ARTÍCULO ORIGINAL

Gac Méd Caracas 2022;130(Supl 1):S143-S148
DOI: 10.47307/GMC.2022.130.s1.26

Phytochemical Screening and Evaluation of Red Ginger Extracts on Aphrodisiac Activity

Detección y evaluación fitoquímica de extractos de jengibre rojo sobre la actividad afrodisíaca

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SUMMARY

Introduction: *One of the breeding processes is influenced by reproduction factors. Herbal therapy can relieve male infertility, such as red ginger. This study aimed to determine the effect of red ginger extract on aphrodisiac activity.*
Methods: *This study was designed to evaluate the effect of red ginger extract on aphrodisiac activity in Wistar rats. Twenty-five male Wistar rats and fifteen two-month-old females weighing 150-200 g were purchased and placed in cages at the Faculty of Health Sciences, University of Muhammadiyah Surabaya. Wistar rats were given light and dark cycles for 12 hours, fed pellet starter broiler, and drank water ad libitum. Mice were acclimatized for 14 days and randomly divided into five groups A, B, C, D, and E. Groups B, C, and D were the treatment groups, while groups A and E were negative and positive controls, each with five rats. in each group and each kept singly in a separate cage. Groups A and E received 1 mL of distilled water and 5 mg of sildenafil citrate orally for the positive control group. Groups B, C, and D received red ginger extract suspension orally at doses of 100, 200, and 300 mg/kg, respectively, between 9:00 - and 10:00 daily for 21 days. Female mice were paired with male mice in a 1:1 ratio, and mating behaviour was recorded.*
Results: *Male rats in groups C and E each showed a significant increase ($p < 0.05$) in mounting frequency (MF), intromission frequency (IF), and ejaculatory frequency (EF).*
Conclusion: *Red ginger extract has activity as an aphrodisiac, but it is necessary to conduct tests related to toxicity tests.*

Keywords: *Aphrodisiac, mating, rat, red ginger, sex stimulant.*

DOI: https://doi.org/10.47307/GMC.2022.130.s1.26

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Recibido: 1 de mayo 2022
Aceptado: 6 de mayo 2022

RESUMEN

Introducción: *Uno de los procesos de crianza está influenciado por factores de reproducción. La terapia a base de hierbas puede aliviar la infertilidad masculina, como el jengibre rojo. Este estudio tuvo como objetivo determinar el efecto del extracto de jengibre rojo sobre la actividad afrodisíaca.*
Métodos: *Este estudio fue diseñado para evaluar el efecto del extracto de jengibre rojo sobre la actividad afrodisíaca en ratas Wistar. Se compraron veinticinco ratas Wistar macho y quince hembras de dos meses de edad que pesaban entre 150 y 200 g y se colocaron*

en jaulas en la Facultad de Ciencias de la Salud de la Universidad de Muhammadiyah Surabaya. A las ratas Wistar se les dieron ciclos de luz y oscuridad durante 12 horas, se alimentaron con pollos de engorde iniciados con gránulos y bebieron agua ad libitum. Los ratones se aclimataron durante 14 días y se dividieron aleatoriamente en cinco grupos A, B, C, D y E. Los grupos B, C y D fueron los grupos de tratamiento, mientras que los grupos A y E fueron controles negativos y positivos, cada uno con cinco ratas. en cada grupo y cada uno mantenido individualmente en una jaula separada. Los grupos A y E recibieron 1 mL de agua destilada y 5 mg de citrato de sildenafilo por vía oral para el grupo de control positivo. Los grupos B, C y D recibieron suspensión de extracto de jengibre rojo por vía oral en dosis de 100, 200 y 300 mg/kg, respectivamente, entre las 9:00 y las 10:00 diariamente durante 21 días. Los ratones hembra se emparejaron con ratones macho en una proporción de 1:1 y se registró el comportamiento de apareamiento.
Resultados: *Las ratas macho de los grupos C y E mostraron un aumento significativo ($p < 0,05$) en la frecuencia de monta (MF), frecuencia de intromisión (IF) y frecuencia eyaculatoria (EF).*
Conclusión: *El extracto de jengibre rojo tiene actividad como afrodisíaco, pero es necesario realizar pruebas relacionadas con pruebas de toxicidad.*

Palabras clave: *Afrodisíaco, apareamiento, rata, jengibre rojo, estimulante sexual*

INTRODUCTION

Reproduction is one of the most important factors influencing the breeding process, and its success is highly dependent on factors such as genetics, physical environment, nutrition, and management (1,2). Evidence from the literature suggests that nutritional factors are the most vital ingredients that directly affect reproductive performance and potentially moderate the effects of other factors (3-6). Herbal therapy can relieve male infertility, regardless of the etiology (7). A large number of plants have been tested for their possible fertility regulating properties (8,9). Some medicinal plants are widely used as aphrodisiacs to relieve sexual dysfunction or fertility-enhancing agents (10). Stress interferes with reproductive function, especially oxidative stress, due to the production of oxygen free radicals that exceed the antioxidant capacity of stressed tissues (11). Each oxidizing radical is a potential agent of oxidative stress (11). The main antioxidant enzymes in mammals are superoxide dismutase (SOD), catalase and glutathione peroxidase, all of which are endogenous (12). Substances such as vitamins A, C, flavonoids and carotenoids are examples of exogenous antioxidants found in food (13).

Red ginger is a well-known spice in Asia for its medicinal properties. The antioxidant and free radical scavenging properties of the ginger extract have been clinically proven (14,15), as well as anticancer (16,17), antibacterial (18,19), and to treat rheumatoid arthritis (20). The ginger extract contains oleoresin and essential oils, yellow, oily, and pungent. Oleoresin contains phenolic compounds that play a role in antioxidant activity, such as gingerol, shogaol, and zingerone (21,22). Gingerol, of which 6-gingerol is the main constituent of oleoresin, is susceptible to heat, light, and oxygen. However, there is still a lack of information about red ginger extract in Wistar rats as an aphrodisiac reserve-enhancing agent. Therefore, it is necessary to determine the effect of red ginger extract on the mounting frequency (MF), intromission frequency (IF) and ejaculation frequency (EF) in Wistar rats.

MATERIALS AND METHODS

Plant Material

Fresh plants were collected from Bilitro (Plant Research Center for Spices and Medicines). Red Ginger Rhizome washed with running water and cut into small pieces. It was air-dried for ten days, and the dried material was then ground into a powder and sifted using a 40 mesh sieve. The cold maceration method carried out Extraction with 96 % ethanol as solvent.

Making Red Ginger Extract

Red Ginger Simplicia powder was weighed as much as 100 g and then extracted with 96 % ethanol 1 000 mL using the maceration method for seven days. With occasional shaking and heating. At the end of the seventh day, a clear filtrate was obtained by filtering through a Buchner funnel. The filtrate was further concentrated using a rotary evaporator, transferred to a Petri dish, and dried

in an oven at 60 °C for five minutes. Finally, the extract was stored in a desiccator for 15 days to reduce humidity (23).

Phytochemical Qualitative Analysis

The Extract of Red Ginger was subjected to different chemical tests to detect phytoconstituents such as alkaloids, saponins, flavonoids, phlobatanins, reducing sugars, and anthranoids, cardiac glycosides, anthraquinones, and polyphenols were carried out according to the method adopted by Harborne (24-26).

Animal Testing

This study used male and female rats aged three months with a bodyweight of 150-200 g. They were obtained from the animal center laboratory of the Faculty of Pharmacy, Universitas Airlangga. Animal testing is designed according to ethical standards for animal use.

Libido Test

The libido test was carried out according to the method described by (27). Twenty-five male Wister rats weighing 150 - 200 g which exhibited faster sexual activity, were selected for this study. They were divided into five groups with five rats each, and each was kept singly in separate cages during the experimental period. Groups A and E were the negative and positive control groups, each of which the negative control received 1 mL/kg aquadest and the positive control 5 mg of sildenafil citrate orally. Groups B, C, and D received red ginger ethanol extract suspension orally at doses of 100, 200, and 300 mg/kg, respectively, daily for 21 days between 9:00 - 10:00 am. Male Wistar rats were not tested under unusual circumstances. The animals were brought to the laboratory from cages and exposed to low light between 16:00 and 18:00 every day for six days before the experiment. Fifteen female rats were made receptive by oral administration of ethyl estradiol suspension (Lynoral® tablets Organon Pharm) at a dose of 100 g/rat 48 hours before the experiment. Acceptance of female animals was confirmed before testing by exposing them to male Wister rats. In addition to controls, the animals were familiarised with the test conditions. Each male Wister rat was placed individually in one cage with receptive female rats in the same cage. Receptive female mice were put into male cages with a ratio of 1 female to 1 male. Observation of mating behaviour started for 3 hours (28). The test will be stopped if the male fails to show sexual interest. If the female does not show a receptive attitude, it will be replaced by another female from the female Wistar rat cluster. Male Wister rats will be observed for mounting frequency (MF), intromission frequency (IF), and ejaculation frequency (EF).

Statistical Analysis

The data obtained were expressed as the mean ± standard error mean (SEM) of the six animals in each group. Data from all groups were analysed using a one-way analysis of variance (ANOVA) followed by Dunnet's t-test.

RESULTS

A total of 100 g of the dried red Ginger powder yielded 28.8 g of the crude extract. The extracts of Red ginger were subjected to qualitative phytochemical screening for the detection of phytoconstituents like alkaloids, saponins, flavonoids, phlobatanins, reducing sugars, anthranoids, cardiac glycosides, anthraquinones, and polyphenols. The results revealed the presence of cardiac glycosides, alkaloids, saponin, flavonoids, and polyphenols (Table 1).

The results obtained for sexual behaviour between groups B, C, and D were treated with aqueous seed extract of M. oleifera at 100 mg, 200 mg, and 300 mg, respectively. Meanwhile, groups A and E were given distilled water and sildenafil citrate (Embragra-100®) at 1 mL/kg and 5 mg/kg, respectively, as shown in (Table 5). As a result, groups B, C, and D showed a significant increase in mounting frequency (MF), intromission frequency (IF), and ejaculatory frequency (EF) compared to control groups A and E, as presented in (Table 2).

Table 1

Phytochemical screening of extract Red Ginger

Chemicals Constituents	Extract Red Ginger
Cardiac glycosides	+
Alkaloids	+
Saponins	+
Tannins	-
Flavanoids	+
Polyphenols	+
Reducing sugars	+
Phlobatanins	-
Anthraquinones	-
Anthranoids	-

Legend: + = Present; - = Absent

Table 2

Evaluation of Aphrodisiac Effects of Red Ginger on Wistar Rats

Treatment Group	Mounting Frequency	Intromission Frequency	Ejaculation Frequency
A	1.0 ± 1.0a	0 ± 0a	0 ± 0a
B	4.6 ± 0.8b	3.2 ± 0.8b	2.2 ± 0.4b
C	9.0 ± 0.7b	6.8 ± 0.8b	3.4 ± 0.5b
D	18.8 ± 0.8b	13.0 ± 1.0b	7.8 ± 0.8b
E	13.2 ± 0.8b	11.4 ± 1.1b	7.0 ± 0.7b

Mean ± SE with different superscripts a, b within columns are significantly different at $P < 0.05$ compared to the negative control group.

DISCUSSION

It has been reported that steroids and saponins present in many plants have fertility potentiating properties and are useful in treating impotence that occurs due to an imbalance of reproductive hormones (29). Saponins found mainly in the leaves of *Tribulis Terrestris L.* have been used as aphrodisiac agents in rats (30). It is also known to stimulate testicular androgenesis and is important for testicular differentiation, integrity, and steroidogenic function steroidogenic (31,32) in goats and rams, respectively. This study also agrees with the findings reported by Mukhallad et al. (2009), who studied the effect of Nigella sativa on spermatogenesis and fertility activity of male albino rats (33).

This study indicates that the aqueous extract of Moringa seeds at 300 mg/kg can increase the libido of male Wistar rats. This is in agreement with the findings reported by Wattanathorn et al., who reported the aphrodisiac effect induced by Allium tuberosum seed extract in male rats treated at a dose of 500 mg/kg for 21 days, which significantly increased the frequency of MF, frequency of IF intromission and frequency of EF ejaculation (34).

The significant increase in EF indicated that the extract and standard drug prolonged interest in intercourse, which is an indicator of increased sexual arousal as reported in rats Wattanathorn et al. Therefore, the increase in spermatogenic activity is a reflection of the pharmacological properties of red ginger extract that may play a role in stimulating the secretion of testosterone which allows the availability of the hormone along the pituitary-gonadal axis. In addition to the intensity of orgasm and ejaculation, which is in accordance with the conclusions noted by Watcho et al. when working with *Mondia whitei* hexane extract on the reproductive performance of male rats. Similar findings were observed in rats given red ginger extract in this study (35).

CONCLUSION

Phytochemical screening of red ginger rhizome showed the presence of cardiac glycosides, alkaloids, saponins, flavonoids, polyphenols, and reducing sugars. As a rich source of phytochemicals and minerals, Zingiber officinale can be considered a potential traditional medicine source. Furthermore, the results of this study confirm that the consumption of red ginger extract has the potential to improve the libido quality of male rats. This also supports the herbalist's claim that red ginger has sexual arousal properties. Thus, red ginger extract proves to be an effective and safe alternative for increasing libido.

ACKNOWLEDGEMENT

I really appreciate the support from the Dean of the Faculty of Health Sciences, University of Muhammadiyah Surabaya. Personally Dr.

Mundakir, M. Kep., N.S. The same appreciation was also given to the Majelis Pendidikan Tinggi Penelitian dan Pengembangan Pimpinan Pusat Myhammadiyah. Thank you to all of you.

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