

# Activation of alpha-4 beta-2 nicotinic receptor against cardiopulmonary bypass surgery induced brain injury by regulating NLRP3 pathway

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## Original article

**Keywords:** Alpha-4 beta-2 nicotinic receptor, Cardiopulmonary bypass, Brain Injury, NLRP3, Oxidative stress

**Posted Date:** May 6th, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-25339/v1>

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# Abstract

Present report evaluates the role of alpha-4 beta-2 nicotinic receptor ( $\alpha 4\beta 2$  nAChRs) in the development of cardiopulmonary bypass (CPB) surgery induced brain injury. Brain injury was induced by CPB and animals were treated with  $\alpha 4\beta 2$  nAChRs agonist (DH $\beta$ E 9 mg/kg, s.c.) and  $\alpha 4\beta 2$  nAChRs antagonist (MLA 10 mg/kg, i.p.) 3 hr before the induction of CPB in the separate groups. Effect of  $\alpha 4\beta 2$  nAChRs agonist was determined on the neurological function in CPB induced brain injured rats. Level of cytokines, ROS and expression of NLRP3, ZO-1 and Occluding proteins were estimated in CPB induced brain injured rats. Effect of  $\alpha 4\beta 2$  nAChRs agonist was determined on the neuronal apoptosis and histopathological changes in the brain tissue. Result of the study suggest that neurological score was reversed in  $\alpha 4\beta 2$  nAChRs agonist treated group than CPB group. Level of cytokines and ROS was reduced in  $\alpha 4\beta 2$  nAChRs agonist treated group than CPB group. Neuronal apoptosis in  $\alpha 4\beta 2$  nAChRs agonist treated group was found to be reduced compared to CPB group of rats. Moreover Activation of  $\alpha 4\beta 2$  nAChRs ameliorates the altered expression of NLRP3, ZO-1 and Occluding protein in the brain tissue of CPB induced brain injured rats. In conclusion, Data of report suggest that treatment with  $\alpha 4\beta 2$  nAChRs agonist protects the brain injury in cardiopulmonary bypass surgery induced brain injury by regulating NLRP3 pathway.

## Introduction

Open heart surgery is performed by using cardiopulmonary bypass (CPB) technology, which has reduces the rate mortality occurs due to postoperative fatal arrhythmia, heart failure, myocardial infarction and other cardiac issues (Hausenloy et al, 2012). However patients with cardiac surgery have been appearing to be cognitive dysfunction which causes mental and neuropsychiatric disorders. Literature suggests that CPB induced neuropsychiatric and neurological disorders leads to enhance the mortality rate up to 19.6% (Salameh et al, 2016). Management of several heart disorders by surgery using CPB induces hypoxia and lower perfusion in the brain tissues, which causes occurrence of oxygen debt (Lee et al, 2008). These alterations in the oxygen supply causes activation of inflammatory cascade and thereby enhances the inflammatory response. Systemic inflammatory response syndrome occurs due to cerebral embolism in CPB (Sarkar and Prabhu, 2017). There is a need of development of novel therapy for the management of it.

Nicotinic acetylcholine receptors (nAChRs) is activated by exerting nicotine and homomeric  $\alpha 7$  and heteromeric  $\alpha 4\beta 2$ - containing receptors were the identified subunit of nAChR (Albuquerque et al, 2009). nAChRs is widely spread and abundantly present in the brain tissue and activation of it regulates the production of cytokines. Literature reveals that nAChR ameliorates immune response and inflammation by regulating the activity of NF- $\kappa$ B and JAK/STAT pathway (Ibrahim et al, 2018) and also reported to posses' neuroprotective effect due to its anti inflammatory property (Hone and McIntosh, 2018).  $\alpha 4\beta 2$  nAChRs agonist reported to be beneficial effect in cessation of smoking (Singh and Budhiraja, 2008). Moreover  $\alpha 4\beta 2$  nAChRs also reduces the NF- $\kappa$ B activity which is induced by LPS by regulating JAK/STAT3 pathway (Zdanowski et al, 2015). Thus present report determines the role of  $\alpha 4\beta 2$  nAChRs in cardiopulmonary bypass surgery induced brain injury.

# Material And Methods

## Animal

Male Sprague–Dawley rats weighing 250–300 g were kept under a 12-h light/dark cycle at  $60 \pm 5\%$  humidity and  $24 \pm 3$  °C. Protocols used in the animals were approved by Institutional Animal Ethical Committee of First Hospital of Hebei Medical University, China (IAEC/FH-HMU/2018/05).

## Experimental

Cardiopulmonary bypass surgery induced brain injury was performed as per earlier reported study (Grogan et al, 2008). 22G and 24G trocar was inserted in the left femoral artery and right femoral vein respectively after anesthetizing all the animals with isoflurane. Further extracorporeal venous outflow ends by inserting 18G puncture needle through right internal jugular vein to the right atrium. CPB model was established by connecting the connecting pipe, filter, arterial infusion tube, blood reservoir and drainage tube to 1.6 mm internal diameter PVC tube. Mechanical ventilation was terminated at the beginning and flow rate was maintained by inserting the circulating solution (lactate Ringer's solution, hydroxyethyl starch, mannitol 20%, sodium bicarbonate 5% and heparin) at 80 ml/kg/min and lung rate was maintained at 800 ml/min by passing oxygenair mixture (1:4 ratio) through the membrane lung. Diameter of venous flow was reduced to decrease the flow rate and later restarted the mechanical ventilation. Later incision was sutured after the removal of catheter and once the berating was resumed animals were fed in laboratory for observation.

Forty rats were separated into four different groups each group carries ten animals such as control group; CPB group;  $\alpha 4\beta 2$  nAChRs agonist group which receives DH $\beta$ E 9 mg/kg, s.c. and  $\alpha 4\beta 2$  nAChRs antagonist treated group which receives MLA 10 mg/kg, i.p. 3 hr before the induction of CPB.

## Neurological dysfunction

Neurological dysfunction was assessed by determining neurological scores in I/R-induced neuronal injury rats using Longa's method, as follows: 0, no deficit; 1, forelimb failed to extend entirely and weak; 2, circling to the contralateral side; 3, weight-bearing capacity reduced on the injured side; and 4, no spontaneous locomotor activity.

## Estimation of cytokines

The levels of the inflammatory mediators interleukin (IL)-1 $\beta$ , IL-6, nuclear factor kappa B (NF- $\kappa$ B) and tumour necrosis factor (TNF)- $\alpha$  were determined in the brain tissue homogenates by ELISA using commercial kits in accordance with the manufacturer's protocols (R&D Systems).

## Estimation of ROS

MitoSOX red mitochondrial superoxide indicator was used to estimate the levels of reactive oxygen species (ROS) in the brain tissues. Briefly, tissue homogenates were stained at 37 °C in the dark for 30 minutes with 5 µM MitoSOX red. A fluorescent plate reader was used to estimate the intracellular ROS levels at excitation and emission wavelengths of 510 and 580 nm, respectively.

## **Western blotting**

The protein expression levels of Bax, Bcl-2, caspase-3, pDrp1, Drp1, NLRP3, ZO-1 and occludin were assessed by Western blotting analysis of brain tissue homogenates. A BCA assay kit was used to quantify the protein from each tissue homogenate, and 10% sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed to separate the proteins, which were electroblotted onto nitrocellulose membranes. Subsequently, each membrane was blocked with 5% blocking solution (non-fat milk) and incubated in blocking buffer with primary antibodies overnight at 4 °C. Goat secondary antibodies conjugated with horseradish peroxidase were added to the blocking buffer, and a chemiluminescence kit was used to detect the proteins.

## **TUNEL assay**

Terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) assay was used to determine apoptosis of myocardial cells. The apoptosis index was calculated as follows: no. of stained myocytes/total no. of myocytes × 100%.

## **Histopathology study**

Hippocampus was isolated from each animal after the 6 hr of CPB and tissue was fixed in formalin solution. Ethanol was used to dehydrate the brain tissue and seed it into liquid paraffin. Microtome was used to section the hippocampus tissue and stained it with H&E staining.

## **Results**

### **α4β2 nAChRs activation ameliorates the neurological functions**

Neurological function was estimated in α4β2 nAChRs agonist and antagonist treated CPB induced brain injured rats by determining the neurological score. Neurological function score was enhanced up to 3.7 score in CPB group than control group (0.1 score). There was reduction in the neurological score to 1.06 in α4β2 nAChRs agonist treated group than CPB group. However α4β2 nAChRs antagonist treated group shows the enhancement of neurological score compared to α4β2 nAChRs agonist and control group of rats (Figure 1).

### **α4β2 nAChRs activation reduces cytokines**

Effect of  $\alpha 4\beta 2$  nAChRs activation was observed on the level of cytokines in the serum of CPB induced brain injured rats as shown in Figure 2. There was increase in the level of cytokines such as IL-1 $\beta$ , IL-8, TNF- $\alpha$  and NF- $\kappa$ B in the serum of CPB group than control group of rats. Level of cytokines was found to be reduced in the serum of  $\alpha 4\beta 2$  nAChRs agonist treated group and enhances in the serum of  $\alpha 4\beta 2$  nAChRs antagonist treated group compared to CPB group.

## **$\alpha 4\beta 2$ nAChRs activation reduces the level of ROS**

Level of ROS was estimated in the brain tissue of  $\alpha 4\beta 2$  nAChRs agonist and antagonist treated CPB induced brain injured rats. There was increase in the level of mitochondrial ROS in the brain tissue of CPB group than control group of rats. Treatment with  $\alpha 4\beta 2$  nAChRs agonist reduces the level of ROS in the brain tissue of CPB induced brain injured rats. However level of ROS was enhanced in the brain tissue of  $\alpha 4\beta 2$  nAChRs antagonist treated group compared to control and  $\alpha 4\beta 2$  nAChRs agonist treated group of rats (Figure 3).

## **$\alpha 4\beta 2$ nAChRs activation reduces the neuronal apoptosis**

Western blot assay was performed to determine the effect of  $\alpha 4\beta 2$  nAChRs activation on the expression of proteins responsible for apoptosis in the neuronal tissue of CPB induced brain injured rats and apoptosis index by TUNEL assay (Figure 4 A&B). There was increase in the expression of caspase-3 and Bax protein and decrease in the expression of Bcl-2 protein in the brain tissue of CPB group than control group of rats. Treatment with  $\alpha 4\beta 2$  nAChRs agonist ameliorates the altered expression of caspase-3, Bax and Bcl-2 protein in the neuronal tissue of CPB induced brain injured rats (Figure 4A). Moreover apoptosis in the neuronal tissue was enhanced in CPB group than control group of rats. There was reduction in the apoptosis of neuronal tissue in  $\alpha 4\beta 2$  nAChRs agonist treated group compared to CPB group (Figure 4B).

## **$\alpha 4\beta 2$ nAChRs activation ameliorates the NLRP3 pathway**

Expression of NLRP3, ZO-1 and Occluding protein was determined in the brain tissue of  $\alpha 4\beta 2$  nAChRs agonist and antagonist treated CPB induced brain injured rats. Expression of ZO-1 and occluding was reduced significantly and enhances the expression of NLRP3 in the brain tissue of CPB group than control group of rats. There was increase in the expression of ZO-1 and occluding protein and decrease in the expression of NLRP3 in the  $\alpha 4\beta 2$  nAChRs agonist treated group than CPB group. However treatment with  $\alpha 4\beta 2$  nAChRs antagonist further enhances the expression of NLRP3 and reduces the expression of ZO-1 and occluding proteins in CPB induced brain injured rats (Figure 5).

## **$\alpha 4\beta 2$ nAChRs activation ameliorates the pathological changes**

Effect of  $\alpha 4\beta 2$  nAChRs activation was observed on the histopathological changes in the hippocampal tissue of CPB induced brain injured rats as shown in Figure 6. Hippocampus tissue was observed to be normal structured with clear boundary and regular. In CPB group hippocampal neurons were found to be damaged with change in regular structure and cell band. However treatment with  $\alpha 4\beta 2$  nAChRs agonist reverses the histopathological changes occurs due to CPB induced brain injury (Figure 6).

## Discussion

Cardiopulmonary bypass (CPB) technology is used for the treatment of several types of cardiovascular disorder by surgery and reported to reduces the mortality occurs due to cardiovascular issues (Ranganath et al, 2019). However CPB reported to induces the injury to different organs of the body including brain (Kim et al, 2016). There is no specific treatment is available for the management of it, moreover exact pathogenesis remain to establish. Thus present report evaluates the role of  $\alpha 4\beta 2$  nAChRs in the development of CPB induced brain injury. Effect of  $\alpha 4\beta 2$  nAChRs agonist was determine on the neurological function in CPB induced brain injured rats. Level of cytokines, ROS and expression of NLRP3, ZO-1 and Occluding proteins were estimated in CPB induced brain injured rats. Effect of  $\alpha 4\beta 2$  nAChRs agonist was determined on the neuronal apoptosis and histopathological changes in the brain tissue.

Postoperative cognitive dysfunction was found to be higher in the geriatric patients, in which loss of social integration, language comprehension, concentration and memory (Berger et al, 2015). All these leads to loss of neurological function (Rundshagen, 2014) and data of the report also suggest it, as CPB group shows enhancement in the neurological score compared to control group of rats. However activation of  $\alpha 4\beta 2$  nAChRs with  $\alpha 4\beta 2$  nAChRs agonist reverses the neurological score to normal. Literature suggest that Ach release enhances in several conditions such as embolism, injury or infection by the activation of vagus nerve and activation of nAChRs reported to inhibit the release of cytokines and also enhances the formation of anti inflammatory factor (de Jonge and Ulloa, 2007). All these lead to produces the anti inflammatory property and data of presented report suggest that activation of  $\alpha 4\beta 2$  nAChRs reduces the level of cytokines in the serum of CPB induced brain injured rats.

Literature suggest that NLRP3 has a proven role in the neuronal dysfunction by enhancing the apoptosis through the activation of caspase pathway and NLRP3 inflammasome pathway reported to be involved in CPB surgery induced brain injury (Eren and Özören, 2019). ROS also contributes in the development of neuronal injury by regulating NLRP3 pathway (Abais et al, 2015). Result of the study suggests that level of ROS was reduced in the brain tissue of  $\alpha 4\beta 2$  nAChRs agonist treated group compaired to CPB group rats. Further treatment with  $\alpha 4\beta 2$  nAChRs agonist ameliorates the altered level of NLRP3 pathway in the brain tissue of CPB induced brain injured rats. In conclusion, Data of report suggest that treatment with  $\alpha 4\beta 2$  nAChRs agonist protects the brain injury in cardiopulmonary bypass surgery induced brain injury by regulating NLRP3 pathway.

## Declarations

## **Ethics approval and consent to participate**

Protocols used in the animals were approved by Institutional Animal Ethical Committee of First Hospital of Hebei Medical University, China (IAEC/FH-HMU/2018/05). Present investigation didn't include human participant and thus consent from the participant not applicable.

## **Consent for publication**

Not applicable

## **Availability of data and material**

The supporting data for present findings is under ethics restrictions and is hence not presented here.

## **Competing interests**

The authors declare no competing interests.

## **Funding**

Presented study got the funding from Hebei Province Health and Family Planning Commission Project (No: 20160698).

## **Acknowledgement**

All the author of this manuscript is thankful to First Hospital of Hebei Medical University, China for providing sufficient and necessary facility and Hebei Province Health and Family Planning Commission Project for providing fund to conduct the presented protocol.

## **Authors' contributions**

XL and LY performed the experimental work, collect the data and supported for the preparation of manuscript for presented study. LW involve in the statistical analysis and histopathology study. QG supervised and designed the work and drafted the manuscript, although all the authors contribute for the preparation of manuscript. Final manuscript is approved by all the authors of the presented report.

## **References**

- Abais JM, Xia M, Zhang Y, Boini KM, Li PL. (2015) Redox regulation of NLRP3 inflammasomes: ROS as trigger or effector? *Antioxid Redox Signal.* 22(13):1111-29.
- Albuquerque EX, Pereira EF, Alkondon M, Rogers SW. (2009) Mammalian nicotinic acetylcholine receptors: from structure to function. *Physiol Rev.* 89(1):73-120.

- Berger M, Nadler JW, Browndyke J, Terrando N, Ponnusamy V, Cohen HJ, Whitson HE, Mathew JP. (2015) Postoperative Cognitive Dysfunction: Minding the Gaps in Our Knowledge of a Common Postoperative Complication in the Elderly. *Anesthesiol Clin.* 33(3):517-50.
- de Jonge WJ, Ulloa L. (2007) The alpha7 nicotinic acetylcholine receptor as a pharmacological target for inflammation. *Br J Pharmacol.* 2007 Aug;151(7):915-29.
- Eren E, Özören N. (2019) The NLRP3 inflammasome: a new player in neurological diseases. *Turk J Biol.* 43(6):349-359.
- Grogan K, Stearns J, Hogue CW. (2008) Brain protection in cardiac surgery. *Anesthesiol Clin.* 26(3):521-38.
- Hausenloy DJ, Boston-Griffiths E, Yellon DM. (2012) Cardioprotection during cardiac surgery. *Cardiovasc Res.* 94(2):253-65.
- Hone AJ, McIntosh JM. (2018) Nicotinic acetylcholine receptors in neuropathic and inflammatory pain. *FEBS Lett.* 592(7):1045-1062.
- Ibrahim SM, Al-Shorbagy MY, Abdallah DM, El-Abhar HS. (2018) Activation of  $\alpha 7$  Nicotinic Acetylcholine Receptor Ameliorates Zymosan-Induced Acute Kidney Injury in BALB/c Mice. *Sci Rep.* 8(1):16814.
- Kim J, Villarroel JP, Zhang W, Yin T, Shinozaki K, Hong A, Lampe JW, Becker LB. (2016). The Responses of Tissues from the Brain, Heart, Kidney, and Liver to Resuscitation following Prolonged Cardiac Arrest by Examining Mitochondrial Respiration in Rats. *Oxid Med Cell Longev.* 2016:7463407.
- Lee JK, Blaine Easley R, Brady KM. (2008) Neurocognitive monitoring and care during pediatric cardiopulmonary bypass-current and future directions. *Curr Cardiol Rev.* 4(2):123-39.
- Ranganath NK, Nafday HB, Zias E, Hisamoto K, Chen S, Kon ZN, Galloway AC, Moazami N, Smith DE. (2019) Concomitant temporary mechanical support in high-risk coronary artery bypass surgery. *J Card Surg.* 34(12):1569–1572.
- Rundshagen I. (2014) Postoperative cognitive dysfunction. *Dtsch Arztebl Int.* 111(8):119-25.
- Salameh A, Dhein S, Dähnert I, Klein N. (2016) Neuroprotective Strategies during Cardiac Surgery with Cardiopulmonary Bypass. *Int J Mol Sci.* 17(11):1945.
- Sarkar M, Prabhu V. (2017) Basics of cardiopulmonary bypass. *Indian J Anaesth.* 61(9):760-767.
- Singh J, Budhiraja S. (2008) Partial nicotinic acetylcholine ( $\alpha 4\beta 2$ ) agonists as promising new medications for smoking cessation. *Indian J Pharmacol.* 2008 Oct;40(5):191-6.
- Zdanowski R, Krzyżowska M, Ujazdowska D, Lewicka A, Lewicki S. (2015) Role of  $\alpha 7$  nicotinic receptor in the immune system and intracellular signaling pathways. *Cent Eur J Immunol.* 40(3):373-9.

# Figures

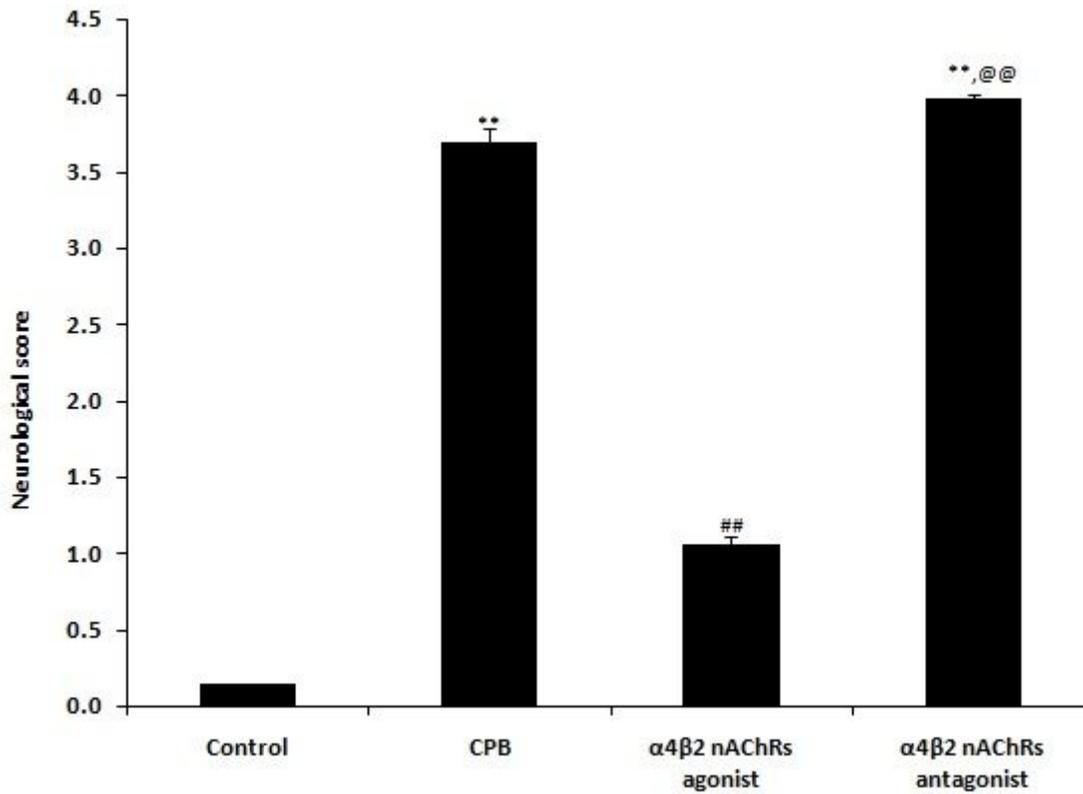
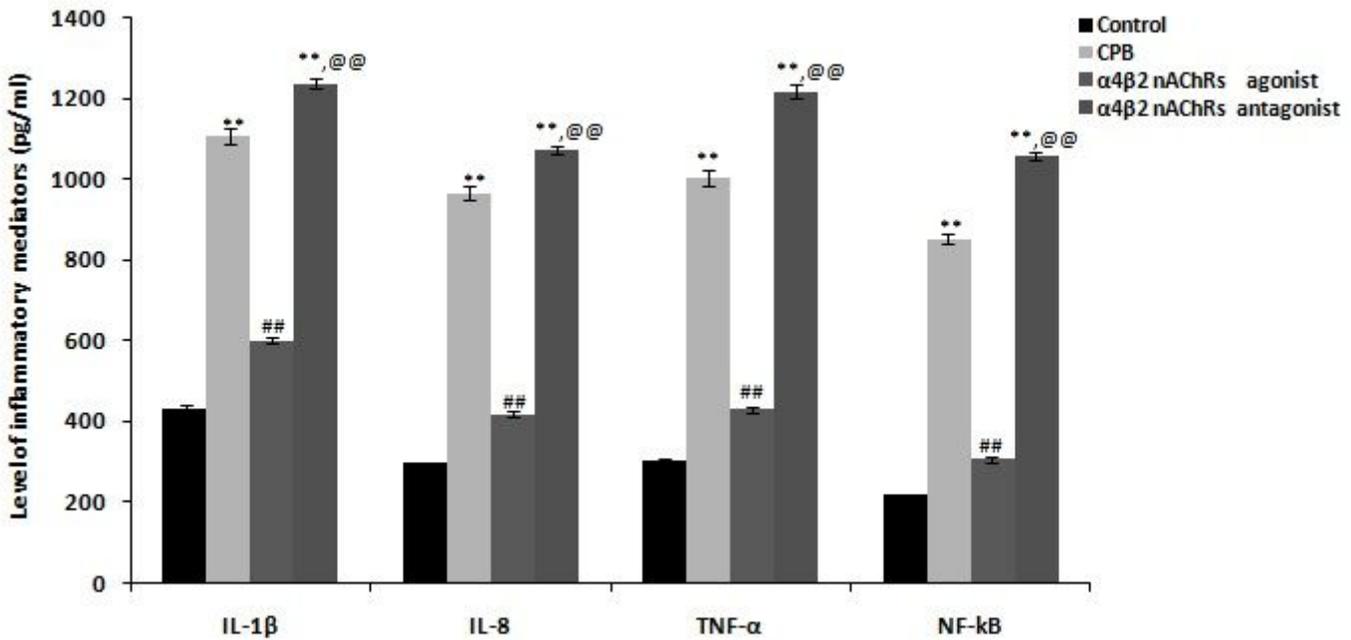


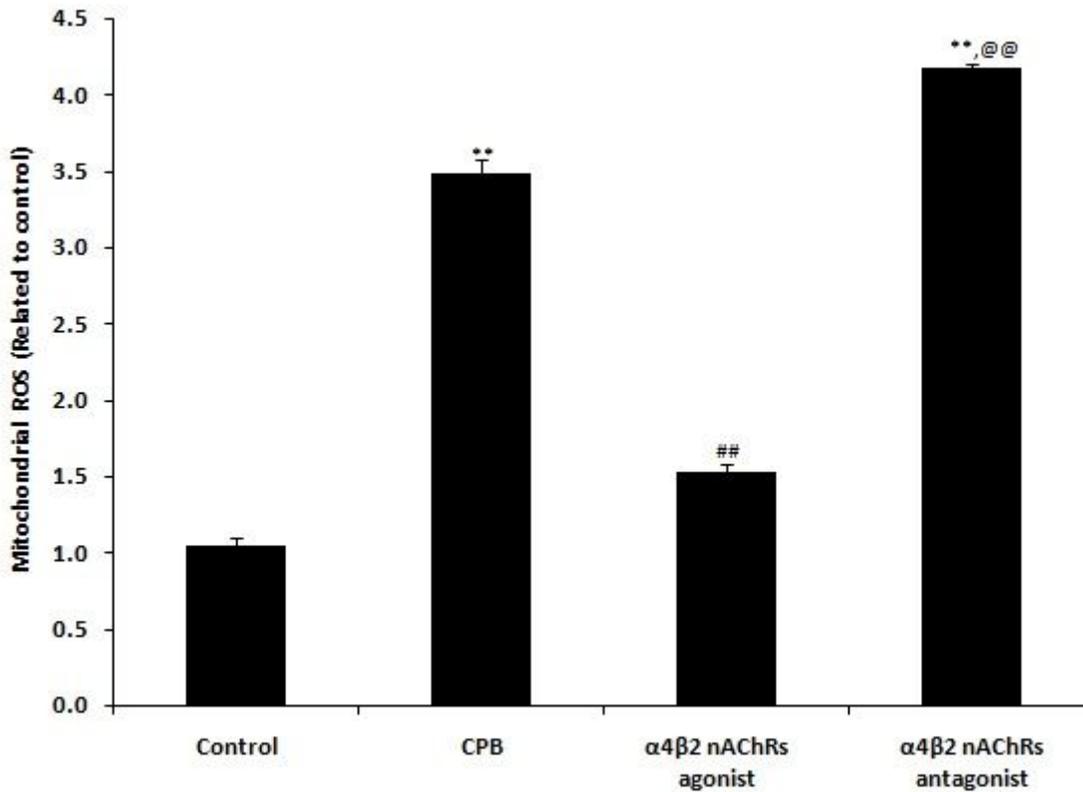
Figure 1

Effect of α4β2 nAChRs activation on the neurological function in CPB induced brain injured rats mean±SEM (n=10); \*\*p<0.01 compared to control group; ##p<0.01 compared to CPB group; @@p<0.01 compared to α4β2 nAChRs agonist



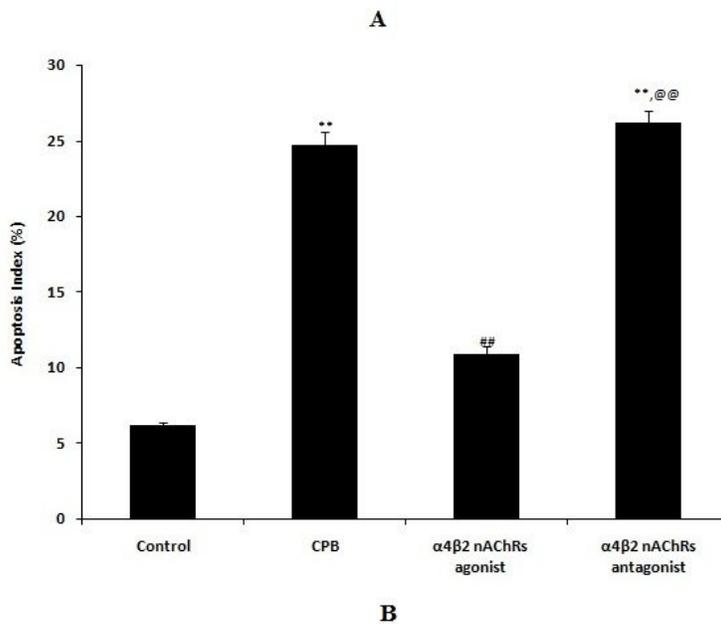
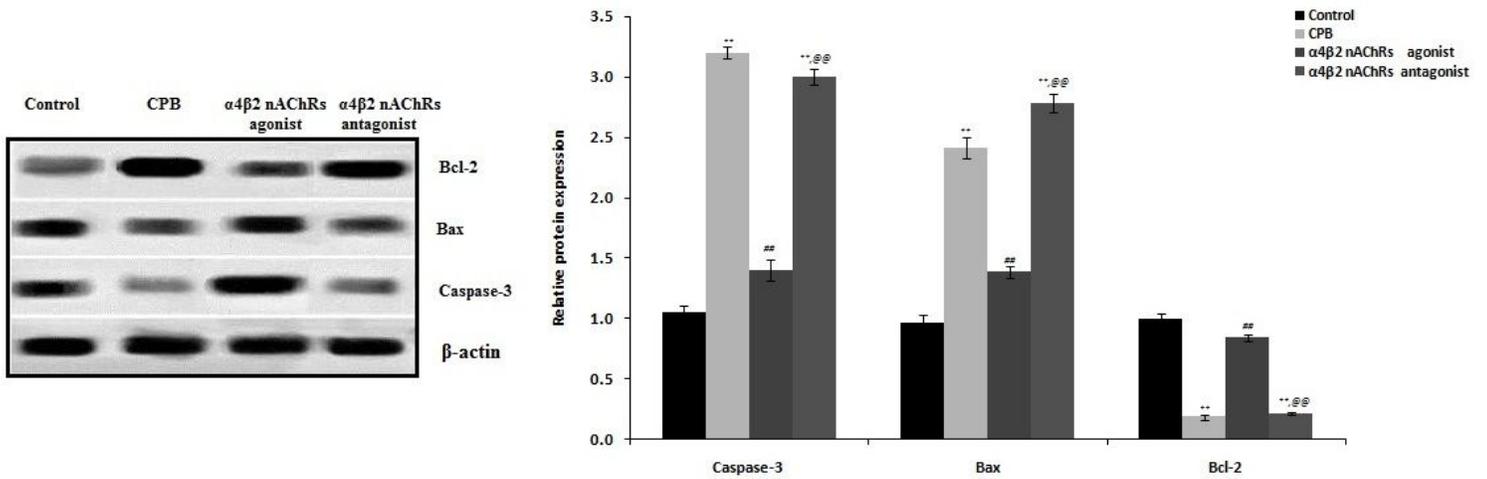
**Figure 2**

Effect of  $\alpha 4\beta 2$  nAChRs activation on the cytokine level in the serum of CPB induced brain injured rats mean $\pm$ SEM (n=10); \*\*p<0.01 compared to control group; ##p<0.01 compared to CPB group; @p<0.01 compared to  $\alpha 4\beta 2$  nAChRs agonist



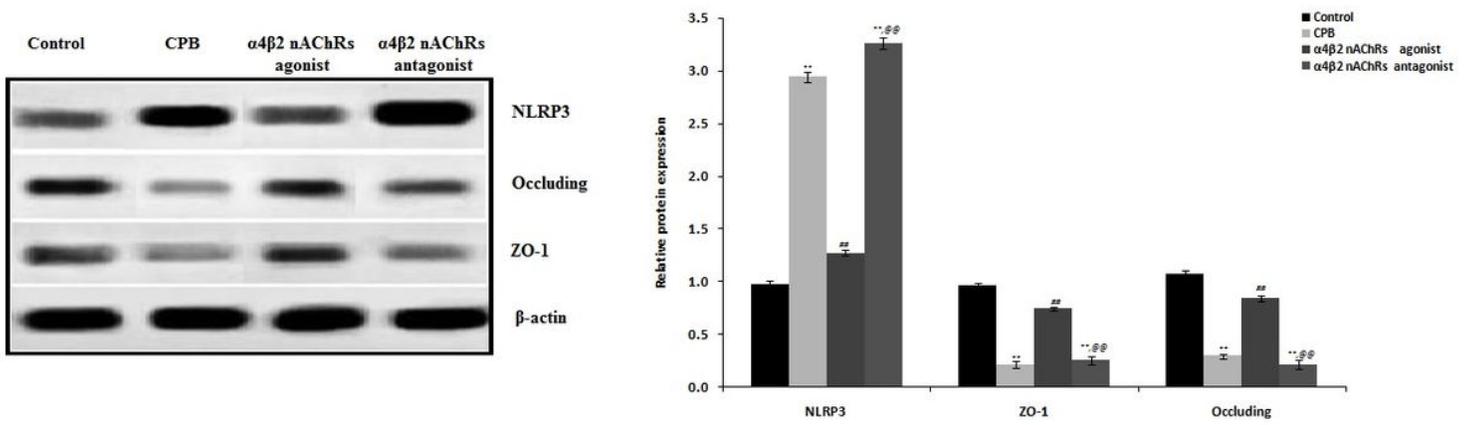
**Figure 3**

Effect of  $\alpha 4\beta 2$  nAChRs activation on the level of ROS in the brain tissue of CPB induced brain injured rats mean $\pm$ SEM (n=10); \*\*p<0.01 compared to control group; ##p<0.01 compared to CPB group; @p<0.01 compared to  $\alpha 4\beta 2$  nAChRs agonist



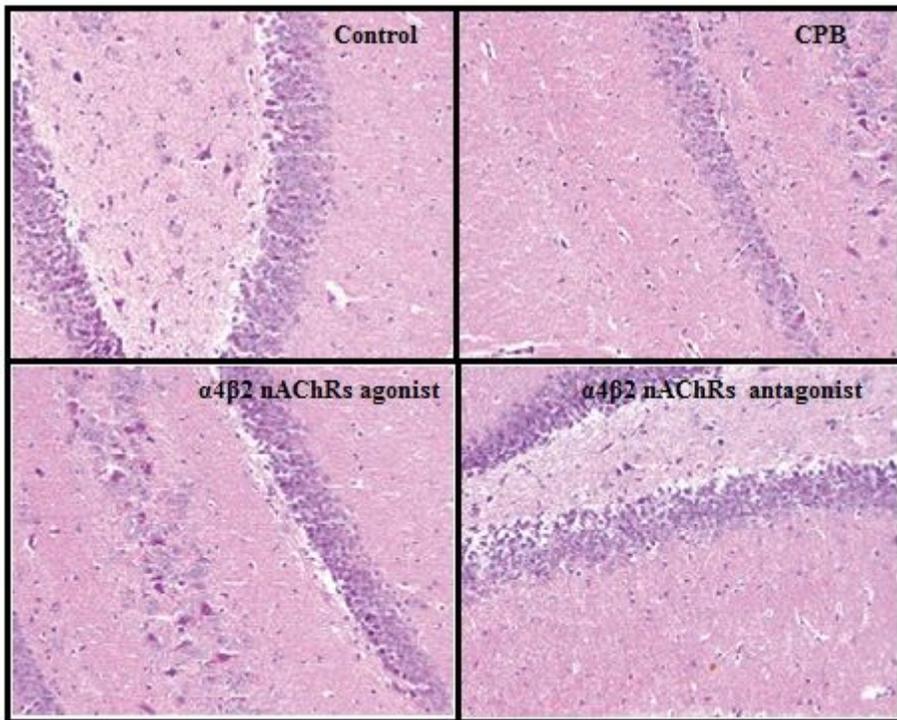
**Figure 4**

Effect of  $\alpha 4\beta 2$  nAChRs activation on the neuronal apoptosis in the brain tissue of CPB induced brain injured rats. A: Expression of Caspase-3, Bax and Bcl-2 protein in the brain tissue of CPB induced brain injured rats by western blot assay; B: Apoptosis index by TUNEL assay mean $\pm$ SEM (n=10); \*\*p<0.01 compared to control group; ##p<0.01 compared to CPB group; @@p<0.01 compared to  $\alpha 4\beta 2$  nAChRs agonist



**Figure 5**

Effect of  $\alpha 4\beta 2$  nAChRs activation on the expression of NLRP3, ZO-1 and Occluding protein in the brain tissue of CPB induced brain injured rats mean $\pm$ SEM (n=10); \*\*p<0.01 compared to control group; ##p<0.01 compared to CPB group; @p<0.01 compared to  $\alpha 4\beta 2$  nAChRs agonist



**Figure 6**

Effect of  $\alpha 4\beta 2$  nAChRs activation on the histopathological changes in the brain tissue of CPB induced brain injured rats