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Original Research Article

Alkaloids from *Peganum harmala* attenuated contractile responses of vascular smooth muscle cells

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Abstract

Purpose: *To investigate the contractile responses of vascular smooth muscle cells (VSMCs) to spasmogens after incubation with harmaline, harmine, and harmalol, which are alkaloids obtained from Peganum harmala L., a member of the Zygophyllaceae family.*
Methods: *Contractile responses of VSMCs to norepinephrine (NE; 1 µmol/L) and potassium chloride (KCl; 60 mmol/L) were recorded in rat aortic ring preparations pre-incubated with 0.5, 1, 5 and 10 µmol/L of each alkaloid for 15 min. Responses were expressed as mean values of contractions in incubated preparations, relative to the recorded tension prior to treatment with alkaloids.*
Results: *Pre-incubation with harmaline at concentration of 10 µmol/L significantly reduced contractile responses to NE by 69.0 ± 3.0 % ($p < 0.00002$), and decreased KCl-induced contraction by 34.0 ± 9.0 % ($p < 0.05$). Harmalol was the most effective in inhibiting contractions to KCl (48.0 ± 9.0 %, $p < 0.01$). However, harmalol produced relatively moderate inhibitory effects on NE-induced contractions (46.0 ± 4.0 %, $p < 0.005$), followed by harmine (52.0 ± 8.0 %, $p < 0.02$), but it did not significantly affect contractile responses to KCl.*
Conclusion: *These results highlight the differential effects of pre-incubation with alkaloids from P. harmala and their potential effects on the prevention of VSMC spasms induced by either chemicals or stimuli that change the membrane potential.*

Keywords: *P. harmala, Harmaline, Harmalol, Harmine, Alkaloids, Vascular smooth muscle cells, Contractile response*

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INTRODUCTION

Hypertension is considered a serious medical condition that affects about 20 - 25 % of the world population, and it significantly increases the risks for heart, kidney, brain and other diseases [1]. The World Health Organization (WHO) has stated that cardiovascular diseases (CVDs) are leading causes of death worldwide, with higher incidents in developed countries, and

some of the identified pre-disposing risk factors are tobacco use, inappropriate diet, hypertension and diabetes [2]. Cardiovascular diseases (CVDs) are often associated with functional disorders related to motor activity of VSMCs. Thus, VSMCs play a major role in integration of responses involved in the regulation of vascular tone, peripheral resistance and blood pressure [3]. Therefore, targeting VSMCs by relaxant compounds may improve cardiovascular functions and reduce risk factors associated with various diseases related to hypertension.

It has been postulated that many plant extracts, alkaloids and non-alkaloidal compounds affect the contractility of VSMCs by interfering directly or indirectly with contractile mechanisms of smooth muscle cells (SMCs) or by pertubing sarcolemna ionic channels, thereby inducing vasodilation and improving cardiovascular functions [4].

One of the most available plants which is widely distributed in the Middle East, Central Asia and Northern Africa is *Peganum harmala L*. The plant belongs to the Zygophyllaceae family. The seeds and roots of *P. harmala* are rich in β-carbolines such as harmine, harmaline, harman and harmalol [5]. It has been reported that *Peganum harmala* exerts broad pharmacological effects such as amelioration of cardiovascular, gastrointestinal and respiratory problems, as well as possessing antioxidant, antimicrobial, antiviral, antihelmintic, antifungal, antineoplastic properties and pain-relieving potential [5,6]. In previous studies, the anti-spasmogenic activities of ethanolic extract of *P. harmala* and its alkaloids on intestinal SMCs, were demonstrated [7,8].

In humans, beside other symptoms, hypotension has been reported in case studies after intoxication due to ingestion of more than 50 *g* of *P. harmala* seeds [9]. Attempts to elucidate the pharmacological effects of the bioactive compounds isolated from *P. harmala* on cardiovascular parameters started early in the last century. *Peganum harmala* contains the alkaloids harmaline, harmine, and harmalol, in addition to other phytoconstituents. Aaron and his colleagues found that the administration of harmine reduced systemic blood pressure and peripheral vascular resistance in dogs [10]. Later studies have suggested the mechanisms underlying the vasorelaxation induced by harmalol, harmaline and harmine. These studies demonstrated alkaloid-induced effects on the release of nitric oxide (NO) from endothelial cells, and/or direct inhibition of VSMCs contractions by interfering with receptor mediated or voltage-induced contractile responses. The effects of these alkaloids on the activity of calcium (Ca^{2+}) channels and activation of inhibitory mechanisms involved in relaxation have also been demonstrated [11-13]. These studies have shown differences in mechanisms through which these alkaloids from the same plant induce relaxation in pre-contracted preparations.

The current study was conducted to evaluate the inhibitory potential of harmaline, harmine and harmalol on KCl- and NE-induced contractile responses of rat thoracic aorta preparations, and to assess the percentiles of inhibition in pre-treated preparations.

EXPERIMENTAL

Preparation of bioactive compounds

Harmaline, harmine, and harmalol (Sigma Chemicals Co. (USA) were dissolved in 5% aqueous dimethyl sulphoxide (DMSO). Two concentrations were prepared as stock for each alkaloid (10^{-3} and 10^{-4} M). From these stocks, 75 or 150 µL was added to the tissue bath.

Animals and preparation of aortic rings

Male Wistar rats weighing 250 – 350 g were obtained from the animal house of The University of Jordan. The rats were kept under automatically controlled temperature conditions (23 - 25 °C) in an atmosphere with a 12-h light/12-h dark photoperiod, and were allowed free access to standard feed and water. All animal experiments were approved by the Research and Ethics Committee of The University of Jordan (approval no. SRF/JU/13-14:218) and conducted in line with *the Regulations and Ethical Guidelines for the Care and Use of Laboratory Animals* of The University of Jordan.

To obtain aortic rings, the rats were handled according to the suggested ethical guidelines for the care of laboratory animals so as to minimize pain and discomfort. On the day of the experiment, rats fully anesthetized with ether-soaked cotton balls in a closed container were sacrificed and thereafter their chest cavities were opened up [14]. The thoracic aorta was dissected out, freed of fat and connective tissue, and cut into 3 - 4 mm long ring segments.

The isolated thoracic aortic rings were suspended in an organ bath containing Krebs buffer consisting of NaCl (118.1 mM), KCl (4.7 mM), $CaCl_2$ (2.5 mM), $MgSO_4$ (2.5 mM), KH_2PO_4 (1.2 mM), $NaHCO_3$ (25 mM) and glucose (11

mM), with pH adjusted to 7.4. The preparation was maintained at 37 °C with a bubbling gas mixture of 95 % O_2 and 5 % CO_2 [13].

Assessment of isometric tension

Isometric tension was recorded using TRI201AD isometric force transducers (Panlab) with computerized data acquisition system (PowerLab 8/30, AD Instruments International). Before the start of experiment, all preparations were allowed to equilibrate for at least 30 min under a resting tension of 2 g [11].

To investigate the inhibitory effects of harmaline, harmine and harmalol, alkaloids were added directly to the organ bath in volumes usually not exceeding 1 % of the bath volume (approximately 15 mL). Tension was recorded in the preparation stimulated twice with 60 mM KCl before treatment. Then, after washing, contraction was evoked once, either with 1 µM norepinephrine or with 60 mM KCl, and the tension recorded without alkaloid treatment served as control [8,11].

After washing, the aortic preparations were incubated for 15 min with various concentrations of harmaline, harmine, or harmalol (0.5, 1, 5 and 10 µM). A 20 min-washing step between treatments was applied at least twice after inducing contractions with each of the spasmogens. To get the final concentration of each of alkaloids; 75 or 150 µl of the appropriate stock was added to tissue bath. Contractile responses to NE or KCl were recorded as tension in grams after treatment with various concentrations of each alkaloid (n = 5 - 7).

Statistical analysis

The results are presented as mean ± standard error of the mean (SEM). Percentiles of contractions for each experiment in treated alkaloid groups were calculated relative to the control of each experiment. Paired-sample statistical analyses were performed with *t*-test using Microsoft Excel 10 worksheet. Values of *p* for contractile responses between concentration groups were obtained using two-tail analysis for recorded tensions in alkaloid treated groups, relative to control tension induced by the last application of spasmogen before addition of alkaloids. Moreover, two-tail analyses of variance for *p*-values of percentiles of inhibition were done between concentration groups for each alkaloid. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Contractile responses evoked by 60 mM KCl and 1 µM NE were evaluated after incubation of aortic preparation with 0.5, 1, 5 and 10 µM harmaline, harmine and harmalol. The results showed different inhibitory effects on VSMCs responses from rat aorta to either KCl or NE after 15 min of incubation with harmaline, harmine or harmalol.

Effect of alkaloids on KCl-induced contractions

Figure 1 A shows that incubation of aortic ring preparation with 10, 5, and 1 µM harmaline led to reduced responses to KCl, with significant differences in percentiles of contractions (66.0 ± 9.0, 60.0 ± 5.0 and 74.0 ± 4.0 %), relative to control (100 % tension recorded after the first application of KCl without alkaloid; $p < 0.05$, *vs*. 10 µM harmaline; $p < 0.01$, *vs*. 5 µM harmaline; $p < 0.02$, *vs*. 1 µM harmaline). However, no significant differences were observed in percentiles of contractions on incubation with 0.5 µM of each alkaloid (84.0 ± 10.0 % *vs*. control).

Preparations incubated with 10, 5, 1 and 0.5 µM harmine also showed lower contractile responses to KCl than control, and the percentiles of contractions were 71.0 ± 20.0, 74.0 ± 15.0, 86.0 ± 16.0 and 89.0 ± 12.0%, respectively (Figure 1 B). Nevertheless, no significant differences were observed between recorded tension and control tension in preparations incubated with any concentration of harmine.

However, as shown in Figure 1 C, preparations incubated with harmalol produced concentration-dependent decreases in contractile responses to KCl (90.0 ± 4.0, 85.0 ± 4.0, 72.0 ± 9.0 and 52.0 ± 9.0 % after incubation with 0.5, 1, 5 and 10 µM harmalol, respectively). The decrease was significant in all preparations after incubation with the indicated concentrations, when compared to control ($p < 0.05$, $p < 0.03$, $p < 0.03$, $p < 0.01$, respectively). Moreover, the differences in contractile responses between the highest and all lower concentrations of harmalol, were significant ($p < 0.02$, $p < 0.01$, $p < 0.02$ for 10 µM *vs*. 5, 1 and 0.5 µM, respectively).

Effects of alkaloids on NE-induced contractions

Alkaloids used in this study also elicited significant differences in effects on contractile responses to NE. The lowest contractile responses to NE were recorded after incubation with 10 µM harmaline (31.0 ± 3.0 % contraction), relative to control ($p < 0.00002$), representing the

most significant effect. On incubation with 0.5, 1 and 5 µM harmaline, contractile responses were significantly reduced to 85.0 ± 3.0, 77.0 ± 5.0 and 56.0 ± 9.0 % ($p < 0.005$, $p < 0.01$ and $p < 0.003$, respectively, relative to control). These results are shown in Figure 2 A. Moreover, there were significant differences between the highest and all lower harmaline concentrations ($p < 0.0001$, $p < 0.0005$, $p < 0.02$; 10 µM *vs.* 0.5, 1 and 5 µM harmaline, respectively). These results indicate concentration-dependent inhibition of contractile responses after incubation of thoracic aorta from rat with the alkaloid harmaline.

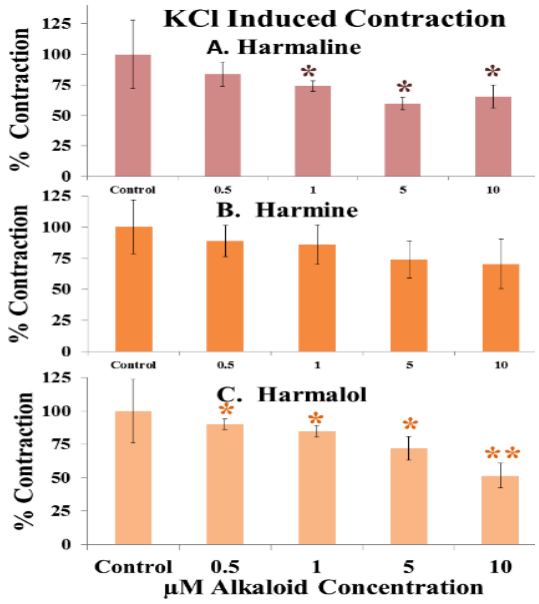

Figure 1: Effect of different concentrations of alkaloids from *P. harmala* on contractile responses elicited by 60 mM KCl in rat thoracic aorta. *$P < 0.005$, **$p < 0.01$, *vs*. control and between concentration groups. Control: **A:** harmaline (n = 6 experiments), **B:** harmine (n = 7 experiments), **C:** harmalol (n = 5 experiments). The first columns are controls (100 % of contractions after the first application of KCl without alakloids). The other columns represent contractile responses after incubation with 0.5, 1, 5 and 10 µM of alkaloids. Data are expressed as mean of percentiles of contractions ± SEM. For controls, SEM was calculated relative to the mean percentage control tension for each group. *****Statistical significance between concentration group and control; **significance *vs*. control and all lower concentrations

As shown in Figure 2 B, the alkaloid harmine at concentrations of 10 and 5 µM, significantly reduced contractile responses to about 48.0 ± 8.0 and 76.0 ± 8.0 %, respectively, when compared to control ($p < 0.02$; $p < 0.05$, respectively). Similar to harmaline, significant differences were also observed between recorded tensions at 10 µM harmine and tensions produced by incubation with concentrations of 5, 1 and 0.5 µM harmine ($p < 0.02$, $p < 0.02$, $p < 0.01$, respectively). Although, at lower concentrations, harmine elicited reduced contractile responses (88.0 ± 6.0 and 89.0 ± 13.0 % on incubation with 0.5 and 1 µM, respectively), there were no statistically significant differences between these responses and control responses.

Inhibitory profile of harmalol on NE-induced contraction of VSMCs showed reductions in responses at 0.5, 1, 5 and 10 µM, with values of 63.0 ± 11.0, 54.0 ± 13.0, 61.0 ± 6.0 and 54.0 ± 4.0%, respectively, when compared to control (Figure 2 C). Although there were significant differences in contractile responses after incubation with 0.5, 1, 5 and 10 µM harmalol ($p < 0.05$, $p < 0.03$, $p < 0.01$, $p < 0.005$, respectively) *vs*. control, no significance was observed between any two concentrations of the alkaloid.

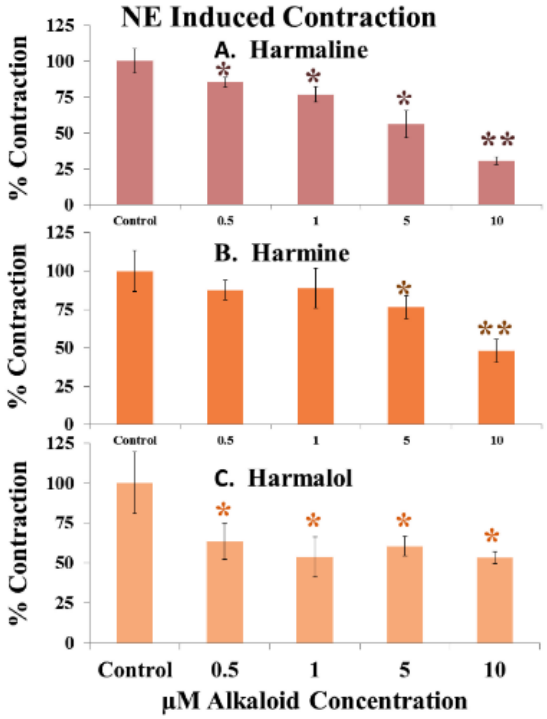

Figure 2: Effect of different concentrations of alkaloids from *P. harmala* on contractile responses elicited by 1 µM norepinephrine (NE) in rat thoracic aorta. **A:** Harmaline (n = 7 experiments), **B:** harmine (n = 6 experiments), and **C:** harmalol (n = 5 experiments). First columns are control (100 % contractions). The other columns are contractile responses after incubation with the alkaloids at concentrations of 0.5, 1, 5 and 10 µM. Data are expressed as means of percentiles of contractions ± SEM. SEM was calculated relative to the mean percentage control tension for each group. *****Statistical significance between concentration group and control; **statistical significance versus control and all lower alkaloid concentrations

These results indicate that harmalol produced a relatively higher inhibitory effect than harmaline or harmine on KCl-induced contraction in aortic preparations treated with the highest concentration (48.0 ± 9.0 *vs.* 34.0 ± 9.0 or 29.0 ± 20.0 %, respectively; Figure 3 A). However, relatively higher inhibitory effect was observed with lower concentrations of harmaline than with harmalol and harmine, with percentile of inhibitions of 40.0 ± 5.0 *vs.* 28.0 ± 9.0 and 26.0 ± 15.0, respectively at 5 µM; 26.0 ± 4.0 *vs*. 15.0 ± 4.0 and 14.0 ± 16.0 at 1 µM, and 16.0 ± 10.0 *vs*. 10.0 ± 4.0 and 11.0 ± 12.0% at 0.5 µM. These results are presented in Figure 3 B. These effects are suggestive of high degrees of potential interference of harmalol and harmaline with the activity of Ca^{2+}-gated voltage channels, with harmine being the least effective in preventing KCl-induced contractile responses due to changes in resting membrane potential.

In this study, harmaline produced higher inhibitory effect on NE-induced contractions than harmine and harmalol at the highest concentrations (69.0 ± 3.0, relative to 52.0 ± 8.0 and 47.0 ± 4.0 %, respectively; Figure 3 B). However, haramalol at concentrations of 1 and 0.5 µM was more effective in inhibiting NE-induced contractions of VSMCs, with % inhibition values of 46.0 ± 13.0 and 37.0 ± 11.0, respectively, when compared to 1 and 0.5 µM harmaline (23.0 ± 5.0 and 15.0 ± 3.0 %, respectively) and 1 and 0.5 µM harmine (11.0 ± 13.0 and 12.0 ± 6.0 %, respectively). These data are shown in Figure 3 B. Significant differences were also observed between percentiles of inhibition at 10 µM harmaline, when compared with all lower concentrations used in this study ($p < 0.03$, *vs* 5 µM; $p < 0.0005$ *vs* 1 µM; $p < 0.0001$ *vs* 0.5 µM). Moreover, percentile of inhibition at 5 µM harmaline was higher than that at 0.5 µM harmaline ($p < 0.02$). These results are presented in Figure 3 B).

The pattern of percentile inhibition by harmine was relatively similar to that of harmaline with respect to higher inhibitory effects at the highest concentration than at all lower concentrations ($p < 0.01$, $p < 0.03$, $p < 0.003$ *vs* 5, 1 and 0.5 µM harmine, respectively; Figure 3 B). However, there was no significant difference in percentile of inhibition by harmine at concentrations of 0.5 and 1 µM, when compared with the control group. Harmine produced relatively low degree of inhibition of contractile response. These results are suggestive of the high potential of harmaline and harmalol to prevent VSMCs responses via mechanisms that are not related to the inactivation of voltage-gated channels (Figure 2 and Figure 3 B).

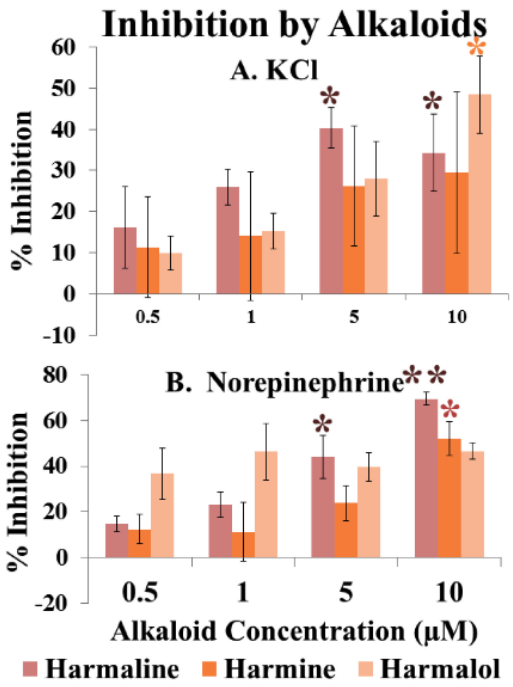

Figure 3: Percentiles of inhibition of NE and KCL-induced contractile responses in rat thoracic aorta on incubation with alkaloids. **A:** Inhibition of KCl-evoked contractions (n = 5 – 7 experiments). **B:** Inhibition of norepinephrine (NE)-evoked contraction (n = 5 - 7 experiments). Each set of columns represents percentiles of inhibitions by harmaline, harmine and harmalol (from left to right). Data are expressed as mean ± SEM of percentiles of inhibition, relative to controls. ***p* > 0.001; < 0.05; ****p* < 0.0005, 5 or 10 µM *vs.* 1.0 and 0.5 µM

DISCUSSION

The responses of VSMCs *via* decreases or increases in tension might have great impact on physiological functions related to the cardiovascular system. Various and complex mechanisms are involved in controlling motor activity of VSMCs [15]. It is well known that NE-induced contraction depends on elevation of sarcoplasmic Ca^{2+} concentration from both extracellular and intracellular sources [15,16]. In addition, KCl-induced contraction depends on the influx of extracellular Ca^{2+} through L-type voltage-operated channels [17]. The inhibition of VSMCs activity is enhanced through mechanisms that increase intracellular levels of cyclic adenosine monophosphate (cAMP) or cyclic guanosine monophosphate (cGMP), reduce sarcoplasmic Ca^{2+} concentration, or increase endothelial-derived factors, which may interfere with receptor-mediated contractile transduction mechanisms or promote hyperpolarization of VSMCs [18,19].

It seems that the results obtained in this study are generally in agreement with data from other reports about the vasorelaxant effects of harmaline, harmine, and harmalol, although the individual inhibitory potential of the alkaloids on NE- or KCl-induced contractile responses were different. For instance, harmalol was more potent than harmaline and harmine in inhibiting KCl-induced contraction, while harmaline had the highest potency in inhibiting contractile responses induced by NE. This is at variance with the findings of Karaki and his colleagues who reported different inhibitory profiles for these alkaloids in a study on rabbit aorta [12]. In the study by Karaki *et al* [12], the IC_{50} of harmine on 1 µM noradrenaline (NA)- and 65.4 mM KCl-sustained contractions were 60 and 22 µM respectively, while those of harmaline and harmalol were 76 and 46 µM, and 38 and 220 µM, repectively, indicating the higher potency of harmine in relaxing KCl-contraction, as well as higher potency of harmalol in relaxing NA pre-contracted preparation [12].

In another study, Shi and his colleagues demonstrated that the values of IC_{50} on phenylephrine (PE)- and KCl-pre-contracted preparations with intact endothelium for harmine were 8 and 10 µM, respectively, while the corresponding values for harmaline were 41 and 33 µM, respectively, with values of 109 and >1000 µM for harmalol, respectively. It was concluded that the relaxation potency was highest for harmine and lowest for harmalol on both PE- and KCl-sustained contractions [13]. In all these reports, the IC_{50} was determined in preparations pre-contracted with spasmogens. Nevertheless, the differences in response to these alkaloids between the present study and other studies could be attributed to the differences in study designs, animal species used, alkaloid purification methods, and other conditions related to incubation time with the alkaloids. In this study, the effect of these alkaloids was assessed 15 min after incubation.

Studies by Berrougui and Shi have also reported the effects of alkaloids on PE response curves after pre-incubation with low concentrations (3 - 30 µM) [11,13]. Berrougui incubated preparations with harmine or harmaline for 30 min and showed that harmine caused a greater shift of the PE response curve to the right, when compared to harmaline in preparations with intact endothelium [11]. Shi and his colleagues have also constructed response curve to PE after 10 min of incubation of endothelium-denuded aorta with 3 - 30 µM harmine, harmaline and harmalol, and the results obtained indicated a greater shift to the right by harmalol [13]. However, apart from contractile responses by chemical stimulation after incubation with these alkaloids, none of these studies have shown the effect of pre-incubation with the alkaloids on KCl-elicited contractile responses. Results from these studies are, in part, in agreement with the results obtained in the present study where incubation with low concentrations of these alkaloids (3 - 30 µM) inhibited contractile responses. From these studies, it seems that higher concentrations of alkaloids are needed to induce relaxation of pre-contracted preparations, while incubation with lower concentrations of the alkaloids is enough to inhibit contractile responses of VSMCs. Thus, these results may suggest the high efficacy of *P. harmala* extracts or its alkaloids as prophylactic therapy for improving the quality of life of hypertensive patients and minimizing toxicity concerns on administration of lower doses that may be helpful in controlling VSMCs responses to spasmogens.

Although the contractile behaviors of VSMCs are dependent on various mechanisms, an understanding of the mechanisms involved in the induction of vaso-relaxation by bioactive compounds from *P. harmala* will augment their therapeutic benefits through combinational use in order to improve vascular functions. This is expected to alleviate symptoms and reduce risks related to hypertension in humans.

CONCLUSION

Incubation of VSMCs with alkaloids from *P. harmala* for 15 min reduces contractile responses to NE and KCl. However, subsequent studies should be carried out on the differences in the mechanisms of inhibition by *P. harmala* alkaloids on VSMCs responses, and the associated potential benefits in the formulation of prophylactic therapy for hypertensive patients.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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REFERENCES

1. *Hypertension, WHO 2019; Geneva. Available from https://www.who.int/news-room/fact-sheets/detail/hypertension.*
2. *Cardiovascular Diseases (CVDs), WHO 2017; Geneva. available from https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).*
3. *Touyz RM, Alves-Lopes R, Rios FJ, Camargo LL, Anagnostopoulou A, Arner A, Montezano AC. Vascular smooth muscle contraction in hypertension. Cardiovasc Res 2018; 114(4): 529-539.*
4. *Knox M, Vinet R, Fuentes L, Morales B, Martínez JL. A review of endothelium-dependent and -independent vasodilation induced by phytochemicals in isolated rat aorta. Animals 2019; 9(9): 623.*
5. *Moloudizargari M, Mikaili P, Aghajanshakeri S, Asghari MH, Shayegh J. Pharmacological and therapeutic effects of Peganum harmala and its main alkaloids. Pharmacogn Rev 2013; 7(14): 199-212.*
6. *Sharifi-Rad J, Quispe C, Herrera-Bravo J, Semwal P, Painuli S, Özçelik B, Hacıhasanoğlu FE, Shaheen S, Sen S, Acharya K, et al. Peganum spp.: a comprehensive review on bioactivities and health-enhancing effects and their potential for the formulation of functional foods and pharmaceutical drugs. Oxid Med Cell Longev 2021; 2021: 5900422.*
7. *Shatarat A, Abuhamdah S, Al-Essa M, Mohammed F, Al-Olimat S. Pharmacological effects of Peganum harmala L. root extract on isolated rat small intestine. Pharmacogn Commun 2014; 4(3): 56-61.*
8. *Shatarat AT, Abuhamdah S, Alefishat E, Al-Essa M, Altaweel RR, Mohammed F, Badran D, Jafar H. Effects of beta-carboline alkaloids of Peganum harmala on induced rat ileum contractions. Pharmacogn J 2020; 12(2): 260-265.*
9. *Moshiri M, Etemad L, Javidi S, Alizadeh A. Peganum harmala intoxication, a case report. Avicenna J Phytomed 2013; 3(3): 288-292.*
10. *Aarons DH, Rossi GV, Orzechowski RF. Cardiovascular actions of three harmala alkaloids: harmine, harmaline, and harmalol. J Pharm Sci 1977; 66(9): 1244-1248.*
11. *Berrougui H, Martín-Cordero C, Khalil A, Hmamouchi M, Ettaib A, Marhuenda E, Herrera MD. Vasorelaxant effects of harmine and harmaline extracted from Peganum harmala L. seeds in isolated rat aorta. Pharmacol Res 2006; 54(2): 150-157.*
12. *Karaki H, Kishimoto T, Ozaki H, Sakata K, Umeno H, Urakawa N. Inhibition of calcium channels by harmaline and other harmala alkaloids in vascular and intestinal smooth muscles. Br J Pharmacol 1986; 89(2): 367-375.*
13. *Shi CC, Liao JF, Chen CF. Comparative study on the vasorelaxant effects of three harmala alkaloids in vitro. Jpn J Pharmacol 2001; 85(3): 299-305.*
14. *Greenfield EA. Administering anesthesia to mice, rats, and hamsters. Cold Spring Harb Protoc 2019; 2019(6).*
15. *Allen BG, Walsh MP. The biochemical basis of the regulation of smooth-muscle contraction. Trends Biochem Sci 1994; 19(9): 362–368.*
16. *Misárková E, Behuliak M, Bencze M, Zicha J. Excitation-contraction coupling and excitation-transcription coupling in blood vessels: their possible interactions in hypertensive vascular remodeling. Physiol Res 2016; 65: 173–191.*
17. *Zamponi GW, Striessnig J, Koschak A, Dolphin AC. The physiology, pathology, and pharmacology of voltage-gated calcium channels and their future therapeutic potential. Pharmacol Rev 2015; 67(4): 821–870.*
18. *Lee MR, Li L, Kitazawa T. Cyclic GMP causes Ca2+ desensitization in vascular smooth muscle by activating the myosin light chain phosphatase. J Biol Chem 1997; 272(8): 5063–5068.*
19. *Ozkor MA, Quyyumi AA. Endothelium-derived hyperpolarizing factor and vascular function. Cardiol Res Pract 2011; 2011: 156146.*