Effects of sildenafil on rat irritable bowel syndrome

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Background: Irritable bowel syndrome (IBS) is a disorder with unknown pathophysiology, although it would appear that stress of different types plays an important role in the onset and development of the disorder. It affects 10–15% of the general population. Currently, anticholinergics and prokinetics are the main therapies. **Objective:** The objective of this study was to examine the effects of sildenafil in an animal model of IBS. **Methods:** IBS was induced in rats using the wrap-restraint method and sildenafil was administered intragastrically by gavage at doses of 0.5, 1 and 5 mg/kg. Gastric emptying, small bowel transit and fecal excretion (index of large intestine motility) as well as concentrations of cyclic nucleotides (cGMP and cAMP) and total antioxidant capacity in the large intestine were determined. **Results:** Sildenafil at all doses used (0.5, 1 and 5 mg/kg) significantly reduced gastric emptying up to 120 min postdrug administration. All doses (0.5, 1 and 5 mg/kg) of sildenafil dose-dependently reduced small bowel transit up to 60 and 90 min after sildenafil administration. Treatment of rats using sildenafil (0.5, 1 and 5 mg/kg) increased the concentration of cAMP 40 min post administration. At 90 min postdrug administration, only sildenafil (5 mg/kg) increased cAMP concentration. At 40 min postdrug administration, sildenafil (1 and 5 mg/kg) and at 90 min, all doses of sildenafil increased the concentration of cGMP. Sildenafil (5 mg/kg) increased total antioxidant capacity and reduced fecal excrements in IBS rats. **Conclusion:** Sildenafil administration appears to have beneficial effects in the treatment of IBS in rats, which is in relation with the drugs potential to increase bowel total antioxidant capacity and cyclic nucleotides.

Keywords: cyclic AMP, cyclic GMP, fecal excretion, gastric emptying, irritable bowel syndrome, sildenafil, small bowel transit, total antioxidant capacity

Irritable bowel syndrome (IBS) is a disorder with unknown pathophysiology, although it seems that stress of different types plays an important role in onset and development of the disorder [1]. The prevalence of IBS in Western countries is approximately 10–15% [2,3], which creates a great economic burden on society [4,5]. IBS comprises 25–50% of all referrals to gastroenterologists [6]. IBS is not curable and only symptomatic therapy is available to relieve the patients' manifestations, which are mainly pain and change in bowel habit [7,8]. The most commonly used medications are anticholinergics which act through inhibition of gastrointestinal tract (GIT) motility. Antidepressants and antidiarrheal drugs are also widely used for the treatment of IBS. Reports considering the efficacy of these drugs are extremely inconsistent and in a recent systematic review, none of the mentioned medications have been shown to be more effective than placebo [9]. However, with limited evidence, it is considered that slowing and relaxing of the bowel smooth muscles is beneficial [10].

Sildenafil, the drug of choice in erectile dysfunction [11,12], is a cGMP phosphodiesterase (PDE) inhibitor which inhibits catabolism of cGMP and increases its availability leading to smooth muscle relaxation in the corpus cavernosum and GIT [13–15]. This effect is achieved by stimulation of the nitric oxide (NO)/cGMP pathway [16]. This study investigates the influence of sildenafil on GIT motility, and large intestine cGMP and cAMP concentrations and total antioxidant capacity (TAC) in an established animal model of IBS to examine whether the drug has any advantage.

Methods

Animals

Male Wistar rats weighing 200–250 g were used. The animals were kept in four groups consisting of six rats on a 12 h light–dark cycle (8 AM to 8 PM) and were given food at specific hours during the day with open access to water. Animals were maintained in this condition for 1 week prior to experimentation. The experiments were carried out on fasted animals (24 h) in the afternoon; except for the fecal excretion test, for which animals were fed.

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Table 1. Effects of sildenafil on small bowel transit in the animal model of IBS

Dose of sildenafil (mg/kg in rat)	Time (min) expressed as a percent (%)		
	60	*90*	*120*
0	18.8 ± 1.3	22.4 ± 0.9	28.4 ± 2
0.5	27.2 ± 2	27.6 ± 0.3§	26.2 ± 1.65
1	28.9 ± 1.9§	28.2 ± 0.2§	21.5 ± 1.5
5	39.9 ± 1§	30.1 ± 1§	25 ± 3

Sildenafil was administered at doses of 0.5, 1 and 5 mg/kg by gavage. Small bowel transit was calculated at 60, 90 and 120 min after phenol red administration in rats. Data are mean ± SEM of six animals in each group. Difference between the concentration of phenol red recovered in the first and third intestinal segment was chosen as small bowel transit and expressed as the percent of the total amount recovered.

§Represents that difference between treated and control (dose 0 mg/kg) is significant at $p < 0.05$.

Study protocol

As a model for IBS, the mild-restraint (wrap-restraint) method, which has been shown to be that which most resembles humans in comparison to other models of IBS, was used [17,18]. Animals were lightly anesthetized with ether and wrapped in masking tape to restrict their movement and then animals were transported into their cages. A total of 18 rats were classified into three groups of six rats each, to examine different doses of sildenafil and six rats received physiologic saline as controls. After 90 min, sildenafil was dissolved in water and administered at doses of 0.5, 1 and 5 mg/kg intragastrically by gavage. After 45 min, phenol red was administered and the animals were sacrificed and the concentration of phenol red in the stomach and three equal segments of small bowel were measured. To indicate gastric emptying, the percent of phenol red recovered was calculated. To assess small bowel transit, differences between the amount of phenol red in the first and third intestinal segment expressed as a percent of the total amount recovered was calculated.

In the second part of the experiment, fed animals in the above-mentioned groups were used to evaluate the effect of sildenafil on fecal excretion. Fecal pellet output in fed animals was counted for 4 h after sildenafil administration. For measuring concentrations of cAMP, cGMP and TAP of the large intestine, after sacrificing, the rats' large intestine was carefully removed, placed in physiologic saline (50 ml) and homogenated (5 sec). After incubation for 1 h, 10 ml of the upper solution was centrifuged (15 min, rpm = 3000 g) and supernatant was used for analysis.

Determination of cAMP & cGMP

Levels of cAMP and cGMP were determined by enzyme-linked immunosorbent assay (ELISA) technique kits. In this technique, a peroxidase-labeled cAMP conjugate, a specific antiserum (immobilized on to precoated microtiter plates), and a single substrate solution were used. The assay is based on the competition between unlabelled cAMP or cGMP and a fixed quantity of peroxidase-labeled cAMP or cGMP, for a limited number of binding sites on a cAMP- or cGMP-specific antibody.

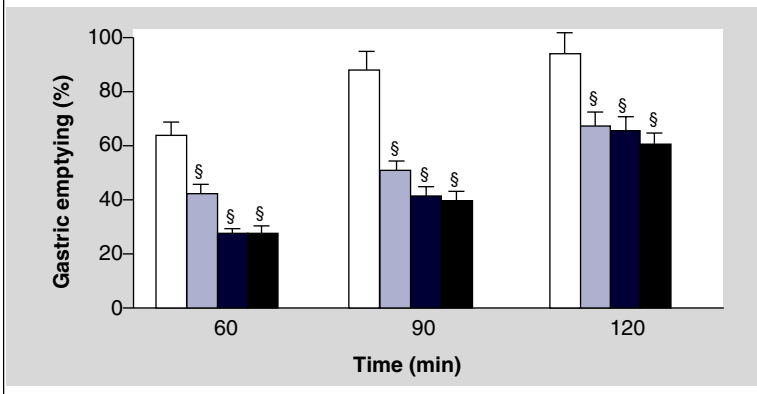
Determination of total antioxidant capacity

The total antioxidant capacity of intestine homogenate was determined by measuring the ability of the sample to reduce Fe^{3+} to Fe^{2+} established as the Fluorescence Recovery After Photo bleaching (FRAP) test [19]. Briefly, in this test, the medium is exposed to Fe^{3+} and the antioxidants present in medium start to produce Fe^{2+} as an antioxidant activity. The reagent included 300 mmol/l acetate buffer, pH 3.6 and 16 ml acetaldehyde/l of buffer solution, 10 mmol/l 2,4,6-tripyridyl-S-triazine (TPTZ) in 40 mmol/l HCl, and 20 mmol/l $FeCl_3.6H_2O$. Working FRAP reagent was prepared as required by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution and 2.5 ml $FeCl_3.6H_2O$ solution. 10 µl of H_2O-diluted sample was then added to 300 µl freshly prepared reagent warmed at 37°C. The complex between Fe^{2+} and TPTZ gives a blue colour with absorbance at 593 nm.

Measurement of gastric emptying & small bowel transit

The phenol red method was used [20,21]. Briefly, animals received 1 ml of a 1.5% methylcellulose solution containing 0.5 mg of phenol red intragastrically (by gavage). The stomach and small intestines were then removed carefully, rinsed in 0.9% saline and the small intestine divided into three equal segments. The stomach and three intestinal segments were placed in 100 ml of 0.1 N

Figure 1. Effects of different doses of sildenafil on gastric emptying.



Sildenafil was administered at doses of 0.5, 1 and 5 mg/kg by gavage. Gastric emptying was calculated at 60, 90 and 120 min after phenol red administration in rats. Data are mean ± SEM of six animals in each group.
§Difference between treated and control (dose 0 mg/kg) groups is significant at $p < 0.01$. Bars respectively represent control and sildenafil (0.5, 1 and 5 mg/kg).

NaOH and homogenized for 30 sec. The suspension was stored at room temperature for 60 min and 5 ml of supernatant was added to 0.5 ml of 20% w/v trichloroacetic acid. After centrifugation at 3000 rpm for 20 min, the supernatant was added to 4 ml of 0.5 N NaOH. Finally, the absorbance of the samples was read by an ultraviolet (UV)-Visible Spectrophotometer (Shimadzu, UV-160A, Japan) at 560 nm. A calibration curve was used to measure the concentration of phenol red.

The percentage of gastric emptying was calculated according to the following formula:

$$\frac{1 - \text{phenol red recovered from test stomach}}{\text{average phenol red recovered from standard stomachs}} \times \frac{100}{1}$$

Statistics

Statistical analysis was performed by the use of the SPSS statistical software package, version 10. The ANalysis Of VAriance (ANOVA) followed by *post hoc* test for multiple comparisons was used. Results are expressed as mean ± SEM. A value of $p < 0.05$ was considered statistically significant.

Results

Changes in gastric emptying after administration of sildenafil at 1, 1.5 and 2 h after phenol red administration are indicated in **Figure 1**. Sildenafil at all doses used (0.5, 1 and 5 mg/kg) significantly reduced gastric emptying in comparison with the control group. This inhibitory effect was not abolished during the time and was observed even after 120 min in comparison with the control group. **Table 1** demonstrates the effect of sildenafil on small bowel transit. All doses (0.5, 1 and 5 mg/kg) of sildenafil dose-dependently reduced small bowel transit compared with the control group up to 60 and 90 min after sildenafil administration. This effect subsided after 120 mins.

The concentrations of cAMP, cGMP and TAP are presented in **Table 2**. Treatment of rats by sildenafil (0.5, 1 and 5 mg/kg) increased the concentration of cAMP 40 min postadministration. At 90 min postdrug administration, only sildenafil (5 mg/kg) increased the cAMP concentration. At 40 min postdrug administration, sildenafil (1 and 5 mg/kg) and at 90 min postdrug administration, all doses of sildenafil increased the concentration of cGMP. The TAP was increased by sildenafil (5 mg/kg) compared with the control group. The effect of sildenafil on fecal excrements is shown in **Figure 2**. Only high doses of sildenafil (5 mg/kg) reduced fecal excrements in IBS rats.

Expert opinion

Overall results of the present study demonstrate that sildenafil reduces gastric emptying and small bowel transit, two important parameters of IBS. The effect observed by sildenafil was interestingly dose-dependent for small bowel transit. The most effective dose of sildenafil for inhibiting large intestine motility was 5 mg/kg. At this dose, added to inhibition of large bowel motility, the TAP, as well as concentrations of cAMP and cGMP, increased.

It is known that PDE-isozymes are highly distributed in the human body and sildenafil has shown a somehow relative affinity for other PDE subtypes, as well as PDE-5 [22], which could explain the increase in cAMP concentrations shown in the present study. Interestingly, PDE-5 is present in both large and small intestines and this implies that the pharmacologic effects of sildenafil predominantly on smooth muscle relaxation in GIT, such as the corpus cavernosum smooth muscle, are expectable. The relaxing effect of sildenafil on smooth muscles is thought to occur via an increase in intracellular cGMP, which then modulates intracellular calcium content and subsequent contractility of smooth muscle [23]. In addition, sildenafil can interestingly reduce rectal hypersensitivity, a common problem in patients with IBS [16]. Supporting the present results, there is a meta-analysis study indicating that myorelaxants such as cimetropium bromide (five trials),

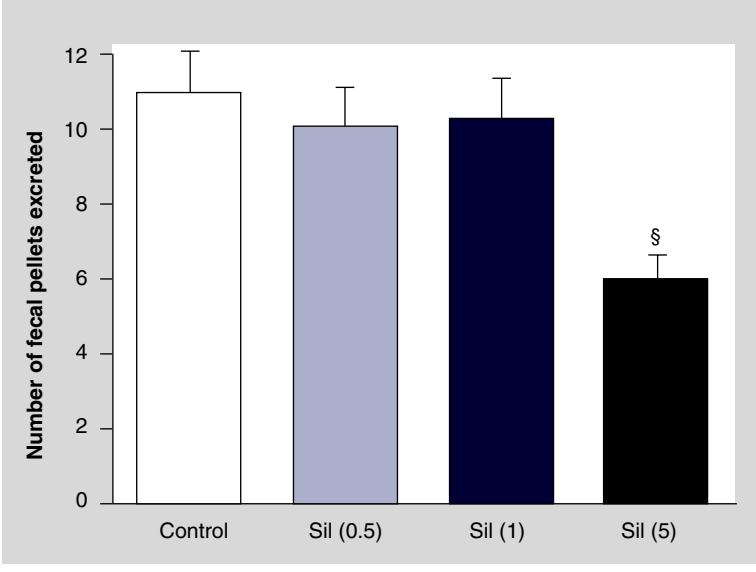
Table 2. Effects of sildenafil administration on large intestine concentrations of cyclic nucleotides and total antioxidant capacity

Sildenafil (mg/kg)	cAMP (pmol/ml); time (min)		cGMP (pmol/ml); time (min)		Total antioxidant capacity (µmol/l); time (min)	
	40	*90*	*40*	*90*	*40*	*90*
0	19.2 ± 0.1	21.25 ± 2.45	3022 ± 13.4	2931.7 ± 12	201.1 ± 16.5	199.5 ± 13
0.5	23.8 ± 0.2§	24.2 ± 1.2	3102.4 ± 12.4	3092.3 ± 12.3§§	206.1 ± 31.2	203.2 ± 15.2
1	28.8 ± 0.2§§	25.8 ± 2.8	3180 ± 22§	3132.5 ± 6.1§§	208 ± 18.4	204.5 ± 22.5
5	35 ± 1§§	37 ± 1.5§	3453.8 ± 33.8§§	3423.7 ± 18.1§§	223.6 ± 15.9§	229.5 ± 16.2§

Sildenafil was administered at doses of 0.5, 1 and 5 mg/kg by gavage. Determination of cAMP, cGMP and total antioxidant capacity were performed at 40 and 90 min post administration of sildenafil. Data are mean ± SEM of six animals in each group.
§ and §§ represent that the difference between treated and control (dose 0 mg/kg) is significant at $p < 0.05$ and $p < 0.01$, respectively.

hyoscine butyl bromide (three trials), mebeverine (five trials), otilium bromide (four trials), pinaverium bromide (two trials) and trimebutine (four trials) are superior to placebo for the global improvement of IBS and reducing pain [10]. Regarding the role of psychologic issues on IBS [1], positive effects of NO on psychologic stress has been proposed [24,25] and it is prudent to expect the same effect for sildenafil, which acts through the same pathway. NO is also involved in nociception and the analgesic property of sildenafil, especially on visceral smooth muscle has been reported [26–28]. Therefore, sildenafil might reduce visceral pain, a main complaint in IBS patients. The present results indicate that the inhibitory effect of sildenafil on small bowel transit, unlike gastric emptying, is dose dependent. The duration of action of sildenafil in the reduction of small bowel transit continued up to 90 min post administration of drug. This finding supports previous reports on the short duration of action of sildenafil on small bowel smooth muscle [13,29]. The present study demonstrated that reduction in fecal excretion by sildenafil continued up to 4 h after sildenafil administration. This supports the potential of sildenafil for reduction of large bowel motility. Unlike the small bowel, which responded well to all doses of sildenafil, only high doses of sildenafil affected large bowel motility. This seems to be due to the unselectivity of sildenafil for PDEs present in the large bowel.

Figure 2. Effects of different doses of sildenafil on rat fecal pellets excretion.



Sildenafil was administered at doses of 0.5, 1 and 5 mg/kg by gavage. Fecal pellets excretion was evaluated up to 4 h post administration of sildenafil. Data are mean ± SEM of six animals in each group.
§Difference between treated and control groups is significant at $p < 0.01$.

An imbalance between the activity of free radicals and TAP in inflammatory bowel diseases (IBDs) has been reported [30]. Although IBS has a different pathophysiology to IBD, the possibility of lipid-peroxidation in IBS has been suggested [31]. A parallel increase in TAP and cyclic nucleotides with decreased large intestine motility are in support of this suggestion. This means that lipid peroxidation may have a role in IBS that should be examined in further studies. Further, in supporting the present results, recent studies have confirmed that prevention of cyclic nucleotide catabolism by the use of PDE inhibitors cope with oxidative stress and decrease oxidative stress [32–34]. This is important and can be considered as a possible mechanism of action of sildenafil's positive effects in IBS observed in the present study.

Outlook

In conclusion, regardless of the mechanisms involved, the present data confirm positive effects of sildenafil in remission of rat IBS. Clinically, this would be promising in symptomatic therapy

Executive Summary

- Irritable bowel syndrome (IBS) is a disorder with unknown pathophysiology affecting approximately 10–15% of the general population.
- Sildenafil reduced gastric emptying, small bowel transit and fecal excrements while increasing intestinal levels of cyclic nucleotides (cAMP and cGMP) and total antioxidant capacity in experimental IBS rats.
- The beneficial effects of sildenafil on rat IBS may be due to its potential in increasing bowel total antioxidant capacity and cyclic nucleotides levels.
- Clinically, this would be promising in symptomatic therapy of IBS. However, there are some limitations such as the short duration of action on small bowel smooth muscle.
- Discovery of new agents acting by the same mechanism as sildenafil but with a high duration of action on small bowel smooth muscle and less unwanted effects, mainly reduction in blood pressure and gastric dyspepsia observed with sildenafil, would be a suitable option.

of IBS, but there are some limitations such as a short duration of action on small bowel smooth muscle, which has been proven herein. Therefore, discovery of new agents acting by the same mechanism, but with a high duration of action on small bowel smooth muscle and less unwanted effects, mainly reduction in blood pressure and gastric dyspepsia observed with sildenafil, would be a suitable option.

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Bibliography

1. Mayer EA, Naliboff BD, Chang L, Coutinho SV. Stress and irritable bowel syndrome. *Am. J. Physiol. Gastrointest. Liver Physiol.* 280, G519–G524 (2001).
2. Thompson WG, Irvine EJ, Pare P, Ferraz S, Rance L. Functional gastrointestinal disorders in Canada: first population-based survey using Rome II criteria with suggestions for improving the questionnaire. *Dig. Dis. Sci.* 47, 225–235 (2002).
3. Hungin AP, Whorwell PJ, Tack J, Mearin F. The prevalence, patterns and impact of irritable bowel syndrome: an international survey of 40,000 subjects. *Aliment. Pharmacol. Ther.* 17, 643–650 (2003).
4. Longstreth GF. Irritable bowel syndrome – a multibillion-dollar problem. *Gastroenterology* 109, 2029–2031 (1995).
5. Awad R, Dibildox M, Ortiz F. Irritable bowel syndrome treatment using pinaverium bromide as a calcium channel blocker. A randomized double-blind placebo-controlled trial. *Acta Gastroenterol. Latinoam.* 25, 137–144 (1995).
6. Everhart JE, Renault PF. Irritable bowel syndrome in office-based practice in the United States. *Gastroenterology* 100, 998–1005 (1991).
7. Koloski NA, Talley NJ, Boyce PM. Predictors of health care seeking for irritable bowel syndrome and nonulcer dyspepsia: a critical review of the lature on symptom and psychosocial factors. *Am. J. Gastroenterol.* 96, 1340–1349 (2003).
8. van Zanten SV. Diagnosing irritable bowel syndrome. *Rev. Gastroenterol. Disord.* 3, S12–S17 (2003).
9. Brandt LJ, Bjorkman D, Fennerty MB *et al*. Systematic review on the management of irritable bowel syndrome in North America. *Am. J. Gastroenterol.* 97, S7–S26 (2002).
10. Poynard T, Regimbeau C, Benhamou Y. Meta-analysis of smooth muscle relaxants in the treatment of irritable bowel syndrome. *Aliment. Pharmacol. Ther.* 15, 355–361 (2001).
11. Cartledge J, Eardley I. Sildenafil. *Expert Opin. Pharmacother.* 1, 137–147 (1999).
12. Boyce EG, Umland EM. Sildenafil citrate: a therapeutic update. *Clin. Ther.* 23, 2–23 (2001).
13. Bortolotti M, Lopilato C, Porrazzo G, Miglioli M. Effect of sildenafil on esophageal motility. *Gastroenterology* 118, A4770–A4770 (2000).
14. Bortolotti M, Pandolfo N, Giovannini M, Mari C, Miglioli M. Effect of sildenafil on hypertensive lower oesophageal sphincter. *Eur. J. Clin. Invest.* 32, 682–685 (2002).
15. de Rosalmeida MC, Saraiva LD, da Graca JR *et al*. Sildenafil, a phosphodiesterase-5 inhibitor, delays gastric emptying and gastrointestinal transit of liquid in awake rats. *Dig. Dis. Sci.* 48, 2064–2068 (2003).
16. Fritz E, Hammer J, Schmidt B, Eherer AJ, Hammer HF. Stimulation of the nitric oxide-guanosine 3',5'-cyclic monophosphate pathway by sildenafil: effect on rectal muscle tone, distensibility, and perception in health and in irritable bowel syndrome. *Am. J. Gastroenterol.* 98, 2253–2560 (2003).
17. Williams CL, Villar RG, Peterson JM, Burks TF. Stress-induced changes in intestinal transit in the rat: a model for irritable bowel syndrome. *Gastroenterology* 94, 611–621 (1998).
18. Kobayashi S, Ikeda K, Suzuki M, Yamada T, Miyata K. Effects of YM905, a novel muscarinic M3-receptor antagonist, on experimental models of bowel dysfunction *in vivo*. *Jpn J. Pharmacol.* 86, 281–288 (2001).
19. Iris F, Benzi F, Strain S. Ferric reducing antioxidant assay. *Method Enzymol.* 292, 15–27 (1999).
20. Izbeki F, Wittmann T, Csati S, Jeszenszky E, Lonovics J. Opposite effects of acute and chronic administration of alcohol on gastric emptying and small bowel transit in rat. *Alcohol* 36, 304–308 (2001).
21. Tache Y, Maeda-Hagiwara M, Turkelson CM. Central nervous system action of corticotropin-releasing factor to inhibit gastric emptying in rats. *Am. J. Physiol.* 253, G241–G245 (1987).
22. Glossmann H, Petrischor G, Bartsch G. Molecular mechanisms of the effects of sildenafil (V AGRA). *Exp. Gerontol.* 34, 305–318 (1999).
23. Carvajal JA, Germain AM, Huidobro-Toro JP, Weiner CP. Molecular mechanism of cGMP-mediated smooth muscle relaxation. *J. Cell Physiol.* 184, 409–420 (2000).
24. Suzuki E, Kageyama H, Nakaki T, Kuba S, Inoue S, Miyaoka H. Nitric oxide induced heat shock protein 70 mRNA in rat hypothalamus during acute restraint stress under sucrose diet. *Cell Mol. Neurobiol.* 23, 907–915 (2003)
25. Sanches DB, Steiner AA, Branco LG. Involvement of neuronal nitric oxide synthase in restraint stress-induced fever in rats. *Physiol. Behav.* 75, 261–266 (2002).

26. Luo ZD, Cizkova D. The role of nitric oxide in nociception. *Curr. Rev. Pain* 4, 459–466 (2000).
27. Abdollahi M, Nikfar S, Abdoli N. Potentiation by nitric oxide synthase inhibitor and calcium channel blocker of aspartame-induced antinociception in the mouse formalin test. *Fundam. Clin. Pharmacol.* 15, 117–123 (2001).
28. Asomoza-Espinosa R, Alonso-Lopez R, Mixcoatl-Zecuatl T, Aguirre-Banuelos P, Torres-Lopez JE, Granados-Soto V. Sildenafil increases diclofenac antinociception in the formalin test. *Eur. J. Pharmacol.* 418, 195–200 (2001).
29. Bortolotti M, Mari C, Lopilato C, La Rovere L, Miglioli M. Sildenafil inhibits gastroduodenal motility. *Aliment. Pharmacol. Ther.* 15, 157–161 (2001).
30. Jahanshahi G, Motavasel V, Rezaie A, Hashtroudi AA, Daryani NE, Abdollahi M. Alterations in antioxidant power and levels of epidermal growth factor and nitric oxide in saliva of patients with inflammatory bowel diseases. *Dig. Dis. Sci.* 49(11–12), 1752–1757 (2004).
31. Schempp H, Toth A, Weiser D, Elstner EF. Antioxidative properties of Iberis amara extracts in biochemical model reactions. *Arzneimittelforschung* 53, 568–577 (2003).
32. Abdollahi M, Chan TS, Subrahmanyam V, O'Brien PJ. Effects of phosphodiesterase 3,4,5 inhibitors on hepatocyte cAMP levels, glycogenolysis, gluconeogenesis and susceptibility to a mitochondrial toxin. *Mol. Cell Biochem.* 252, 205–211 (2003).
33. Abdollahi M, Fooladian F, Emami B, Zafari K, Bahreini-Moghadam A. Protection by sildenafil and theophylline of lead acetate-induced oxidative stress in rat submandibular gland and saliva. *Hum. Experim. Toxicol.* 22, 587–592 (2003).
34. Abdollahi M, Bahreini-Moghadam A, Emami B, Fooladian F, Zafari K. Increasing intracellular cAMP and cGMP inhibits cadmium-induced oxidative stress in rat submandibular saliva. *Comp. Biochem. Physiol. Toxicol. Pharmacol.* 135, 331–336 (2003).

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