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Neurobehavioral Impacts of Xylazine and Piracetam Interaction on NADH Oxidase in the Rat Brain

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ABSTRACT

In the present study, the effects of different doses of xylazine combined with piracetam were investigated through different behavioral experiments. Xylazine is alpha 2 adrenergic agonist a veterinary sedative, and analgesic agent. Piracetam is gamma-aminobutyric acid, a nootropic compound, are known to affect brain function and behavior individually.

Design: Twenty adults female Wistar albino rats were randomized divided into four equal groups: control, xylazine (5 mg/kg i.p.) group, piracetam (600 mg/kg i.p.), xylazine and piracetam combination group, motor performance was assessed by Open field. Marble Burying, Wire hanging assay tests were done for cognitive and behavioral evaluation. and biochemical parameters NADH oxidase in the brain were done by ELISA.

Results: The results of neurobehavioral tests showed significant changes in the treated three groups compared to the control group. While, NADH oxidase level in the brain if there was a significant decrease in the Xylazine group, Piracetam group, and Xylazine & Piracetam group Comparison with the control group.

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INTRODUCTION

Analgesic drugs exert complex effects on the central nervous system (CNS), modulating pain perception while also influencing neural activity, mood, and cognition. Understanding these pharmacological actions is crucial for optimizing pain management [1,2]. Numerous studies have investigated the behavioral activity of laboratory rat, leveraging this animal model's well-established behavioral repertoire to advance pharmacological and neuroscientific understanding effectively [3]. The research methodologies encompass multiple stages, which include the observation and characterization of general manifestation, reflexes, animals' behaviors, locomotion, learning ability, sensorimotor function. In our investigation, we utilized Piracetam, Xylazine to explore potential behavioral alterations and analgesic effect.

Piracetam, synthesized and introduced for clinical use since 1972 used as a medicine in Alzheimer's disease and dementias. Is chemically described as 2-oxo-1-pyrrolidineacetamide. Structurally, it is derived from gamma-aminobutyric acid GABA [4]. As the pioneering member of the nootropic drug class, Piracetam can have analgesic and anti-inflammatory effects. It has been reported to enhance cognition, particularly in conditions of hypoxia, and to augment memory and learning capacities. Resulting in notable improvements in memory and learning processes. Despite its established pharmacological and therapeutic effects, the precise mechanism of action of piracetam remains elusive [5].

Xylazine is an α2 adrenergic receptor agonist, when administered parenterally to animals, induces sedation, analgesia, and muscle relaxation [6]. Its antinociceptive effects are mediated through the activation of presynaptic α2 receptors, which decreases the release of norepinephrine and dopamine, subsequently leading to central nervous system depression [7]. The objective of the research is to evaluate the neurobehavioral impacts that occur in experimental animals and explore pharmacological interactions between Xylazine and piracetam using biochemical measures. This is a result of the medications' central nervous system analgesic effects.

MATERIALS AND METHODS

Ethical approval

All procedures in this study were reviewed and approved by the Scientific Committee of the Faculty of Veterinary Medicine at the University of Kufa, in accordance with ethical principles of animal welfare, under reference number UK.VET.2023.27152.

Experimental animals

A total of, twenty female adult's Albino rats were used, their weights ranged between (180 - 300 g), the rats were housed in the animal house of the house of faculty of Veterinary Medicine / University of Kufa. They were maintained under standard water, feed, ventilation conditions, with 12:12 h. light/dark cycle, at 22-26 ˚C room temperature. They were divided into four equal groups G1 (control giving distal water), G2 group was administrated xylazine in dose 5 mg/kg B.W, the G3 group administrated Piracetam in dose 600mg/kg B.W, by injection intraperitoneal, the G4 group administrated xylazine 5 mg/kg B.W & piracetam 600 mg/kg [8,9].

Drugs and reagents

Xylazine HCl 20 mg/ml was obtained from Alfas A Company, Holland, Piracetam (Nootropil®) was obtained from UCB pharma, Turkey. Measuring Elisa kits for NADH oxidase were obtained from BT lab, China.

Behavioral experiments

By using four groups each group have 5 females. The groups were placed together in the same environment with consistent access to food and water to study the behavioral changes among them by;

Open field area

The open field test assesses the efficacy of neurobiological manipulations, as well as anxiety-related and locomotor effects, by recording the changes observed in rats in the cage number of square crossings by four legs of animal, rearing and defecation [10].

Marble burying

This test assesses perseverative behavior and anxiety by recording the number of marbles buried by rats in a clean cage [11].

Hanging wire test

This measure evaluates the forepaw strength of a rat's front paws, its ability to grasp, and records the time until it releases and falls [12].

Determination NADPH oxidase level in brain.

NADH oxidase was measured at 30 and 60 minutes using four groups, each consisting of five female rats. These groups were kept together in the same environment with consistent access to food and water. The rats received intraperitoneal injections, and at 30 and 60 minutes post-injection, the animals were euthanized using an overdose of Ketamine (90 mg/kg) Xylazine (10 mg/kg) [13]. The brain tissue was immediately extracted for molecular assessments. NADH oxidase activity was measured using a fluorometric assay kit. (ab273345, BT lab, China).

Statistical analysis

The statistical programs SPSS and GraphPad Prism 8 were used with a one-way ANOVA and were expressed as mean ± standard error [14]. The level of significant difference for all tests was at a probability level of less than 0.05 ($p \leq 0.05$).

RESULTS

Open field, the number of squares within 5 minutes decreased in the G3 group (36±2.67). while the decrease was significant in the G2 group (28±1.24) and in the G4 group (25±1.70) Comparison with the G1 group) 48±4.17). Also, the increase was significant in G3 group Comparison with G4 group **(Figure .1. A)**. There was also a significant decrease in the number of times standing on the hind legs in G2 group (2.8±0.96) and G3 group (5.4±1.16) and G4 group (3.4±0.67) Comparison with the G1group (14.6±1.88 **(Figure1.B)** As well as a decrease in the number of stool occurrences in G2 group (2.2±0.20), G3 group (1.6±0.40), G4 group (2±0.31), Comparison with the control group (2.4±0.24; **Figure 1.C).**

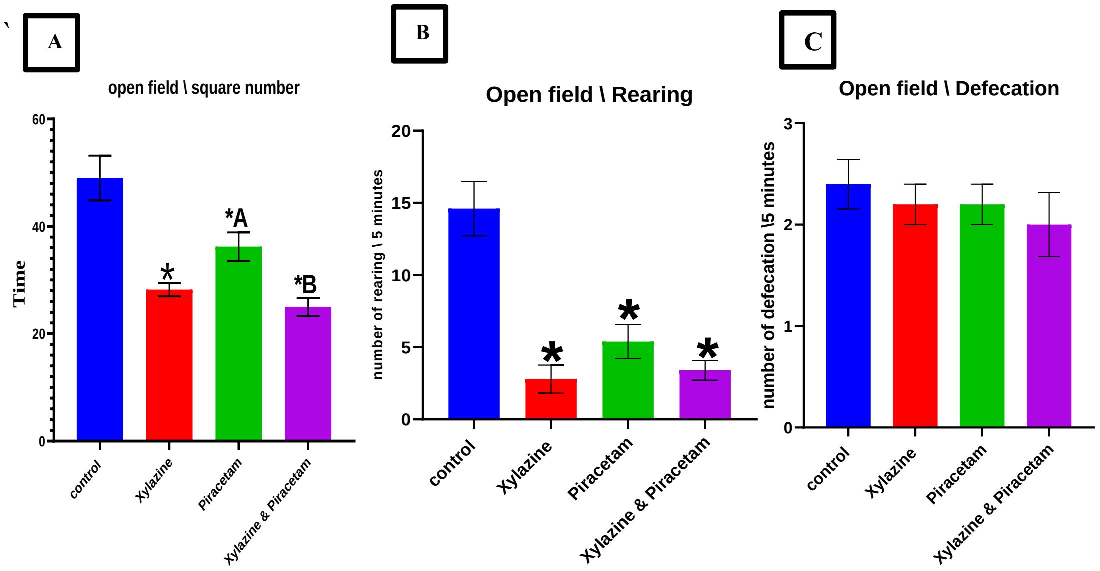

Figure 1. Explained the open field results A) number of square cross through 5 minutes. B) times of rearing during 5 minutes. C) number of defecations through 5 minutes.

The values represented mean ±SE for 5 rat/group Piracetam was injected alone (600mg/kg, i.p.) or with Xylazine (5 mg/kg, i.p.)..

(*): Significantly change with the control group at $P \leq 0.05$

(A): Significantly change with the Xylazine & Piracetam group at $P \leq 0.05$.

(B): Significantly change with the Piracetam group at $P \leq 0.05$

The marble burning test, there was a significant decrease in the number of moving balls during 5 minutes in a G2 group (4±0.63) and G4 group (3.6±0.74). comparison with the G1 group (7±1.51) There was also a decrease in G3 (5.6±0.60) compare with control **(Figure 2.A).** There was also significant decrease in the number of times standing on the hind legs during 5 minutes in G2 group

(3.8±0.80) and G3group (6.2±0.58), G4 group (4.40±0.87), Comparison with the G1 group (13.2±0.86). additionally, the decrease in G2 group was significant compare with G3 group. **(Figure 2.B).** In wire suspension test there was decrease in time required to suspended during 2 minutes in G3 group (84±15), also there was a significant decrease in G2 group (67±11.4) and G4 group (58±13.9), comparison with the G1 group (113±7) **(Figure. 3)**

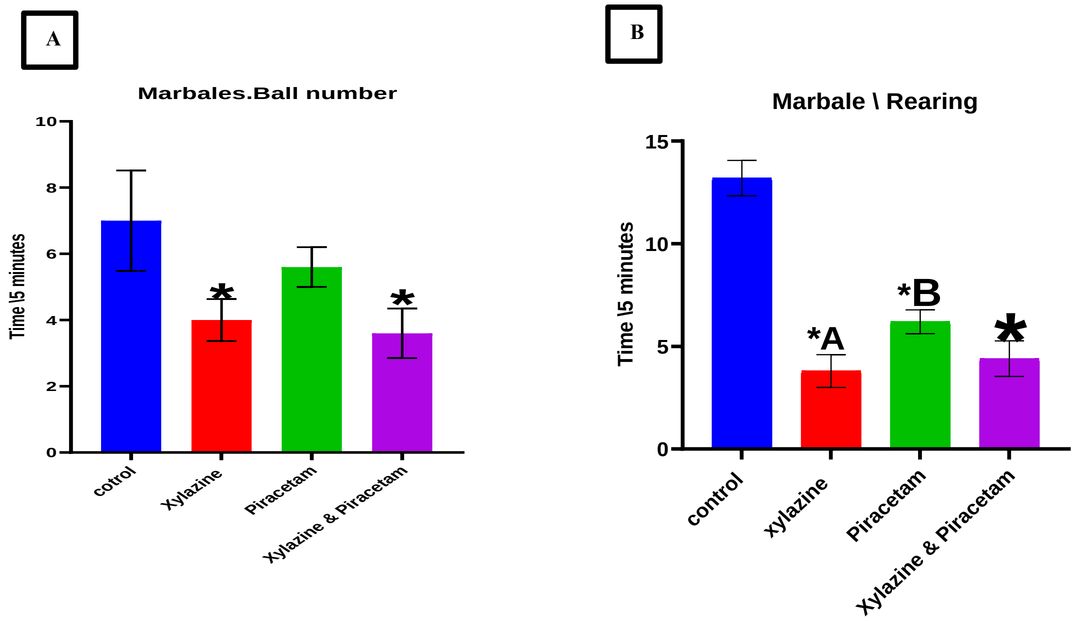

Figure 2. Show the results of Marbale buyring test A) number of ball moved through 5 minutes. B) times of rearing during 5 minutes.

The values represented mean ±SE for 5 rat/group Piracetam was injected alone (600mg/kg, i.p.) or with Xylazine (5 mg/kg, i.p.).
(*): Significantly change with the control group at P ≤0.05
(A): Significantly change with the Piracetam group at P ≤0.05.
(B): Significantly change with the Xylazine group at P ≤0.05.

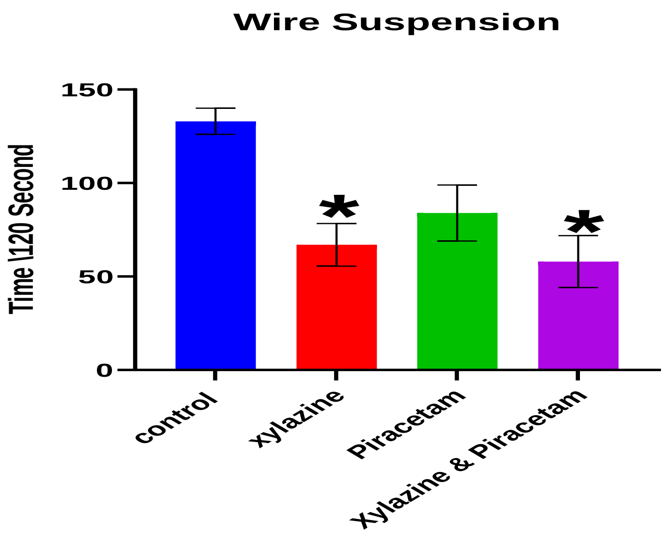

Figure 3. Show the result of wire suspension test

(*): Significantly change with the control group at P ≤0.05

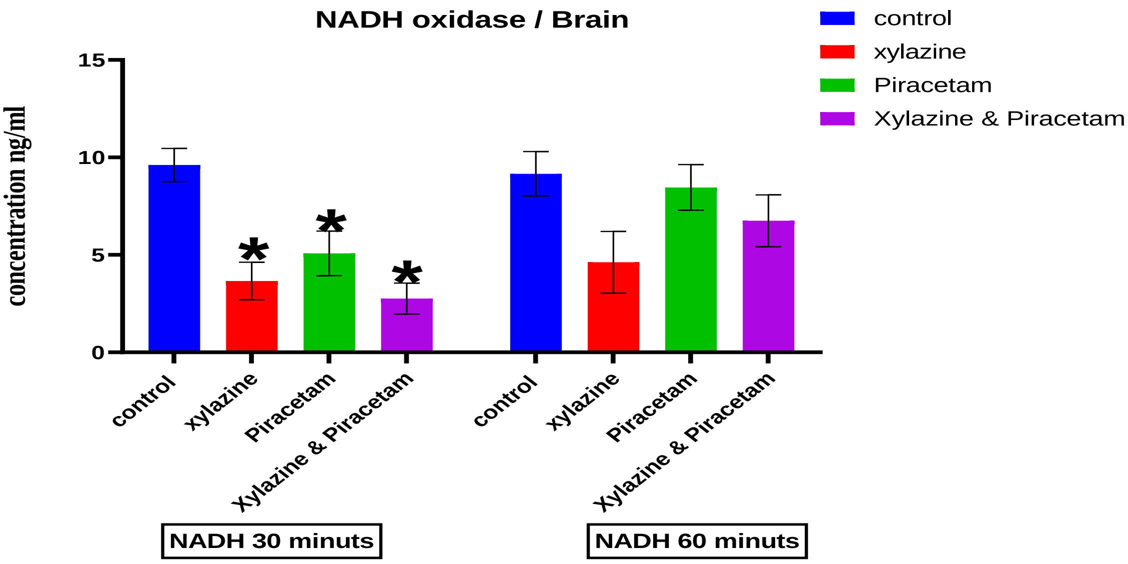

Figure 7. Determine the effects of Xylazine and/ or with Piracetam after 30,60 minutes of injection on brain (NADH) oxidase level.

The values represented mean ±SE for 5 rat/group Piracetam was injected alone (600mg/kg, i.p.) or with Xylazine (5 mg/kg, i.p.).
(*): Significantly change with the control group at P ≤0.05

DISCUSSION

The interaction between xylazine and piracetam produced complex neurobehavioral outcomes. Xylazine-induced sedation and anxiogenesis were partially alleviated by piracetam, suggesting that piracetam can modulate the neurobehavioral effects of xylazine [15][16].

The cognitive impairments induced by xylazine were also mitigated by piracetam, indicating potential therapeutic benefits of combining these agents in scenarios where both sedation and cognitive function need to be managed. The beneficial effects of piracetam mitigate aging-induced alterations in brain structures that affect cognitive functioning [17]. These effects include increased plasma membrane fluidity, improved erythrocyte rheology, and reduced platelet aggregation, which enhance vascular perfusion and improve mitochondrial function and dynamics, as well as providing antioxidant properties [18][19]. In rat primary cortical cells subjected to oxygen and glucose deprivation, piracetam reduced neuronal damage and exhibited antioxidant effects [20].

Xylazine inhibits the release of neurotransmitters, such as norepinephrine and glutamate, which are involved in the transmission of signals from motor neurons to skeletal muscles. Piracetam binds selectively to a synaptic vesicular protein ' SV2 A', and this may reduce the release of the excitatory neurotransmitter glutamate during trains of high-frequency activity. and/ or GABA across the synapse, thereby exeing anti-seizure effect [21].

Xylazine-induced changes in NADH oxidase activity may be attributed to direct interactions with the enzyme or indirect effects mediated through α2-adrenergic receptor signaling pathway [22]. Xylazine has been reported to modulate mitochondrial respiration and oxidative phosphorylation in various tissues, including brain, and liver [23]. The increase in NADH oxidase serum concentration in brain reported in this study may be caused by the mechanisms by which piracetam modulates NADH oxidase activity in the brain are not fully understood but may involve direct and indirect effects on mitochondrial function and cellular signaling pathways[24] .

CONCLUSION

The level of sedation and analgesia increases with the combination of piracetam and xylazine. Additionally, in comparison to either medication alone, through neurobehavioral effects

CONFLICT OF INTEREST

Regarding the publication of this article, the authors have declared that they have no conflicts of interest.

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