Neuroprotective Effect of Vinpocetine against Lead Acetate-Instigated Neurotoxicity in Rats by Evaluation Tumor Necrosis Factor-Alpha, Interleukin-1Beta and Interleukin-10

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Abstract
Lead toxicity elicits neurological damage which is a well-known disorder that has been considered to be a major cause for multiple condition such as behavioral defect; mental retardation; and nerve insufficient activity.

This research is designed to estimate potential protective effect of vinpocetine on neurotoxicity stimulated by lead acetate in rats.

Eighteen adult rats of both sexes were randomly enrolled into three groups. Each group includes 6 rats as followings: Group I- Rats were given 0.3ml normal saline solution orally; then intraperitoneal injection of 100μl of the normal saline was given 1h later; this group was considered as control. Group II- Rats were given an intraperitoneal injection of 20mg/kg lead acetate for 5 days. Group III- Rats were orally given 3mg/kg vinpocetine, which was given 1hr before [(the IP injection of Pb every 24 hours at a dose of 20mg/kg) for 5 days and continued for 10 days]. On 11th day of the study, the brain of each animal has been surgically cut to make homogenate preparation to estimate tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-10 (IL-10) levels.

Lead significantly elevated TNF-α and IL-1beta; while, it significantly decreased IL-10 levels. Vinpocetine significantly minimized IL-1beta and TNF-α; furthermore, vinpocetine significantly raise IL-10 levels at (P<0.05).

Vinpocetine may have a neuro-protective activity against lead-stimulated toxicity brain of rats.

Keyword: Lead, Vinpocetine, Rats, Neuroprotective, Cytokines.

The Institutional Review Board of the College of Pharmacy, University of Basrah, Iraq approved the experimental protocol. The study was conducted in accordance with the guidelines of the European Communities for the care and use of laboratory animals. Each animal was treated humanely and did not suffer in any way that was not necessary for the purpose of the study.

The author declares that there is no conflict of interest.

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Introduction

Exposure to Lead (Pb) can occur through many routes involving contaminated water, air, food, soil, and other public pathway; and the secure threshold for Pb exposure has not been specified, as there is no accurate amount for toxicity of such element (1). Pb is available in various forms and is considered as a basic ingredient of different organic compounds, which have been directly penetrated to skin, respiratory, and brain; where, toxic effect of central nervous system is considered as a dominant effect of such element (2). Thus; Pb can cause significant public health problems, although its concentration in ecosystem has been decreased after several trials (3). Researchers published that neurological destruction that stimulated by Pb toxicity can urge various disorders like mental defect, Alzheimer’s disease (AD), behavioral problems, loss of nerve activity, Parkinson’s disease (PD), and probably schizophrenia (4); Pb has the ability to pass through blood-brain barrier (BBB) and can substitute calcium (Ca$^{2+}$) ions. Accordingly, such element can interfere with activity of Ca$^{2+}$ on cell functions and perturb several biological actions (5). The pro-inflammatory pathway of neurotoxicity instigated by Pb has not been completely evaluated (6,7).

Vinpocetine is an alkaloid vincamine derivative. In many countries, vinpocetine has been utilized for the treatment of central nervous system disorders such as stroke and dementia for more than 30 years. Up to date, It is also obtainable in the market as a nutrition supplement to boost memory and cognition. The safe and marvelous activity of vinpocetine result in discovering the novel remediation and mechanism actions of it in disease pattern and diverse cell types (8). Vinpocetine, has boosting the cognitive function that it has been utilized as a nootropic agent for patients with central nervous system disorder; where, it increases glucose uptake and cerebral blood flow (9). Moreover, it can reduce the peril of strokes and temporary ischemic attacks in chronic cerebrovascular insufficiency patients (10). As well as, vinpocetine is an efficacious antioxidant and then inhibit lipid peroxidation (11). Furthermore, such drug exhibit memory-protective and memory-boosting properties and potent anti-inflammatory activity (12). Besides, vinpocetine is a phosphodiesterase-1 ([PDE]-1) inhibitor (13) and a blocker of voltage-gated Na$^+$ channels (14). Previously, in vitro studies approved that vinpocetine inhibited the blockage of the mitochondrial complexes (II, III, and IV) as well as entirely negated the deduction of pyruvate levels and the assemble of free radical-stimulated by noxious concentrations of amyloid peptides in PC12 cells (15). It is a potential choice for the management of various neurodegenerative diseases that related to cognitive improvement properties and the anti-inflammatory effect of vinpocetine (16).

The current study is designed to estimate probable protective action of vinpocetine against neurotoxicity instigate by Pb in rats through the estimation of tumor necrosis factor-alpha (TNF-alpha), interleukin-10 (IL-10) and, interleukin-1beta (IL-1β) levels in brain tissue homogenate.

Materials and Methods

Experimental animals

Eighteen male and female Albino adult rats (weighing 160-250gm) were selected for this study. Rats were obtained from House Animal for College of Pharmacy, Basra University. Commercial pellets and tap water ad libitum were dependent in feeding of rats during experiment period.

Materials

Lead acetate powder was purchased from Fluka Chemical, Turkey. Vinpocetine pure powder was purchased from America medic science (USA).

Experimental design

Adult rats were randomly distributed into three equal groups (6 animals for each group) as follows: Group I - each rat was given 0.3ml normal saline orally for 10 days, 5days before IP injection of normal saline and then 100μl of the normal saline solution injected IP 1hr later for 5day; this group considered as control. Group II - each rat was given 0.3ml normal saline orally for 10 days, 5days before IP injection of pb acetate which freshly prepared (20 mg/kg/day body wt.) for 5days (17). Group III - Each rat was orally given 3mg/kg/day vinpocetine (dissolve in normal saline for 5 days by oral gavage before starting Pb injection and continued for 10 days; where it was given 1hr before Pb, which was injected IP every day at a dose 20 mg/kg for 5 days (18).

Twenty four hour after the end of the treatment duration; i.e. at day six, each animal was euthanized by diethyl ether, and then by cervical dislocation. Thereafter, the skull was crushed by surgical scissor and then the brain of each rat has been cutout surgically for homogenate preparation.

Preparation and estimation of homogenate biochemical parameters

The preparation of brain tissue homogenate involved removal of excess blood by rinsing in ice-cold phosphate buffer saline (PBS,pH=7.4), followed by desiccation using filter paper and then measuring the weight of each brain tissue before homogenization was performed. Then each of rats' brain tissue minced to small pieces and put in 15ml plastic test tube containing chilled PBS solution (pH=7.4); where ratio of tissue weight in g to PBS volume in mL is 1:9. Homogenization was performed using cell lab homogenizer in icy condition. Then, the homogenate was centrifuged for approximately 15 minutes at 2000xg. The supernatant liquid was accurately collected and kept...
at -20 °C until the time for the evaluation of TNF-α, IL-1β, and IL-10 cytokines levels by automated biochemistry analyzer (Elabscience, USA) (19).

**Statistical analyses**

Data were explicated as mean±standard error (SEM). ANOVA –post hoc test was utilized for estimating the significant difference among groups. Differences were statistically considerable for \( P \) value less than 0.05 (\( P<0.05 \)).

**Results**

**Effect of vinpocetine against lead acetate on tumor necrosis factor-alpha (TNF-α) in rats’ brain tissue homogenate.**

Table 1 and figure 1 showed that rats injected with 20 mg/kg of Pb acetate IP every day for 5 days lead to a significant elevation in the level of TNF-α in homogenate tissue of brain compared to those level in control rats. The TNF-α level in brain tissue homogenate were respectively, 307.5±17.1 and 83.3±5.3.

Furthermore, there were significant reduction in TNF-α level in homogenate tissue of brain in group of rats treated with 3mg/kg vinpocetine prior to 20 mg/kg of Pb acetate compared to the corresponding level in group of rats injected IP with 20 mg/kg of Pb acetate every day for 5 days . The TNF-α level in brain tissue homogenate were respectively, 173.3±4.4 and 307.5±17.1.

**Effect of vinpocetine against lead acetate on interleukin-1beta (IL-1β) in rats’ brain tissue homogenate.**

Table 1 and figure 2 showed that rats injected with Pb acetate every 24hours at a dose of 20mg/kg for 5 days IP led to significant rising in the level of IL-1β in homogenate tissue of brain compared to control rats. The level of such cytokine in homogenate tissue of brain were respectively, 125.5±6.6 and 61.3±4.9.

Moreover, table 1, and figure 2 showed that, there were significant reduction in IL-1β level in homogenate tissue of brain for the groups of rats treated with 3mg/kg vinpocetine prior to 20 mg/kg Pb acetate compared to corresponding levels in group of rats injected IP with 20 mg/kg of Pb acetate every day for 5 days. The level of IL-1β in homogenate tissue of brain were respectively, 84±3.9 and 125.5±6.6.

**Effect of vinpocetine against lead acetate on interleukin-10 (IL-10) in rats’ brain tissue homogenate.**

Table 1 and figure 3 showed that rats injected with 20 mg/kg of Pb acetate every day for 5 days IP, there was a significant reduction in the level of IL-10 in brain tissue homogenate compared to those levels in control rats. The level of IL-10 in homogenate tissue of brain were respectively, 40±2.8 and 205±7.6.

Furthermore, there were significant raising in the level of IL-10 in homogenate tissue of brain in rats treated with 3mg/kg vinpocetine prior to 20 mg/kg lead acetate compared to corresponding levels in group of rats injected with 20 mg/kg of Pb acetate every day for 5 days IP. The level of IL-10 in homogenate tissue of brain were respectively, 63.8±3.9 and 40±2.8.

Table 1. Effect of vinpocetine on TNF-α, IL-1β, and IL-10 levels in brain tissue homogenate of rats after IP injection of lead acetate

<table>
<thead>
<tr>
<th>Treatment Groups n=6</th>
<th>Treatment Type</th>
<th>TNF-alpha (pg/ml) (Mean±SEM)</th>
<th>IL-1Beta (pg/ml) (Mean±SEM)</th>
<th>IL-10 (pg/ml) (Mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Negative control/normal saline</td>
<td>83.3±5.3</td>
<td>61.3±4.9</td>
<td>205±7.6</td>
</tr>
<tr>
<td>II</td>
<td>lead acetate (20 mg/kg)</td>
<td>307.5±17.1( ^a )</td>
<td>125.5±6.6( ^a )</td>
<td>40±2.8( ^a )</td>
</tr>
<tr>
<td>III</td>
<td>3mg/kg vinpocetine prior to 20 mg/kg of lead acetate</td>
<td>173.3±4.4 ( ^b )</td>
<td>84±3.9 ( ^b )</td>
<td>63.8±3.9 ( ^b )</td>
</tr>
</tbody>
</table>

* Mean significant different compared to control rats at P<0.05.
Differences letters mean there are significant different in the same column at P<0.05.
Neuroprotective effect of vinpocetine

In developing animals, Pb can cross blood-brain barrier (BBB) and deactivate the basic structural components by damaging the brain glial cells. Moreover, such element stimulated devastation that mainly-occur in most area of brain that include cerebellum, cerebral cortex, and hippocampus, that may consequently cause morphological change in the brain. The destructive effect of lead may be related to its creation of ROS or its confliction with calcium inactivation of protein kinase C (PKCs), which may have a critical role in signal transduction, differentiation and cell development. Furthermore, lead vies with calcium for prevalent binding sites and is integrated into neurotransmission systems of calcium.

The major findings of previous studies that approved raising cytokine creation and axonal destruction with astrocytic activation by Pb effect in immature rat brain. Furthermore, researchers mentioned that Pb can cause increment in level of inflammatory cytokines.

Glial cells have basic role in local inflammatory processes by creation cytokines such as TNF-α, IL-1β, IL-6. Furthermore, the neuroinflammation was controlled by activation of glial cells which participated in destructive and progression of several disorders. In Alzheimer's disease, inflammatory and oxidative induction effect that were created by chronically glial cells activation which result destruction of neurons.

In this study, IP injection of Pb acetate every 24 hours (20mg/kg) for five days to rats in group II, significantly increase level of inflammatory markers such as TNF-α and IL-β levels of these cytokines, and a significant reduction in IL-10 level each when compared with control group rats at (P<0.05). Results of this work are agreed with previously-mentioned studies.

Furthermore, this study showed the preventive effect of vinpocetine on inflammatory pathway of brain through suppressing the TNF-α and IL-1β elevation; where, oral-administration of vinpocetine for 5 days prior to IP injection of lead acetate for 5 days and vinpocetine continued for 10 days in group III significantly decrease levels of inflammatory markers such as cytokines TNF-α and IL-β levels, but with significant increase in IL-10 level when compared with those levels in rats IP injected with Pb acetate at (P<0.05). Researchers reported that, in the CNS; where, the PDE inhibitor (vinpocetine) can down-regulate the following inflammatory cytokines [TNF-α, IL-1, and IL-6], however, it can up-regulate the suppressor cytokines such as IL-10 due to the effects of lipopolysaccharides.

Conclusion

Vinpocetine may have a neuro-protective action against lead-instigate neurotoxicity in rats.
Acknowledgements
This is the first study to estimate in vivo neuro-protective action of vinpocetine on lead-instigate neurotoxicity in rats.

Competing interests
There are no competing interests to declare.

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