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Effects of intraperitoneal and intracerebroventricular injection of cinnamaldehyde and yohimbine on blood glucose and serum insulin concentrations in ketamine-xylazine induced acute hyperglycemia

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Abstract

This study was designed to investigate the effects of peripheral [intraperitoneal (IP)] and central [intracerebroventricular (ICV)] administration of cinnamaldehyde on concentrations of blood glucose and serum insulin in the acute hyperglycemia induced by ketamine/xylazine. Yohimbine (a α_2-adrenoceptor antagonist) was used alone and in combination with cinnamaldehyde to explore the α_2-adrenergic receptor contribution. A total of 48 rats were divided into eight groups with six rats in each for IP administration of normal saline, vehicle, cinnamaldehyde (25.00, 50.00 and 100 mg kg^{-1}), yohimbine (0.50 and 2.00 mg kg^{-1}) and cinnamaldehyde plus yohimbine. These rats were used again for ICV administration 15 days after the completion of IP experiment. During this 15 days period, the lateral ventricle of the brain was surgically cannulated for ICV administration of normal saline, vehicle, cinnamaldehyde (25.00, 50.00 and 100 µg per rat), yohimbine (5.00 and 20.00 µg per rat) and cinnamaldehyde plus yohimbine. Blood glucose levels were measured from tail blood using a glucometer and serum insulin concentrations were determined via enzyme-linked immunesorbent assay kit. The increased levels of blood glucose and the decreased concentrations of serum insulin were significantly decreased and increased, respectively, by separate and combined IP and ICV administrations of cinnamaldehyde and yohimbine. The systemic effects of these chemical compounds were significantly greater than the central ones. Based on the results, it can be argued that cinnamaldehyde has a potential to induce anti-hyperglycemic and antihypoinsulinemic effects. Peripheral and central α_2-adrenegic receptors might be involved in these effects of cinnamaldehyde.

Introduction

Body physiological functions depend on the availability of substrates such as glucose for energy production.[1] Blood glucose concentration is accurately regulated and abnormal levels of blood glucose (hypoglycemia and hyperglycemia) result in deleterious effects at the whole organism level.[2] The central nervous system (CNS) areas such as the hypothalamus along with autonomic nervous system (ANS) and the peripheral endocrine (pancreas) and metabolic organs (liver, fat and skeletal) involve in glucose homeostasis.[3,4] Both the parasympathetic and sympathetic divisions of ANS innervate and regulate endocrine and metabolic organ functions that involved in glucose production and utilization.[5] A variety of hormones can regulate glucose homeostasis directly, by modulating glucose uptake, storage and release, or indirectly, by interacting with other hormones that are important for glucose regulation such as insulin and glucagon.[6]

Cinnamon is a spice obtained from the inner bark of several tree species from the genus *Cinnamomum*. It is used mainly as an aromatic condiment and flavoring additive in a wide variety of cuisines, sweet and savory dishes, breakfast cereals, snack foods, tea and traditional foods. The available *in vitro* and *in vivo* evidence suggests that cinnamon has anti-diabetic, anti-microbial, anti-parasitic and anti-oxidant properties.[7] Cinnamaldehyde (C_9H_8O), a major component of cinnamon, is responsible of

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aroma and flavor of cinnamon.[8] Clinical and animal experiments have confirmed that cinnamaldehyde exerts obvious anti-diabetic effects by blood glucose reducing, insulin-mimetic and anti-oxidant properties.[9,10]

Yohimbine (17a-hydroxy yohimbine-16a-carboxylic acid methyl ester), an indole alkaloid found in a variety of botanical sources such as Rauwolfia root, is a principal alkaloid extracted from the bark of the *Pausinystalia yohimbe* tree.[11] Yohimbine has the highest affinity for the α_2-receptor; but, also antagonizes the α_1-receptor as well as some serotonin and dopamine receptors and is used for treatment of erectile dysfunction, memory impairment and anxiety disorders.[12] This drug decreased blood glucose and increased serum insulin concentrations in a rat model of type 2 diabetes, GK rats.[13]

It is known that cinnamaldehyde has anti-hyperglycemic and insulin mimetic properties.[10] In addition, peripheral and central α_2-adrenoceptors are involved in glucose homeostasis.[14] In the present study, the effects of intra-peritoneal (IP) and intracerebroventricular (ICV) administration of cinnamaldehyde and yohimbine were investigated on hyperglycemia and hypoinsulinemia induced by ketamine-xylazine in rats.

Materials and Methods

Animals. In the present study, healthy adult male Wistar rats (220-250 g) were used. In a laboratory under controlled 12 hr light-dark cycle and ambient temperature (22.00 ± 0.50 ˚C), the animals were maintained with *ad libitum* food and water. All experiments were performed between 9:30 and 13:00. The Animal Ethics Committee of Faculty of Veterinary Medicine of Urmia University (Ref No.: AECVU-195-2019) approved the research protocol and animal care procedures.

Chemicals. The used chemical compounds including cinnamaldehyde and yohimbine were purchased from Sigma-Aldrich (St. Louis, USA). Ketamine (Alfasan, Woerden, The Netherlands) and xylazine (Alfasan) were purchased from native veterinary pharmacy. Chemical compounds were prepared before IP and ICV administrations.

Animal groups. Forty-eight rats were divided into eight groups with six rats in each as follows: 1) V(IP)+NS (IP) group was treated with IP injections of cinnamaldehyde vehicle plus normal saline. 2) V(IP)+KX(IP) group was treated with IP injections of cinnamaldehyde vehicle plus ketamine-xylazine. 3) C25(IP)+KX(IP) group was treated with IP injections of 25.00 mg kg^{-1} cinnamaldehyde plus ketamine-xylazine. 4) C50(IP)+KX(IP) group was treated with IP injections of 50.00 mg kg^{-1} cinnamaldehyde plus ketamine-xylazine. 5) C100(IP)+KX (IP) group was treated with IP injections of 100 mg kg^{-1} cinnamaldehyde plus ketamine-xylazine. 6) Y0.5(IP)+KX (IP) group was treated with IP injections of 0.50 mg kg^{-1} yohimbine plus ketamine-xylazine. 7) Y2(IP)+ KX(IP) group was treated with IP injections of 2.00 mg kg^{-1} yohimbine plus ketamine-xylazine. 8) C25(IP)+Y0.5(IP)+ KX(IP) group was treated with IP injections of 25.00 mg kg^{-1} cinnamaldehyde plus 0.50 mg kg^{-1} yohimbine plus ketamine-xylazine.

To reduce the number of used animals, the IP injected animals were used again for ICV injection 15 days after the end of IP injection experiments. During this period, rats were undertaken the lateral ventricle of the brain cannulation. These rats were divided into eight groups with six rats in each as follows: 1) V(ICV)+NS(IP) group was treated with ICV administration of cinnamaldehyde vehicle plus IP injection of normal saline. 2) V(IP)+KX(IP) group was treated with ICV administration of cinnamaldehyde vehicle plus IP injection of ketamine-xylazine. 3) C25(IP)+KX(IP) group was treated with ICV administration of 25.00 µg per rat cinnamaldehyde plus IP injection of ketamine-xylazine. 4) C50(IP)+KX(IP) group was treated with ICV administration of 50.00 µg per rat cinnamaldehyde plus IP injection of ketamine-xylazine. 5) C100(IP)+KX(IP) group was treated with ICV administration of 100 µg per rat cinnamaldehyde plus IP injection of ketamine-xylazine. 6) Y0.5(IP)+KX(IP) group was treated with ICV administration of 5.00 µg per rat yohimbine plus IP injection of ketamine-xylazine. 7) Y2(IP)+KX(IP) group was treated with ICV administration of 20.00 µg per rat yohimbine plus IP injection of ketamine-xylazine. 8) C25(IP)+Y0.5(IP)+KX(IP) group was treated with ICV administrations of 25.00 µg per rat cinnamaldehyde plus 5.00 µg per rat yohimbine plus IP injection of ketamine-xylazine.

The doses of cinnamaldehyde and yohimbine used here were in accordance with the other investigations in which cinnamaldehyde at doses of 50.00 and 100 mg kg^{-1} for IP injection and yohimbine at doses of 1.00 - 4.00 mg kg^{-1} for IP injection and 20.00 µg per rat for ICV administration have been used.[15-17]

Surgical procedure. The lateral ventricle of the brain was surgically implanted with a permanent guide cannula for delivering the chemical agents.[18] In brief, each rat was anaesthetized with IP injection of a mixture of ketamine (80.00 mg kg^{-1}; Alfasan) and xylazine (10.00 mg kg^{-1}; Alfasan). A 23-gauge, 13.00-mm stainless-steel guide cannula was stereotaxically (Stoelting Stereotaxic Apparatus, Wood Dale, IL, USA) placed in the lateral ventricle of the brain according to the following coordinates: 1.40 mm posterior to the bregma**,** 2.00 mm lateral to the midline and 4.00 mm below the top of the skull.[19] Cerebrospinal fluid (CSF) exit from the tip of cannula confirmed the presence of cannula inside the ventricle (Fig. 1). The guide cannula was anchored with two screws and dental acrylic. A 13.00-mm stylet was inserted into the guide cannula to keep it patent prior to injection. All animals were allowed to recover from surgery for 10 days.

The IP and ICV injections. Cinnamaldehyde and yohimbine were dissolved in normal saline with adding one drop of Tween 80 and ketamine-xylazine was diluted in normal saline. The IP injections were done using 25-guage syringes at a fixed volume of 1.00 mL kg^{-1}. The ICV injections were performed using a 10.00-µL Hamilton syringe over a period of 30 sec at a constant volume of 2.00 µL per rat. After completion of each ICV injection, the injection needle was left in place for further 30 sec to facilitate the diffusion of drug solution. Cinnamaldehyde and yohimbine were administered IP 40 and 30 min, respectively, before ketamine-xylazine injection. This time schedule for ICV administration was 10 and 5 min for cinnamaldehyde and yohimbine, respectively. Although yohimbine and cinnamaldehyde cross the blood-brain-barrier after systemic administration,[20,21] we used ICV administration for exploring the direct effects of yohimbine and cinnamaldehyde on CNS structures involved in glucose homeostasis. In this context, peripheral and central contributions of α_2-adrenergic system in urine flow during ketamine-xylazine anesthesia have been reported by intra-venous (IV), ICV and intra-para-ventricular nucleus administration of yohimbine in rats.[15]

Induction of hyperglycemia and hypoinsulinemia. We used ketamine-xylazine model of hyperglycemia and hypoinsulinemia. Briefly, a mixture of ketamine (100 mg kg^{-1}) and xylazine (10.00 mg kg^{-1}) was injected IP at a constant volume of 1.00 mL kg^{-1}. Blood glucose level was measured before and after induction of anesthesia. Serum insulin level was determined at the end of experiment. Hyperglycemia and hypoinsulinemia induced by ketamine-xylazine may be related to xylazine, because alone IP injection of ketamine did not alter blood glucose and serum insulin levels in fed rats.[16] Hyperglycemia and hypoinsulinemia induced by anesthetics such as ketamine-xylazine have been considered as a pre-clinical model for evaluating acute glucose homeostatic mechanisms.[16,22,23]

Blood glucose measurements. Blood glucose concentration was determined using a digital glucometer (Glucocard 01-Mini; Arkray, Kyoto, Japan). For this purpose, the end of tail was punctured with a 30-guage needle, 10.00 µL blood was introduced to the strip of glucometer and 6 sec later the amount of glucose was read from the monitor. Blood glucose concentrations were recorded 45 and 0 min before and at 30, 60, 90 and 120 min after ketamine-xylazine injection. Blood glucose concentrations were expressed as mg dL^{-1}.

Serum insulin determination. After the last blood glucose measurement, a 25-guage, injection needle was inserted into the heart through 7th and 8th intercostals muscles.[24] Blood samples (0.40 mL) were collected from the heart into the non-heparin containing tubes. These tubes were centrifuged at 3,500 rpm for 10 min and serum samples were separated and transferred to Eppendorf tubes for insulin determination. Serum insulin concentration was detected using a rat insulin enzyme-linked immunosorbent assay kit (Mercodia AB, Sylveniusgatan 8A SE-75450, Uppsala, Sweden) after the serum samples were thawed at room temperature. Serum insulin levels were expressed as µg L^{-1}.

Cannula verification. After ICV injection of 2.00 µL blue ink, the animals were euthanized under ether-induced deep anesthesia and the brains were removed and placed in a 10.00% formalin solution. Twenty-four hr later, transverse and longitudinal sections of the brains were viewed to observe the distribution of methylene blue in the lateral ventricle (Fig. 1) according to the figure plates (Fig. 1) of the atlas of Paxinos and Watson.[19]

Statistical analysis. Statistical comparisons were performed using GraphPad Prism (version 5.3; GraphPad software Inc., San Diego, USA). The data obtained from glucose measurement at time points before and after anesthesia were analyzed by two-way analysis of variance (ANOVA) followed by the Bonferroni post hoc test. Data obtained from insulin concentrations and area under curve (AUC) of blood glucose levels were analyzed using one-way ANOVA followed by Tukey's post hoc test. Area under the curve (AUC) for blood glucose levels was calculated by the trapezoidal method.[25] In figures, data are expressed as the Mean ± SEM. A value of $p < 0.05$ was considered statistically significant.

Results

The distributed blue ink in a lateral ventricle of the brain and a drop of CSF out of the cannula are shown in Figure 1. The rat brain sections (Fig. 1) were adopted from the atlas of Paxinos and Watson.[19]

No significant differences were observed among all IP and ICV groups at the −45 and 0 min time points before anesthesia induction. In addition, IP and ICV vehicle groups showed no significant differences at 30, 60, 90 and 120 min time points. In the both IP and ICV groups, ketamine-xylazine injection significantly ($p < 0.001$) increased blood glucose levels at 30, 60, 90 and 120 min time points (Figs. 2A-2C and 3A-3C).

Figure 2 shows the effects of IP injections of cinnamaldehyde (A), yohimbine (B), their combination (C) and AUC (D) on blood glucose level changes induced by ketamine-xylazine in rats. There were significant differences among treatments ($F_{(4,150)} = 169.051$; $p < 0.001$), across times ($F_{(5,150)} = 145.112$; $p < 0.001$) and between interactions ($F_{(20,150)} = 17.241$; $p < 0.001$) in the effects of cinnamaldehyde on blood glucose level changes induced by ketamine-xylazine (Fig. 2A). Cinnamaldehyde at a dose of 25.00 mg kg^{-1} significantly ($p < 0.05$) decreased blood glucose levels at 30 and 60 min post-anesthesia; whereas, at doses of 50.00 and 100 mg kg^{-1}, it significantly ($p < 0.001$) reduced the elevated blood glucose levels at all post-anesthesia time points (Fig. 2A). The IP-injected

yohimbine produced significant differences among treatments ($F_{(3,120)}$ = 270.703; p < 0.0001), across times ($F_{(5,120)}$ = 102.108; p < 0.001) and between interactions ($F_{(15,120)}$ = 26.151; p < 0.001) on blood glucose level changes induced by ketamine-xylazine (Fig. 2B). Yohimbine at a dose of 0.50 mg kg^{-1} significantly (p < 0.05) decreased 30 and 60 min time point hyperglycemia; whereas, a dose of 2.00 mg kg^{-1}, it significantly (p < 0.001) reduced anesthesia-induced elevation of blood glucose at all-time points (Fig. 2B). Significant differences among treatments ($F_{(3,120)}$ = 217.705; p < 0.0001), across times ($F_{(5,120)}$ = 64.658; p < 0.001) and between interactions ($F_{(15,120)}$ = 22.433; p < 0.001) were observed in the effects of co-administered cinnamaldehyde and yohimbine on blood glucose level changes induced by ketamine-xylazine (Fig. 2C). This combined treatment significantly (p < 0.01) decreased the increased blood glucose levels at all post-anesthesia time points (Fig. 2C). One-way ANOVA blood glucose levels AUC revealed significant ($F_{(7,39)}$ = 16.934; p < 0.001) differences among groups. Ketamine-xylazine significantly (p < 0.001) increased the AUC. Cinnamaldehyde at a dose of 25.00 mg kg^{-1} and yohimbine at a dose of 0.50 mg kg^{-1} did not alter AUC. The AUC was significantly decreased by cinnamaldehyde at doses of 50.00 mg kg^{-1} (p < 0.05) and 100 mg kg^{-1} (p < 0.01) and by yohimbine at a dose of 2.00 mg kg^{-1} (p < 0.001). A combined treatment with cinnamaldehyde (25.00 mg kg^{-1}) and yohimbine (0.50 mg kg^{-1}) significantly (p < 0.05) decreased AUC (Fig. 2D).

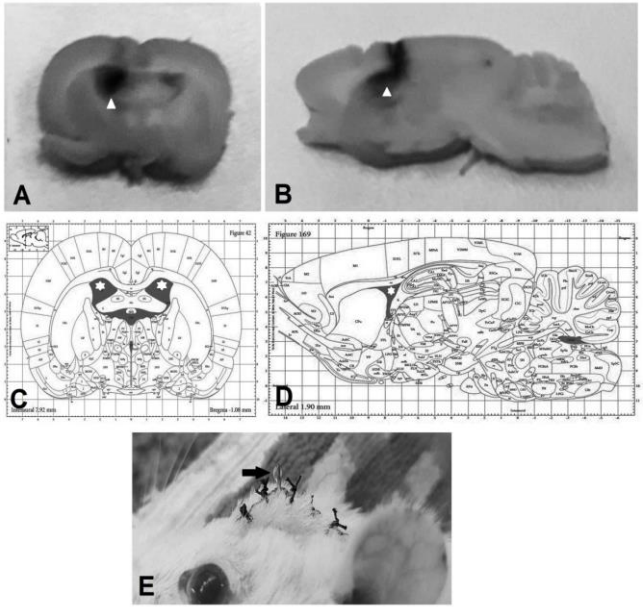

Fig. 1. Transverse (A) and longitudinal (B) sections of the rat brain showing the distribution of ink in the lateral ventricle (white arrowheads). The rat brain sections (C and D) were adopted from the atlas of Paxinos and Watson[19] to show the location of lateral ventricle (white asterisks). Figure E shows a drop of cerebrospinal fluid (black arrow) out of the cannula.

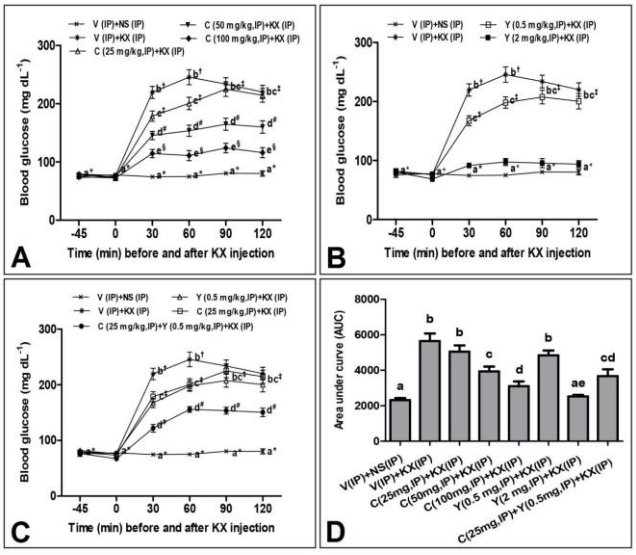

Fig. 2. Effects of IP administration of cinnamaldehyde (A), yohimbine (B), their combination (C) and area under curve (D) changes induced by ketamine-xylazine. Similar letters indicate no significant differences in respective measured times. Similar symbols indicate no significant differences regarding used chemical compounds doses. Non-similar letters and non-similar symbols indicate significant differences regarding measured times and used chemical compounds doses, respectively. IP: Intraperitoneal; V: Vehicle; NS; Normal saline; C: Cinnamaldehyde; Y; Yohimbine; KX: Ketamine-xylazine.

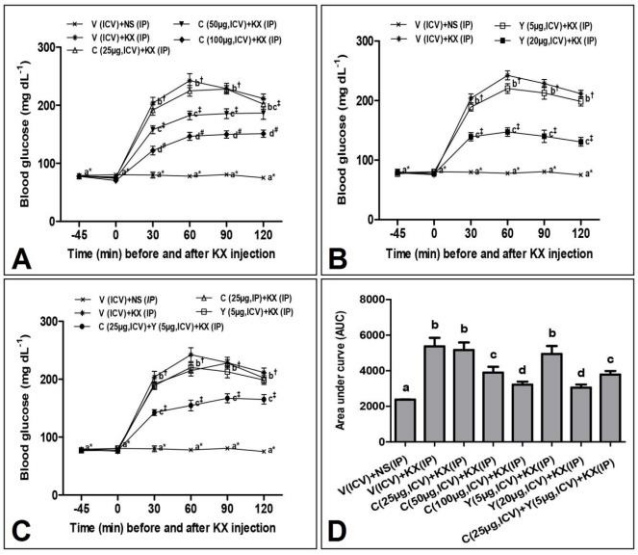

Fig. 3. Effects of ICV administration of cinnamaldehyde (A), yohimbine (B), their combination (C) and area under curve (D) changes induced by ketamine-xylazine. Similar symbols indicate no significant differences regarding used chemical compounds doses. Non-similar letters and non-similar symbols indicate significant differences regarding measured times and used chemical compounds doses, respectively. ICV: Intracerebroventricular; IP: Intra-peritoneal; V: Vehicle; NS: Normal saline; C: Cinnamaldehyde; Y: Yohimbine; KX: Ketamine-xylazine.

Figure 3 shows the effects of ICV injections of cinnamaldehyde (A), yohimbine (B), their combination (C) and AUC (D) on blood glucose level changes induced by ketamine-xylazine in rats. The ICV-administered cinnamaldehyde produced significant differences among treatments ($F_{(4,150)}$ = 204.302; p < 0.0001), across times ($F_{(5,150)}$ = 253.912; p < 0.0001) and between interactions ($F_{(20,150)}$ = 22.533; p < 0.01) on blood glucose level changes induced by ketamine-xylazine (Fig. 3A). Serum insulin concentrations in vehicle-treated groups in both IP and ICV route administration were 0.65 ± 0.03 µg L^{-1} and 0.68 ± 0.03 µg L^{-1}, respectively. Ketamine-xylazine injection significantly (p < 0.001) decreased these values to 0.29 ± 0.02 µg L^{-1} and 0.26 ± 0.03 µg L^{-1} in IP and ICV groups, respectively. Cinnamaldehyde at a dose of 25 µg per rat had no effect; whereas, at doses of 50 and 100 µg per rat, it significantly (p < 0.01) reduced the anesthesia-induced hyperglycemia (Fig. 3A). There were significant differences among treatments ($F_{(3,120)}$ = 305.214; p < 0.0001), across times ($F_{(5,120)}$ = 201.918; p < 0.0001) and between interactions ($F_{(15,120)}$ = 33.041; p < 0.01) regarding the effects of yohimbine on blood glucose level changes induced by ketamine-xylazine (Fig. 3B). Yohimbine at a dose of 5.00 µg per rat produced no significant effect; whereas, anesthesia-induced hyperglycemia was significantly (p < 0.01) decreased by 20.00 µg per rat yohimbine at all post-anesthesia time points (Fig. 3B). Significant differences among treatments ($F_{(4,150)}$ = 196.921; p < 0.001), across times ($F_{(5,150)}$ = 276.605; p < 0.0001) and between interactions ($F_{(20,150)}$ = 21.831; p < 0.01) were observed considering the effects of co-administered cinnamaldehyde and yohimbine on blood glucose level changes induced by ketamine-xylazine (Fig. 3C). Hyperglycemia induced by ketamine-xylazine was significantly (p < 0.01) decreased by a combined treatment with cinnamaldehyde and yohimbine at all post-anesthesia time points (Fig. 3C). One-way ANOVA of blood glucose levels AUC revealed significant ($F_{(7,39)}$ = 11.582; p < 0.001) differences among groups. The ACU was significantly (p < 0.001) increased by ketamine-xylazine. Cinnamaldehyde at a dose of 25.00 µg per rat and yohimbine at a dose of 0.50 µg per rat did not change AUC. Cinnamaldehyde at doses of 50.00 µg per rat (p < 0.05) and 100 µg per rat (p < 0.01) and yohimbine at a dose of 20.00 µg per rat (p < 0.001) decreased AUC. A combined treatment with cinnamaldehyde (25.00 µg per rat) and yohimbine (5.00 µg per rat) significantly (p < 0.05) decreased AUC (Fig. 3D).

Figure 4 shows the effects of IP (A) and ICV (B) administrations of cinnamaldehyde, yohimbine and their combination on the serum insulin level alterations induced by ketamine-xylazine. One-way ANOVA revealed significant ($F_{(7,47)}$ = 87.671; p < 0.0001) differences among groups. Serum insulin level was significantly (p < 0.001) decreased by ketamine-xylazine. The IP injection of cinnamaldehyde (25.00 mg kg^{-1}) and yohimbine (0.50 mg kg^{-1}) had no significant (p > 0.05) effects; whereas, IP injection of cinnamaldehyde at doses of 50 mg kg^{-1} (p < 0.05) and 100 mg kg^{-1} (p < 0.01), yohimbine at a dose of 2.00 mg kg^{-1} (p < 0.01) and a combined treatment with 25.00 mg kg^{-1} cinnamaldehyde and 0.50 mg kg^{-1} yohimbine (p < 0.05) significantly increased the decreased levels of serum insulin (Fig. 4A). In addition, ICV injection of cinnamaldehyde (25.00 µg per rat) and yohimbine (5.00 µg per rat) had no significant (p > 0.05) effects; whereas, ICV injection of cinnamaldehyde at doses of 50.00 µg per rat (p < 0.05) and 100 µg per rat (p < 0.01), yohimbine at a dose of 20.00 µg per rat (p < 0.01) and a combined treatment with 25.00 µg per rat cinnamaldehyde and 5.00 µg per rat yohimbine (p < 0.05) significantly increased the decreased levels of serum insulin (Fig. 4B).

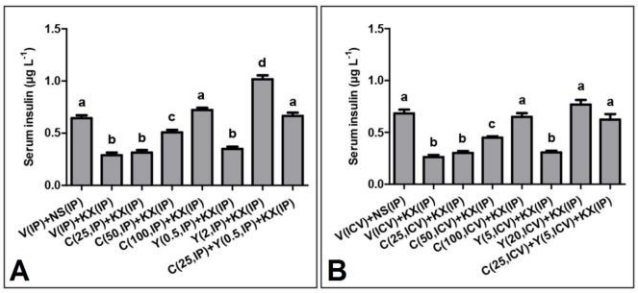

Fig. 4. Effects of IP (A) and ICV (B) administration of cinnamaldehyde, yohimbine and their combination on serum insulin changes induced by ketamine-xylazine. Similar letters indicate no significant differences. Non-similar letters indicate significant differences. ICV: Intracerebroventricular; IP: intra-peritoneal; V: Vehicle; NS: Normal saline; C: Cinnamaldehyde; Y: Yohimbine; KX: Ketamine-xylazine.

Discussion

The results of the present study showed that IP injection of ketamine-xylazine produced hyperglycemia and hypoinsulinemia. In addition, hyperglycemic and hypoinsulinemic effects of ketamine-xylazine were reversed by IP and ICV administrations of yohimbine. These results indicate that peripheral and central α_2-adrenoceptors are involved in glucose homeostasis. These results are in accordance with the findings of other investigations. For example, intra-muscular injection of ketamine (100 mg kg^{-1}) and xylazine (10.00 mg kg^{-1}) produced potent hyperglycemia and hypoinsulinemia in fed rats and these effects were blocked by prior administration of yohimbine.[16,23] In addition to well-known dissociative anesthetic property, ketamine has many pharmacological effects such as analgesic and anti-depressant properties.[26] Anesthetic and analgesic properties of ketamine are attributed to direct inhibition of N-methyl-D-aspartate receptors; whereas, other functions are related to dopamine, serotonin, opioids and voltage-gated sodium channels.[27] Xylazine, 2-[2,6-(Dimethyl-d6) phenylamino]-5,6-dihydro-4H-1,3-thiazine hydrochloride,

is widely used as a sedative, analgesic and relaxant in veterinary medicine.[28] Xylazine is a typical α_2-adrenergic receptor agonist and this receptor is distributed in the sympathetic nervous system (SNS)-innervated cells with high densities in hypothalamic neurons and endocrine pancreas.[14,29,30] Therefore, SNS through α_2-adrenoceotprs at the peripheral, spinal and supra-spinal levels influences insulin release from beta cells of the pancreas and subsequent blood glucose concentration and increased α_2-adrenoceptor signaling in these regulatory systems may result in beta-cell dysfunction leading to metabolic syndrome.[14,31,32] In this context, IV injection of dexmedetomidine, an α_2-adrenergic receptor agonist, increased and decreased blood glucose and serum insulin levels in dogs, respectively, and these effects were prevented by MK-467, an α_2-adrenergic receptor antagonist.[33] In addition, intra-thecal administration of clonidine, an α_2-adrenoceptor agonist, produced hyperglycemia and hypoinsulinemia in mice and these effects were attenuated by yohimbine pre-treatment.[31] The ICV injection of idazoxan (an α_2-adrenergic receptor antagonist) and yohimbine reversed adrenaline-induced inhibition of insulin secretion in rats and suggested the involvement of centrally-mediated α_2-adrenergic receptor in glucose homeostasis.[34] The above-mentioned findings and the results of this study confirm the involvement of peripheral and central α_2-adrenergic receptor in blood glucose homeostatic mechanisms.

Our results showed that IP and ICV injections of cinnamaldehyde reduced the elevated level of blood glucose and increased the decreased concentration of insulin induced by ketamine-xylazine. Although there are no reports showing the effects of cinnamaldehyde on hyperglycemia and hypoinsulinemia induced by ketamine-xylazine, antihyperglycemic and antihypoinsulinemic effects of IP-injected cinnamaldehyde may be associated with stimulatory effects on insulin secretion and peripheral glucose utilization. It has been reported that cinnamaldehyde produces antidiabetic effect by protecting pancreatic beta-cells from histopathological changes.[35] The *in vitro* incubated pancreatic islets with cinnamaldehyde showed an elevation in insulin secretion when compared with glibenclamide.[36] Cinnamaldehyde up-regulated the expression of glucose transporter 4 (GLUT4) in C2C12 mouse muscle cells.[37] The GLUT4 is the major glucose transporter in skeletal muscle and adipose tissue, which is under control of insulin.[38] To date, we did not find any report demonstrating the ICV effects of cinnamaldehyde. However, medicinal plant extracts and their biologically active substances have ability to produce beneficial effects when centrally administered. For example, ICV injection of crocin, a constituent of saffron, attenuated centrally-administered penicillin-induced epileptiform activity through a $GABA_A$-benzodiazepine receptor mediated mechanism.[39] In addition, ICV or intra-basolateral amygdale infusion of Ginkgo biloba leaf extract (EGb761) produced memory-enhancing effects in rats.[40] It seems that cinnamaldehyde can affect glucose homeostasis through peripheral and central mechanisms.

In the present study, IP and ICV administrations of cinnamaldehyde and yohimbine produced comparable antihyperglycemic and antihypoinsulinemic effects with superior effects of yohimbine. In addition, a synergistic effect was observed between cinnamaldehyde and yohimbine when used together. This indicates that cinnamaldehyde influences glucose homeostatic mechanisms through interaction with peripheral and central α_2-adrenoceptors. Although there are no reports showing the interaction between cinnamaldehyde and α_2-adrenoceptors receptors, pre-treatment with cinnamaldehyde prevented the contractile effect of phenylephrine, an α1-adrenoceptor activator, in the aorta of rats.[41] Cinnamaldehyde was found to have ability to interact with cell membrane proteins such as transient receptor protein ankyrin-1 and toll-like receptor 4.[42-44] Therefore, based on all data collected here, there is enough evidence to support that glucose homeostasis could be affected by cinnamaldehyde in contribution with α_2-adrenergic system.

In conclusion, the results of the present study showed that ketamine-xylazine produced acute hyperglycemia and hypoinsulinemia. Systemic and central administrations of cinnamaldehyde prevented hyperglycemia and hypoinsulinemia induced by ketamine-xylazine. Peripheral and central blockade of α-$_2$adrenoceptor by yohimbine reversed ketamine-xylazine induced hyperglycemia and hypoinsulinemia. A synergistic effect was observed when a combined treatment with low doses of cinnamaldehyde and yohimbine was used. Interaction with peripheral and central α-$_2$adrenergic system may be related to antihyperglycemic and antihypoinsulinemic effects of cinnamaldehyde.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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