Title:

The effects of hydroalcholic extract of*crocus sativus*and crocin on blood pressure and heart rate in acute hypertensive rats induced by angiotensin II

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ABSTRACT:

Introduction: Hypertension is one of the most important health problems that many factors including renin-angiotensin system (RAS) are involved in its phatogenesis.In present time blockers of RAS are most antihypertensive drugs. It is seems that antihypertensive effect of many herbs such as *Crocus sativus (C. sativus)*is mediated via this system.Therefore present study was designed to evaluate the effect of C. *sativus* and crocin on acute hypertension induced by angiotensin II (Ang II).

Materials and methods: In present study, 96 rats were used. After anesthetized by urethane, a heparinized polyethylene catheter was inserted into femoral artery. Then systolic blood pressure (SBP), mean arterial pressure (MAP) and heart rare (HR) were continuously recorded by power lab system. Three doses of Ang II (50, 150 and 300ng/kg), losartan (10mg/kg), three doses of hydro-alcoholic extract of *C. sativus* (10, 20 and 40 mg/kg) and crocin (50, 100 and 200mg/kg) were injected intraperitoneally (i.p) and intravenously (i.v) through a catheter inserted into femoral vein. In i.p injection, 30 min after the injection of *C. sativus* and crocin, Ang II was injected every 5 min and in i.v injection it was adminesterated 10 min after injection of *C. sativus* and crocin. The changes of SBP, MAP and HR were calculated and analyzed by instat software. Changes of SBP, MAP and HR in C. sativus and crocin compare to Ang II group.

Results: All three doses of Ang II significantly increased SBP and MAP compared to control group ($P < 0.01$ and $P < 0.001$). in addition dose 50ng/kg of Ang II () decreased HR compare to control group, whereas in dose 300ng/kg, increased HR in compared to control group.All effects of AngII were attenuate by losartan. Three doses of hydro-alcoholic extract of *C. sativus* in both i.v and i.p injections significantly reduced SBP and MAP induced byAng II ($P < 0.05$ and $P < 0.001$). However, i.v injection was more effective than i.p injection.The *C. sativus* (40mg/kg) attenuate tachycardia induced by highest dose of Ang II (300) ($P < 0.01$). Intravenous and intraperitoneal administration of crocin (50, 100 and 200mg/kg) significantly decrease effect of AngII on SBP and MAP ($P < 0.05$ and $P < 0.01$). The results of present study also indicated that crocin

reverse the effects of angiotensin II (50 and 300mg/kg) on HR. Comparison the effect of the highest dose of *C. sativus* and crocin on changes of SBP and MAP induced by Ang II showed that extract is more effective than crocin while there was not any significant difference between them on HR.
Conclusion: Regarding to modulatory effects of hydro-alcoholic extract of *C. sativus* and crocin on Ang II -induced hypertension, it seems that the cardiovascular effects of *C. sativus* and crocin partly mediated via suppression of renin-angiotensin system. It is also possible that the cardiovascular effect of crocin mostly mediated by effect on HR.
Key word: *Crocus sativus*, Crocin, Hypertension, Heart rate, Angiotensin II

2. Title:

The evaluation of cardiovascular effects of cholinergic system in pedunculopontine tegmentum nucleus of rat

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Abstract:

Introduction: The pedunculopontine tegmentum nucleus (PPT) is a mesancephalic nucleus that involved in several function including regulation of central cardiovascular system. Although, presence of cholinergic system in PPT nucleus has been indicated but its effects on cardiovascular system is uncertain. Therefore, in this study the effect of cholinergic system of the PPT and its both muscarinic and nicotinic receptors on cardiovascular system was evaluated .
Material and Methods: This study was done on 80 male rats. After anesthesia with urethane (1.4g/kg, i.p), a polyethylene catheter filled by heparinized saline was inserted in the femoral artery. The catheter connected to a pressure transducer and mean arterial pressure (MAP), heart rate (HR), and systolic blood pressure (SBP) were continuously recorded by a power lab system. The drugs including Acethlcholine (Ach), Atropine (Atr; a nonselective muscarinic receptor antagonist), and Haexamethonium (Hexa; a nonselective nicotinic receptor antagonist) were microinjected into nucleus. For drugs microinjection, animals were placed in a stereotaxic apparatus and a small hole drilled in the skull over the PPT nucleus .The stereotaxic coordination of PPT were (AP: 7.44-8.64 mm; L: 1.6-2.2 mm; H: 6.8-7.8 mm). Microinjections were performed by a single barreled micropipette that was

connected through a PE-10 tube to an injector syringe and carefully introduced into the PPT .
Two doses of Ach (90 and 150 nmol), Atr (3 and 9 nmol) and Hexa (100 and 300 nmol) and coinjection of Atr (9 nmol) + Hexa(100nmol) were used . In Atr and Hexa and Atr+Hexa groups, 2 min after microinjection of drugs, Ach (150 nmol) was microinjected. Then maximum changes of MAP, SBP and HR were obtained and compared with control group (unpaired t-test) and pre injection (paired t test.(
Results: Microinjection both doses of Ach (90 and 150 nmol) significantly decreased the MAP ($p<0.05$ and $P<0.01$ respectively) and SBP ($P<0.01$ and $P<0.001$ respectively) but had no significant effect on HR. Atropine itself did not alter MAP or HR in rats. To make sure of cardiovascular effect of the PPT muscarinic receptors, Atr (3 and9 nmol) separately microinjected and 2 min later, Ach (150 nmol) was microinjected. Dose 3 nmol of Atr significantly increased HR compare to Ach (150nmol) group. But its effect on MAP and SBP was not significant. Dose 9 nmol significantly decreased hypotensive MAP ($P<0.01$) and SBP ($P<0.001$) compare to Ach (150nmol) but its effect on HR was no significant. Hexa also itself did not alter BP or HR .Therefore, same as atropine two doses of hexa (100 and 300 nmol) were separately microinjected 2 min before microinjection of Ach (150 nmol). Hexa cannot attenuate the hypotensive effect of Ach on MAP and SBP. It also had no significant effect on the HR. Co injection of Atr + Hexa also strongly inhibited hypotensive effect of Ach on MAP ($P<0.01$) and SBP ($P<0.001$) with no significant effect on HR .
Conclusion: Results of this study indicate involvement of cholinergic system of PPT nucleus on central regulation of cardiovascular system. This system caused decreased of MAP and SBP with no obvious effect on HR that strongly mediated through muscarinic receptors .

Key words: PPT, Acetylcholine, Microinjection, MAP, Atropine, Hexametunium

3. Title:

The effect of Gabaergic system of Pedunculopontine tegmentum nucleus on central cardiovascular function in rat

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ABSTRACT:

Introduction:

The pedunculopontine tegmentum (PPT) nucleus located in the upper part of the pons and involved in several functions, including central cardiovascular regulation. Presence of GABAergic system in the PPT has been shown but its effects on the cardiovascular system is not clear. Therefore, in present study the cardiovascular effects of GABAergic system of PPT were investigated.

Materials and Methods:

In this study 100 adult male Wistar rats were used. Animal groups were divided into following groups: 1) Control ,2) and 3) two doses of 1.5 and 2.5 nmol muscimol, 3 and 4) two doses (0.1 and 0.2 nM) of bicuculline, 5 and 6) two doses (0.5 and 1 nM) of baclofen , 7 and 8) two doses (0.5 and 1 nmol) of phaclophen 9) hexamethonium(Hexa;10mg/kg; i.v) and bicuculline (2.5 nM) 10) atropine(10) and bicuculline (2.5 nM). To perform the test, after anesthesia a polyethylene catheter filled with heparinized saline inserted in the femoral artery.the catheter is connected to a pressure transducer and mean arterial pressure (MAP), heart rate (HR) and systolic blood pressure (SBP) was recorded continuously by powerlab system.

For drugs injection A small hole was drilled in the skull over the PPT using a stereotaxic system with coordinats (AP; 7.44-8.64mm: L; 1.6-2.2mm, H; 6.8-7.8mm), the barrel micropipette filled with drugs insereted into the nucleus and drug microinjected by injector system.changes of cardiovascular responses (Δ HR, SBP, MAP) induced by injection were

calculated and compared with control group (One way ANOVA) and pre injection value (paire t-test).

Results:

Microinjection two doses of muscimol into the PPT reduce HR, SBP and MAP. However, only reduction of SBP in dose 2.5 was significant ($P < 0.05$). Two doses of bicuculline significantly increased SBP, MAP and HR compared to the control group ($P < 0.05$, $P < 0.001$). Comparing two doses of bicuculline also showed that effect of dose 2.5 was significantly greater than dose 1.5 ($P < 0.05$, $P < 0.01$). Microinjection baclofen at doses of 0.5 and 1 nmol has no significant on cardiovascular parameters. Microinjection phaclophen at a dose of 0.5 and 1 nmol increased SBP, MAP and HR. However, these effects were not significant compare to the control group. Intravenous injection of Haxamethonium (Hexa) significantly reduced SBP, MAP and HR compared to the control group ($P<0.01$). Microinjection of bicuculline after injection haxa not only reversed cardiovascular effect of Hexa, but also significantly increase these parameters compare to control group ($P < 0.05$, $P < 0.01$). Intravenous injection of atropine cause increased heart rate with no significant effect on blood pressure. Microinjection bicuculline after atropine significantly increased heart rate ($P < 0.01$) but has no significant effect on blood pressure.

Conclusion:

This study showed that GABAergic system of PPT has a tonic inhibitory effect on central cardiovascular system and this effect mainly done through $GABA_A$ receptors. In addition, peripheral effect of GABAergic system on blood pressure mediated by sympathtic and effect on HR mediated by both sympathtic and parasymphatic systems.

Keywords: Mean arterial pressure, Heart rate, Microinjection, the pedunculopontin tegmentum nucleus (PPT), GABA, Bicuculline

4.The role of inducible and neuronal nitric oxide synthase on lippolysaccharid-induced memory impairment in male rats

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ABSTRACT:

Introduction: Nitric oxide (NO) has been considered to have modulatory role in both memory and neuro-inflammation. Lipopolysaccharide (LPS) deteriorates learning and memory through triggering immune responses and oxidative stress. The present study was designed to evaluate the role of inducible (iNOS) and neuronal nitric oxide synthase (nNOS) on LPS-induced learning and memory impairment.

Materials and methods: The rats were divided into : (1) control, (2) LPS (1mg/kg), (3) Aminoguanidine (AG; 100 mg/kg)-LPS, (4) AG, (5) 7-nitroindazole (7NI; 30 mg/kg)-LPS, (6) 7NI, (7) L- arginine (LA; 200 mg/kg)-LPS, (8) LA, (9) Sodium nitroprusside (SNP; 2 mg/kg)-LPS and (10) SNP. 7NI was dissolved in saline supplemented with DMSO. The other drugs were dissolved in saline. LPS was administered (i.p.) 2h before the behavioral and electrophysiological experiments. AG, 7NI, LA and SNP were injected 30 minutes before LPS. Morris water maze (MWM), passive avoidance (PA) tests and high frequency stimuli (HFS) protocol of 100 Hz were carried out. The serum level of TNFα and the hippocampus tissue concentration of malondialdehyde (MDA), total thiol, NO metabolites, and the activities of superoxide dismutase (SOD) and catalase (CAT) were determined.

Results: In MWM, LPS enhanced the latency and traveled distance to find the platform ($P<0.05$ and $P<0.001$) while, reduced the time spent in target quadrant ($P<0.05$ and $P<0.001$) in probe day. In PA test, LPS decreased the latency to enter the dark compartment at 3, 24, 48 and 72 hours after receiving a shock ($P<0.01$ and $P<0.001$). In addition, LPS lessened amplitude and slope of field excitatory post synaptic potential (fEPSP) ($P< 0.05$ and $P<0.01$). These effects were associated with significant enhancement in serum TNFα ($P<0.01$), the hippocampus tissue concentration of MDA ($P<0.001$) and

NO metabolites (P<0.05) and reduction of total thiol (P<0.001), SOD and CAT activities (P<0.05). Administration of AG and 7NI before LPS decreased the latency and traveled distance to find the platform (P<0.001), while increased the time spent in target quadrant (P<0.01) in probe day in MWM. They also increased the latency to enter the dark compartment at 3, 24, 48 and 72 hours after receiving a shock (P<0.001) in PA test. The amplitude and slope of fEPSP enhanced in AG-LPS and 7NI-LPS groups compared to LPS group (P <0.05). According to biochemical tests, AG and 7NI significantly reduced the serum TNFα (P<0.01), the hippocampus tissue concentration of MDA (P<0.001) and NO metabolites (P<0.01) while, increased the level of total thiol (P<0.001), and the activities of SOD and CAT (P<0.05) in AG-LPS and 7NI-LPS groups compared to LPS group. Administration of LA and SNP before LPS decreased the latency and traveled distance to find the platform (P<0.001) while, increased the time spent in target quadrant (P<0.05 and P<0.01) in probe day in MWM test. They also increased the latency to enter the dark compartment at 3, 24, 48 and 72 hours after receiving a shock (P<0.05 and P<0.001) in PA test. Significant difference was not observed in amplitude and slope of fEPSP in LA-LPS and SNP-LPS groups compared to LPS group. On the basis of biochemical tests, LA and SNP reduced the serum TNFα (P<0.01) and the hippocampus tissue concentration of MDA (P<0.01 and P<0.001) while, increased the total thiol content (P<0.001) and the activities of SOD and CAT (P<0.05) in LA-LPS and SNP-LPS groups in comparison to LPS group. In addition, the hippocampus tissue concentration NO metabolites in LA-LPS group was lower than LPS group (P<0.05) whereas, no significant difference was seen in these metabolites between SNP-LPS and LPS groups.

Concussion: Briefly overproduction of NO and the brain tissue oxidative damage followed by LPS-induced inflammation involve in detrimental effects of LPS on learning, memory and synaptic plasticity. Regarding protective effects of AG and 7NI against memory and synaptic plasticity impairment caused by LPS which was accompanied with an improvement in inflammatory responses, oxidative stress and reduction of NO metabolites, the possible role of both iNOS and nNOS in LPS-induced memory and neuronal plasticity impairment might be postulated.

Key words: Learning, Memory, Synaptic plasticity, Lipopolysaccharide, Aminoguanidine, 7-nitroindazol

5. Title:

Study of the effect of *Curcuma Longa* and *Nigella Sativa* hydro alcholic extract on Adriamycin-induced proteinuria and oxidative stress in the rat kidney.

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Background: Adriamycin (ADR) is one of the important anti-cancer drugs that use in treatment of head and neck, testis, prostate and bladder tumors. Proteinuria and nephrotoxicity are important side effects of Adriamycin that develop by Oxidative stress, inflammation and apoptosis. Curcuma Longa (C. Longa) and nigella sativa (N. sativa) are annual herbaceous plants traditionally have been used in the treatment of many diseases. Several pharmacological effects such as anti-diabetes, anti-inflammatory, anti-oxidant and anti-bacterial and anti-cancer also have been reported for these plants. In the current study the effects of hydroalchoholic extracts of C. Longa and N Sativa on ADR-induced proteinuria and oxidative stress and tissue injuries of the kidney have been investigated.

Method: 80 male Wistar rats were randomly divided into 10 groups: 1-control 2-Adriamycin(5 mg/kg,IV) 3-Vit C(100mg/kg) 4-N. Sativa(200 mg/kg) 5- C. Longa(1000mg/kg) 6-Vit C (100 mg/kg) + ADR(5 mg/kg) 7-N. Sativa 200mg/kg + ADR 8-C. Longa 1000mg/kg + Adriamycin 9- C. Longa1000mg/kg + N. Sativa 200mg/kg 10- C. Longa1000mg/kg +N. Sativa 200mg/kg+ ADR .

Extracts were given orally for 35 days and ADR was administered intravenously on day 7. The extract administrated in 6 days before and 28 days after the injection of ADR. On days 0,6,10,14,21,28 and 35, serum and urine samples were collected and at the end of the experiments the kidneys were removed. Serum concentration of urea, creatinine, glucose, albumin and osmolarity and the urine concentration of glucose, albumin and osmolarity, urine output, GFR, urea clearance and urinary excretion of glucose, albumin and osmolarity were

determined. In end of experiment kidneys were removed for renal index, pathology and oxidative stress markers including malon dialdehide, total thiol groups and superoxide dismutase were evaluated.

Results: Serum concentration of Cr, urea and osmolarity did not changed compare to control group. However, serum albumin concentration decreased, cholesterol level increased, serum glucose and GFR decreased compare to control group. Both C. Longa and N. Sativa extracts increased albumin concentration compare to the day 0 and this effect is higher in co-administration of extracts. Cholesterol concentration increased by both extract compare to ADR group. The glucose of urine, clearance of urea did not changed in ADR group. Proteinuria due to ADR decreased by both extracts and this effect potentiate by co-administration of extracts. Tissue injuries of the kidney significantly were decreased by extracts. The MDA, total thiol and superoxide dismutase activity in kidney on day 35 in Adriamycin group was significantly decreased compared to day 35 in control group .

Conclusion: The results of present study showed that the hydro-alcoholic extracts of C. Longa and N. Sativa improved renal functional test, pathological and oxidative stress dysfunction tests induced by ADR administration. Mechanism(s) of the effects these plants on decreasing the renal ADR toxic effect is not clarified and requires further investigations.

Keywords: Adriamycin, Nigella Sativa, Curcuma Longa, Vitamin C. Proteinuria, Oxidative Stress