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RESEARCH ARTICLE •

Relaxant Effect of Purwoceng (*Pimpinella pruatjan* Molk.) Extract on Isolated Wistar Rat Corpus Cavernosum

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Abstract

Purwoceng (*Pimpinella pruatjan Molk.)* is an Indonesian medicinal plant traditionally used to support male vitality, but its direct effect on corpus cavernosum tone remains unclear. This exploratory study evaluated the relaxant response of ethanolic Purwoceng root extract in isolated Wistar rat corpus cavernosum and compared it with Krebs solution and sildenafil. Eleven biological preparations from 11 male Wistar rats were allocated sequentially to Krebs control (n = 4), sildenafil (n = 4), and Purwoceng extract (n = 3). Isolated tissues were mounted in an organ bath for isometric tension recording, precontracted with phenylephrine, and exposed to nominal concentrations of 0.01, 0.03, 0.10, and 0.30 mg/mL, corresponding to log10 concentrations of −2.0, −1.5, −1.0, and −0.5. Between-group comparisons were performed separately at each concentration using one-way ANOVA followed by Tukey post hoc testing. Purwoceng extract produced relaxation values of −20.11 ± 4.60%, −22.95 ± 3.81%, −25.32 ± 3.67%, and −30.32 ± 2.31%. The response was greater than the Krebs control at all tested concentrations ($p = 0.005$, 0.003, 0.002, and < 0.001, respectively) but lower than sildenafil (all $p < 0.001$). Control relaxation also increased over time, so the findings do not establish a concentration–response relationship. Ethanolic Purwoceng root extract showed direct relaxant activity in isolated Wistar rat corpus cavernosum under the tested conditions. Because the sample was small, allocation was nonrandom, and the extract was not chemically standardized, the findings should be interpreted as preliminary ex vivo evidence requiring confirmation.

KEYWORDS Purwoceng; pimpinella pruatjan; corpus cavernosum; relaxation; organ bath

HIGHLIGHTS

- Ethanolic Purwoceng root extract produced greater corpus cavernosum relaxation than the Krebs control at all four tested concentrations.
- The relaxant response remained lower than sildenafil, supporting preliminary peripheral activity rather than clinical equivalence.
- Small nonrandom groups and time-related change in the control require cautious interpretation and confirmation in larger, standardized studies.

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INTRODUCTION

Erectile dysfunction is the persistent inability to achieve or maintain an erection sufficient for sexual activity. It is a multifactorial condition associated with vascular, metabolic, neurological, hormonal, and psychological factors, as well as the use of certain medications. Diabetes mellitus, hypertension, dyslipidemia, obesity, smoking, and cardiovascular disease commonly coexist with erectile dysfunction. Because its causes vary, assessment and management should consider the patient's overall clinical condition (Burnett et al., 2018; European Association of Urology [EAU], 2026; Köhler et al., 2024; Mazzilli, 2022; McMahon, 2019).

Penile erection is a neurovascular process that depends on relaxation of the smooth muscle of the corpus cavernosum. Sexual stimulation triggers the release of nitric oxide (NO) from nonadrenergic, noncholinergic nerves and the endothelium. NO activates soluble guanylyl cyclase, increases cyclic guanosine monophosphate (cGMP), reduces intracellular calcium, and ultimately causes cavernosal smooth muscle relaxation. This relaxation increases blood flow and fills the penile sinusoidal spaces (Argiolas et al., 2023; Burnett, 2024; Hashimoto et al., 2023; MacDonald & Burnett, 2021; Sangiorgi et al., 2021).

Phosphodiesterase type 5 (PDE5) inhibitors, including sildenafil, are a mainstay of pharmacological treatment for many patients with erectile dysfunction. Sildenafil inhibits cGMP degradation, enhancing the relaxation response mediated by the NO–cGMP pathway. Clinical guidelines and systematic reviews support the effectiveness of sildenafil and other PDE5 inhibitors, although patient responses vary. Side effects, treatment expectations, access, cost, and continued use may affect long-term adherence (Burnett et al., 2018; EAU, 2026; McMahon, 2019; Nik-Ahd & Shindel, 2022; Pyrgidis et al., 2021; Williams et al., 2021).

These considerations have encouraged research into natural products as potential sources of compounds that may influence erectile function. One plant that has received attention in Indonesia is Purwoceng (*Pimpinella pruatjan Molk.*). Purwoceng roots have traditionally been used as a tonic and aphrodisiac. Animal experiments and reviews of Indonesian medicinal plants provide a basis for further investigation, but the available evidence is insufficient to establish Purwoceng as an effective treatment for erectile dysfunction in humans (Kanedi et al., 2017; Prasetya et al., 2024).

Chemical studies of Purwoceng indicate that extract composition may vary according to the plant part and growing conditions. Studies of the roots have reported phenolic compounds, flavonoids, betaine, bergapten, and other constituents associated with antioxidant activity. Analyses of root essential oil have also identified various volatile compounds, while LC–MS/MS analysis of the aerial parts has identified several flavonoids and phenolic derivatives. Findings from aerial parts cannot be assumed to represent the root extract. Taken together, these data show that Purwoceng contains a complex mixture of compounds and requires standardization before further development (Batubara et al., 2023a, 2023b; Nurcahyanti et al., 2018; Timotius et al., 2023).

Evidence of the direct effect of Purwoceng root extract on corpus cavernosum tone remains limited. An isolated corpus cavernosum preparation in an organ bath allows the peripheral tissue response to be examined directly, without the effects of gastrointestinal absorption, systemic metabolism, or acute regulation by the central nervous system. Similar approaches have been used to assess the relaxant effects of natural extracts and compounds on cavernosal tissue (Alan et al., 2022; Hashimoto et al., 2023; Karna et al., 2022).

To address this gap, the present study aimed to assess the relaxation response to ethanolic Purwoceng root extract in isolated Wistar rat corpus cavernosum and compare it with Krebs solution as the control and sildenafil as a pharmacological comparator. The study was designed as an initial exploratory investigation to obtain functional evidence, not to establish clinical efficacy, molecular mechanisms, or the EC50 or Emax of Purwoceng.

METHODS

Design and setting

This exploratory ex vivo laboratory experiment used isolated corpus cavernosum preparations from Wistar rats mounted in an organ bath with isometric tension recording. The study was conducted from March to July 2026 at the Pharmacology Laboratory, Faculty of Medicine, Mulawarman University. The response to ethanolic Purwoceng root extract was compared with Krebs solution as the control and sildenafil as a pharmacological comparator.

The experimental animals were healthy male Wistar rats (Rattus norvegicus), aged 8–12 weeks and weighing 200–250 g. They were acclimatized for seven days, housed in groups, provided unrestricted access to food and water, monitored for health, and handled as little as possible. Eleven animals were used, with one animal providing one biological replicate. Animals were assigned nonrandomly in the order of experimentation: the first four to the Krebs control group, the next four to the sildenafil group, and the final three to the Purwoceng extract group. This sequential allocation is reported as a limitation because it may introduce order-related bias.

The number of animals was limited in accordance with the Reduction principle, while applying several concentrations to the same preparation reduced the need for additional animals. Replacement was not used because the contraction–relaxation response examined in this study required corpus cavernosum tissue that retained local interactions among smooth muscle, endothelium, intracavernosal nerves, and the extracellular matrix. Refinement measures included acclimatization, group housing, health monitoring, minimal handling, anesthesia before euthanasia, and prompt tissue collection after death was confirmed. Reporting of the 3Rs principles and animal study design followed the transparency principles of ARRIVE 2.0 (Percie du Sert et al., 2020).

Krebs solution and organ bath preparation

One liter of Krebs solution was prepared using 6.9 g NaCl, 0.35 g KCl, 0.29 g MgSO4, 0.16 g KH2PO4, 2.1 g NaHCO3, 2 g glucose, and 0.28 g CaCl2. Corpus cavernosum preparations were placed in an organ bath at 37°C, aerated with a mixture of 95% O2 and 5% CO2, subjected to an initial tension of 0.5 g, and equilibrated for 20–30 minutes. The administration records indicated an initial fluid volume of approximately 15 mL in the organ bath before cumulative addition of the test substances.

The functional viability of each preparation was assessed through phenylephrine-induced contraction and methacholine-induced relaxation. Phenylephrine and methacholine were each prepared as 10^{-2} M stock solutions. Phenylephrine (50 µL) and

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methacholine (100 µL) were added. According to the experimental records, their final concentrations were approximately 3.32×10^{-5} M and 6.60×10^{-5} M, respectively. Following the viability assessment, the tissue was washed with Krebs solution and re-equilibrated before testing.

Relaxation testing procedure

The tissue was precontracted with 50 µL of 10^{-2} M phenylephrine stock solution until a plateau was reached. Once stable, the test substances were administered cumulatively. The four concentrations analyzed in this article were nominal concentrations of 0.01, 0.03, 0.10, and 0.30 mg/mL, reported as log10 concentrations (mg/mL) of −2.0, −1.5, −1.0, and −0.5. Changes in tension were recorded in real time using an ADInstruments isometric transducer system and Chart 5 software.

Krebs solution served as the control. Responses were reported as percentage changes in tone relative to the condition after phenylephrine precontraction. Negative values indicate a decrease in tone, or relaxation, so a greater absolute negative value represents greater relaxation.

Analysis and evaluation

Data are presented as mean ± standard error (SE). Normality at each concentration was assessed using the Shapiro–Wilk test, and variance homogeneity was examined using the Brown–Forsythe test. Because group sizes were very small (n = 3–4), the assumption tests were treated as descriptive checks rather than strong evidence that the data met the assumptions of parametric analysis. The Krebs control, sildenafil, and Purwoceng groups were compared exploratorily using a separate one-way analysis of variance (ANOVA) at each concentration, followed by Tukey's post hoc test. This analysis compared three independent groups at each concentration; it did not model the repeated measurements across concentrations within the same preparation. Accordingly, the results were not used to test an overall concentration–response curve or calculate EC50 or Emax. A p-value below 0.05 was considered statistically significant.

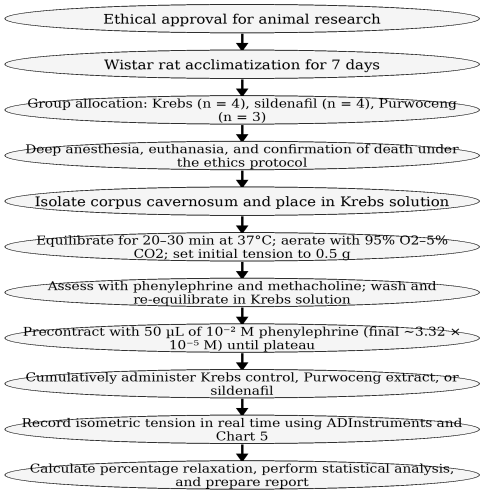


Figure 1. Study flowchart

Ethical approval and anesthesia procedure

Research involving experimental animals requires approval from an ethics committee. Anesthesia was administered using Ketamil (ketamine hydrochloride, 100 mg/mL) at a dose of 1 mg/kg body weight via the intracardiac route. Euthanasia was then performed, and tissue was collected immediately after death was confirmed.

The plant material was identified under specimen number 107/UN17.4.08/TA.00.01/2026. Extraction of 200 g of dried root material yielded 4.68 g of extract, corresponding to a yield of 2.34%. The solid extract was covered, labeled, stored at 4°C, and used the following day.

RESULTS

TABLE 1. Relaxation response of isolated Wistar rat corpus cavernosum in the control, sildenafil, and Purwoceng extract groups.

log10 nominal conc. (mg/mL)	Krebs control (mean ± SE)	Sildenafil (mean ± SE)	Purwoceng extract (mean ± SE)	p (ANOVA)
-2.0	-4.49 ± 1.05	-59.97 ± 1.23	-20.11 ± 4.60	< 0.001
-1.5	-8.60 ± 0.32	-74.05 ± 1.52	-22.95 ± 3.81	< 0.001
-1.0	-9.90 ± 0.61	-82.26 ± 1.48	-25.32 ± 3.67	< 0.001
-0.5	-14.18 ± 0.45	-94.89 ± 1.52	-30.32 ± 2.31	< 0.001

Table 1 presents mean ± SE values from 11 biological preparations: Krebs control (n = 4), sildenafil (n = 4), and Purwoceng extract (n = 3). Negative values indicate a decrease in tone, or relaxation. A greater absolute negative value indicates greater tissue relaxation. The values −2.0, −1.5, −1.0, and −0.5 are the log10 values of the nominal concentrations of 0.01, 0.03, 0.10, and 0.30 mg/mL.

The Shapiro–Wilk test yielded p-values of 0.508, 0.173, 0.506, and 0.846 at the four respective concentrations. The corresponding Brown–Forsythe p-values were 0.052, 0.145, 0.188, and 0.057. These tests did not detect violations of their assumptions. However, because each group included only three or four preparations, the tests had low statistical power, and their results were interpreted with caution.

A separate one-way ANOVA at each concentration showed between-group differences at all tested points (all $p < 0.001$). These point-by-point analyses compared groups at the same concentration and were not intended to test a concentration–response relationship across concentrations.

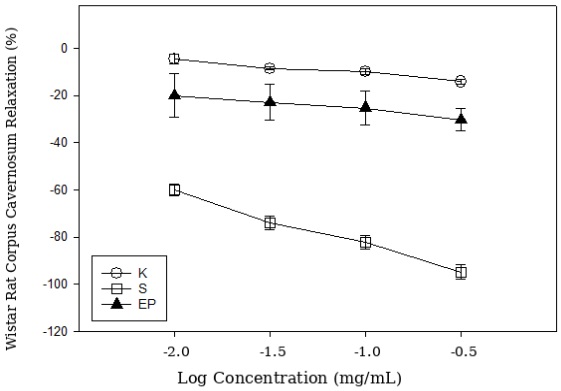


Figure 2. Percentage relaxation curve of Wistar rat corpus cavernosum after phenylephrine precontraction in the Krebs control, sildenafil, and Purwoceng extract groups.

Note: K = Krebs control; S = sildenafil; EP = Purwoceng extract. K and S each had n = 4, while EP had n = 3. Data are presented as mean ± SE. Negative values indicate relaxation. One-way ANOVA showed overall differences among groups at each concentration ($p < 0.001$). An asterisk (*) indicates that EP differed significantly

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from both control and sildenafil at the same concentration according to Tukey's test ($p < 0.05$).

At the log10 concentration of −2.0, mean relaxation in the control, sildenafil, and Purwoceng groups was −4.49 ± 1.05%, −59.97 ± 1.23%, and −20.11 ± 4.60%, respectively. Tukey's test showed differences between control and sildenafil ($p < 0.001$) and between control and Purwoceng ($p = 0.005$). Purwoceng also differed from sildenafil ($p < 0.001$).

At the log10 concentration of −1.5, the corresponding mean values were −8.60 ± 0.32%, −74.05 ± 1.52%, and −22.95 ± 3.81%. Control differed from sildenafil ($p < 0.001$) and Purwoceng ($p = 0.003$), while Purwoceng differed from sildenafil ($p < 0.001$).

At the log10 concentration of −1.0, the corresponding mean values were −9.90 ± 0.61%, −82.26 ± 1.48%, and −25.32 ± 3.67%. Control differed from sildenafil ($p < 0.001$) and Purwoceng ($p = 0.002$), while Purwoceng differed from sildenafil ($p < 0.001$).

At the log10 concentration of −0.5, the corresponding mean values were −14.18 ± 0.45%, −94.89 ± 1.52%, and −30.32 ± 2.31%. Control differed from both sildenafil and Purwoceng (both $p < 0.001$), and Purwoceng also differed from sildenafil ($p < 0.001$).

Descriptively, the magnitude of relaxation in the Purwoceng group increased across the tested concentrations. However, the Krebs control group also showed an increase in relaxation magnitude over the observation period. Therefore, this pattern was not taken as evidence of a concentration–response relationship.

DISCUSSION

The main finding of this study is that ethanolic Purwoceng root extract produced greater corpus cavernosum relaxation than the Krebs control at all four tested concentrations, although its response remained lower than that of sildenafil. This indicates direct relaxant activity in isolated tissue under the conditions of the experiment. It does not, however, establish the clinical efficacy of Purwoceng for erectile dysfunction.

The magnitude of the response should be considered in the context of the study design. The control and sildenafil groups each included four preparations, whereas the Purwoceng group included only three. Small sample sizes reduce the precision of estimates and limit the power of tests for normality and variance homogeneity. Thus, statistically significant p-values indicate differences in the observed data but do not remove uncertainty arising from the small samples, nonrandom allocation, and biological variability. The ARRIVE guidelines emphasize transparent reporting of sample size, allocation, experimental units, and analysis so that readers can judge the strength of the evidence appropriately (Percie du Sert et al., 2020).

Changes in the control group are also important. The magnitude of control relaxation rose from 4.49% at the first time point to 14.18% at the last. This change may reflect time-related drift, a gradual decline in phenylephrine-induced contraction, mechanical changes after fluid addition, tissue conditions during observation, or vehicle effects if the test-substance vehicle differed from Krebs solution. The difference in mean relaxation magnitude between Purwoceng and control remained within a relatively narrow range of approximately 14.35–16.14 percentage points. Therefore, an increase in the absolute relaxation of Purwoceng across time points is not sufficient to demonstrate a concentration–response pattern without an analysis that accounts for control changes and the repeated-measures structure.

Sildenafil produced the greatest response at all tested points. This is consistent with its action as a PDE5 inhibitor that preserves cGMP in cavernosal smooth muscle. The NO–cGMP pathway is a major mechanism of erectile tissue relaxation and remains an important physiological basis for current research on erection and erectile dysfunction (Argiolas et al., 2023; Burnett, 2024; MacDonald & Burnett, 2021; Sangiorgi et al., 2021). The effectiveness of PDE5 inhibitors, including sildenafil, is supported by clinical guidelines and recent systematic reviews (Burnett et al., 2018; EAU, 2026; Nik-Ahd & Shindel, 2022; Pyrgidis et al., 2021). In this study, sildenafil primarily served as a comparator demonstrating that the preparations and organ bath system could detect a strong relaxation response.

A key feature of this study is that Purwoceng extract was applied directly to corpus cavernosum tissue. In an isolated-organ system, the acute response occurs without gastrointestinal absorption, first-pass metabolism, systemic distribution, or acute influence from the central nervous system. Therefore, the difference from control supports the possibility that one or more extract components act directly on peripheral tissue. However, this design cannot determine whether the effect involves the endothelium, smooth muscle, ion channels, the NO–cGMP pathway, or other targets.

Chemical data on Purwoceng provide a biological basis for further study but should be interpreted cautiously. Studies of the roots have identified phenolic compounds, flavonoids, betaine, bergapten, and variation in constituent levels associated with growing conditions (Batubara et al., 2023a, 2023b). Root essential oils also have a varied chemical composition (Nurcahyanti et al., 2018), while hydroponic studies indicate that cultivation conditions may affect the antioxidant capacity of Purwoceng extracts (Batubara et al., 2023a, 2023b). LC–MS/MS analysis of aerial parts has identified several flavonoids and phenolic derivatives (Timotius et al., 2023). Because roots were used in the present study, findings from aerial parts can only provide supporting information and should not be treated as the chemical profile of the tested extract.

Studies of other natural products suggest that corpus cavernosum tissue can relax through several pathways. Root extracts of Prangos heyniae and P. uechtritzii affected relaxation in mouse corpus cavernosum through mechanisms involving NO, hydrogen sulfide, and calcium flux, with differences between the extracts (Alan et al., 2022). Rubus occidentalis and ellagic acid also affected rabbit corpus cavernosum contractility through NO–cGMP and cAMP signaling (Karna et al., 2022). More recent reviews of natural products and bioactive compounds for erectile dysfunction identify NO–cGMP and PDE5 as frequent targets in mechanisms of erectile tissue relaxation (Kulshrestha et al., 2024; Niu et al., 2022). These findings cannot be directly applied to Purwoceng, but they support the possibility that plant extracts may regulate tissue tone through more than one mechanism.

The NO–cGMP pathway and PDE5 are two mechanistic hypotheses worth investigating, not conclusions of the present study. Recent reviews indicate that certain nutraceuticals and flavonoids, including icariin and its derivatives, can influence PDE5 and NO–cGMP signaling in experimental models (Kulshrestha et al., 2024; Meng & Jiang, 2026; Niu et al., 2022). Potassium channels, calcium channels, and other ion channels

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also contribute to the regulation of cavernosal tone (Diniz et al., 2020). However, this study did not measure NO, cGMP, PDE5 activity, ion channels, eNOS, nNOS, or soluble guanylyl cyclase. These mechanisms therefore remain topics for future experiments.

Evidence from flavonoids in other models also suggests possible effects on cavernosal tissue, but it does not establish that flavonoids are the principal active components of Purwoceng. Reviews of icariin and icariside II have reported improvements in several measures of erectile function in preclinical models through various pathways, including NOS/NO/cGMP and PDE5 (Meng & Jiang, 2026; Niu et al., 2022). Quercetin has also been reported to increase NO levels and modulate enzymes related to erectile function in the corpus cavernosum of cyclosporine-treated rats (Olabiyi et al., 2022). Purwoceng extract is a complex mixture; without fractionation and chemical analysis of the tested batch, the contributions of individual components cannot be determined.

The traditional use of Purwoceng as an aphrodisiac should also be distinguished from evidence of tissue-level activity. A study in mice found that Purwoceng root extract could influence certain behaviors but did not consistently increase libido (Kanedi et al., 2017). A review of Indonesian medicinal plants identified the need for stronger preclinical evidence, standardization, safety assessment, and clinical research on Purwoceng (Prasetya et al., 2024). Thus, the relaxation response observed here adds a layer of preclinical evidence but does not support recommending Purwoceng for patients.

Another limitation is the sequential rather than random allocation of animals. The first four animals were assigned to control, the next four to sildenafil, and the final three to Purwoceng. If preparation conditions, instruments, laboratory temperature, or other technical factors changed over time, this sequence could introduce bias. Applying the same organ bath procedures to all groups helps reduce technical variation but cannot replace randomization. Future studies should use random allocation and, where possible, blinded outcome assessment.

Applying several concentrations to the same preparation also means that measurements across concentrations were not independent. Separate ANOVA tests at each concentration can serve as exploratory between-group comparisons at that concentration, but they do not account for correlations within the same preparation. Accordingly, this study does not establish a concentration–response curve or calculate EC50 or Emax. Future studies may use more preparations and appropriate mixed-effects or repeated-measures models.

Extract standardization is important before responses can be compared across studies. The extraction yield of 2.34% indicates only how much extract was obtained, not the concentration of active compounds. Ethanol concentration, plant-material-to-solvent ratio, extraction procedure, vehicle, and batch-specific chemical profile should be fully reported. Environmental and cultivation conditions may influence Purwoceng composition, so marker-compound analysis or chromatographic fingerprinting would improve reproducibility (Batubara et al., 2023a, 2023b).

Overall, the data support direct relaxant activity of ethanolic Purwoceng root extract in isolated corpus cavernosum, with a response between those of Krebs control and sildenafil. Further work should include a larger sample, randomization, an appropriate vehicle control, extract standardization and fractionation, testing of preparations with and without endothelium, and use of inhibitors targeting the NO–cGMP pathway, ion channels, or other mechanisms. Measurements of NO, cGMP, and other molecular markers could help identify the mechanisms involved. In vivo studies and safety assessments are needed before clinical relevance can be evaluated.

LIMITATIONS

This study has several limitations, including a relatively small and unequal sample size across groups and nonrandom allocation of experimental animals, which may introduce bias. Furthermore, the statistical analysis did not account for repeated measurements within the same tissue preparations, making it difficult to establish a definitive concentration–response relationship. The Purwoceng extract was not standardized based on its active compound content, and the molecular mechanisms underlying its relaxant effects were not investigated. As this study was conducted ex vivo using isolated corpus cavernosum tissues from Wistar rats, the findings cannot be directly generalized to humans. Therefore, further studies employing more rigorous experimental designs, extract standardization, and mechanistic evaluations are needed to confirm these preliminary findings.

CONCLUSION

Ethanolic Purwoceng (*Pimpinella pruatjan Molk.)* root extract produced greater relaxation in isolated Wistar rat corpus cavernosum than Krebs control, but less relaxation than sildenafil, at all tested concentrations. This finding supports direct peripheral relaxant activity under ex vivo conditions. However, the results should be viewed as preliminary exploratory evidence because the numbers of preparations were small and unequal, group allocation was nonrandom, the control response changed over the observation period, and the extract was not chemically standardized. Further studies with randomization, larger samples, a confirmed vehicle control, extract standardization, appropriate repeated-measures analysis, and mechanistic testing are needed before the therapeutic potential of Purwoceng can be assessed further.

DECLARATIONS

Author contributions. All authors contributed to the conception, design, conduct, and preparation of this study. All authors reviewed and approved the final manuscript.

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Ethics, participation, and publication. The study involved experimental Wistar rats and was reported as ethically acceptable. Details of the approving ethics committee, approval number, and approval date require confirmation. Consent to Participate and Publication is not applicable, as this study did not involve human participants or identifiable personal information.

Data availability and conflicts of interest. The data supporting the findings of this study are available from the corresponding author upon reasonable request. The authors declare no conflicts of interest related to this study.

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