



Pharmacological Effect of *Panax ginseng* Extract Standardized with Ginsenoside Rg3 on Mating Behavior of Male Rats Treated with Dopamine Antagonists

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AAEK and AAEN carried out the experimental work and shared in writing the first draft of the manuscript. Author OJ managed the literature searches and performed the statistical analysis and shared in writing the first draft of the manuscript. Authors YTK, WJY and MHP prepared and identified the extract. Authors MAAW and BHR wrote the protocol, managed the project, managed the analyses of the study and wrote the final draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article

Received 22nd February 2014

Accepted 8th April 2014

Published 13th May 2014

ABSTRACT

Aim: The aim of this study was to evaluate the effects of *Panax ginseng* extract standardized with ginsenoside Rg3 (PGRg3) on the mating behavior of sexually active or inactive male rats treated with dopamine antagonists.

Methodology: Animals were treated with PGRg3 (50,150 and 450mg/kgb.w) with or without dopamine antagonists. The penile erection, motor activity and stretching-yawning episode were evaluated in animals treated with PGRg3 alone or in combination with

lisurode or SND 919. Testosterone and sperm counts were also evaluated in different treatment groups.

Results: The results showed that (-) Eticlopride counteracted PGRg3-induced penile erection but not motor hyperactivity. PGRg3 treatment enhanced lisuride-induced behavioral effects. Moreover, PGRg3 plus SND 919 showed a marked stretching-yawning behavior compared to the animals received SND 919 alone. PGRg3 also succeeded to increase testosterone level and sperm count in a dose dependent fashion.

Conclusion: It could be concluded that DAD2 receptors are involved in PGRg3-induced mating behavior and testicular function improvement. PGRg3 could be used to improve sexual function and mating behavior in people suffering from erectile dysfunction.

Keywords: *Phoenix ginseng*; *ginsenoside Rg3*; *sexual behavior*; *dopamine D₂ receptors*; *mating behavior*.

1. INTRODUCTION

Ginseng refers to the root of several species in the plant genus *Panax* (C. A. Meyer Araliaceae) and it is a widespread herbal medicine [1]. Ginseng is commonly taken by itself or with an herbal formula to enhance sexual performance in traditional Chinese medical practices. The beneficial effects have been scientifically evaluated and confirmed in meta-analyses of randomized clinical trials [2]. Ginsenosides or ginseng saponins are the principle active ingredients in ginseng and more than thirty different ginsenosides have been identified [3]. Ginsenosides have a four-ring steroid-like structure with sugar moieties attached and exert their diverse effects in central and peripheral nervous systems [4].

Male sexual behavior is mainly governed by a well-organized neural circuit that connects a variety of brain areas, including the medial preoptic area (MPOA), nucleus accumbens (NAc) and the bed nucleus of stria terminalis (BNST), which appear to control different components of mating behaviors [5]. Several reports have indicated that the catecholamines, dopamine (DA) and norepinephrine (NE), play an important role in the regulation of male sexual behavior and the administration of DA or its agonists facilitate male sexual behavior [6,7]. DA-mediated enhancement of sexual behavior was first recognized when the precursor to DA, 3, 4-dihydroxyl-phenylalanine, was administered to men suffering from Parkinson's disease and resulted in increased libido and sexual potency [8]; however this increase was not related to improvements in locomotor function [9]. Moreover, treatments with DA agonists enhanced male sexual behavior and also restored sexual behavior in animals displaying sexual impairments. However, treatments with DA antagonists impaired behavior [10]. Treatment with apomorphine restored mounting behavior in socially stressed rats [11]. Moreover, SDN 919 is a potent D2/D3 dopamine receptor agonist and it was reported to restore sexual arousal and ejaculatory ability in sexually sluggish rats [12]. Furthermore, Scaletta and Hull [13] reported that apomorphine partially restored sexual activity in animals with sexual deficits resulting from castration and fully restored copulation in mice lacking the gene for the estrogen receptor-alpha [14]. The aim of the current study was to evaluate the effects of *Panax ginseng* extract standardized with ginsenoside Rg3 (PGRg3) on the mating behavior, hormonal level and sperm count of sexually active or inactive male rats treated with dopamine antagonistst.

2. MATERIALS AND METHODS

2.1 Chemicals and Hormones

Lisuride hydrogen maleate, (-) eticlopride hydrochloride, Estradiol benzoate and progesterone were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ketamine and xylazine were purchased from Bayer Co. (Cairo, Egypt). SND 919 was purchased from Boehringer Ingelheim, Ingelheimam Rhein (Germany).

2.2 Plant Materials

Panax ginseng C. A. Mayer extract EFLA400 (Batch No. 303298) was supplied by LOTTE Group R & D Center (Seoul, Korea) and was prepared according to the procedure of Korean patent 0425022, PCT/KR2003/000003. The ginsenoside Rg3 (PGRg3) of *Panax ginseng* was 3.6% (w/w; i.e. 36µg/mg *p. ginseng* extract) as determined by HPLC [15].

2.3 Preparation of PGRg3 Doses

PGRG3 was dissolved in saline at concentrations of 50, 150 and 450mg/ml. The solutions were sterilized by membrane filtration and administrated orally to the rats at a volume of 1ml/rat.

2.4 Preparation of Chemicals Reagents

Ketamine xylazine, Lisuride hydrogen maleate, (-) eticlopride hydrochloride and SND 919 were freshly dissolved in saline as described by Ferrari and Giuliani [16] to prepare the corresponding doses.

2.5 Experimental Animals

Mature male Sprague-Dawley rats (140-150g) were purchased from Animal House Colony, Giza, Egypt. The animals were maintained on standard lab diet (Protein: 16.04%; Fat: 3.63%; Fiber: 4.1%, and metabolic energy: 0.012 MJ) and water *ad libitum* at the Animal House Lab., National research center, Dokki, Cairo, Egypt in a 12/12hr light-dark cycle (lights on from 07:00 to 19:00hr) for 2 weeks prior to the experiments. All animals were received humane care in compliance with the guidelines of the Ethical Committee of Medical Research, National Research Centre, Egypt.

2.6 Behavioral Procedure

All the experiments were performed between 09:00 and 14:00hr in a soundproof, air-conditioned room. The animals were monitored by trained observers unaware of the treatment schedule. All behavioral evaluations, apart from those on copulatory behaviour, were carried out on groups of animals (six homogeneous rats/ treatment) which were transferred to glass observation cages (40x30x34cm) on the day of the experiment and were left to settle down for 10min before the administration of the extract and the start of the tests. Different groups of rats and different cages were used in each series of experiments.

2.7 Penile Erection (PE) and Motor Activity

Seventy two male rats were used in this experiment and were divided into 12 groups and treated as shown in Table 1. The tests were started immediately after the treatment with the respective treatments. PE and motor activity were monitored simultaneously for each rat within each group for 30min. PE was counted only when the rat bent down to lick its penis in full erection. However, motor activity was scored as described by Ferrari and Giuliani [17]. Each rat was observed for 30sec at 5min intervals and was rated on a scale 0-2 where: 0=absent, 1=exploratory behavior for no more than 15 sec and 2=uninterrupted locomotor activity for at least 25 sec. PE and motor activity values for each rat were calculated as the sum of all the numbers or scores respectively, attributed to the animal during the test period.

Table 1. Experimental design for the penile erection (PE) and motor activity experiment

Groups (6rats/group)	Treatments
1	Control (saline only)
2	s.c injected with (-) eticlopride as antagonist(0.025mg/kgb.w) (E1)
3	s.c injected with (-) eticlopride as antagonism(0.05mg/kgb.w) (E1)
4	PGRg3(50mg/kgb.w)
5	PGRg3(150mg/kg b.w)
6	PGRg3(450mg/kg b.w)
7	E1, 25min before PGRg3(50mg/kgb.w)
8	E1, 25min before PGRg3(150mg/kgb.w)
9	E1, 25min before PGRg3(450mg/kgb.w)
10	E2, 25min before PGRg3(50mg/kgb.w)
11	E2, 25min before PGRg3(150mg/kgb.w)
12	E2, 25min before PGRg3(450mg/kgb.w)

s.c: subcutaneous; E1: (-) Eticlopride at low dose (0.025 mg/kg b.w);E2: Eticlopride at high dose (0.05 mg/kg b.w)

2.8 Lisuride-induced Mounting and Motor Activity

Other 48 male rats were used in this study and were randomly assigned to two groups (24rats/group). Each group was further subdivided into four sub-groups (6 rats/ sub-group) and treated as shown in Table 2 and the two tests followed the same schedule. Mounting and motor activity were evaluated for each rat:mounting episodes were counted up when one rat mounted another rat and exhibited pelvic thrusts, with or without PE. Motor activity was scored as described previously. Mounting and motor activity values for each rat were calculated as the sum of all the numbers or scores respectively, attribute to the animal during the test period.

2.9 SND 919-induced Stretching-yawning (SY)

In this test, other 24 male rats were randomly divided into four groups (6 rats/ group). The first group was treated with saline whereas; the second group was treated with PGRg3 (150mg/kg b.w) once daily for 7 consecutive days; the third group was i.p injected with a single dose of SND 919 (0.1mg/kgb.w) at the last day of experiment (day 8) and the fourth group was treated with PGRg3 (150mg/kgb.w) for 7 consecutive days then i.p injected with a single dose of SND 919 (0.1mg/kgb.w) 24h after the last dose of PGRG3. The dose of SND 919 was selective for auto receptors since it did not elicit stereotype but only SY and hype-motor activity [18,19]. After the last dose, all the four groups were transferred to glass

observation cages and the tests were started immediately and each SY episode was counted for each rat over a 30min period. A SY episode consists of opening the mouth widely accompanied by stretching of the limbs and/or body, as manifested in different species of animals [19].

Table 2. Experimental design for the Lisuride-induced mounting and motor activity experiment

Groups	Treatment (for 7 consecutive days)	Experimental day (day 8)
Group 1 (24 rats)	Subgroup 1 Saline for 7 days	i.p. injected with lisuride (0.2mg/kgb.w.)
	Subgroup 2 PGRg3 (50mg/kgb.w.) for 7 days	
	Subgroup 3 PGRg3 (150mg/kgb.w.) for 7 days	
	Subgroup 4 PGRg3 (450mg/kgb.w.) for 7 days	
Group 2 (24 rats)	Subgroup 1 Saline then i.p. injected with lisuride (0.2mg/kgb.w.)	
	Subgroup 2 PGRg3 (50mg/kgb.w.) then i.p. injected with lisuride (0.2mg/kgb.w.)	
	Subgroup 3 PGRg3 (150mg/kgb.w.) then i.p. injected with lisuride (0.2mg/kgb.w.)	
	Subgroup 4 PGRg3 (450mg/kgb.w.) then i.p. injected with lisuride (0.2mg/kgb.w.)	

i.p.: intraperitoneal

2.10 Serum Testosterone and Sperm Counts

In this test, another 24 male rats were divided into four groups including the control group and the groups treated daily with PGRg3 at the three tested doses (50,150 and 450mg/kgb.w) for two weeks. On day 15, blood samples were collected from retro-orbital venous plexus. Blood samples were left to clot, centrifuged at 5000 rpm under cooling for 10 min and serum was separated for testosterone determination by enzyme linked immunoassay procedure as described by Parker [20]. After the collection of blood samples, all animals were sacrificed by cervical dislocation and testes were removed for the determination of sperm counts. Epididymis (free of fats, vas deferens and other tissues) from each side of testis of either control or treated rats were dissected out and the inner content squeezed out into 10ml of 0.87% normal warmed saline separately and spermatozoa were counted using heamocytometer.

2.11 Statistical Analysis

All data were statistically analyzed using the General Linear Model Procedure of the Statistical Analysis System [21]. The significance of the differences among treatment groups were determined by Waller-Duncan k-ratio [22]. All statements of significance were based on probability of $p \leq 0.05$.

3. RESULTS

3.1 Penile Erection (PE) and Motor Activity

The current data clearly indicated that (-) Eticlopride at the two tested doses (0.025 and 0.05mg/kgb.w) did not elicit PE Table 3 and decreased rat motor activity especially at the high dose. Treatment with PGRg3 alone or plus E1 or E2 resulted in a significant increase in PE. Moreover, the groups received E2 plus PGRg3 showed a pronounced improvement

compared to those received E1 plus PGRg3. The data also revealed that motor activity score was significantly higher in the group received 150mg/kgb.w. PGRg3 alone. Moreover, there was insignificant difference between the animals received PGRg3 at 150mg/kgb.w. alone and those received PGRg3 at 50mg/kg plus E1 or those received PGRg3 at 450mg/kgb.w. plus E1 or those received PGRg3 at 50mg/kgb.w plus E2.

Table 3. Effect of (-) eticlopride alone or in combination with PGRg3 extract on number (no) of penile erection (PE) and motor activity of sexually active male rats (means±SE)

Treatment Parameter	PE (no)	Motor Activity (score)
S	0.19±0.01 ^a	6.3±0.3 ^a
E1	0.09±0.005 ^b	5.2±0.3 ^a
E2	0.05±0.004 ^b	4.7±0.2 ^b
PGRg3(50mg)	1.52±0.09 ^c	5.3±0.5 ^a
PGRg3(150mg)	1.68±0.06 ^d	5.7±0.5 ^a
PGRg3(450mg)	2±0.12 ^e	5.3±0.7 ^a
E1 + PGRg3(50mg)	2.22±0.09 ^e	5.5±0.4 ^a
E1 + PGRg3(150mg)	2.65±0.08 ^f	4±0.4 ^b
E1 + PGRg3(450mg)	2.95±0.08 ^f	3.3±0.3 ^c
E2 + PGRg3(50mg)	3.42±0.12 ^g	3.3±0.3 ^c
E2 + PGRg3(150mg)	4.03±0.08 ^h	2.7±0.3 ^d
E2 + PGRg3(450mg)	4.11±0.09 ⁱ	2.5±0.4 ^d

Within each column, means average superscript with different letters (b,c,d,...) are significantly different than the control (a) at p≤0.05; E1: (-) eticlopride 0.025mg/kgb.w; E2: (-) eticlopride 0.05mg/kgb.w; (-) eticlopride was injected s.c (subcutaneous)

3.2 Lisuride-induced Mounting and Motor Activity

Data presented in Table 4 showed that rats treated with saline and lisuride at 0.2mg/kgb.w showed few mounts percentage. The same pattern was found when the DA agonist was administrated to rats which had received repeated saline injections. Acute and subchronic treatments with PGRg3 at the three tested doses stimulated the phenomenon over a certain dose range; it increased the percentage of animals mounting and the number of mounts per animal. This increase was significantly higher in the group received 450 mg/kg PGRg3. On the other hand, the response of the animals to lisuride differed according to the mode of PGRg3 treatment (acute or subchronic) as well as the dose. The effect was maximal after acute or subchronic treatment with PGRg3 at 450mg/kgb.w, and significant differences were found between acute and subchronic treatments within the same dose. It is of interest to mention that lisuride-induced motor activity was always potentiated by PGRg3 in a dose response manner. Although the subchronic treatment with PGRg3 plus lisuride showed a higher score values in motor activity, the difference between the two modes was not significantly different for each tested doses of PGRg3.

3.3 SND 919-induced Stretching-yawning (SY)

The current results indicated that injection of saline alone did not exert any significant effect on SY in the rats. As expected, the DA D2 agonist SND 919 at the tested dose (0.1mg/kgb.w) elicited SY in both the saline and the PGRg3-treated rats Fig. 1. Moreover, the behaviour being more marked in those animals received PGRg3 at 150mg/kgb.w. once daily for 7 consecutive days before the SND 919 injection.

Table 4. Effect of Lisuride-induced mounting and motor activity in rats treated with PGRg3 extract (means±SE)

Treatment parameter	Acute				Subchronic			
	Saline+Lis	PGRg3 (50mg)+Lis	PGRg3 (150mg)+Lis	PGRg3 (450mg)+Lis	Saline+Lis	PGRg3 (50mg)+Lis	PGRg3 (150mg)+Lis	PGRg3 (450mg)+Lis
Mounting (no)	14.3 ^a ±1.58	49.5 ^b ±1.38	55.3 ^c ±1.74	68 ^d ±1.06	13.17 ^a ±1.7	42.5 ^b ±0.76	63.5 ^d ±1.67	73.5 ^e ±1.84
Motor activity (score)	5.5 ^a ±0.4	10.5 ^b ±0.9	18 ^c ±0.9	26.5 ^d ±0.8	6.17 ^a ±0.6	11.17 ^b ±0.9	15.8 ^c ±0.6	25 ^d ±0.9

Within each row, means average superscript with different letters (b,c,d,...) are significantly different than the control (a) at $p \leq 0.05$; Lisuride was injected intraperitoneal (ip) at 0.2mg/kg.b.w. Acute treatment: after the first injection; Sub chronic treatment: after the last injection

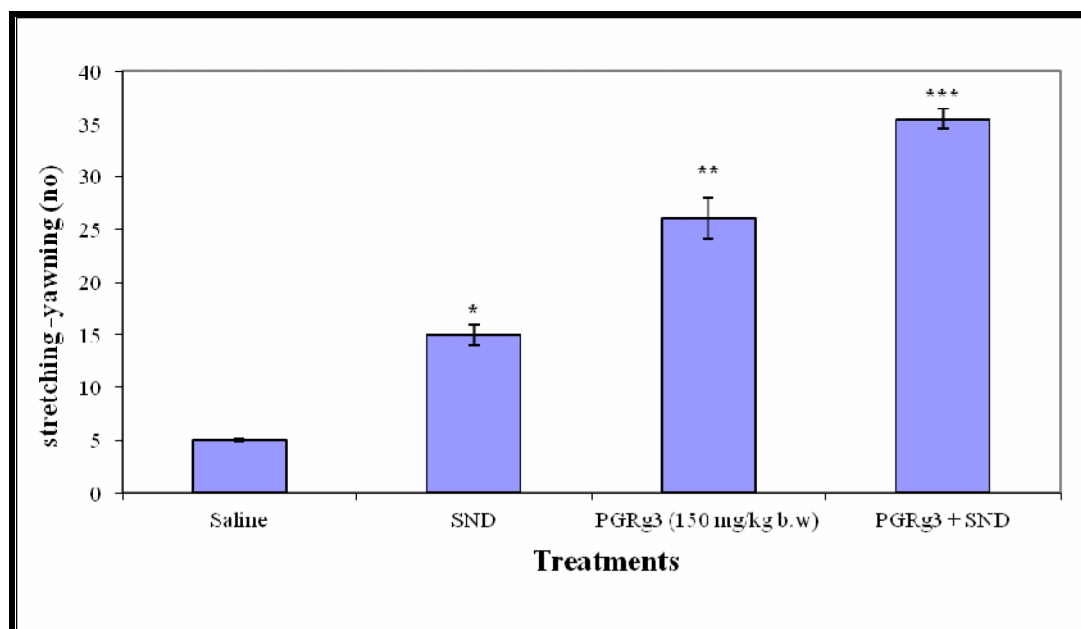


Fig. 1. Effect of PGRg3 extract (150mg/kg.b.w.) alone or in combination with SND919 (0.1mg/kg.b.w) on stretching -yawning in male rats (means±SE)

3.4 Testosterone and Sperm Counts

The present study showed that serum testosterone levels were significantly increased in the rats treated with PGRg3 at the three tested doses in a dose-dependent manner Fig. 2. Moreover, the sperm count was also increased significantly in the rats treated with PGRg3 in a dose dependent fashion Fig. 3.

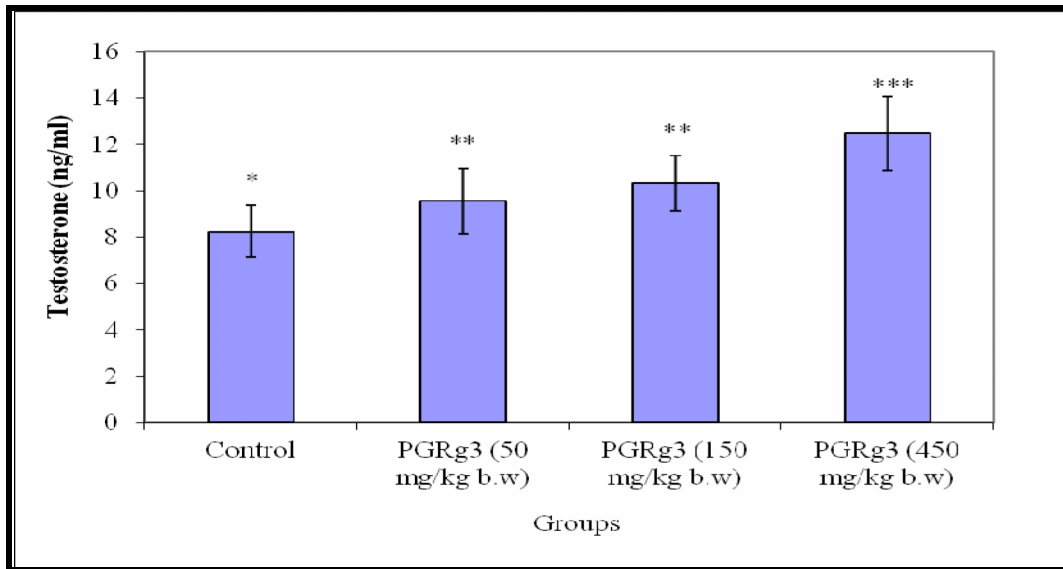


Fig. 2. Concentration of testosterone in serum of rats treated with PGRg3 at three tested doses for 14 days (means±SE)

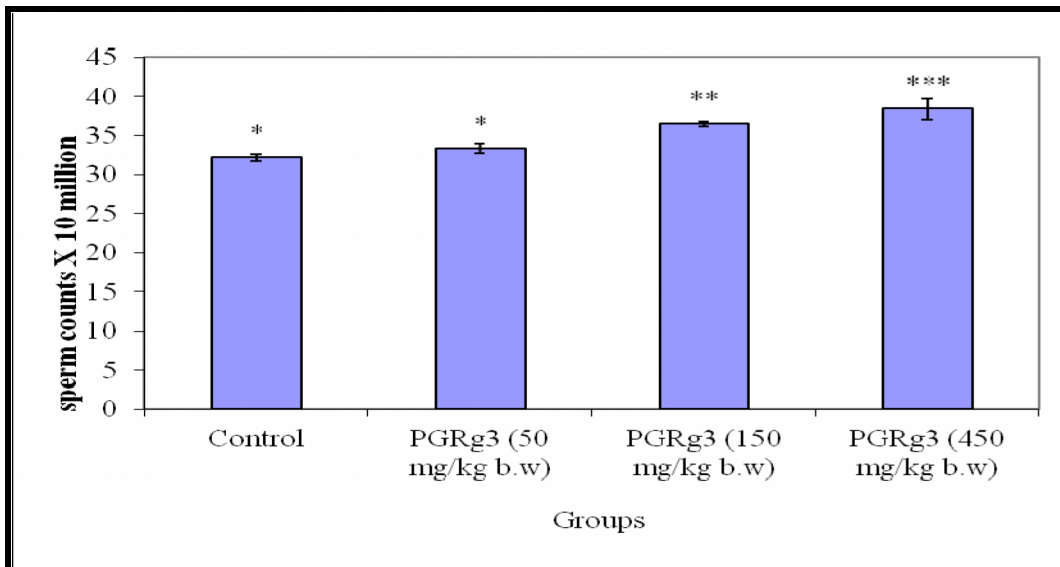


Fig. 3. Sperm counts in rats treated with PGRg3 for 14 days (means±SE)

4. DISCUSSION

The present study provides new information about the influence of the PGRg3 on the copulatory pattern of male rats and the behavioral effects induced by DA agonists. In the mating tests, the most striking effect after acute PGRg3 administration at 50, 150 and 450mg/kg.b.w was the increase in mounts preceding ejaculation. Such a result could suggest an enhancement of libido [23] and therefore, an aphrodisiac-like effect of the PGRG3 extract. Alternatively, an increase in mounts before ejaculation could be interpreted as a decrement in copulatory behaviour, whereby the rats need more stimulation in order to ejaculate. It is well known that the copulatory behaviour of rats comprises a pre copulatory phase; the most common measure is the mount latency, and a consummatory phase, mainly represented by the intromission frequency and ejaculation latency [23]. However, the mount latency as well as the post-ejaculatory interval are considered indexes of rat sexual appetitive behavior [24,25].

The DA D2 antagonist (-) eticlopride significantly decrease Penile erection (PE) when injected in the normal rats at the two tested doses and this decrease was pronounced in the group injected with the high dose. However, (-) eticlopride antagonized PGRg3-induced motor hyperactivity did not inhibit PE which was actually stimulated in the high dose of the extract. This finding is in accordance with Ferrari et al. [16] who stated that DA D2 antagonist has been found to counteract behavioral sensitization to certain drug-induced motor hyperactivity and to reverse the inhibitory effect on PE. Administration of PGRg3 at moderate doses facilitated indiscriminate mounting, typically elicited by lisuride at doses higher than 0.2mg/kg [26].

The principal active constituents of *Panax ginseng* are thought to be the saponin glycosides, ginsenosides. Ginsenosides have been shown to cause a dose-dependent relaxation of the corpus cavernosal smooth muscle in rabbits by increasing release of nitric oxide [27-29]. Similar to the current results Ryu et al. [30] reported that Korean red ginseng preserve potency in non-insulin-dependent diabetes rats through its antioxidant activity. Moreover, McKay [31] stated that several herbs including ginseng have some degree of evidence that they may be helpful for erectile dysfunction and improve penile endothelial L-arginine-nitric oxide activity appear to be unifying explanation for the actions of these agents. In this regards, Achike and Kwan [32] reported that ginsenosides from ginseng have been shown to relax blood vessels (probably contributing to the antifatigue and blood pressure-lowering effects of ginseng) and corpus cavernosum. Although these authors stated that the legendary aphrodisiac of ginseng may be an overstatement for men who are suffering from erectile dysfunction; the current results suggested that the tested extract has a strong effect in enhancing sexual behaviour in sex active and in improving both sexual behaviour and penile erection in sex inactive rats.

In agreement with the current results, Hong et al. [33] concluded that Korean red ginseng can be as effective alternative for treating male erectile dysfunction. Moreover, Choi et al. [27] reported that the long-term administration of Korean red ginseng enhances erectile capacity and its action is mediated by endothelium-derived relaxing factor and peripheral neurophysiologic enhancement. Clinical studies have also supported the use of *Panax ginseng* to assist the phalldynamically challenged. Choi et al. [34] demonstrated that *Panax ginseng* was superior to placebo for the treatment of erectile dysfunction (ED) in ninety patients divided into three groups and given *Panax ginseng*, placebo, or trazodone orally. These authors reported that frequency of intercourse, premature ejaculation, and morning erections after treatment were unchanged in all three groups. However, in the *Panax*

ginseng-treated group a significant improvement in erectile parameters such as penile rigidity, girth, duration of erection, improved libido, and patient satisfaction were reported. Moreover, Hassan et al. [35] reported that treatment with PGRg3 extract increased testosterone level and improve tests function. A double-blind, placebo-controlled, crossover study conducted by Hong et al. [33] showed that *Panax ginseng* is an effective alternative for treating ED. Moreover, the same authors speculated that therapeutic efficacy of *Panax ginseng* for ED is not mediated through improvements in hemodynamics alone or a hormonal effect, but through several mechanisms that have not yet been completely elucidated. From the current data it appears PGRg3 may possess the ability to improve erectile function. Preliminary conclusions suggest its primary mechanism is mediated through increased NO levels, resulting in improved penile hemodynamics. This mechanism suggests therapeutic success of PGRg3 for the treatment of ED may be dependent on the presence of impaired endothelial L-arginine-NO activity [32].

The mating pattern observed in rats after subchronic administration of PGRg3 at the three tested doses differed significantly from that obtained after the first oral dose and showed that all tested parameters including ejaculation mechanisms are affected [25]; moreover, it seems to reflect a premature ejaculation reminiscent of that produced by acute systemic DA agonists [17,23,24] This similarity between subchronic PGRg3 and DA agonist-induced effects would, therefore, suggest that the neuronal substrates in the two cases are quite similar, though different from those affected by acute administration of PGRg3. The role of the specific DA receptors involved in the facilitation of ejaculation by DA agonists is questioned [23]. What is more, any hypothesis as to the mechanisms involved in the various behavioral effects exerted by such compounds now seems suspect [24] in view of the proliferation of DA receptor subtypes [36] and of the yet limited understanding of specific affinity for them of the DA agonists and antagonists [37]. According to Zhang et al. [38], the mechanism of antipsychotic drug (eticlopride as well)-induced sexual dysfunction is a common and serious clinical side effect. This depends on blocking both central neuronal nitric oxide (nNOS) and dopamine D2 receptor (DRD2) in the medial preoptic area (MPOA) and the paraventricular nucleus (PVN) of the hypothalamus. (-) Eticlopride, itself previously described as a selective DA D2 antagonist, has been reported as having considerable affinity for the DA D3 receptor [39]. However, leaving aside any specific neurochemical interpretation of the effects of PGRg3 extract on rat sexual behaviour, our findings support the notion that, although the drug might heighten sexual potency and urge in the short term (hence its popular reputation as one of the best aphrodisiacs). In fact, just as drug-induced hyper activity sensitizes, certain sexual stimulant effects rapidly decline [40]. A functional as well as neurochemical interdependence of the two phenomena is possible and would also be indirectly confirmed by the results obtained on PGRg3 extract-induced PE and motor activity after (-) eticlopride pretreatment.

There is conflicting evidence regarding DA D2 autoreceptor function after chronic drugs e.g. cocaine [40]; some authors have found a decrease [41], some an increase [42] and others no change [43]. The present study clearly shows a significant enhancement of SND 919-induced SY after 7 days of PGRg3 treatment. If it is true that the SY syndrome reflects the selective stimulation of DA D2 autoreceptors [19,44] or DA D3 receptors [17], the results of our behavioral study would indirectly confirm that one of these two DA receptor subtypes is supersensitive, at least in our experimental conditions.

The present results revealed that PGRg3 at the three tested doses induced a significant increase in testosterone level and the total sperm counts. Similar results were rats fed with *Panax ginseng* have shown significantly increased blood testosterone levels [45].

Ginsenoside, the major active constituent in *Panax ginseng*, is responsible for the increase of serum testosterone levels and improvement of copulatory behavior [46]. Moreover, ginsenoside is found to increase the secretion of LH by acting directly on the anterior pituitary gland which is responsible for testosterone production [47]. The initial evidence that ginseng may have positive effects on spermatogenesis and stimulate DNA and protein syntheses in rat testes [48]. Moreover, Kumar et al. [49,] and Kang et al. [50] observed that *ginseng* extract increased testicular acid phosphatase activity. These effects may be attributed to the active components ginsenosides which are well known to suppress lipid peroxidation and reduce the cellular damage. Zhao [51] has also observed that anti lipid peroxidative activity was increased and thus lipid peroxidation was inhibited in *ginseng*-treated rats. On the other hand, rats treated with ginseng showed an increased rate of spermatogenesis via glial cell-derived neurotrophic factor (GDNF) expression elevation in Sertoli cells [52], and activation of testicular cAMP-responsive element modulator (CREM) [53]. Moreover, Kim et al. [29] reported that water fraction and alkaloid fraction of *ginseng* may reduce cell damage, especially the damage to DNA molecules, caused by gamma rays and thus playing a role in the repair of regeneration process of damaged cells. They also concluded that it is possible that *ginseng* reduces DNA damage by antiradical action. Taken together, the improvement of testicular function reported in the current study and the results of the behavior studies indicated that PGRg3 might be a useful agent to improve sexual behavior in people who suffer from erectile dysfunction or other sex problems. This action may be involved the neuroendocrine axis.

5. CONCLUSION

It could be concluded that PGRg3 enhanced the sexual behavior of the sexual active or inactive male rats and markedly improved the rats' copulatory ability in a dose and mode-dependent manner. However the dose required for the sexually active rat was less than those for the sexually inactive. Acute administration of PGRg3 stimulated penile erection and induced behavioral effects but not on motor hyperactivity in (-) Etilopride-treated sexually-native male rats. PGRg3 displayed a more marked stretching-yawning behaviour in animals injected with DA D2 agonist SND 919.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "principles of laboratory animal care" (NIH publication no. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rhim H, Kim H, Lee DY, Oh TH, Nah SY. Ginseng and ginsenoside Rg3, a newly identical active ingredient of ginseng, modulate Ca²⁺ channel currents in rat sensory neurons. *Eur J Pharmacol.* 2002;463:151-158.
2. Jang DJ, Lee MS, Shin BC, Lee YC, Ernst E. Red ginseng for treating erectile dysfunction: a systematic review. *Br J Clin Pharmacol.* 2008;66:444-450.
3. Back NI, Kim DS, Lee YH, Park JD, Lee CB, Kim SI. Ginsenoside Rh4, a genuine dammarane glycoside from Korean red ginseng. *Planta Med.* 1996;62:86-87.
4. Nah SY, Kim DH, Rhim H. Ginsenosides: Are any of them candidates for drugs acting on the central nervous system? *CNS Drug Rev.* 2007;13:381-404.
5. Tsai HW, Chen JC, Yeh KY, Tai MY, Tsai YF. Male sexual behavior and brain catecholamine levels in middle-aged rats. *Chin J Physiol.* 2009;52(4):215-222.
6. Hull EM, Meisel RL, Sachs BD. Male sexual behavior. In: *Hormones, Brain and Behavior*, edited by Pfaff, D., Arnold, A.P., Etgen, A.M., Fahrbach, S.E. and Rubin, R.T. New York, NY, USA: Academic Press. 2002;1:3-137.
7. Hull EM, Muschamp JW, Sato S. Dopamine and serotonin: Influences on male sexual behavior. *Physiol Behav.* 2004;83:291-307.
8. Shapiro SK. Hypersexual behavior complicating levodopa (L-dopa) therapy. *Minn Med.* 1973;56:58-59.
9. Brown WA, Monti PM, Corriveau DP. Serum testosterone and sexual activity and interest in men. *Arch Sex Behav.* 1978;7:97-103.
10. Dominguez JM, Hull EM. Dopamine, the medial preoptic area, and male sexual behavior. *Physiol Behav.* 2005;86:356-368.
11. Niikura S, Yokoyama O, Komatsu K, Yotsuyanagi S, Mizuno T, Namiki M. A causative factor of copulatory disorder in rats following social stress. *J Urol.* 2002;168:843-849.
12. Giuliani D, Ottani A, Ferrari F. Influence of sildenafil on copulatory behaviour in sluggish or normal ejaculator male rats: a central dopamine mediated effect? *Neuropharmacol.* 2002;42: 562-567.
13. Scaletta LL, Hull EM. Systemic or intracranial apomorphine increases copulation in long-term castrated male rats. *Pharmacol Biochem Behav.* 1990;7:471-475.
14. Wersinger SR, Rissman EF. Dopamine activates masculine sexual behavior independent of the estrogen receptor alpha. *J Neurosci.* 2000;20:4248-4254.
15. Panwar M, Kumar M, Samarth R, Kumar A. Evaluation of chemo preventive action and anti mutagenic effect of the standardized panax ginseng extract, EFLA400, in Swiss albino mice. *Phytother Res.* 2005;19:65-71.
16. Ferrari F, Giuliani D. Influence of eticlopride on cocaine- and DA D2 agonist-induced behavioral effects in rats. *Pharmacol Biochem Behav.* 1996;53:230-252.
17. Ferrari F, Giuliani D. Behavioral assessment in rats of the antipsychotic potential of the potent dopamine D2 receptor antagonist (-) eticlopride. *Pharmacol Res.* 1995b;31:261-267.
18. Matsumoto S, Yamada K, Domae M, Shirakawa K, Furukawa T. Occurrence of yawning and decrease of prolactin levels via stimulation of dopamine D2-receptors after administration of SND 919 in rats. *Naunyn-Schmiedeberg's Arch Pharmacol* 1989;340:21-25.
19. Ferrari F, Pelloni F, Giuliani D. Behavioral evidence that different neurochemical mechanisms underly stretching-yawning and penile erection induced in male rats by SND 919, a new selective D2 dopaminergic agonist. *Psychopharmacol.* 1993;113:172-176.

20. Parker F. Skin and hormones. In: Text Book of Endocrinology. Williams, R.H. (Hrsg), W.B. Saunders Co., Philadelphia. 1981;1080-1098.
21. SAS, SAS User's Guide: Statistics. SAS Institute, Cary, N.C; 1982.
22. Waller RA, Duncan DB. A Bayes rule for the symmetric multiple comparison problems. J Am Stat Assoc. 1969;64:1484-1503.
23. Melis MR, Argiolas A. Dopamine and sexual behavior. Neuroscience and Biobehavioral Rev. 1995;19:19-38.
24. Ferrari F, Giuliani D. Sexual attraction and copulation in male rats: Effects of the dopamine agonist SND 919. Pharmacol Biochem Behav. 1995a;50:29-34.
25. Abdel-Wahhab MA, El-Nekeety AA, Aly SE, Yoon WJ, Kim YT, Park MH. Improvement of sexual behavior in male rats via dietary supplementation with Panax ginseng extract standardized with ginsenoside Rg3. J Med Sci. 2013;13(5):337-345.
26. Baggio G, Ferrari F. The role of dopaminergic receptors in the behavioral effects induced by lisuride in male rats. Psychopharmacol. 1983;80:38-42.
27. Choi YD, Rha HK, Choi HK. *In vitro* and *In vivo* experimental effect of Korean red ginseng on erection. J Urol. 1999;162:1508-1511.
28. Choi YD, Xin ZC, Choi HK. Effect of Korean red ginseng on the rabbit corpus cavernosal smooth muscle. Int J Impot Res. 1998;10:37-43.
29. Kim HJ, Woo DS, Lee G, Kim JJ. The relaxation effects of ginseng saponin in rabbit corporal smooth muscle: Is it a nitric oxide donor? Br J Urol. 1998;82:744-748.
30. Ryu JK, Lee T, Kim DJ, Park IS, Yoon SM, Lee HS, Song SU, Suh JK. Free radical-scavenging activity of Korean red ginseng for erectile dysfunction in non-insulin-dependent diabetes mellitus rats. Urol. 2005;65:611-615.
31. McKay D. Nutrients and botanicals for erectile dysfunction: Examining the evidence. Altern Med Rev. 2004;9:4-16.
32. Achike FI, Kwan CY. Nitric oxide, human diseases and the herbal products that affect the nitric oxide signaling pathway. Clin Pharmacol Physiol. 2003;30:605-615.
33. Hong B, Ji YH, Hong JH, Nam KY, Ahn TY. A double-blind crossover study evaluating the efficacy of Korean red ginseng in patients with erectile dysfunction: A preliminary report. J Urol. 2002;168:2070-2073.
34. Choi HK, Seong DH, Rha KH. Clinical efficacy of Korean red ginseng for erectile dysfunction. Int J Impot Res. 1995;7:181-186.
35. Hassan AM, Abbès S, Park MH, Kim YT, Yoon WJ, Abdel-Wahhab MA. Panax ginseng extract standardized with ginsenoside Rg3 counteracts the reproduction disturbance in male rats received Zearalenone mycotoxin. 9th International Symposium on Ginseng. Korea. 2006;25-28.
36. Levesque D, Diaz J, Pilon C, Martres MP, Giros B, Souil E, Schott D, Morgat JL, Schwartz JC, Sokoloff P. Identification, characterization and localization of the dopamine D3 receptor in rat brain using 7-(3H) hydroxy-N, N di-n-propyl-2-aminotetralin. Proc Natn Acad Sci USA. 1992;89:8155-8159.
37. Ahlenius S, Sahni P. Behavioral and biochemical effects of the dopamine D3 receptor-selective ligand, 7-OHDPAT, in the normal and reserpine-treated rat. Eur J Pharmacol. 1994;260:177-181.
38. Zhang XR, Wang YX, Zhang ZJ, Li L, Reynolds GP. The effect of chronic antipsychotic drug on hypothalamic expression of neural nitric oxide synthase and dopamine D2 receptor in the male rat. PLoS ONE. 2012;7(4):e33247.
39. Baldessarini RJ, Kula NS, McGrath CR, Bakthavachalam V, Keabian JW, Neumeyer JL. Isomeric selectivity at dopamine D3 receptors. Eur J Pharmacol. 1993;239:269-270.

40. Kalivas PW, Duffy P, DuMars LA, Skinner C. Behavioral and neurochemical effects of acute and daily cocaine administration in rats. *J Pharmacol Exp Ther.* 1988;245:485-492.
41. Ackerman JM, White FJ. A 10 somatodendritic dopamine autoreceptor sensitivity following from repeated cocaine treatment. *Neurosci Lett.* 1990;117:181-187.
42. Dwoskin LP, Peris J, Yasuda RP, Philpott K, Zahniser NR. Repeated cocaine administration results in supersensitivity of striatal D2 dopamine autoreceptors to pergolide. *Life Sci.* 1988;4:255-262.
43. Fitzgerald JL, Reid TJ. Chronic cocaine treatment does not alter rat striatal D2 autoreceptor sensitivity to pergolide. *Brain Res.* 1991;541:327-333.
44. Ferrari F, Baggio G, Mangiafico V. The dopamine autoreceptor agonist B-HT 920 markedly stimulates sexual behaviour in male rats. *Experientia.* 1985;41:636-638.
45. Fahim MS, Fahim Z, Harman JM, Clevenger TE, Mullins W, Hafez ES. Effect of *Panax ginseng* on testosterone level and prostate in male rats. *Arch Androl.* 1982;8:261-263.
46. Wang X, Chu S, Qian T, Chen J, Zhang J. Ginsenoside Rg1 improves male copulatory behavior via nitric oxide/cyclic guanosine mono phosphate pathway. *J Sex Med.* 2010;7:743-750.
47. Tsai SC, Chiao YC, Lu CC, Wang PS. Stimulation of the secretion of luteinizing hormone by ginsenoside-Rb1 in male rats. *Chin J Physiol.* 2003;46:1-7.
48. Yamamoto M, Kumagai A, Yamamura Y. Stimulatory effect of *Panax ginseng* principles on DNA and protein synthesis in rat testes. *Arzneimittelforschung.* 1977;27:1404-1405.
49. Kumar M, Sharma MK, Saxena PS, Kumar A. Radio protective effect of *Panax ginseng* on the phosphatases and lipid peroxidation level in testes of Swiss albino mice. *Biol Pharm Bull.* 2003;26:308-312.
50. Kang J, Lee Y, No K, Jung E, Sung J, Kim Y, Nam S. Ginseng intestinal metabolite-I (GIM-I) reduces doxorubicin toxicity in the mouse testis. *Reprod Toxicol.* 2002;16:291-298.
51. Zhao ZD. Experimental treatment of smoke inhalation injury with anti-lipid peroxidation agents. *Zhonghua Zheng Xing Shao Shang Wai Ke Za Zhi.* 1990;6:294-298.
52. Yang WM, Park SY, Kim HM, Park EH, Park SK, Chang MS. Effects of *Panax ginseng* on glial cell derived neurotrophic factor (GDNF) expression and spermatogenesis in rats. *Phytother Res.* 2011;25:308-311.
53. Park WS, Shin DY, Kim R, Yang WM, Chang MS, Park SK. Korean ginseng induces spermatogenesis in rats through the activation of cAMP-responsive element modulator (CREM). *Fertil Steril.* 2007;88:1000-1002.

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