Sexually mature male mice (Mus musculus; 12–14 weeks; 30–40 g; n = 34) were obtained from the Pharmacology Unit and randomly assigned to a control group (0.1 mL distilled water) or a treatment group receiving bitter melon (Momordica charantia L.) fruit extract (800 mg/kg b.w., in 0.1 mL) by oral gavage once daily. At the end of the intervention, spermatozoa concentration was assessed using an Improved Neubauer hemocytometer, and testicular StAR protein expression was quantified by immunohistochemistry. Between-group differences were analyzed using an independent-samples t-test, and a regression-based mediation (path) analysis was performed to estimate direct and indirect effects through StAR.* **Results:** *Shapiro–Wilk test showed that spermatozoa concentration and StAR protein expression were normally distributed in both groups ($p > 0.05$). Accordingly, independent-samples t-tests demonstrated that the treatment group had a significantly lower spermatozoa concentration than the control group (4,941,176 ± 875,874 vs. 6,852,941 ± 243,746; $p = 0.0001$) and a significantly lower StAR protein expression (25.702 ± 6.261 vs. 34.623 ± 12.898; $p = 0.015$). To further evaluate the hypothesized pathway, regression-based path analysis*

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indicated that bitter melon extract exerted direct effects on StAR protein (0.463) and on spermatozoa concentration (0.380), with total effects of 0.413 and 0.612, respectively. StAR protein also showed an effect on spermatozoa concentration (direct 0.047; total 0.178), suggesting that the association between bitter melon extract and reduced spermatozoa concentration is partly conveyed through changes in StAR protein expression. **Conclusion:** *Bitter melon (Momordica charantia L.) fruit extract significantly reduced spermatozoa concentration and testicular StAR protein expression in male mice. Path analysis suggested that the reduction in spermatozoa concentration was partly mediated through changes in StAR protein, supporting a potential steroidogenic mechanism and highlighting bitter melon extract as a candidate lead for plant-based male contraceptive research.*

Keywords*: Momordica charantia L., bitter melon, male contraception, spermatozoa concentration, StAR protein, fertility*

RESUMEN

Antecedentes*: El rápido crecimiento poblacional sigue siendo un desafío importante en muchos países en desarrollo, incluida Indonesia. El fortalecimiento de los programas de planificación familiar requiere una mayor participación masculina y la disponibilidad de opciones anticonceptivas masculinas aceptables. El melón amargo (Momordica charantia L.), una planta ampliamente distribuida en Indonesia se ha utilizado tradicionalmente en varias comunidades asiáticas y africanas y podría tener efectos antifertilidad.* **Objetivos:** *Evaluar el efecto del extracto de melón amargo (Momordica charantia L.) sobre la concentración de espermatozoides y la expresión testicular de la proteína StAR en ratones macho, y examinar si la expresión de StAR media el efecto del extracto sobre dicha concentración.* **Métodos:** *Se realizó un estudio experimental, aleatorizado, con grupo control posterior a la prueba, durante 36 días. Se utilizaron ratones macho sexualmente maduros (Mus musculus; 12-14 semanas; 30-40 g; n = 34) de la Unidad de Farmacología y se asignaron aleatoriamente a un grupo control (0,1 mL de agua destilada) o a un grupo de tratamiento que recibió extracto de fruta de melón amargo (Momordica charantia L.) (800 mg/kg de peso corporal, en 0,1 mL) por sonda oral una vez al día. Al final de la intervención, se evaluó la concentración de espermatozoides mediante un hemocitómetro de Neubauer mejorado y se cuantificó la expresión de la proteína StAR testicular mediante inmunohistoquímica. Las diferencias entre grupos se analizaron mediante una prueba t de muestras independientes, y se realizó un análisis de mediación (path) basado en regresión para estimar los efectos directos e indirectos a través de StAR.* **Resultados***: La prueba de Shapiro-Wilk mostró que la concentración de espermatozoides y la expresión de la proteína StAR se distribuyeron normalmente en ambos grupos (p > 0,05). En consecuencia, las pruebas t de muestras independientes demostraron que el grupo de tratamiento presentó una concentración de espermatozoides significativamente menor que el grupo control (4.941.176 ± 875.874 frente a 6.852.941 ± 243.746; p = 0,0001) y una expresión de la proteína StAR significativamente menor (25,702 ± 6,261 frente a 34,623 ± 12,898; p = 0,015). Para evaluar más a fondo la vía hipotética, el análisis de regresión indicó que el extracto de melón amargo ejerció efectos directos sobre la proteína StAR (0,463) y la concentración de espermatozoides (0,380), con efectos totales de 0,413 y 0,612, respectivamente. La proteína StAR también mostró un efecto sobre la concentración de espermatozoides (directo: 0,047; total: 0,178), lo que sugiere que la asociación entre el extracto de melón amargo y la concentración reducida de espermatozoides se transmite en parte a través de cambios en la expresión de la proteína StAR.* **Conclusión:** *El extracto de melón amargo (Momordica charantia L.) redujo significativamente la concentración de espermatozoides y la expresión testicular de la proteína StAR en ratones macho. El análisis de la ruta sugirió que la reducción en la concentración de espermatozoides se debió en parte a cambios en la proteína StAR, lo que respalda un posible mecanismo esteroidogénico y destaca al extracto de melón amargo como candidato principal para la investigación de anticonceptivos masculinos de origen vegetal.*

Palabras clave: *Momordica charantia L., melón amargo, anticoncepción masculina, concentración de espermatozoides, proteína StAR, fertilidad.*

INTRODUCCIÓN

One of the major challenges faced by developing countries such as Indonesia is the high population growth rate, which can increase demographic and socio-economic pressures. One key factor contributing to population growth is the high birth rate. Indonesia's population was recorded at 261.8 million in 2017, and with an annual growth rate of 1.38 %, it is projected to reach 306 million by 2035 (1). Therefore, the government continues to optimize the Family Planning Programme (KB) to control population growth. Unfortunately, population growth

remains a persistent concern, indicating the need for more effective and inclusive fertility control strategies.

Women still dominate family planning participation, while male participation remains very low at only 4.4 % (2). Low male involvement in the family planning program is partly attributable to the limited availability of male contraceptive options, primarily condoms, vasectomy, and hormonal methods, and the fact that the community has not fully accepted these options. Expanding access to safe, effective, and socially acceptable male contraceptives is therefore essential to encourage men to take a more active role in family planning. However, the limited choice of male contraceptives and low community acceptance continue to be major barriers to increasing men's participation in the KB program.

Indonesia has rich biodiversity with strong potential for medicinal development, including plant-based candidates for male antifertility agents. One such plant is bitter melon (*Momordica charantia L.*) (3). Bitter melon (*Momordica charantia L.*) has traditionally been used for family planning in several Asian and African countries (4). Its natural antioxidants are mainly phenolic and polyphenolic compounds found in the fruit and seeds, and phytochemical screening of methanolic extracts has demonstrated the presence of alkaloids, tannins, saponins, glycosides, and steroids (5). Nevertheless, despite its ethnomedicinal use and bioactive constituents, the development of bitter melon as a male contraceptive candidate still requires stronger scientific evidence, particularly regarding its mechanism of action.

The characteristic bitter taste of bitter melon is attributed to cucurbitacins (*Momordicoside K and L*), which have been reported to inhibit cell growth and development. Cucurbitacins are triterpene glycosides that share a cyclopentano-perhydrophenanthrene backbone with steroids; thus, steroids may act as reversible inhibitors of spermatogenesis (6). In addition, bitter melon (*Momordica charantia L.*) extract has been shown to have anti-mitotic activity (7). However, the specific molecular targets that explain how these bioactive compounds ultimately reduce male fertility have not been clearly identified.

Several experimental animal studies have demonstrated the antifertility potential of bitter melon (*Momordica charantia L*). For example, it has been shown to reduce the percentage of normal sperm morphology in male mice (8) and to affect sperm quality, including sperm count, motility, morphology, and viability (9). Moreover, bitter melon extract has been reported to influence spermatogenic cells during spermatogenesis by decreasing the numbers of spermatogonia, spermatocytes, and spermatids (10). Unfortunately, most existing studies emphasize reproductive outcomes, while mechanistic evidence explaining how bitter melon induces fertility reduction remains limited.

Although previous studies have shown that bitter melon (*Momordica charantia L.)* extract can reduce fertility across various indicators, the existing evidence remains largely limited to changes in reproductive organs, hormone levels, and organ function. The mechanism underlying reduced fertility remains poorly understood. To better understand the effects of bitter melon (*Momordica charantia L.)* extract on the male reproductive system and to clarify its mechanism of action, both hormonal and cytotoxic, further in-depth studies are needed that examine additional underexplored biomarkers, including StAR protein expression. Therefore, this study aims to investigate the mechanism of fertility reduction in male mice administered bitter melon (*Momordica charantia L.)* extract, focusing on changes in StAR protein expression and their association with spermatozoa concentration.

MATERIALS AND METHODS

Study design

This study employed an experimental design with a randomized post-test–only control group, in which male mice were randomly allocated to either an intervention or a control group, and outcomes were assessed exclusively at the end of the intervention period.

The population comprised sexually mature adult male mice (*Mus musculus*), 12–14 weeks old (approximately 3–4 months old) and weighing 30–40 g, healthy mice without physical abnormalities, obtained from the Pharmacology Unit at the Faculty of Medicine, Udayana

University, with normal feeding and drinking behavior. Mice with >10 % body weight loss during the acclimatization period and those that died during the study were excluded.

Sample size was calculated using the Federer formula: $(t - 1)(r - 1) \geq 15$, where t is the number of treatment groups and r is the number of replications. With two groups ($t = 2$), the minimum replication per group was 16, yielding a minimum total sample size of 32. To account for potential attrition, an additional 10 % was included (one mouse per group), bringing the total to 34 mice.

The bitter melon fruit extract was prepared from fresh gajih-type bitter melon fruits, characterized by a yellowish-green color and a length of approximately 15–20 cm. The fruits were processed and extracted by maceration using 96 % ethanol as the solvent. The resulting ethanolic extract was subsequently used for the experimental intervention.

Reagents for testicular histological preparation

Reagents used for testicular histological processing and staining included 10 % formalin for tissue fixation, 96 % ethanol for dehydration, liquid paraffin (Histosac) for embedding, xylol for clearing, and balsam for mounting. Histological staining reagents included hematoxylin–eosin (H&E) and Von Gieson stain.

Study Procedure

Animal Housing and Acclimatization

Male mice (*Mus musculus*) were acclimatized in the laboratory animal facility before the experiment. A total of 34 healthy male mice aged 12–14 weeks with body weights of 30–40 g was selected. Animals were housed in individual cages and provided standard chow and water ad libitum. The animal room was maintained in clean, dry conditions with adequate ventilation, a stable temperature, and minimal noise. Mice showing signs of illness were separated and managed in accordance with laboratory procedures.

After eligibility screening, mice were randomly assigned into two groups: (1) a control group receiving standard chow and water plus 0.1 mL distilled water (Aquadest) daily, and (2) a treatment group receiving standard chow and water plus bitter melon (*Momordica charantia L.*) fruit extract at 800 mg/kg body weight, prepared in 0.1 mL aquadest daily.

The intervention was administered once daily for 36 consecutive days. Bitter melon extract was delivered orally via feeding gavage, while the control group received Aquadest at the same volume. Body weight was recorded before and after the intervention period, and the administered dose was adjusted according to the current body weight.

Sample Collection and Termination

Blood samples were collected from the lateral tail vein and placed into Eppendorf tubes for ELISA-based analysis. One day after the final administration, animals were euthanized by cervical dislocation. Testes were harvested for histopathological and immunohistochemical analyses, and spermatozoa were obtained from the epididymal duct for concentration assessment. All remaining biological materials were disposed of in accordance with institutional biosafety procedures.

Spermatozoa Concentration

Sperm suspension was prepared and initially observed on a glass slide to determine the appropriate dilution and counting area. Sperm concentration was measured using an Improved Neubauer hemocytometer under 400× magnification. Samples were diluted with a fixative solution consisting of 50 g $NaHCO_3$ and 10 mL 35 % formalin in 1 000 mL distilled water; gentian violet was optionally added to enhance visualization of sperm heads. After loading the chamber, sperm were allowed to settle for 10–15 minutes before counting. At least 200 spermatozoa were counted per assessment to minimize sampling error. Concentration was calculated from the number of sperm counted, the chamber volume, and the dilution factor, and reported as spermatozoa per unit volume.

StAR Protein Expression (Immunohistochemistry)

StAR protein expression was evaluated by immunohistochemistry on formalin-fixed, paraffin-embedded (FFPE) testicular sections based on antigen–antibody interactions (11). Sections were deparaffinized in xylol and rehydrated through 96 % ethanol, then washed with phosphate-buffered saline (PBS). Antigen retrieval was performed using citrate buffer (pH 6.0) at 90°C, followed by blocking of endogenous peroxidase activity with 3 % H_2O_2. Sections were incubated with an anti-StAR primary antibody (AA 101–200; Proteintech®), followed by biotin conjugate and streptavidin–HRP. Immunoreactivity was developed using DAB and counterstained with Mayer hematoxylin, then dehydrated and cleared before mounting. StAR expression was quantified as the number of positively stained cells per 100 cells at 400× magnification across five fields of view, and the mean value was recorded.

Data Analysis

Data were tabulated and analyzed using statistical software. Continuous variables (spermatozoa concentration and StAR protein expression) were summarized as mean ± standard deviation (SD). Before hypothesis testing, normality was assessed using the Shapiro–Wilk test. Between-group comparisons (control vs. treatment) were performed using an independent-samples t-test when normality assumptions were met; otherwise, the Mann–Whitney U test was applied. Statistical significance was set at $p < 0.05$.

To examine the hypothesized mechanistic pathway linking bitter melon (*Momordica charantia L.*) fruit extract exposure to changes in spermatozoa concentration through StAR protein expression, a regression-based path (mediation) analysis was conducted. Effect decomposition was used to estimate direct, indirect, and total effects for the key paths (extract → StAR, extract → spermatozoa concentration, and StAR → spermatozoa concentration).

Ethical Approval

The Ethics Committee approved this study under reference No: 11/UN14.2.9/PT.01.04/2020.

RESULTS

Normality Test

Normality testing was performed on the study variables after administration of bitter melon extract, such as spermatozoa concentration and StAR protein expression. All outcomes were measured on a continuous (numeric) scale. As a prerequisite for applying the independent-samples t-test, the data must be normally distributed. Normality was assessed using the Shapiro–Wilk test. The results are presented in Table 1.

Table 1. Shapiro–Wilk normality test results

Variable	Treatment group (n = 14)	Control group (n = 14)
Spermatozoa concentration	0.623	0.303
StAR protein expression	0.088	0.420

Note: Shapiro–Wilk test; data were considered normally distributed when $p > 0.05$.

All variables showed p-values > 0.05 in both groups, indicating that the data were normally distributed. Therefore, subsequent between-group comparisons were conducted using the independent-samples t-test.

Comparability Test

Statistical testing to determine differences in spermatozoa concentration and StAR protein expression between the treatment and control groups is presented in Table 2.

Table 2. Differences in Spermatozoa Concentration and StAR Expression Between the Treatment and Control Groups

Variable	Treatment (Mean ± SD)	Control (Mean ± SD)	t	p
Spermatozoa concentration	4941176 ± 875874	6852941 ± 243746	-5.913	0.0001
StAR protein expression	25.702 ± 6.261	34.623 ± 12.898	-2.568	0.015

Note: p-values are considered statistically significant at $p < 0.05$.

Table 2 shows that the mean spermatozoa concentration in the treatment group was lower than in the control group, and the independent t-test indicated a significant difference ($p = 0.0001$, $p < .0\ 05$). The mean StAR protein expression was also lower in the treatment group than in the control group, with a significant difference ($p = 0.015$, $p < 0.05$).

Multivariate Analysis

Multivariate analysis was conducted using a regression-based path (mediation) approach to evaluate the hypothesized relationships among bitter melon (*Momordica charantia L.*) fruit extract, StAR protein expression, and spermatozoa concentration. The model estimated both direct effects and indirect effects of the extract on spermatozoa concentration through StAR expression. The direct, indirect, and total effect estimates are presented in Figure 1.

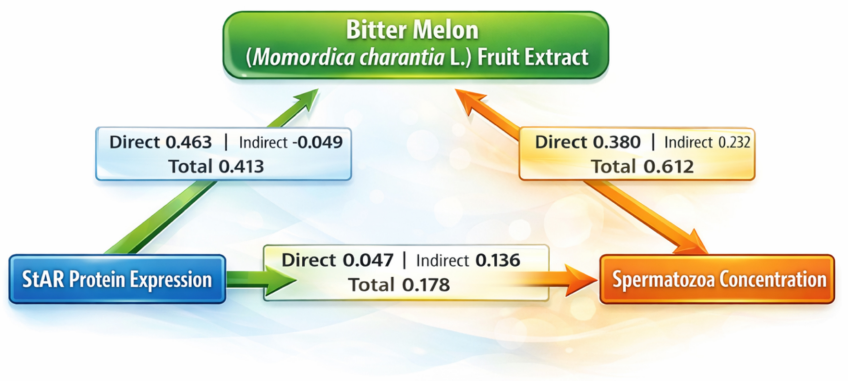

Figure 1. Effects of Bitter Melon (*Momordica charantia L.*) Fruit Extract on Spermatozoa consentration and StAR Expression.

Figure 1 shows that bitter melon extract presents a direct effect of 0.463 and a total effect of 0.413 on StAR protein expression. The extract also showed a direct effect of 0.380 and a total effect of 0.612 on spermatozoa concentration. StAR protein expression had a direct effect of 0.047 and a total effect of 0.178 on spermatozoa concentration, indicating that the total effect exceeded the direct effect and suggesting the presence of mediated pathways within the specified model. Overall, these findings indicate that bitter melon extract is associated with reduced spermatozoa concentration and that changes in StAR protein expression partly mediate this effect.

DISCUSSION

The qualitative analysis of bitter melon (*Momordica charantia L.*) extract revealed the presence of saponins, flavonoids, alkaloids, and triterpenes. Phytochemical screening of extracts was performed to determine the levels of active compounds and to ensure that the extraction process did not degrade them into bitter melon fruit. The results of this phytochemical screening are similar to previous research (12) on the functional components and biological activities of bitter melon fruit, which found that it contains active substances such as flavonoids, triterpenes, saponins, ascorbic acid, and steroids. Similar phytochemical results were also reported by Bakare et al. (13), who found that the nutritional components and secondary metabolites of bitter melon fruit have potential medicinal benefits.

The pharmacological effects of bitter melon extract are closely related to the substances it contains, especially secondary metabolites, which include compounds from the alkaloid, terpenoid, steroid, and flavonoid groups (14). The results of phytochemical screening of petroleum extracts of bitter melon fruit (*Momordica charantia L.*) showed the presence of steroids, alkaloids, oil, and oil (15). At the same time, the ethanol extract showed positive tests for alkaloids, flavonoids, glycosides, phenols, tannins, oils, and fats (16). In the Gas Chromatography Mass Spectrometry results, ethanol extracts were also identified as containing 2,4-Pentanediol (23.61 %), Eucalyptol (1,8-Cineole) (20.41 %), Trans (Beta)-Caryopyllene (10.07 %), 3-Decyn-2-ol (9.54 %), Epiglobulol (7.82 %), Methyleugenol (7.15%), Beta-Selinene (5.80 %), Alpha-Selinene (5.43 %), and Alpha-Humulene (5.29 %), showing the presence of compounds which have medicinal properties (17).

The present results showed a significant difference in spermatozoa cell concentration between the treatment and control groups ($p<0.001$). The mean concentration of spermatozoa in the treatment group given bitter melon extract was lower than that of the control group given distilled water. This difference in spermatozoa concentration occurred because bitter melon extracts reduced StAR protein expression. Previous studies have shown that *Momordica charantia L.* can reduce sperm concentration across multiple animal models, including guinea pigs (7), mice, and rats, suggesting a consistent biological effect. In a study in which the effect of the bitter melon extract (*Momordica charantia L.*) on the ejaculated sperm quality of mice (*Mus musculus L.)* was determined, at doses of 0.2, 0.4, and 0.6 g, it was observed a decreased the average sperm count when compared with the control, indicating that the extract of *Momordica charantia L.* can decrease the quality of the ejaculated sperm of mice (*Mus musculus L.)* (18). Meanwhile, in Wistar rats, at doses of 400 and 800 mg/kg body weight, there was also a decrease in spermatozoa concentration (19). Also, at a dose of 50 mg/kg, there was a 20-30 % reduction in spermatozoa in seminiferous tubules (20). At doses of 10, 25, and 50 mg/kg, the number of spermatozoa decreased significantly with increasing dose (21). As noted by Neto et al. (22), spermatogenesis is influenced by both endogenous factors—such as hormonal balance, genetic background, age, and oxidative stress—and exogenous factors, including chemicals, temperature, radiation, nutrition, trauma, and environmental pollutants. Bitter melon extract, classified as an exogenous nutritional factor, may exert its effects by modulating endogenous pathways, particularly hormonal regulation and oxidative stress levels.

Sperm concentration is one of the key parameters used to assess spermatozoa's functional capacity. This parameter largely depends on the process of spermatogenesis that occurs in the seminiferous tubules. Any disruption to spermatogenesis will inevitably lead to a reduction in sperm concentration. The present results demonstrated that StAR protein expression in the treatment group was lower than in the control group. The decrease in protein expression was due to the bitter melon extract, which had an effect of 0.413. The StAR protein plays a central role in steroidogenesis by transporting cholesterol into the mitochondria of Leydig cells, representing one of the key rate-limiting steps in androgen synthesis (23). Its expression and activity are tightly regulated by the gonadotropic hormones FSH and LH through the cAMP/protein kinase A signaling pathway. Disruption of this regulatory axis can impair testosterone production, ultimately

aff ecting spermatogenesis (24). In this study, the observed reduction in sperm concentration may be linked to alterations in these endocrine mechanisms. The convergence of these findings supports the hypothesis that the extract interferes with spermatogenesis, potentially by affecting Leydig cell function, altering StAR expression, or increasing free radical production within the testes. Any of these mechanisms could compromise testosterone synthesis or damage the seminiferous epithelium, ultimately reducing sperm output. Taken together, the evidence indicates that bitter melon extract may impair male reproductive function through endocrine disruption and oxidative mechanisms that interfere with the spermatogenic process. This aligns with the dose-dependent decreases in spermatozoa reported in previous studies and reinforces the importance of evaluating phytochemical exposures that may influence reproductive health. The results of this study support those of Patil and Patil (16), who reported an increase in cholesterol in mice treated with bitter melon extract (*Momordica charantia L.),* indicating a decrease in cholesterol conversion to steroid hormones. This condition is thought to be due to reduced cholesterol transport into mitochondria, which could be related to the lack of StAR protein in Leydig cells. Bitter melon extract contains various active compounds, including alkaloids, saponins, terpenoids, polypeptides, flavonoids, glycosides, and sterols. These active substances can affect StAR protein expression in Leydig cells. Another study conducted by Maneenin et al. (25) assessed the *Momordica charantia L* preventive effects on tissue injuries, antioxidant capacity and protective effect of MC pulp and peel (MCP) assessed the *Momordica charantia L* preventive effects on tissue injuries, antioxidant capacity and protective effect on valproic acid (VPA)-testicular damage. In effect, the antioxidant effect of bitter melon (*Momordica charantia L.*) protected testicular damage and could prevent the decrease of StAR and a 50-kDa phosphorylated protein expression in VPA-treated testis, indicating that the presence of antioxidant activities can prevent male reproductive toxicity in VPA-induced rats. In addition, it can increase antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase. In conclusion, *M. charantia L* has antioxidant activities and can prevent male reproductive toxicity in VPA-induced rats.

Implications

These findings suggest that bitter melon (*Momordica charantia L.)* fruit extract has potential as a candidate male antifertility agent, as it was associated with reduced spermatozoa concentration and decreased StAR protein expression, a key regulator of steroidogenesis. Mechanistically, the results support the possibility that disruption of the steroidogenic pathway involving StAR may contribute to fertility reduction, providing a rationale for further mechanistic and translational studies.

From a research perspective, the study highlights StAR protein expression as a relevant biomarker to include in future evaluations of plant-derived antifertility compounds. Future work should expand to dose–response and time-course studies, quantify active constituents, and incorporate upstream (LH/FSH, cAMP), downstream (steroidogenic enzymes, intratesticular testosterone), and structural (Leydig/Sertoli cell changes) markers to strengthen causal inference.

From a public health perspective, identifying locally available botanical resources with antifertility potential may help broaden the evidence base for male contraception development, particularly in settings where male contraceptive options remain limited.

Limitations

This study has several limitations. First, the experiment used a post-test–only design, so baseline (pre-intervention) values of spermatozoa concentration and StAR expression were not available for within-subject comparison. Second, the sample size was relatively small and derived from a single animal facility, potentially limiting generalizability. Third, only one dose of bitter melon extract (800 mg/kg, BW) and a single exposure duration (36 days) were evaluated; thus, dose–response and time-dependent effects could not be determined. Fourth, the study focused

on StAR protein expression as a mechanistic marker. At the same time, other key steroidogenic regulators and upstream signals (e.g., LH/FSH, cAMP, Leydig cell number, and additional steroidogenic enzymes) were not assessed, limiting mechanistic inference. Fifth, although an indirect effect was estimated in the regression-based path model, not all potential mediators were measured or formally tested, and the pathway estimates should be interpreted within the constraints of the specified model. Finally, the phytochemical screening was qualitative, so the specific concentrations of active constituents responsible for the observed effects could not be established.

CONCLUSIONS

Administration of bitter melon (*Momordica charantia L.*) fruit extract significantly reduced spermatozoa concentration and decreased testicular StAR protein expression in male mice. Regression-based path analysis indicated that the extract exerted both direct effects on spermatozoa concentration and indirect effects through StAR expression, suggesting that disruption of StAR-related steroidogenic pathways may contribute to the observed reduction in fertility. These findings may serve as preliminary evidence supporting bitter melon fruit extract as a potential lead for the development of plant-based male contraceptive candidates and as a reference for future mechanistic studies focusing on steroidogenic biomarkers, particularly StAR.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Declaration of generative AI in scientific writing

No generative AI software was used in the writing of this manuscript. Where applicable, AI tools were used only for language editing and have been appropriately acknowledged.

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