Effect of Aerial Parts and Root Extracts from Four *Ferulago* Species on Erectile Dysfunction in Streptozotocin-Induced Diabetic Rats

Streptozotosin ile Oluşturulan Diyabetik Sıçanlarda Dört *Ferulago* Türünün Toprak Üstü ve Kök Ekstrelerinin Erektil Disfonksiyon Üzerine Etkisi

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Short Title: Effect of Extracts from *Ferulago* spp. on Erectile Dysfunction

ABSTRACT

Objectives: The extracts of *Ferulago* species are used as an aphrodisiacs in Turkey so we aimed to demonstrate *in vivo* and *in vitro* the relaxant effect of four *Ferulago* species extracts on corpus cavernosum (CC).

Materials and Methods: A total of 30 adult male Sprague-Dawley rats were divided into control and diabetic group which were induced by single intraperitoneal injection of 40 mg/kg of Streptozotocin. *In vivo* erectile responses were utilized by the stimulation of cavernosal nerves and repeated after intracavernosal injection of extracts in rats and datas were expressed as intracavernosal pressure (ICP)/ mean arterial pressure (MAP) and total ICP. The relaxant and contractile responses of CC strips were analyzed in the presence or absence of extracts.

Results: It was found that extracts were active both control and diabetic rats. The extracts (especially, methanol extract of root from *Ferulago bracteata*)-induced maximum relaxation responses (98.30 ± 2.6%) were decreased after incubation with L-NAME (44.8 ± 1.8). ODQ, soluble guanylate cyclase inhibitor inhibited 77% of extracts-induced maximum relaxation in CC from control rats.

Conclusion: In conclusion these species can be utilized in erectile dysfunction and may exhibit a herbal alternative to synthetic drugs.
Key words: Aphrodisiacs**,** Apiaceae, *Ferulago*, erectile function

ÖZET

Amaç: *Ferulago* türlerine ait ekstreler Türkiye'de afrodizyak olarak kullanılmaktadır, bu nedenle *in vivo* ve *in vitro* olarak dört *Ferulago* türüne ait ekstrelerin korpus kavernosum (CC) üzerindeki gevşetici etkisini göstermeyi amaçladık.

Gereç ve Yöntemler: Kontrol ve diyabetik gruba ayrılan toplam 30 yetişkin erkek Sprague-Dawley sıçanı, 40 mg/kg Streptozotocin ile intraperitonal olarak tek seferlik enjeksiyon ile indüklenmiştir. Kavernosal sinirlerin uyarılmasıyla *in vivo* erektil yanıtlar elde edildi ve sıçanlarda intrakavernozal ekstraktların enjeksiyonu sonrasında tekrarlandı ve veriler intrakavernozal basınç (ICP)/ortalama arteriyel basınç (MAP) ve toplam ICP olarak ifade edildi. CC striplerin gevşetici ve kasılma yanıtları, ekstraktların varlığında veya yokluğunda analiz edildi.

Bulgular: Ekstraktların hem kontrol hem de diyabetik sıçanlar üzerinde aktif olduğu bulundu. Ekstraktlar (özellikle *Ferulago bracteata* kök metanol ekstresi) ile maksimum gevşeme yanıtları (% 98.30 ± 2.6) L-NAME (44.8 ± 1.8) ile inkübasyondan sonra azalmıştır. ODQ, çözünebilir guanilat siklaz inhibitörü, kontrol sıçanlarından CC'de ekstraktların indüklediği maksimum gevşemenin% 77'sini inhibe ettiği görülmüştür.

Sonuç: Sonuç olarak bu türler erektil disfonksiyonda kullanılabilir ve sentetik ilaçlara karşı bitkisel alternatif oluşturabilir**.**
Anahtar kelimeler: Afrodizyak, Apiaceae, *Ferulago*, erektil disfonksiyon

INTRODUCTION

Diabetes is one of the most prevalent causes of erectile dysfunction (ED), which eminently influences the quality of life and the risk of developing ED in diabetic men is threefold higher than in healthy men.[1, 2] As compared with the other complications of diabetes, the development of ED begins at an earlier age. Moreover, the incidence and severity of ED increase with duration of diabetes[3] and multifactorial mechanisms including neurogenic and vasculogenic factors are involved in diabetic ED. The efficacy of some ED treatments

is limited for diabetes-associated ED. For example, men with diabetes frequently represent a poor response to first-line oral phosphodiesterase type 5 (PDE-5) inhibitors.[4] Alternative therapy choice may be phytotherapy for diabetic ED.

In this study, we purposed to demonstrate effect of the lyophilized aqueous and methanol extracts of *Ferulago* species growing naturally in Turkey on erectile tissue. In Turkey these species known as "Çağşır" or "Çakşır" and utilized conventionally as an aphrodisiac in South and Southeast Anatolia. Actually, many species that belong to *Ferulago*, *Prangos* and *Ferula* genera have been utilized for this aim. These species are utilized in rutting of goat and sheep, and besides the water decoctions of the roots and aerial parts are administered orally as aphrodisiacs.[5] In Turkey *Ferulago* species are usually well known for their aphrodisiac activities like various plants in other countries.[6] Apart from medicinal usage, they have been consumed as salad or spice due to their special odor, otherwise as food for goats and deer.[7]

Ferulago W. Koch. (Apiaceae) is represented 34 taxa in Turkey 19 of which are endemic. For this reason Anatolia is considered to be the gene center of this genus.[8] *F. blancheana* Post ex Boiss., *F. pachyloba* (Fenzl) Boiss. and *F. bracteata* Boiss. & Hausskn. and are endemic perennial species, growing only in Kayseri-Central Anatolia, Niğde-Central Anatolia and Gaziantep-Southeastern Anatolia, Turkey respectively, but *F. trachycarpa* Boiss. is not an endemic species, growing in Antalya.[9] During our studies, we have found that aqueous and methanol extracts of roots and aerial parts from *Ferulago* species produced relaxation in precontracted rat corpus cavernosum.Thereby, we attributed to investigate pharmacological profile of their relaxant effect by using isolated corpus cavernosum tissue *in vivo* and *in vitro*. This study aims to give first report to evaluate the effect of extracts from *F. blancheana*, *F. pachyloba*, *F. trachycarpa* and *F. bracteata* on ED of streptozotocin (STZ)-induced diabetic rats.

MATERIALS AND METHODS

Plant material

Flowering plants of *Ferulago blancheana, F. pachyloba, F. trachycarpa* and *F. bracteata*, were collected in 2014 from Kayseri, Niğde, Antalya and Gaziantep (Turkey), respectively and identified by Prof. Dr. Hayri Duman, a plant taxonomist in the Department of Biology, Faculty of Science, Gazi University. The voucher specimens are kept in the Herbarium of Ankara University, Faculty of Pharmacy (Herbarium numbers are AEF 26673, AEF 26674, AEF 26677 and AEF 26676, respectively).

Extraction

Air-dried roots and aerial parts of these species were powdered and macerated three times with methanol for 8 hours in a water bath not exceeding 45°C (3 × 200 mL) using a mechanical mixer at 300 rpm, separately. The extracts were filtered and concentrated till dryness by rotary evaporator (Heidolph VV2000, Germany). In other respects, 50 g of roots and aerial parts from these plants were grounded and macerated with 200 mL of distilled water for 8 h/3 days at 30 to 35°C, separately. Aqueous extract was filtered, freezed (Sanyo Medical Freezer, Germany) and lyophilized (Christ® Gamma 2-16 LSC, Germany) to give aqueous extracts from roots and aerial parts. Amounts of the powdered plants and obtained extracts are given in Table 1.

Animals

Adult male Sprague-Dawley rats (350-400g) received a dose of streptozotocin (STZ, 40 mg/kg, i.p.) within a citrate buffer (pH= 5.5) at the day of usage.[10] Measurement of blood glucose levels was carried out using an Accu-Chek glucometer (Roche Diagnostics, Indianapolis, IN) after that induction of diabetes. The animals were housed in separate cages on a 12-h light–dark cycle and were fed standard water and chow ad libitum. This study was confirmd by the Institutional Animal Care and Use Committee of Ankara University (2014-15-86).

In Vivo Assessment of Erectile Function

To measure *in vivo* assessment of erectile function, intracavernosal pressure (ICP, mmHg) was monitored in rats. Therefore, the rats were anesthetized with ketamine (50mg/kg, i.p.) and the trachea was cannulated (polyethylene, [PE]-240 tubing) to keep up the airway, and the carotid artery was cannulated (PE-50 tubing) to measure the main arterial pressure (MAP, mmHg), by a transducer (Statham, Oxnard, CA) attached to a data acquisition system (Biopac MP 100 System, Santa Barbara, CA). A 25-gauge needle filled with 250U/ml of heparin and connected to polyethylene-50 tubing was place in the penis right crura connected to a pressure transducer to measure indissolubly ICP. The right major pelvic ganglion and cavernosal nerve (CN) were represented. A stainless-steel bipolar hook electrode for stimulation was installed around the CN postero-lateral to the prostate on one side, and the MAP (mmHg) and ICP (mmHg) were indissolubly measured with pressure transducers. The CN was stimulated (2.5, 5, and 7.5 V, 15 Hz, 30 s train duration) with a square pulse stimulator (Grass Instruments, Quincy, MA) and electrical stimulation was inducted distally to the ligature. The measurements were recapped after intracavernosal administration of extracts (1µM) in groups[10].

Isometric Tension Measurements

Cavernosal tissue (CC) strips were installed in organ bath chambers and maintained in Krebs-bicarbonate solution (containing, mM: KCl; 4.7, NaCl; 118.1, $MgSO_4$; 1.0, KH_2PO_4; 1.0, $NaHCO_3$; 25.0, glucose; 11.1 and CaCl; 22.5, pH:7.4). The strips (1 × 1 × 9 mm3) were dissected and combined under 1 g of resting tension in a 20-ml organ bath. The organ chamber temperature was kept going at 37°C by a circulating water bath and enduring bubbling with a mixture of 95% O_2, 5% CO_2. The tissues were permitted to equilibrate for a minimum of 60 minutes, and the bath solution was changed place every 15 minutes. Electrical field stimulation (EFS) of the autonomic nerves (duration: 15 seconds; amplitude: 50-90 V; frequency: pulse width: 5 ms) was consummated by the use of platinum electrodes, emplaced on the either side of the tissue strip (Grass Instruments, Quincy, MA).

In the first series of trials, CC strips were precontracted with phenylephrine (Phe, 10^{-5} M) and allowed to relax after administration of the extracts. The relaxation response curves to the extracts were also acquired in the presence of the nonspecific nitric oxide synthase (NOS) inhibitor, L-NAME (L-N(G)-Nitroarginine Methyl Ester, 100μM) and soluble guanylate cyclase inhibitor, ODQ (1H-[1,2,4]-oxadiazolo[4,3-a] quinoxaline-1-one, 30μM).

In the second series of trials, acetylcholine (ACh), EFS, sildenafil and sodium nitroprusside (SNP)induced relaxation responses were stimulated after precontraction of CC strips with Phe (10^{-5} M) in the presence or absence of the extracts (100 μM).

Statistical Analysis

All results are expressed as mean ± SE and differences between means were statistically analyzed using One-way analysis of ANOVA followed by Bonferroni's complementary analysis, with $P < 0.05$ considered to indicate statistical significance. At the end of the experiment, each CC strip was weighed. All contractile responses were expressed as mg of tension developed per mg of corporal tissue and relaxant responses were calculated as a percentage of Phe-contraction.

Drugs

All drugs were purchased from Sigma Chemical Co (St. Luis, MO).

RESULTS

Extraction

Methanol and lyophilized aqueous extracts of the roots and aerial parts from *Ferulago* species were evaluated for their effect on erectile dysfunction.

Characteristics of Animals

Body weight in diabetic rats was considerably lower than in control rats (fig. 1A, P<0.001). Blood glucose levels in the diabetic group were considerably higher than in the control group (fig. 1B, P<0.001).

In vivo Erectile Responses in Both Groups

ICP/MAP values in control rats were higher than in diabetic rats (P<0.001; fig. 2), which was returned by intracavernosal administration of the extracts (1μM). Moreover, total ICP values were decreased in the diabetic group contrasted with the control group (P<0.001; fig. 2). After that the intracavernosal administration of the extracts (1μM) total ICP values were restored in the diabetic group at all voltage levels, except for 7.5 voltage level (fig. 2).

In vitro Responses of CC Strips

The extracts (especially, methanol extract of root from *Ferulago bracteata*)-induced maximum relaxation responses (98.30 ± 2.6%) were decreased after incubation with L-NAME (44.8 ± 1.8, Fig. 3A). ODQ, soluble guanylate cyclase inhibitor inhibited 77% of extracts-induced maximum relaxation in CC from control rats (fig. 3).

The endothelial-dependent relaxation response to ACh (1 mM) in control rats was higher than in diabetic rats, which was increased after the incubation of the extracts (100 μM) in control and diabetic groups (fig4).

EFS-induced relaxation response at 20 Hz was decreased in the diabetic group contrasted with the control group, which was restored by the incubation with the extracts (100μM). There was no difference in EFS-induced relaxation response in control rats between the presence and absence of the extracts (fig. 5).

SNP- induced endothelial-independent relaxation response at 0.1 μM dose relaxation was not changed in control rats when compared with diabetic rats (fig. 6). Though, relaxation responses to SNP were enhanced in the presence of the extracts (100μM) in diabetic and control rats.

PDE-5 inhibitor, sildenafil-induced relaxation response at 1μM dose was considerably reduced in diabetic rats when compared with control rats (Fig. 4D). After incubation of the extracts (100μ), relaxation responses to sildenafil were augmented in diabetic and control rats (fig.7).

DISCUSSION

In this study, we aimed to perform the relaxant effect of methanol and lyophilized aqueous extracts of roots and aerial parts from *Ferulago blancheana, F. pachyloba, F.*

trachycarpa and *F. trachycarpa* in corpus cavernosum with *in vivo* and *in vitro* studies. Corporal smooth muscle relaxation plays a significant role in erection. Smooth muscle relaxation, which is interceded by nitric oxide (NO) throughout sexual stimulation, is synthesized in nerve terminals of parasympathetic noncholinergic and nonadrenergic nerves in the penis and besides by the endothelial cells lining blood vessels and lacunar spaces of corpus cavernosum.[11]

The first data provides basic mechanistic information concerning the extracts induced dose-dependent relaxation in rat CC. The major findings of the study show that (i) the extracts relaxes rat CC in a concentration-dependent manner; (ii) the NO-cGMP pathway plays a important role in mediating extract-induced relaxation; and (iii) they partially restored *in vivo* erectile function in diabetic rats.

Penile erection in response to CN stimulation was made firm *in vivo* in a diabetic animal model. Our data showed that diabetes reduced the *in vivo* erectile response and the *in vitro* relaxant response of CC to EFS. Amazingly, erectile responses (ICP/MAP and total ICP) gained after cavernous nerve stimulation except 7.5 V were augmented in the extract-injected diabetic group, as compared with the vehicle-injected diabetic group. In *in vitro* studies, the nitrergic relaxation response to EFS in diabetic rats was augmented by the incubation of extracts. There is no previous data to evaluate the effect of these species on erectile function. Though, the extracts treatment reduced in the diabetes-induced renal damage related to the diabetic nephropathy.[12] Moreover, the treatment improved the activities of enzymatic and non-enzymatic antioxidants,[13] also *in vitro* augmented the glycolytic activities.[14] These results proposes a rationale for more studies using combinations of the extracts and phosphodiesterase-5 inhibitors in diabetes-induced ED.

The present study showed that extract-induced relaxation in CC from the diabetic group was not changed when contrasted with CC from the control group. The data support to the intracavernosal administration of the extracts augmented erectile responses. It seems that the extracts responses serve as the normal activity *in vivo* and *in vitro* under diabetic provisions. Moreover, relaxation to the extracts were calmly inhibited after pre-contraction with KCl. Potential sensitive calcium channels are forced by depolarization of the plasma membrane when the extracellular K^{+1} concentration is augmented. The reduced response by the extracts in high K^{+1} medium indicating that relaxation to they do not important alteration by a calcium channel antagonistic property.

In the current study, we researched the underlying mechanism of the extracts effects on erectile responses that can be mediated by NO/cGMP-dependent pathway

which is damaged in diabetic stipulations. No earlier study appears on the mechanism of the extracts in penile tissue. The extracts are most likely to have a role for the NO-cGMP signaling pathway in mediating CC relaxation responses.

In the isolated CC from the diabetic group, endothelium-dependent relaxation response to ACh was considerably reduced which was potentialized in the presence of the extracts. There is no heretofore supporting data similar to these findings.

There was not the difference between the endothelial-independent relaxation response to SNP in control and diabetic rats, which was enhanced in groups after the incubation of extracts. In previous studies, SNP-induced relaxant responses did not change in diabetic rats when contrasted with controls. [15,16]

In the study, relaxation responses to PDE-5 inhibitor sildenafil in CC strips were decreased in diabetic rats contrasted with control rats. There was no difference relaxant response to sildenafil between contol and diabetic rats CC after incubation of the extracts. The finding has offered that these species have a potential effect on penile function by means of various pathways to contribute erectile function of diabetic rats.

As shown in Figure 1 among the extracts methanol extracts of roots (especially roots of *F. bracteata*) showed the best activity. On the other hand lyophilized aqueous extracts of aerial part (especially *F. blancheana*) showed the worst activity. EFS relaxation responses decreased from 40% at controls rats, to 3% with diabetes rats. However, as a result of a 15-minute incubation of the extracts, the EFS relaxation responses increased to 21%. Similarly, acetylcholine relaxation responses decreased from 38% at controls to 13% with diabetes. However, as a result of a 15-minute incubation of the extracts, acetylcholine relaxation responses were increased by 40% and higher than control groups. Sildenafil relaxation responses were 92% at controls and 74% with diabetes though, as a result of a 15-minute incubation of the extracts, acetylcholine relaxation responses were increased by 95% and higher than control groups. SNP relaxation responses were 90% at controls and 85% with diabetes. Though, as a result of a 15-minute incubation of the extracts, acetylcholine relaxation responses were increased by up to 94% and higher than control groups. The results are shown at fig. 1-7.

CONCLUSION

The study primarily revealed that the useful effect of intracavernosal administration of the extracts in improving erectile function in diabetic rats which is dependent on NO/cGMP pathway. The preclinical findings should extend our information of the prospering effect of the extracts on penile function to develop preventive or therapeutic agents and

combinations of them and phosphodiesterase-5 inhibitors may be a beneficial option for diabetes-induced ED.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Table 1. Amounts of the Powdered Plants and Obtained Extracts.

Species	Used parts	Powdered (g)	MeOH (g)	Lyophilized Aqueous (g)
F. blancheana	root	50	6.62	5.78
	aerial part	50	3.22	4.78
F. pachyloba	root	50	7.25	6.98
	aerial part	50	3.32	4.01
F. trachycarpa	root	50	6.77	7.76
	aerial part	50	3.41	3.67
F. bracteata	root	50	7.94	5.99
	aerial part	50	3.65	4.88

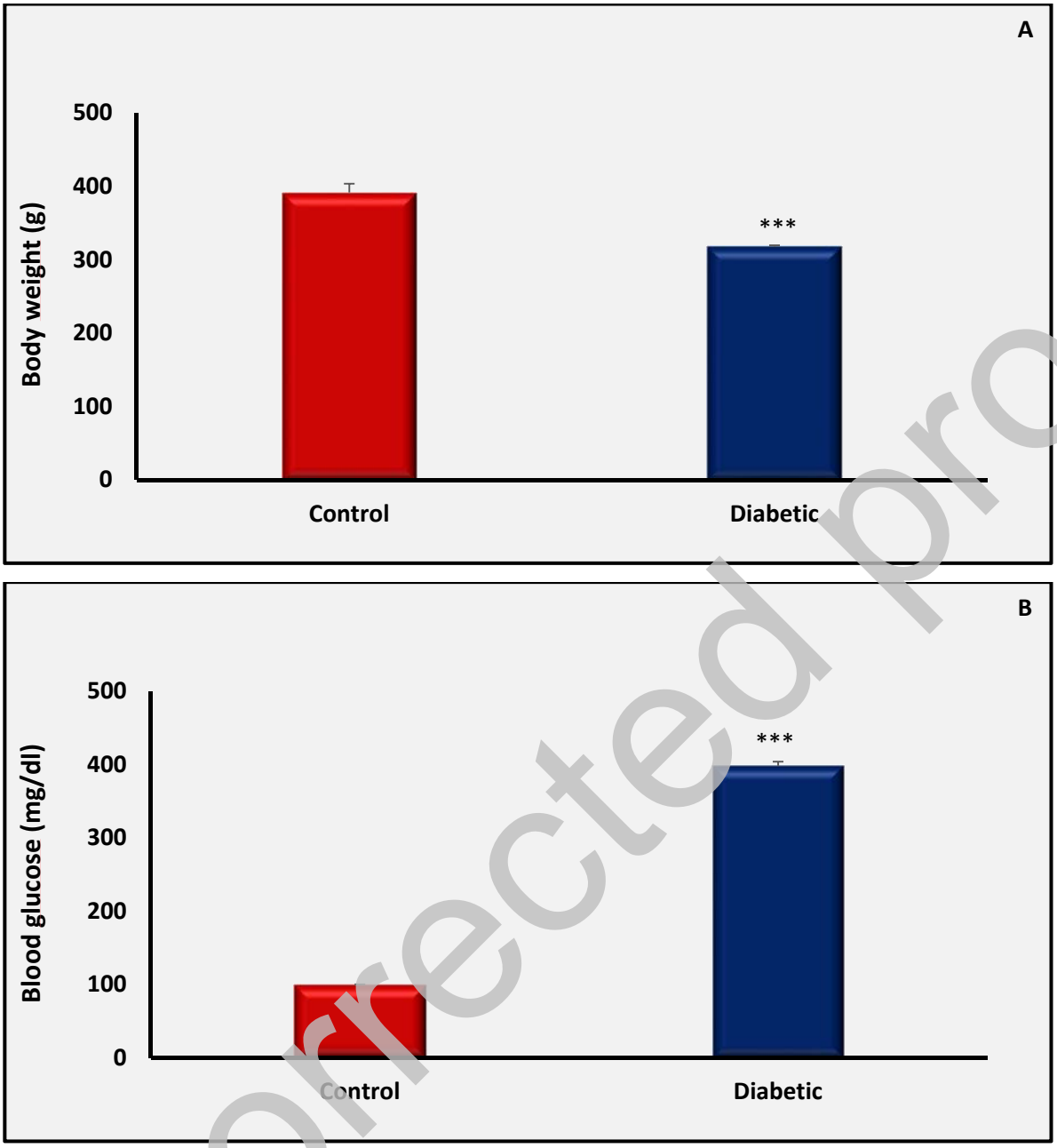

Figure 1. Bar graph showing body weight of control and diabetic groups (A) and glucose levels (B) in control and diabetic groups. Data are mean±SEM (n=6) and ***p<0.001 vs control.

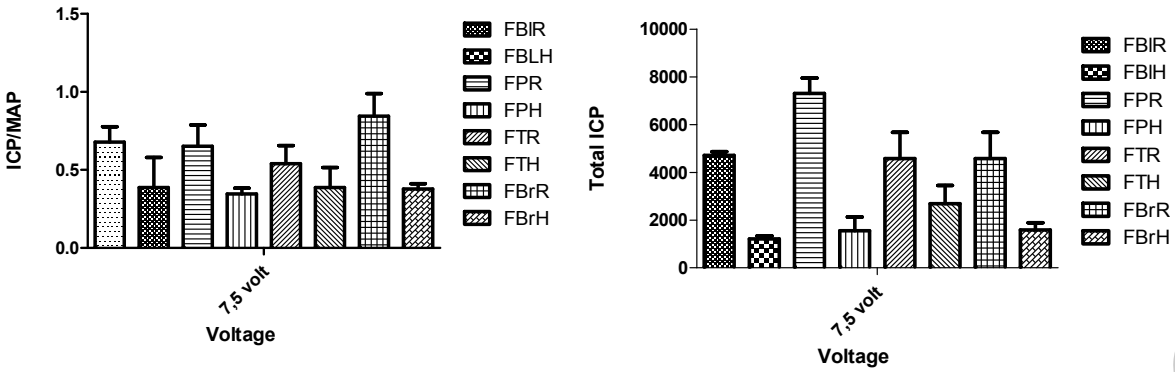

Figure 2. *In vivo* intracavernosal effect of extracts from roots on control and diabetic rat penile erection. Bar graphs showing ICP/MAP total ICP. Data represent mean ± SEM of 6–8 observations (p=0.1413). (FBlR: Root of F. *blancheana*, FBlH: Herba of *F. blancheana*; FPR: Root o *F. pachyloba*, FPH: Herba of *F. pachyloba*; FTR: Root of *F. trachycarpa*, FTH: Herba of *F. trachycarpa*; FBrR: Root of *F. bracteata*, FBrH: Herba of *F. bracteata*).

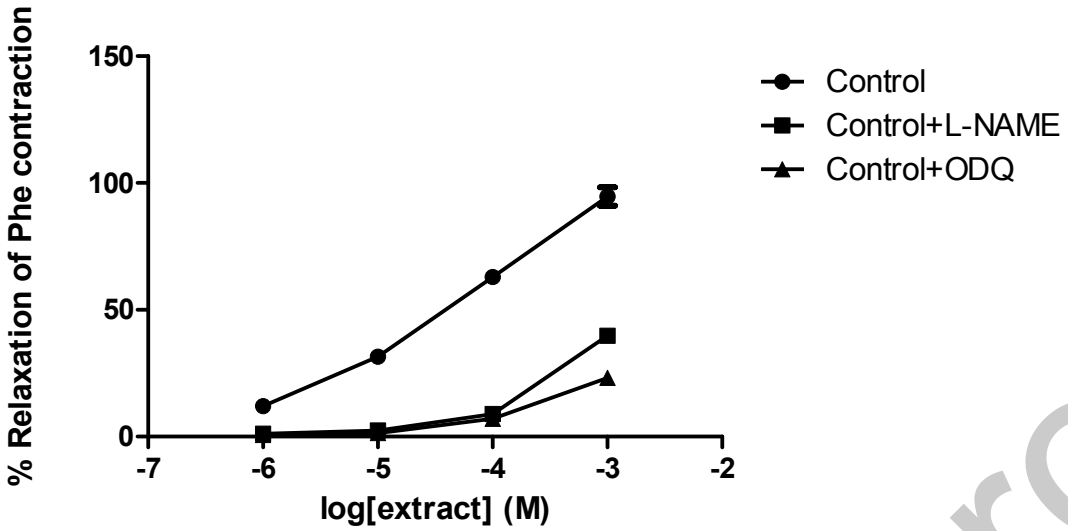

Figure 3. Concentration–response curves to extract (10^{-6}-10^{-3} M) in corpus cavernosum after pre-contraction with phenylephrine (Phe, 10μM) in the presence of L-NAME (100μM, A) and ODQ (30μM, B). Data represent mean ± SEM of 6–8 observations. ****P* <.001 vs control value.

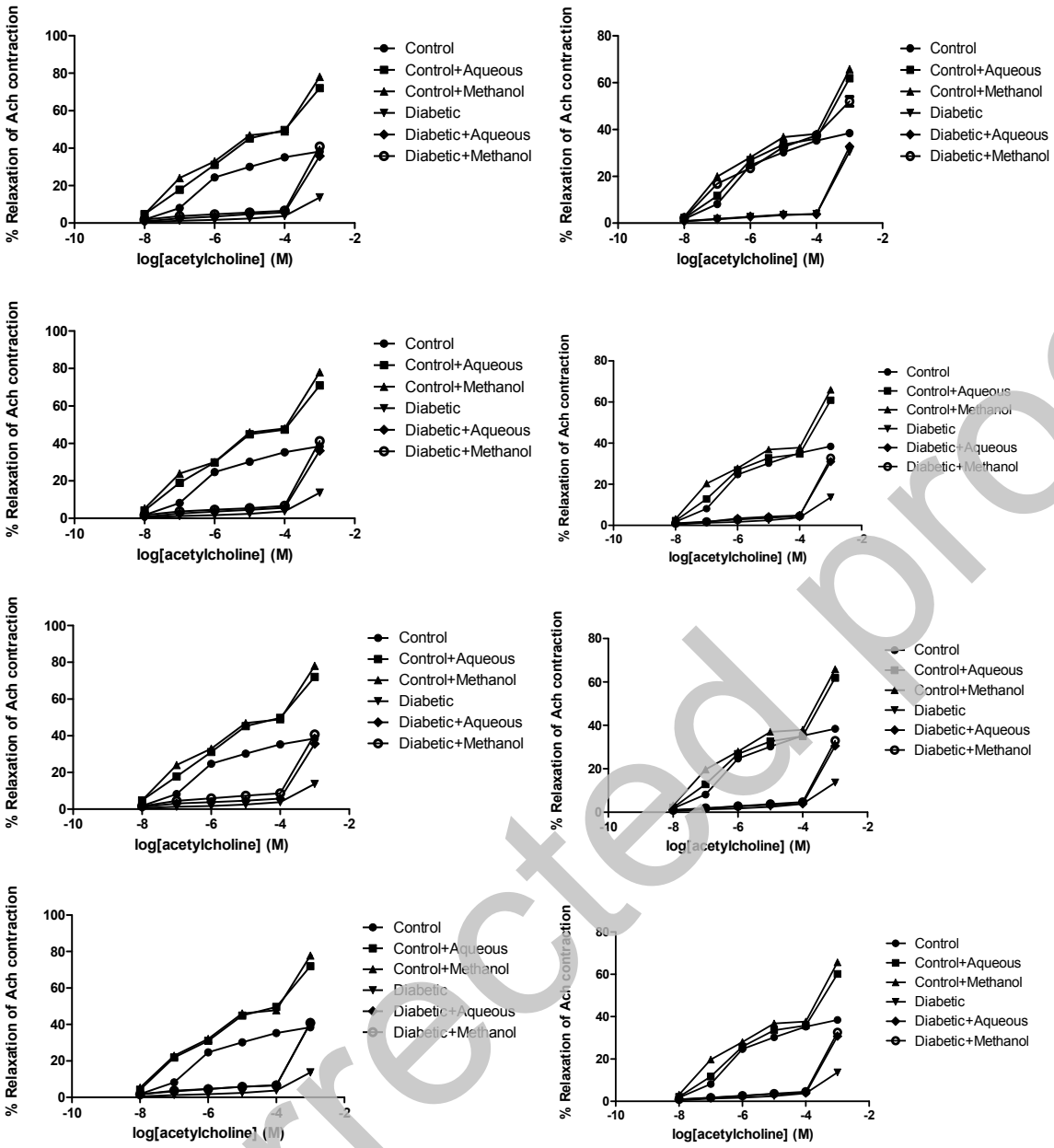

Figure 4. Relaxation responses to single doses of ACh in the presence of extract of FBIR, FBlH, FPR, FPH, FTR, FTH, FBrR and FBrh, respectively. Data represent mean ± SEM of 6–8 observations. **P* < .05, ****P* <.001 vs control value. § *P* < .05, §§ *P*<.01 vs. diabetic value.

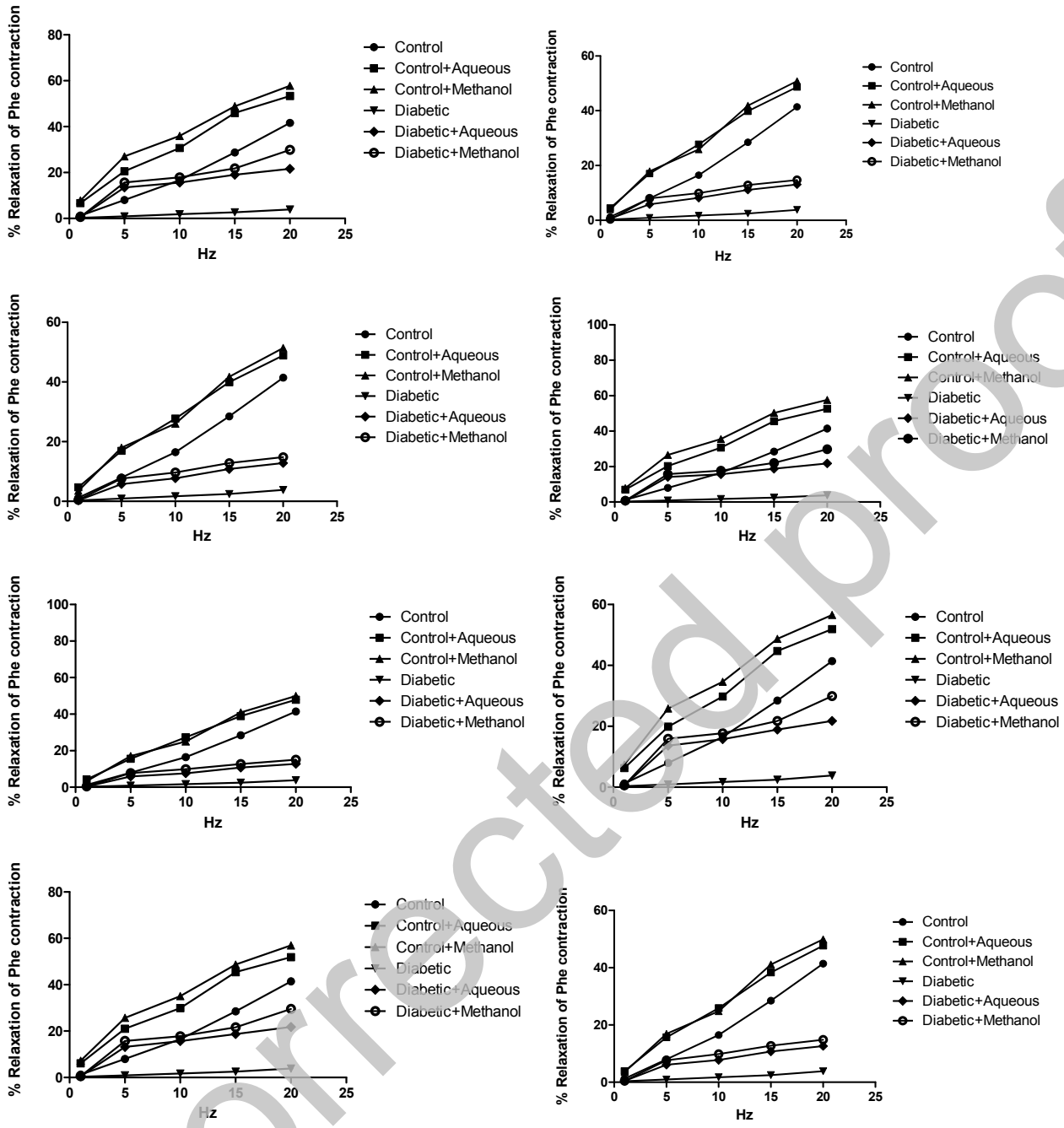

Figure 5. Relaxation responses to single doses of EFS in the presence of extract of FBlR, FBlH, FPR, FPH, FTR, FTH, FBrR and FBrh, respectively. Data represent mean ± SEM of 6–8 observations. *P < .05, ***P <.001 vs control value. § P < .05, §§ P<.01 vs. diabetic value.

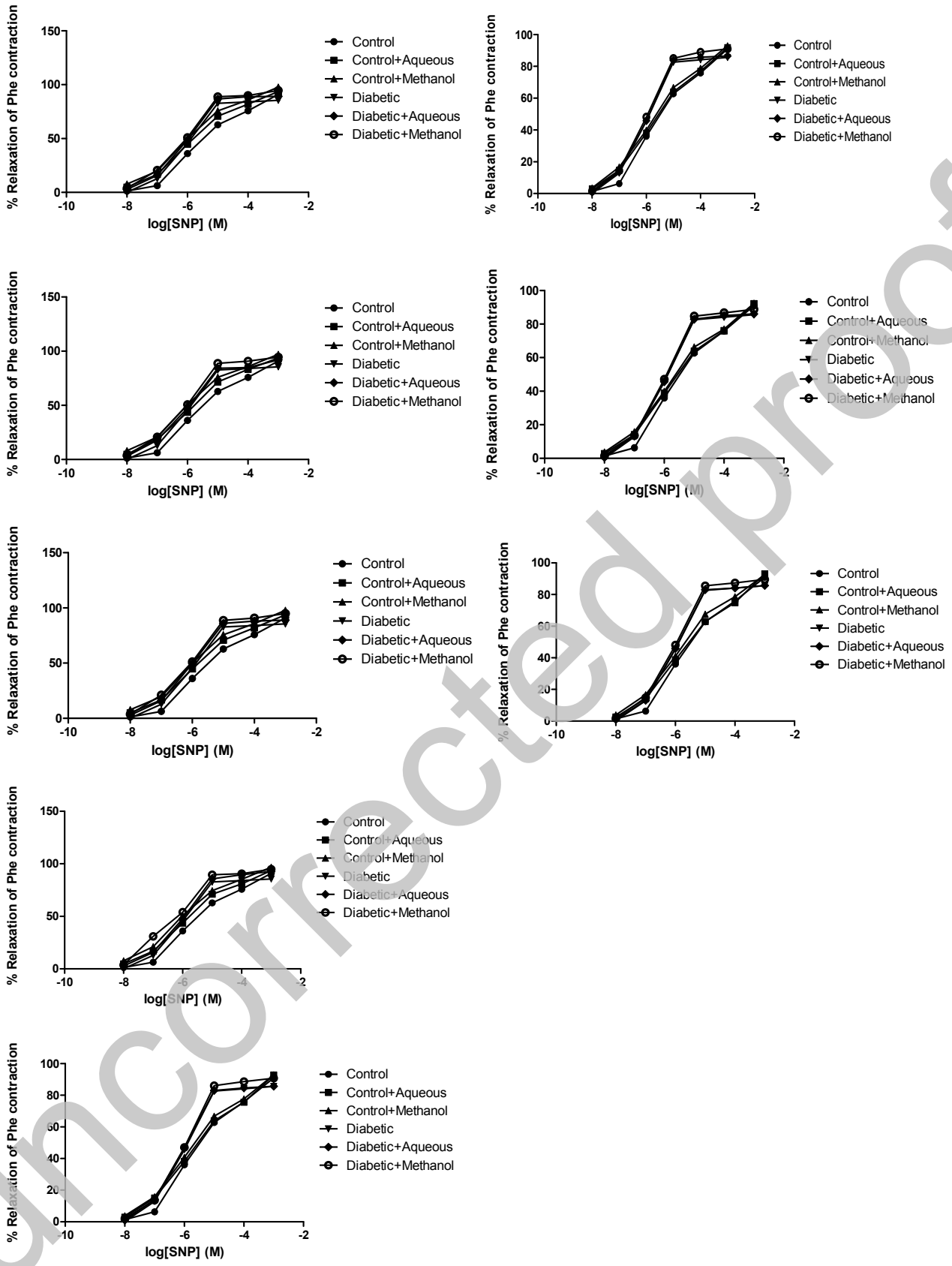

Figure 6. Relaxation responses to single doses of SNP in the presence of extract of FBIR, FBlH, FPR, FPH, FTR, FTH, FBrR and FBrh, respectively. Data represent mean ± SEM of 6–8 observations. *P < .05, ***P <.001 vs control value. § P < .05, §§ P<.01 vs. diabetic value.

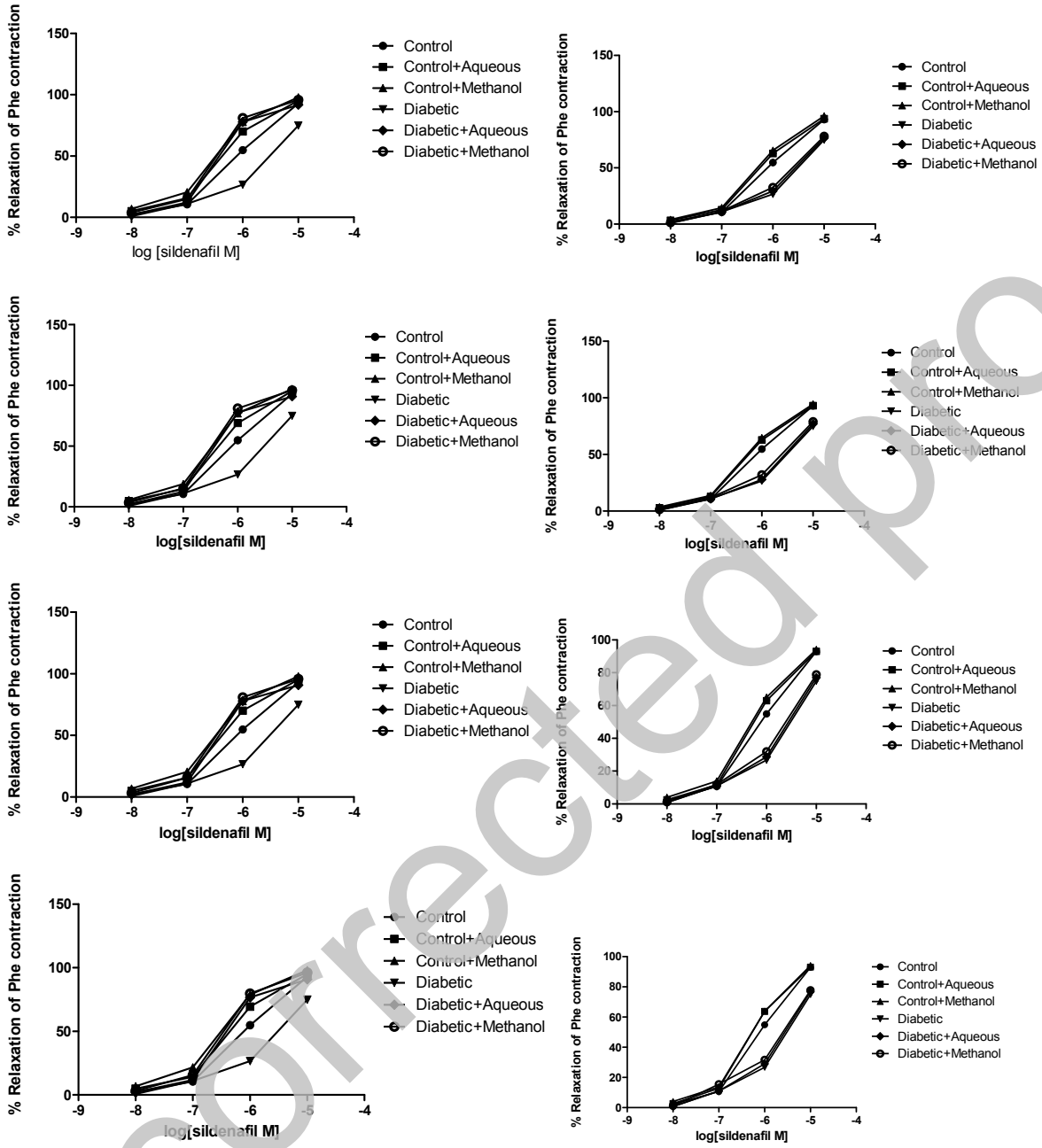

Figure 7. Relaxation responses to single doses of Sildenafil in the presence of extract of FBlR, FBlH, FPR, FPH, FTR, FTH, FBrR and FBrh, respectively. Data represent mean ± SEM of 6–8 observations. *P < .05, ***P <.001 vs control value. § P < .05, §§ P<.01 vs. diabetic value.