Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages 14300-14309
Received 05 March 2021; Accepted 01 April 2021.

Protective impression of Artemisia on Salbutamol -induced hemodynamic alteration in Wister rats via eNOS

Hadeel.Khalaf.Alboaklah[1]

Clinical Laboratory Science/College of Pharmacy, University of Kerbala

Abstract
Salbutamol is named a 'reliever' drug because it swiftly releases patients short breathing. It begins to work through a few minutes and their effect will remain between 3 - 5 hours. like all treatments, salbutamol can show side effects, risks of cardiovascular disease (CVD). However, herbal supplements (Artemisia annua) have reported high effectiveness in numerous against CVD. The plants also have a hypotensive consequence. This paper proposes several hypotheses to explain this property. In endothelium-containing aorta preparations, released the contraction stimulated by phenylephrine (PE) in a concentration sequence-dependent method. This influence is based upon the health of the vascular endothelium as it was eliminated in the endothelium. These outcomes propose that Artemisia stimulate vasorelaxation of the separated rat aorta, a result that looks primarily mediated via stimulation of the endothelial nitric oxide synthase (eNOS).
Keywords: hypertension, Artemisia annua, nitric oxide,

Introduction

Cardiovascular damages after intake Salbutamolat relaxing state are commonly recognised, Short effect of β-adrenergic agonists are commonly beneficial nonetheless usually cause hypertension, augmented heart beatings, and also have an adverse outcome as arrhythmias, tremors, and tenseness (1). Inhaled Salbutamol result in arterial stiffness (2). The vascular response may contribute to the increased cardiovascular risk (2). Many studies suggest that the heart rate(HR) increased with increasing plasma salbutamol concentration. In Total, the effect of salbutamol is cardiac motivation (augmented HR, contractility, transmission velocity, relaxing) and systemic vasorelaxant. Blood pressure may increase (3) and electrocardiographic alterations including T-wave destruction, Q-Tc intermission prolongation, and, in some subjects, U-waves and S-T segment depression (4). Consequently, on high-dose, the beta-1 agonist action of salbutamol leads to cardiovascular harm effects by triggering beta-1 adrenoceptors in the heart to acceleration the power and degree of the contractions by simulation both nodal sinoatrial and atrioventricular, and ventricular muscular contractions (5). As a result of augmented these values, the stroke capacity and heartbeat will also rise. This consequence demonstrates in the heart production equivalence. Cardiac output = stroke volume + HR. Therefore increasing perfusion to tissues in the body. Numerous of the disorders disturbing individuals today are related to oxidative stress that comes from inequality between realization and removal of free radicals (6) ROS are made via all vascular slices, through the endothelium, smooth muscle, and adventitia (7). Endothelial cells have key factors in the signalling of vascular tenor, adaptations of inflammation, and stimulate or block vascular development and platelet accumulation and clotting (8). These cells provide themselves protection against free radical injury by antioxidant substances (9) Plants are a valued source of normal antioxidants and create an essential source of vital natural material, several of which are employed as flavours in serviceable foods or bioactive material in pharmaceutical preparations. Several phytochemical substances of plants are responsible for the antioxidant properties of plants (10). Artemisia has been commonly used as a herbal medicine due to its numerous pharmacological properties, which include

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages 14300-14309
Received 05 March 2021; Accepted 01 April 2021.

antibacterial, anti-inflammatory (11), Artemisinin is extracted from the traditional Chinese medicine 'qinghao' (Artemisia Annua L.) (12). This study is to provide up-to-date information on Artemisia, involving its phytochemistry and pharmacological effects, in exploring therapeutic and scientific potentials.

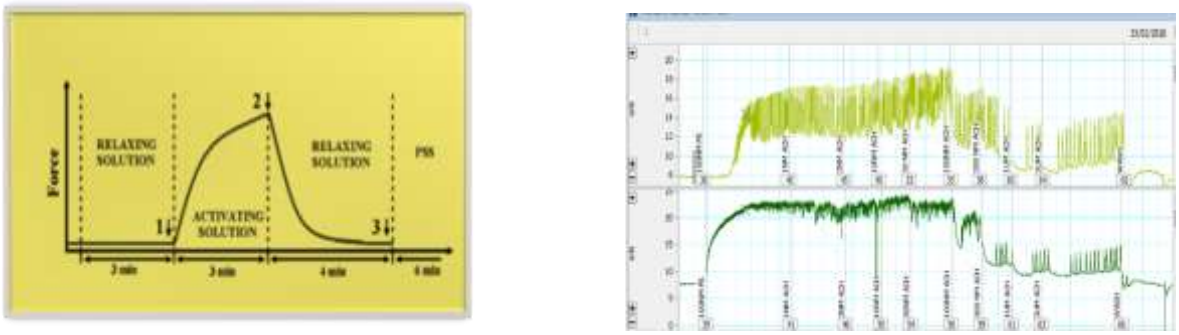
Material and Method

Animal ethics statement

The united kingdom registered with PIL Home Office certificates of description. Animals were killed by schedule 1 (cervical dislocation) licenses are not essential according to UK regulations.

Drugs preparation for myography.

PE and Ach were obtained from
Sigma (Figure1)



A B

Figure 1: ıple of outcomes recordings throughout the estimate of artery viability and endothelium dependability. Lift (A) Illustration of the phase of contraction and relaxation, responding to add phenylephrine and acetylcholine respectively.
Right (B): Lab Chart Visualisation window displays the force records alongside the time in 2 channels. Perpendicular lines relate to explanations that are written by the technician to signpost the material add-on to prompted vasomotor influence. Presented from leftward to right is the relaxation after rinses, the pre-contraction prompted by 1 µM phenylephrine (PE 10-6), and the relaxation prompted by 10 µM (Ach 10-5).



Figure2: Artemisinin (C15H22O5), derived from the sweet wormwood Artemisia annua

Preparation of aqueous Artemisia extract.

The medical plant (Figure2) was taxonomically recognised by botanists in the Branch of (Tehran University, Iran). The gunpowder of air-dried herbs (100 g) was dissolved in (1Liter)of fumed distilled water and maintained the degree at 60°C in a water bath for the fifteenth min and then filtered 3 times by using semipermeable paper. The filtered products were dehydrated at 37°C until obtained a particular concentration (62% w/w). This standard

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages 14300-14309
Received 05 March 2021; Accepted 01 April 2021.

extract was preserved at -20°C for a long time. The experimental design was ready and then the liquid dose of the extract was prepared up by using normal saline (13).

Animal.

Rats weighing 220- 250 g (Male Albino Wistar), were gotten from the University of Kerbala of Iraq (Kerbala) and provided with normal pellet food and water. All sets of rats were sustained in typical room environments for four weeks with unrestricted access to diets and water. rats received 20 mg/kg/day salbutamol orally for 4 weeks, Bodyweight rats were measured before and four weeks after intraperitoneal (IP) injection of saline or Artemisia extract (200 mg kg-1). The rats were subjected to killing by inhalation procedures such as chambers that seal with anaesthetic gases (isoflurane) and then dissection of rat and collect the left anterior descending artery and other organs.

Set 1) Control rats for gaining the standard values.

Set 2) Rats received saline (0.5 ml/24h) for 4 weeks.

Set 3) Rats intakes salbutamol (20 mg kg-1/0.5 ml saline 24h) for 4 weeks.

Set 4) Rats intakes 0.5 ml of Artemisia extract (200 mg kg-1/0.5 ml saline 24h) for 4 weeks.

Group E) Rats received 0.5 ml of Artemisia extract (200 mg kg-1/0.5 ml saline 24h) + salbutamol (20 mg kg-1/0.5 ml saline 24 h) for 4 weeks.

Histological preparation

Aortic rings (n=14: two control, eight salbutamol, and eight artemisias) were diagonally cryosections (10 μm) and stained with H&E to identify cellular components. Immunohistochemistry was achieved, using Human Aortic Endothelial cells (HAEC) gotten from Lonza Ltd. HAEC are cryopreserved at the path by (10 % (v/v) of each DMSO & FCS)and storage in liquid nitrogen. Cells were cultivated. as recommended in their optimal fluid (HumanArotic Endothelial Cell Growth Medium, Lonza Ltd). Endothelial cells plated on glass coverslips were rinsed one time with PBS and then kept at 37 °C with tritiated salbutamol (200 nmol/L) and (3.5 μg per/ml) artemisia for two days. The extreme amount of DMSO supplemented to the culture media was 0.1%, and this amount of the solvent was permanently employed as a standard. Control and treated cells were then washed by PBS and 10 min with 4% (w/v) paraformaldehyde in phosphate buffer for fixations. After fixation, the cells were washed 3x with PBS, using 0.1% Triton X-100 for 2 min for permeabilizing, and washed up 3x in PBS. Here cells were kept for 24 h a 4°C with a primary antibody to phosphorylated eNOS prepared in phosphate buffer containing 1% bovine serum albumin. Cells were rinsed 3x with PBS and then preserved for 60 min a room temperature with the secondary antibody goat anti-rabbit antibody in phosphate buffer plus 1% bovine serum albumin. Each antibody concentration in the recipes was 20 μg/ml. Cells were rinsed three times with phosphate buffer and one time in distilled water and then using Dako Fluorescence mounting media. Cells were examined on an Axioskop epifluorescent microscope. Immunopositive zones were measured on digital capture by the ImageJ programme.

Assessment of rat Vessel Viability

Wire myography (14) is an in vitro study method for examining both dynamic and inactive features of the aorta at widths of between 100 -1000 μm. Blood vessel types used in this technique are large arteries, including mesenteric artery, and aorta (15) from laboratory animal to evaluated it in both physiological and pathological environments (16). The myography procedure implicates the following steps: separating arteries from rat and insertion of these arteries, standardization, measuring of tissue validity, and contraction of a serials dose-reactions curve. The arteries were obtained from 12-14 week-old healthy male Wistar rats (MBW 261±7 g, n=10) and examined by wire myography. Isoflurane is used for anesthetic technic and then head displacement, the thoracic artery was wisely removed and impeded to Kerb's liquid and then dissected arteries to eliminate connective tissue and

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages 14300-14309
Received 05 March 2021; Accepted 01 April 2021.

adjacent fat tissue. Segments sizes are (roughly 2 mm long) were implanted in a wire myography hanger (Dual Wire Myography System, DMT) and were fixed for 30 min. The arteries were exposed to PE (100 nM) and then Ach at different concentrations (Figures 1).

Statistical examination

All outcomes were referred to as the mean ± S.E.M. and statistically interpreted by using 2 - way ANOVA, followed by the post hoc Tukey method. A value of $P<0.05$ was considered statistically significant.

Results

1. Hemodynamic cardiovascular alteration in rat

   Although salbutamol is known to cause asthma release in humans, the hemodynamic characteristics of salbutamol-induced hypertension in the rat remain unclear. Therefore, the current study pointed to evaluate the acute impacts of salbutamol on the cardiac autonomic parameter in the rat model and determined the administration of 200 mg kg-1 Artemisia extract considerably reduced the salbutamol-induced haemodynamic cardiovascular changes using left ventricular measurements (Figure 3) also using incessantly achieved haemodynamic analysis,including blood pressure and heart rate variability.

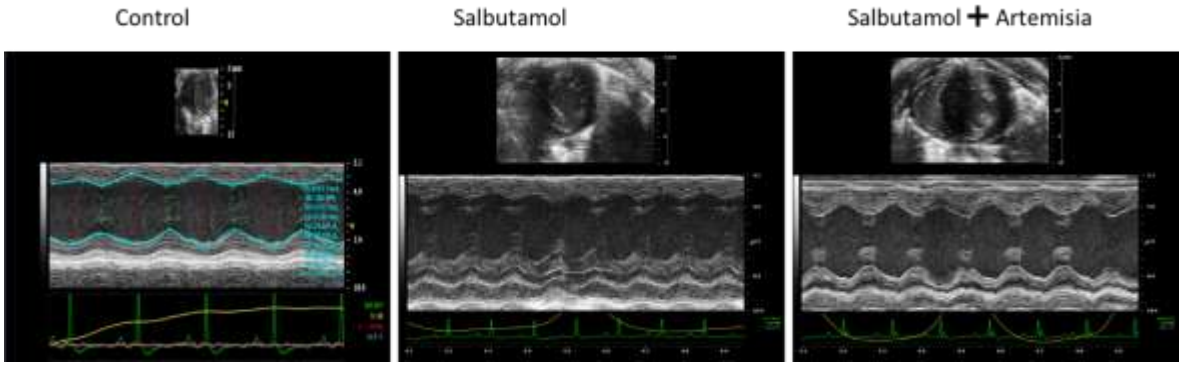


Figure 3: Illustration shows the increasing thickness of left ventricles in healthy rat group treated with Salbutamol compared to the control, and reducing the effect of Artemisia on salbutamol group after treated with aqueous extraction of Artemisia.

Control group injections with sterile saline did not alter SBP (120±5 mm Hg) while the group of salbutamol, increased to 141±4 mm Hg in comparison to Artemisia combined with salbutamol, (130±7mm Hg n=8). Salbutamol significantly increased MBP from 120±2 mm Hg to 141±2 m. Salbutamol administration significantly increased heart rate, blood pressure and cardiac index in the healthy control group. Artemisia significantly reduce MBP from 141±4 mm Hg to 128±7mm Hg on week 4 (n=24, $P<0.001$) (Table1)

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages 14300-14309
Received 05 March 2021; Accepted 01 April 2021.

group	Numbers	Mean systemic blood pressure (mm Hg)	Heart rate Beat per minutes (BPM)	Cardiac output (ml/minutes)
control	8	120.1± 5.1	348±12	15.59±2
Salbutamol	8	141.7±4.1	557±93	22±2
Artemisia Salbutamol&	8	128.6 ±7.1	486±23	16.8±1

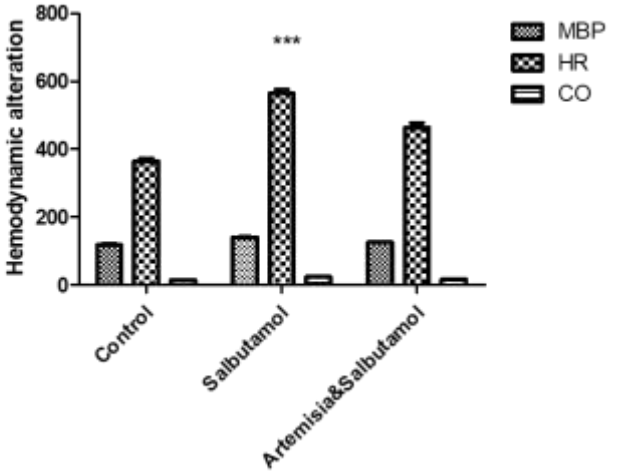


Figure3: shown reducing the effect of artemisia on haemodynamic alteration induced by salbutamol in the rat model

2.Myography results (Relaxation percentage)

Salbutamol caused an intensification in mean blood pressure (MBP) in the rat model in a time-related manner. MBP was considerably higher in the Salbutamol group when corresponding with the vehicle- group as standard and reducing the effect of Artemisia when the salbutamol group treated with Aqueous extraction of Artemisia, thus, In this experiment, we examined whether the aortic vascular response was changed under salbutamol or Artemisia. Data investigation. The vasocontractile reactions of rat aortic segment to PE and vasodilation reactions for Ach were identified as a proportion decrease of the supreme vasocontractile reaction prompted by PE. All data were set as average ± S.E.M. Numerical implication was shown via a *P<0.05, and ***P<0.0001.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages 14300-14309
Received 05 March 2021; Accepted 01 April 2021.

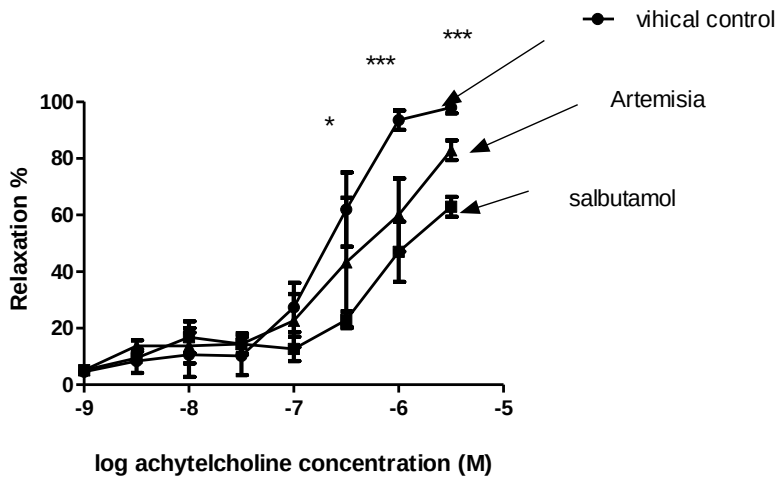


Figure 4: Concentration-response curve of endothelium-dependent relaxation to acetylcholine, with vehicle control and with the presence of Salbutamol and Artemisia separately.Statistical significance is shown as *P<0.05, and ***P<0.0001.

3. Histological examination

Aimed at the light microscopic investigation, the samples were impeded in 10% formaldehyde for fixation, initiated a dehydration procedure with alcohol chains, implanted in paraffin wax. Samples were sliced (5 µm/thin sample) using a microtome (MICRO M HM 340E, WALLDORF, Germany). For the arteries tissue, the sectioning was stained with standard Haematoxylin and Eosin (H&E). The examination at an intensification of ×200 for the aorta. In this study, tunica intima thickness and Endothelial structure did not demonstrate any significant variations (Figure 5). related to the control vehicle group. The innermost tunica thickness (µm) was measured for the control 4.06 ± 1.36 and the salbutamol group 3.87 ± 1.33 and the Artemisia group 5.71 ± 2.31. This might be because of the less effect of H&E to show the smallest detail of tissue structural organisation.

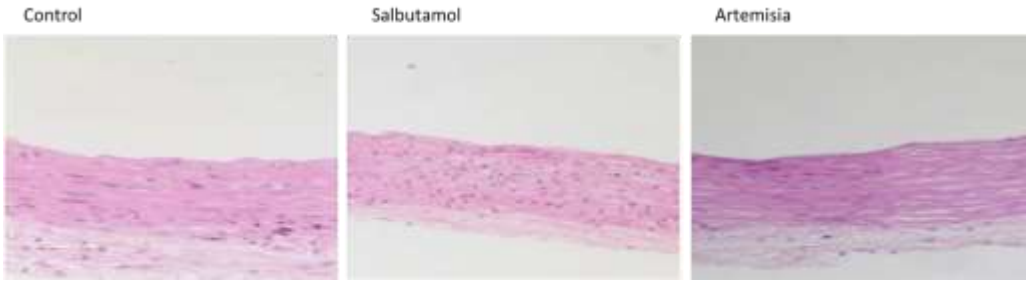


Figure 5: Histologic sections stained with H&E showing the aorta of the experimental group's salbutamol treated rat and artemisia treated rat compared to the control group. There was no significant difference regarding either the degeneration or thickening in the tunica intima.x200

4**. Impact of Artemisia on eNOS activation**

The histological outcomes on the aortic tissue did not show up the expression of endothelial cells, and on the other hand, impairment of endothelium-mediated relaxation and the underlying mechanism involved has not been fully explored in this

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages 14300-14309
Received 05 March 2021; Accepted 01 April 2021.

rat model. Thus, effects of salbutamol on the expression of phosphorylation eNOS in Human aortic endothelial cells. Immunohistochemical staining using (fluorescence microscope) after treatment with solvent or salbutamol and artemisia. The results obtainable are representative of data obtained in four further experiments. Cells were fixed and prepared as described in Methods.

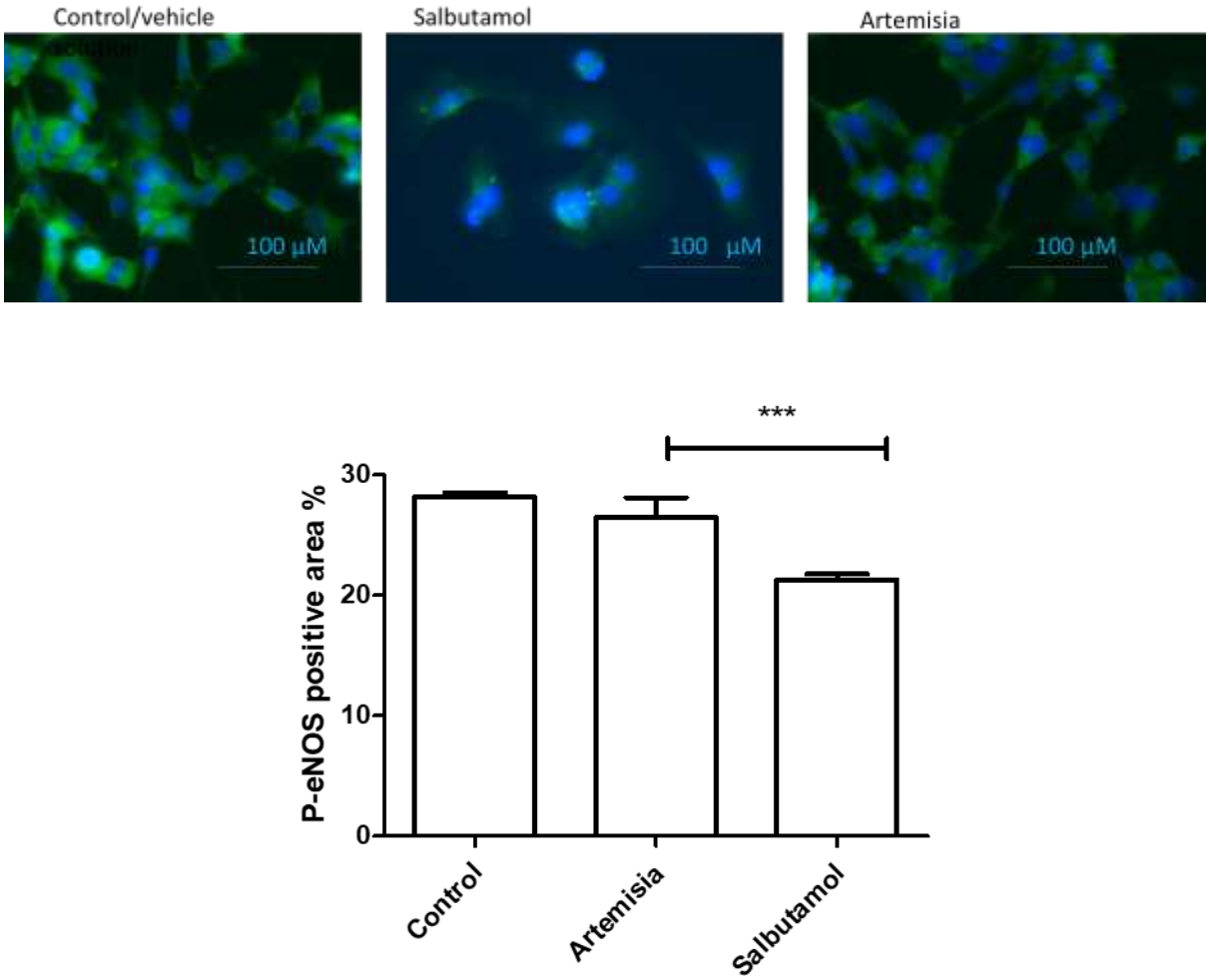


Figure 6: shows endothelial cells (HAEC) with or without salbutamol or artemisia had increased phosphorylated eNOS after incubation with acetylcholine (3 µM) for 8 min.

Discussions

Selecting a β2-agonists, salbutamol, are in general usage for patients with asthma. Although Salbutamol broncho-dilating influence, it is able of leading to adverse properties on the CVS. Several previous pieces of information have labelled a link between either oral or gasped β2-agonist treatment and amplified CV morbidity and mortality (17). Despite the numeral of clinical investigations presenting a link between intake β2-agonist therapy with CV risk, merely few documented have scientifically examined the impacts of β2-agonist intake on the haemodynamic indicator. The basic outcomes of the existing project were the usage of salbutamol concentration influence HR and BP throughout rest condition, in the rat model. This finding agreed with other studies that documented that 200mg of salbutamol augmented HR by 4 BPM in the medium in patients with a current identification of asthma and regardless of drug treatment (18). An augmented HR because of absorption of the medication, which may lead to a reduction of the diastole, (Figure 3), so cumulative

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages 14300-14309
Received 05 March 2021; Accepted 01 April 2021.

myocardial oxygenation is dropping in the period for coronary artery perfusion also a diminution in potassium absorptions that might show pro-arrhythmogenic properties.
In the last years, laboratory information has recorded that medical herbs may show therapeutic worth in inhibit numerous CVD risk elements, hypertension is the silent and strongest agent of these risk factors (19). This study shows Artemisia impact on reducing hypertension (table1) this finding agreed with other study showing Artemisia is a medicinal herb conventionally employed to treat hypertension and many other diseases (20). The histological outcome of this study found no significant differences between the salbutamol and Artemisia group associated with the control model (Figure 5). The second results indicated that the vasocontractile reaction of the aortic segment with endothelium to (PE) in artemisia-treated rats attenuations ($P<0.05$-0.0001) and their endothelium-dependent vasorelaxation reaction to (Ach) rises ($P<0.05$-0.001) significantly in contrast with the salbutamol-treated rat. These data recommend that the extract of Artemisia might diminish contraction reaction and improve the endothelium-dependent relaxation response of aortic segment from normal rats and inhibition impact of salbutamol, which caused a contractual impact on endothelial cells. This finding was agreed with several studies (21)
The lack of information about histological alteration in aortic rat treated with salbutamol and Artemisia by using rotuin stain,may be related to the aim of this experiments focussing on the morphology of endothelial (in tunica intima) rather than smooth muscle cells in tunica media. NO made by eNOS marks a several of biological pathway in the vessel structure. It is essential for the mechanism of blood pressure and shows an important factor in the cluster of platelets, and relocation of vascular smooth muscle cells (21). A diminution in NO obtainability is best of the hallmarks detection of endothelial disorders, which can arise in a severalof cardiovascular disorders, including hypertension and heart failure (22). Vascular endothelium regulates both vascular penetrability, by modifiable the fluidity of blood constituents via the vessel barrier by intracellular with intercellular paths (23) and vascular tone via corresponding the secretion of vasodilators nitric oxide and vasoconstrictors. Therefore,another experiment was employed to investigate about endothelial activity by using immunohistopathological assessments for detection eNOS activity under effect of salbutamol and Artemisia. No significant difference between control and Artemisia while there was significant differences between salbutamol regareding to the control. This finding suggested prtotective effect of Artemisia to the endothelial cell compared to the inhibited impact of salbutamol to the Enos (Figure 6). This outcomes agreed with several studies (22,23).

01000000000000110Conclusion

In conclusion, We show the statement that the capability of extract of artemisia to inhibit the hemodynamic reactions caused by salbutamol was reduced in Artemisia–treated rats. This proposes that the relative status of salbutamol is inhibited by Artemisia. treatment with Artemisia extract ensured a beneficial consequence on experimentally induced rats via salbutamol. Treatment with Artemisia extracts preserved the histological integrity of the aortic tissues by reducing the degenerative changes in the endothelial cells as well as in the. Further research is required to validate such facts. These studies pave the approach for further investigations of artemisia as inhibitory hypertension modulators in humans.

Conflicts of interest

The authors whose names are listed above certify that they have NO conflicts of interest to declare

References

1. Molis MA, Molis WE. Exercise-induced bronchospasm. Sports Health. 2010;2(4):311–7.
2. Moore LE, Kapoor K, Byers BW, Brotto AR, Ghods-Esfahani D, Henry SL, et al. Acute effects of salbutamol on systemic vascular function in people with asthma. Respir Med. 2019;155:133–40.
3. Wang Y-X, Poon KS, Randall DJ, Pang CCY. Endothelium-derived nitric oxide partially mediates salbutamol-induced vasodilatations. Eur J Pharmacol. 1993;250(3):335–40.
4. Lipworth BJ, McDevitt DG, Struthers AD. Electrocardiographic changes induced by inhaled salbutamol after treatment with bendrofluazide: effects of replacement therapy with potassium, magnesium and triamterene. Clin Sci. 1990;78(3):255–9.
5. Ungerer M, Böhm M, Elce JS, Erdmann E, Lohse MJ. Altered expression of beta-adrenergic receptor kinase and beta 1-adrenergic receptors in the failing human heart. Circulation. 1993;87(2):454–63.
6. Sarma A Das, Mallick AR, Ghosh AK. Free radicals and their role in different clinical conditions: an overview. Int J Pharma Sci Res. 2010;1(3):185–92.
7. Ray PD, Huang B-W, Tsuji Y. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal. 2012;24(5):981–90.
8. Kim H, Yun J, Kwon S-M. Therapeutic strategies for oxidative stress-related cardiovascular diseases: removal of excess reactive oxygen species in adult stem cells. Oxid Med Cell Longev. 2016;2016.
9. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010;4(8):118.
10. Aiyegoro OA, Okoh AI. Preliminary phytochemical screening and. vitro; 2010.
11. de Magalhães PM, Dupont I, Hendrickx A, Joly A, Raas T, Dessy S, et al. Anti-inflammatory effect and modulation of cytochrome P450 activities by Artemisia annua tea infusions in human intestinal Caco-2 cells. Food Chem. 2012;134(2):864–71.
12. Bora KS, Sharma A. The genus Artemisia: a comprehensive review. Pharm Biol. 2011;49(1):101–9.
13. Calderone V, Martinotti E, Baragatti B, Cristina Breschi M, Morelli I. Vascular effects of aqueous crude extracts of Artemisia verlotorum Lamotte (Compositae): in vivo and in vitro pharmacological studies in rats. Phyther Res An Int J Devoted to Pharmacol Toxicol Eval Nat Prod Deriv. 1999;13(8):645–8.
14. Mulvany MJ, Halpern W. Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. Circ Res. 1977;41(1):19–26.
15. Khazaei M, Moien-Afshari F, Laher I. Vascular endothelial function in health and diseases. Pathophysiology. 2008;15(1):49–67.
16. Spiers A, Padmanabhan N. A guide to wire myography. In: Hypertension. Springer; 2005. p. 91–104.
17. Au DH, Lemaitre RN, Randall Curtis J, Smith NL, Psaty BM. The risk of myocardial infarction associated with inhaled β-adrenoceptor agonists. Am J Respir Crit Care Med. 2000;161(3):827–30.
18. Cekici L, Valipour A, Kohansal R, Burghuber OC. Short-term effects of inhaled salbutamol on autonomic cardiovascular control in healthy subjects: a placebo-controlled study. Br J Clin Pharmacol. 2009;67(4):394–402.
19. Sawicka K, Szczyrek M, Jastrzebska I, Prasal M, Zwolak A, Daniluk J. Hypertension–the silent killer. J Pre-Clinical Clin Res. 2011;5(2).
20. Dib I, Fauconnier M-L, Sindic M, Belmekki F, Assaidi A, Berrabah M, et al. Chemical

composition, vasorelaxant, antioxidant and antiplatelet effects of essential oil of Artemisia campestris L. from Oriental Morocco. BMC Complement Altern Med. 2017;17(1):1–15.

21. BALUCHNEJADMOJARAD T, ZARE N, ROUGHANI M. EFFECT OF SUBCHRONIC ADMINISTRATION OF AQUEOUS ARTEMISIA ANNUA EXTRACT ON-ADRENOCEPTOR AGONIST-INDUCED CONTRACTION OF ISOLATED AORTA IN RAT. 2005;
22. Tulis DA. Histological and morphometric analyses for rat carotid balloon injury model. In: Vascular Biology Protocols. Springer; 2007. p. 31–66.
23. Mojarad TB, Roghani M, Zare N. Effect of subchronic administration of aqueous Artemisia annua extract on Î±1-adrenoceptor agonist-induced contraction of isolated aorta in rat. Iran Biomed J. 2005;9(2):57–62.